

TURKISH JOURNAL OF FIELD CROPS

Published by the Society of Field Crop Science

Indexed and Abstracted in: Science Citation Index, WOS Core Collection, (SciSearch)
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Publication Date

Turkish Journal of Field Crops is published twice a year (June and December).

Indexing and Abstracting

Turkish Journal of Field Crops is indexed and abstracted in: Science Citation Index (SciSearch), WOS Core Collection (Clarivate), Journal Citation Reports/Science Edition. In CABI Abstracts: Crops Physiology Abstr., Field Crops Abstr., Grasslands and Forage Abstr., Plant Breeding Abstr., Seed Abstr. and the Ulakbim of the Scientific and Technological Research Council of Turkey.

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Printer (Dizgi ve Baskı)	:	OFİS-SER Matbaacılık LTD Gediz Cad. No:12/B, Bornova/Izmir
Tel	:	+90 232 311 1428 +90 232 343 3474
Date of Publication (Basım Tarihi)	:	June, 2025

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The Editorial Board of the Turkish Journal of Field Crops would like to express its sincere thanks and appreciations to the referees and reviewers listed below who reviewed and evaluated the manuscripts sent for publication in this issue
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Reddy, V. R. (1995). Modeling ethephon-temperature interactions in cotton. *Computers and Electronics in Agriculture*, 13, 27–35. [https://doi.org/10.1016/0168-1699\(95\)00012-S](https://doi.org/10.1016/0168-1699(95)00012-S)

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IBAN: TR69 0006 4000 0013 3760 3716 73

For US dollar payments, bank account information is sent via email.

PUBLICATION POLICY

This publication policy statement outlines policy and procedures relative to the Turkish Journal of Field Crops.

1. General Policy

All material intended for publication by the Society should be written in English. Manuscripts for the Journal should be sent to the Editor in Chief.

2. Editor-in-Chief

The Editor in Chief makes recommendations to the Governing Board for the appointment of the editors and serves as a Chairman of the Editorial Board.

3. Editorial Board

Editorial Board, consisting of the Editor-in-Chief and Editors, prepares the Journal. Editorial Board develops procedures for manuscript submission, review and referee criteria, acceptance, release and publication. The Editor in Chief delegates editorial functions to other members of the Editorial Board. The Editor in Chief also processes review and interpretive papers and handles the appeal procedure for rejected manuscripts. The Editor-in-Chief may write editorials or solicit manuscripts on special topics.

4. Editor

Editors are appointed for specific subject matter areas and are responsible for the technical and intellectual content of the journal in these areas. They supervise the registering of manuscripts and other record-keeping activities and direct the work of the assigned referees and Editors in reviewing and evaluating manuscripts submitted to the Turkish Journal of Field Crops.

Editors are responsible for obtaining a minimum of two reviews for each manuscript assigned to them. Each review is an evaluation of the intellectual content of the manuscript for publication. The following steps will be followed for prompt reviews.

a. Editors are expected to act as primary reviewer for papers close to their area of expertise.

b. Editors are encouraged to contact prospective reviewers and request a return of the reviewed paper within two weeks.

c. Editors are advised to read papers carefully before sending them out for review. Editor is encouraged to be one of the primary reviewers. The goal is to have two quality reviews of the manuscript.

5. Manuscript Handling

Three copies of manuscripts should be submitted to the receipt of the Editor in Chief. The Editor notifies the corresponding author of the receipt of the manuscript, sends permission to print and reprint the form to the author, and assigns the registration number to the manuscripts. The registration number must be used in all correspondence regarding the manuscript. The Editor in Chief assigns manuscripts to the Editors on the basis of subject matter. The Editors, in turn, assign manuscripts to the referees and reviewers.

If they recommend publication without change and the Editor agrees, the manuscript and reviewers report are sent to the Editor in Chief for concurrence.

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The editor-in-chief could also assign manuscripts to the editors and two referees. If two referees accept, the manuscript is accepted for publication after the Editor approves it.

If the reviewers and the Editor find the manuscript could be published after some revision, the manuscript is returned to the author to obtain a satisfactory revision. If the author does not return a revised manuscript, the first released manuscript must be submitted to the editor for additional consideration by the journal.

If the reviewers and Editor recommend rejection, the manuscript and reviewers' comments are sent to the Editor in Chief. If the Editor in Chief concurs that the manuscript should be rejected, the manuscript is released to the author. The author of a manuscript rejected has the option of appealing the release to the Editor in Chief. In appealing the release, the author must provide the Editor in Chief with a clean copy of the released version of the manuscript. All editorial correspondence and a letter are stating the reasons why the author is appealing the release.

7. Notes

Short papers covering experimental techniques, apparatus and observations of unique phenomena are published as notes. Review procedures for notes are the same as those for regular articles. The format for notes with less than two printed pages is less formal than that for full-length articles.

8. Letters to the Editor

The journal publishes Letters to the Editor. Letters may contain comments on articles appearing in the Turkish Journal of Field Crops or general discussion about crop science research and are limited to two printed pages. If a letter discusses a published paper, the author of that paper may submit a response to the comments. The Editor-in-Chief must approve published papers and may receive a peer review.

Effect of Slow-Release Nitrogen Fertilizer with NBPT Inhibitor on Popcorn (*Zea mays* L. *everta*) Yield and Quality

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ARTICLE INFO

Research Article

Received: 6 November 2024

Accepted: 10 March 2025

Published: 23 June 2025

Keywords:

Grain yield

NBPT

Popcorn

Popping volume

Slow release nitrogen

ABSTRACT

This study was conducted to determine the effect of slow-release nitrogen with NBPT inhibitor [(N-(n-butyl) thiophosphoric triamide (NBPT)] fertilizer dosages on yield, yield components and quality of popcorn (*Zea mays* L. *everta*) under second crop conditions of Şanlıurfa in 2022 and 2023. The experiment was conducted in a randomized block trial design with three replications. The R-997 popcorn variety was employed as the crop material in the research. Slow-release urea fertilizer doses with NBPT inhibitor were utilized at the following rates: control, 40 kg ha⁻¹, 80 kg ha⁻¹, 120 kg ha⁻¹, 160 kg ha⁻¹, 200 kg ha⁻¹, 240 kg ha⁻¹, 280 kg ha⁻¹, 320 kg ha⁻¹, respectively. The analysis of variance revealed that slow-release nitrogen dose applications had a statistically significant impact on several key traits, including the number of kernels per ear, ear diameter, ear length, thousand grain weight, and grain yield ($P \leq 0.01$). Furthermore, some quality parameters, including protein ratio, popping volume and unpopped kernel ratio, were also found to be statistically significant ($P \leq 0.01$). It was determined that grain yield, yield components and quality parameters increased with increasing slow-release nitrogen (N) doses. However, this increase continued up to 240 kg ha⁻¹ nitrogen application. The analysis of regression indicated that the highest grain yield was achieved with the inclusion of the slow-release N with NBPT inhibitor at a dosage of 240 kg N ha⁻¹, while the lowest grain yield was observed in the treatment where no nitrogen was applied. In similar regions with high evaporation and hot climates where the experiment was conducted, the use of slow-release nitrogen fertilizers may be recommended. Also this study demonstrates that NBPT can be a promising companion to nitrogen fertilizer, as no adverse effects were observed with increasing levels of slow-release nitrogen applications.

Citation: Öktem, A.G., Ağa kesen, M.N., & Öktem, A. (2025). Effect of slow-release nitrogen fertilizer with NBPT inhibitor on Popcorn (*Zea mays* L. *everta*) yield and quality. *Turkish Journal of Field Crops*, 30(1), 1-10. <https://doi.org/10.17557/tjfc.1580477>

1. INTRODUCTION

The world average nitrogen uptake efficiency is approximately 50%, although this figure can vary according to production management practices and crop varieties (Ibrikci et al., 2012). It has been stated that unconscious and excessive fertilizer use not only causes economic losses, but is also an important factor in terms of sustainable soil fertility (Karaman et al., 2007).

The response of crops to fertilizers is dependent upon many factors, including the fertility level of soils, other environmental conditions and genotypes. Nitrogen is a vital element for the vegetative growth, yield, and quality of corn (Yigit et al., 2024). The majority of nitrogen is lost through many processes, including leaching, denitrification, volatilization, soil erosion and immobilization (Cahill et al., 2010).

Losses of gaseous nitrogen (N) to the atmosphere can occur, for example, in the form of greenhouse gases (GHG), nitrous oxide (N₂O) or - especially - the reactive gas ammonia (NH₃) (Guardia et al. 2021). Slow-released fertilizers prevent nitrogen losses in gas form and increase the efficiency of the utilization of fertilizer (Canterella et al., 2018).

Controlled-release fertilizers ensure that conventional fertilizers are coated or microencapsulated, making them continuously available to the plant (Morales-Morales et al., 2019). The long-term effects of slow-release fertilizers, which have been used in recent years, are currently being investigated in a variety of plant species (Guerdal, 2009; Uçgun et al., 2021; Denizhan et al., 2021;). The dissolution rate of these fertilisers is slow, resulting in high efficiency and minimal losses through washing (Wang et al., 2021).

The urease inhibitor (NBPT) in slow-release urea fertilizer minimizes nitrogen losses by delaying the conversion of urea. Because this inhibitor delays the hydrolysis (dissolution) of urea and slows down its conversion to ammonium form, thus preventing ammonia losses to the atmosphere (Trenkel, 2021).

Even if rainfall is insufficient, it can remain on the soil surface for 2 weeks. Barut et al. (2022) reported that statistically significant increase in stem diameter and yield values resulting from the application of Nutrisphere-N-Urea. Ozbek & Akgun (2020) demonstrated that slow-release fertiliser applications effectively improved grain quality.

The production and popularity of popcorn (*Zea mays* L. *everta*) are steadily increasing in Turkey. Popcorn differs from other maize varieties in terms of its grain structure. When exposed to heat, the endosperm within the grain expands, causing the hard outer shell to rupture and resulting in a significant increase in volume. Due to its rich vitamin and mineral content, popcorn is directly consumed as a snack. Additionally, its low carbohydrate content, ability to promote satiety, and capacity to absorb stomach acid make it a preferred choice in various diets (Oktem & Kahramanoglu, 2021).

Popcorn, a C4 plant, produces a considerable quantity of dry matter per unit area. Insufficient fertilizer application during the development stages of corn results in a low yield (Koca, 2013). The nitrogen uptake of the corn plant increases in the middle of vegetative development and reaches a maximum level before the tassel flowering period (Subedi & Ma, 2009). Fertilizer should be applied in a correct, balanced and sufficient manner. Therefore, it is essential for productivity to have sufficient nitrogen in the soil during the development period until the tasseling period.

In traditional popcorn top dressing, all nitrogen should be given when the plants reach 40-50 cm in height. Since the plants grow quickly, applying top dressing with ground equipment in later periods is impossible. In addition, not all of the nitrogen given can be used by the plants, some of it is absorbed by surface runoff, some by seeping into the depths of the soil, or some of it is mixed into the atmosphere in the form of ammonia gas. However, this problem can be eliminated because slow-release nitrogen fertilizer remains in the soil for a long time without being lost and continuously nourishes the plant.

The nitrogen required by the popcorn plant during its vegetative and generative development can be met by using slow-release nitrogen fertilizers that remain in the soil for a long time and do not disappear, and nitrogen use efficiency can be increased. It can also reduce environmental and water pollution. Since nitrogen is not lost from the soil, less fertilizer can be used. Therefore, the amount of slow-release nitrogen fertilizer to be given to popcorn needs to be determined.

The objectives of this study was to determine the most appropriate dose of slow-release nitrogenous fertilizer containing NBPT inhibitor for popcorn and to determine its effects on yield, yield components and quality.

2. MATERIALS AND METHODS

This study was conducted in Sanliurfa (altitude: 465 m; coordinates 37° 08' N and 38°46' E) in 2022 and 2023 on second crop growing conditions. Some climatic and soil characteristics of the study area are given in Tables 1 and Table 2, respectively.

Table 1. Some climate data of the growing season 2022-2023 and long term about the experimental area[†]

Month	Temperature (°C)									Total Rainfall (mm)			Average Relative Humidity (%)		
	Maximum			Minimum			Average								
	2022	2023	LT [†]	2022	2023	LT	2022	2023	LT	2022	2023	LT	2022	2023	LT
6	41.0	39.8	34.6	19.9	17.8	20.5	26.7	28.7	28.1	1.2	0.0	4.3	42.7	31.9	32.6
7	40.7	43.3	38.8	26.8	22.9	24.3	28.9	33.3	32.0	0	0.0	2.0	42.9	22.7	29.3
8	40.4	44.6	38.4	23.3	20.7	23.9	26.9	34.1	31.5	0	0.1	3.4	32.4	26.2	32.0
9	37.0	38.8	34.0	21.7	18.3	20.0	22.7	29.1	27.2	0	0.0	4.6	30.4	29.5	35.0
10	33.0	31.1	27.1	1.9	15.3	14.5	16.5	22.5	20.6	10	2.3	26.5	48.3	42.1	44.1

[†] : LT: Belongs to the average of long term, *; 6 June, 7 July, 8 August, 9 September, 10 October

^{††} : The State Meteorological Service of Turkey (2022, 2023 and Long Term period).

Table 2. Some soil properties of the test area

Deep (cm)	Phosphorus (g cm ³)		Sand (%)		Clay (%)		Silt (%)		pH		Org. Mat. (%)		CaCO ₃ (%)	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
0-30	1.37	1.28	20.4	20.3	54.0	53.0	25.60	25.74	7.15	7.16	1.33	1.28	7.90	7.86
30-60	1.39	1.42	20.4	20.6	52.0	53.0	27.60	27.86	7.44	7.42	1.31	1.20	9.50	9.15

As can be seen in Table 1, during the growing season of corn, temperature degrees exceed 40 °C. The minimum temperatures indicate the values recorded during the night. Relative humidity was under 50 %. The experimental area has from arid to semiarid climatic conditions.

To eliminate the residual effect, the experiment was set up in an area where no crops had been grown for many years, and no fertilization had been applied. The trial area displayed a deficiency in organic matter and a pH level of approximately 7. The concentration of CaCO₃ was found to be at a medium level. In the research, R-997 popcorn variety (*Zea mays* L. *everta*) was used as plant material. The experimental plots were treated with pure phosphorus at a rate of 90 kg ha⁻¹ at the time of sowing, applied via band (Oktem & Ulger, 1998).

Slow-release urea (46% nitrogen) with urease inhibitor NBPT [(N-(n-butyl) thiophosphoric triamide (NBPT)] was used as a source of nitrogen. Thanks to its inhibitor (NBPT), it prevents nitrogen losses in gaseous form. Slow-release nitrogen dosages were organized as control, 40, 80, 120, 160, 200, 240, 280 and 320 kg ha⁻¹. The nitrogen fertilizer was applied in two stages, with half of the dose incorporated at the time of sowing in a band and the remainder applied when the plants had seven to eight leaves.

The land was prepared for planting through plowing and tilling, followed by a single pass of the disc harrow to establish an optimal seedbed. The experiment was conducted using a randomized complete block design (RCBD) with three replications. Sowing took place in the latter half of June in both years. Each plot measured 5 meters in length, with a row spacing of 70 cm and an intra-row spacing of 20 cm. The plots were arranged in four rows.

Weeds were removed when their presence was recognized. The initial hoeing of the plants was conducted when they reached a height of approximately 15-25 cm, and the subsequent hoeing was performed when the plants reached a height of 40-45 cm. The germination of seeds was undertaken using the sprinkler irrigation method. Thereafter, the furrow irrigation method was employed, and an equal volume of water was applied to the plots. In order to prevent the passage of water and fertilizers between the plots, a three-meter space was maintained between them, and each parcel was surrounded by a barrier comprising soil.

Grain moisture was measured at the harvest and grain yield was calculated based on 15% grain moisture. The ears of the plants situated in the central two rows of each plot were harvested. The ear diameter, number of kernels per ear and 1000 grain weight values were calculated by taking the mean of 10 randomly selected ear samples from each plot. The protein content of the seeds obtained from each plot was determined by the Kjeldahl method in accordance with ICC Standard No. 167 (Kacar & Inal, 2008).

To determine the Popping volume ($\text{cm}^3 \text{g}^{-1}$), a total of 150 g of samples were taken from the grains of the sample plants obtained from each plot of the experiment, the grains were operated for approximately 150 seconds using an electric blasting machine until the explosion sound stopped, and then determined according to the formula given below (Dofing et al., 1990).

Popping volume ($\text{cm}^3 \text{g}^{-1}$) = total popped grain volume (cm^3) / sample weight (g)

The percentage of unpopped kernels was determined according to the given below formula,

Unpopped kernels (%) = number of unpopping kernels / total number of kernels X 100.

An analysis of variance (ANOVA) was performed according to randomized block design in order to evaluate statistically significant differences between the results using SAS statistical package. The difference between the means was compared using the least significant difference (LSD) test at a significance level of $P \leq 0.05$. To assess the interaction between grain yield and slow-release N doses, regression analysis was performed.

3. RESULTS AND DISCUSSION

Grain Yield (kg ha^{-1})

The results of variance analysis for the years 2022, 2023 and averages of two years are given in Table 3. According to the analysis of variance, slow-release nitrogen applications were found to be statistically significant in both years ($P \leq 0.01$). According to average of two years, the highest grain yield was obtained from the 240 kg ha^{-1} slow-release N application ($8651.6 \text{ kg ha}^{-1}$), while the lowest value was measured in the control plot ($2502.4 \text{ kg ha}^{-1}$).

Table 3. Results of the analysis of variance for 2022, 2023 and the average of the two years.

Mean of squares values									
Source of Variation	DF	Grain yield	Ear diameter	Ear length	Kernel number per ear	Thousand kernel weight	Protein content	Popping volume	Percentage of unpopped kernel
Growing season of 2022									
Rep.	2	234631.5	0.07	0.178	988.17	2.936	3.506	0.129	0.20
N	8	20290712.88**	101.20**	9.270**	29137.92**	32.86**	1.712**	28.30**	31.95**
Error	16	115538.55	0.25	0.361	508.8	1.62	0.055	0.2397	0.38
Total	26								
Growing season of 2023									
Rep.	2	27708.5	0.36	0.017	523.705	2.82	0.090	3.189	0.08
N	8	140898883.5**	103.92**	5.094**	7946.46**	237.76**	1.351*	23.38**	119.00**
Error	16	99017.543	0.99	0.672	589.16	22.87	0.186	1.7051	0.31
Total	26								
Average of two year									
N	8	3.38e+7**	201.08**	10.387**	32446.3 **	205.92**	2.671**	48.33**	132.82**
Error 1	2	59209.3	0.14	0.0713	1425.58	4.67	0.045	2.3013	0.13
Year X N Dosages	8	624831**	4.03 **	3.976**	4638.12 **	64.70 **	0.391 *	3.34 *	18.13 **
Error 2	32	107278.05	0.62	0.5166	549.0	12.25	0.121	0.9724	0.35

*, ** Statistical significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; DF: Degree of freedom

The mean value of two years indicated an increase in grain yield of up to 240 kg ha^{-1} with the application of slow release nitrogen doses, although a decrease in yield was observed at higher doses after this point (Table 4).

Nitrogen is the most important element required for photosynthesis. In the plant, nitrogen is incorporated into the structure of organic compounds that perform vital functions, including protein, amino acid, amide, nucleic acid, and chlorophyll. Plants generally take nitrogen from the soil through their roots. As the amount of nitrogen dose increases, the vegetative organs of the plant develop better and therefore produce dry matter by photosynthesizing more and more nutrients are transported to the ear, which positively affects yield (Oktem et. al., 2001).

In the study area, air temperature rises above 40°C and relative humidity is below 40% during the maize growing season, which increases nitrogen losses through evaporation. As in the research area, nitrogen losses in the soil increase

due to high evaporation in the summer months. With the application of slow-release nitrogen fertilizer, it is thought that nitrogen is used more effectively by the plant and this has a positive effect on grain yield.

As can be seen from the regression analysis (Figure 1), a polynomial curve was found between grain yield and slow-release nitrogen dosages. The curve demonstrates that the optimal slow-release nitrogen dosage was determined to be 240 kg N ha⁻¹ of application. The relationship between slow-release nitrogen and grain yield was determined as $y = -0.0683x^2 + 42.025x + 1844.1$ with $R^2 = 0.9488$.

Table 4. Effect of slow-release nitrogen with NBPT inhibitor applications on grain yield and ear diameter of popcorn

Applications (kg ha ⁻¹)	Grain Yield (kg ha ⁻¹)			Ear Diameter (mm)		
	2022	2023	Average	2022	2023	Average
Control	2369.9 f	2634.9 g	2502.4 G †	19.20 e	19.52 e	19.36 E
40	2554.0 f	3600.8 f	3077.4 F	19.57 e	20.54 e	20.05 E
80	3818.2 e	4600.8 e	4209.5 E	20.88 d	26.20 d	23.54 D
120	4488.1 d	5958.7 d	5223.4 D	30.92 b	32.37 b	31.64 B
160	7091.3 c	7069.1 c	7080.2 C	31.35 b	32.50 b	31.93 B
200	8288.1 ab	7834.8 b	8061.4 B	31.79 b	33.10 b	32.45 B
240	8760.3 a	8542.9 a	8651.6 A	33.58 a	35.63 a	34.61 A
280	8027.8 b	8205.6 ab	8116.7 B	30.01 c	33.73 b	31.88 B
320	7973.8 b	7804.8 b	7889.3 B	29.93 c	30.43 c	30.18 C
LSD	588.3	544.7	385.2	0.87	1.72	0.84

†: There is no statistically significant difference between the means in the same letter group at 0.05 level according to LSD test

Our findings align with those of other researchers in the field. For instance, Lakshmi et al. (2020) observed that maize grain yield in low-fertility soils increased with nitrogen application until reaching an optimal threshold, beyond which additional nitrogen had no effect or a negative impact on yield. Similarly, Jokela and Randal (1989) found that increasing the nitrogen (N) rate from 0 to 150 kg ha⁻¹ significantly enhanced grain yield, while a further increase to 225 kg ha⁻¹ did not result in a substantial additional yield improvement. Bucak and Akgun (2024) reported that the highest grain yield was achieved with the application of slow-release nitrogen at a rate of 200 kg N ha⁻¹. Additionally, Oktem and Oktem (2006) noted that the highest nitrogen application led to only a marginal yield increase, which they attributed to excessive vegetative growth. Several studies on maize have demonstrated that nitrogen application rates ranging from 150 to 240 kg ha⁻¹ are optimal for maximizing grain yield (Blumental et al., 2003; Mayer et al., 2012; Safdarian et al., 2014; Chen et al., 2015; Avsar et al., 2018).

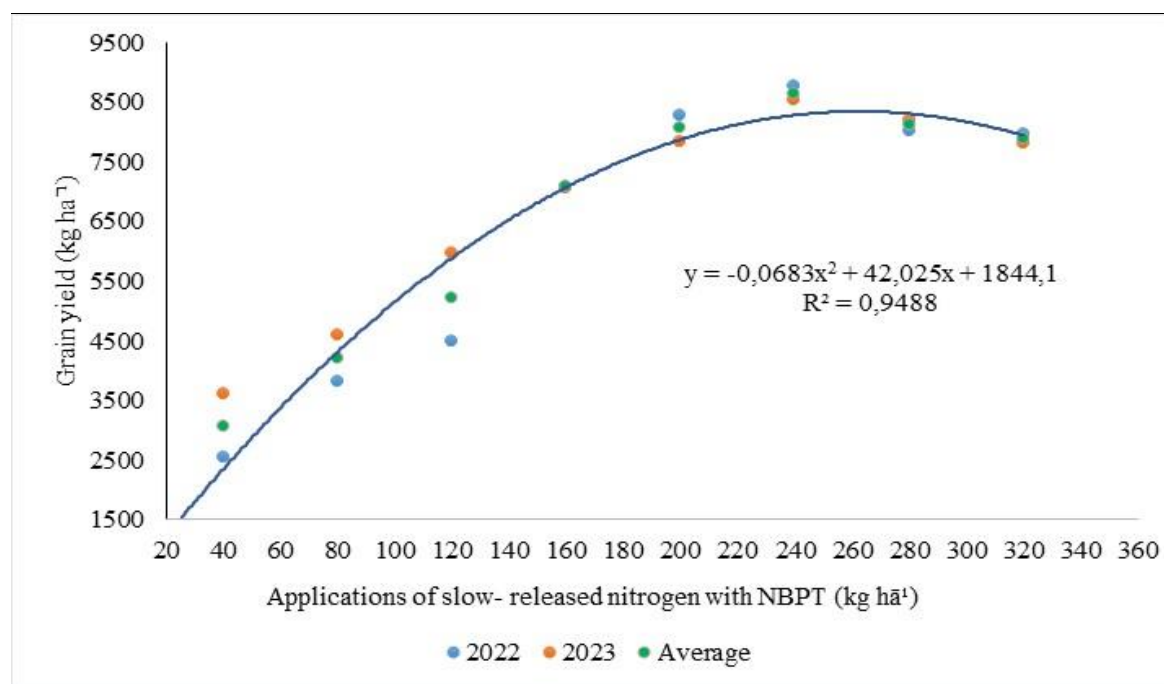


Figure 1. Analysis of regression for grain yield

Ear Diameter (mm)

The analysis of variance revealed that the differences between the slow-release nitrogen applications in terms of ear diameter were statistically significant ($P \leq 0.01$). According to the two-year averages, it was determined that the ear diameter values varied between 19.36 mm (Control) and 34.61 mm (240 kg ha⁻¹) (Table 4). The diameter of the ear is a significant determinant of ear size and, subsequently, grain yield. The heritability of ear diameter has been demonstrated to be relatively low (Saleh et al., 2002), while environmental factors have been shown to exert a more pronounced influence (Oktem & Oktem, 2006; Eser, 2014). The results of the experiment showed that the ear diameter value increased as the nitrogen dose increased. However, this observed trend showed a decrease after the application of 280 kg ha⁻¹ nitrogen dose. In general, the application of slow-release nitrogen fertilizers had a positive effect on ear diameter.

Ear length (cm)

The analysis of variance demonstrated that the impact of the slow-release nitrogen doses on ear length was statistically significant in 2022 and 2023 ($P \leq 0.01$). The ear length values exhibited ranging from 24.33 - 19.02 cm, 23.87 - 19.17 cm, and 23.01 - 19.09 cm in 2022, 2023, and the two-year average, respectively (Table 5).

Table 5. Effect of slow-release nitrogen with NBPT inhibitor applications on ear length and kernel number per ear of pop corn

Applications(kg ha ⁻¹)	Ear length (cm)			Kernel number per ear (number)		
	2022	2023	Average	2022	2023	Average
Control	19.02 g	19.17 d	19.09 D†	479.89 e	539.40 d	509.64 F
40	19.78 fg	21.70 bc	20.74 C	482.52 e	567.71 d	525.12 F
80	20.66 ef	22.40 bc	21.53 BC	573.67 d	618.79 c	596.23 E
120	21.67 de	21.30 c	21.48 C	625.66 c	676.38 ab	651.02 CD
160	23.12 b	21.55 bc	22.33 AB	681.59 b	642.81 bc	662.20 C
200	23.36 ab	22.67 abc	23.01 A	715.70 b	675.23 ab	695.46 B
240	24.33 a	21.63 bc	22.98 A	758.33 a	689.21 a	723.77 A
280	22.82 bc	22.80 ab	22.81 A	690.96 b	660.05 abc	675.51 BC
320	21.84 cd	23.87 a	22.85 A	612.74 c	652.59 abc	632.66 D
LSD	1.04	1.42	0.83	39.04	42.03	27.55

†: There is no statistically significant difference between the means in the same letter group at 0.05 level according to LSD test

The results indicated that based on two-year average the lowest ear length value was recorded in the treatment that did not include nitrogen application, while the highest value was observed in the treatment that included application of a nitrogen dose of 200 kg ha⁻¹.

Nevertheless, it can be observed that the 200, 240, 280 and 320 kg ha⁻¹ slow-release N applications are found to be within the same statistically group. The application of slow-released nitrogen was observed to have a beneficial impact on ear length. As observed by other researchers, an increase in nitrogen dose was found to result in a increase in ear length (Oktem & Oktem, 2005; Safdarian et al., 2014; Saracoglu & Oktem, 2021).

Kernel number per ear

According to analysis of the variance results based on 2022, 2023 and the average of the two years, the number of kernels per ear was found to be statistically significant ($P \leq 0.01$). The results of the study showed that the control plot and 40 kg ha⁻¹ N had the lowest value, while the highest value was observed in the application of 240 kg ha⁻¹ N in both years (Table 5). Some researchers reported a significant increase in the number of seeds per ear with increasing nitrogen doses (Sabir et al. 2000; Mehmood et al. 2001).

Slow release nitrogen fertilizer applications caused a statistically significant increase in the number of kernels per ear. It is thought that the increase in photosynthetic efficiency positively affected yield and yield elements such as the number of kernels per ear. This can be attributed to higher net photosynthesis and dry matter content resulting from improved photosynthetic efficiency. Increased nitrogen application enhances plant growth and development, which subsequently improves yield-related traits, such as ear length, ear diameter, and the number of kernels per ear.

Table 6. Effect of slow-release nitrogen with NBPT inhibitor applications on 1000 kernel weight and protein content of popcorn

Applications (kg ha ⁻¹)	Thousand kernel weight (g)			Protein content (%)		
	2022	2023	Average	2022	2023	Average
Control	136.08 c	135.40 d†	135.74 D	13.13 d	11.90 d	12.52 F
40	140.67 b	153.93 c	147.30 C	13.37 d	12.67 c	13.02 E
80	140.67 b	154.60 c	147.63 C	13.50 d	13.60 ab	13.55 D
120	141.17 b	158.83 abc	150.00 C	14.27 c	13.53 ab	13.90 BCD
160	144.53 a	156.40 bc	150.47 C	14.57 c	13.07 bc	13.82 CD
200	146.01 a	163.77 ab	154.93 AB	14.67 bc	13.43 ab	14.05 ABC
240	146.70 a	165.33 a	156.02 A	14.67 bc	13.97 a	14.32 AB
280	140.83 b	161.40 abc	151.12 BC	15.00 ab	13.95 a	14.48 A
320	140.63 b	160.83 abc	150.73 C	15.23 a	13.70 ab	14.47 A
LSD	2.20	8.28	4.12	0.41	0.75	0.451

†: There is no statistically significant difference between the means in the same letter group at 0.05 level according to LSD test

Thousand kernel weight (g)

According to the results of the variance analysis, statistical significance was determined between the slow release nitrogen doses in terms of thousand kernel weight. (Table 3). Based on the average of two years the maximum thousand kernel weight was obtained from the application of 240 kg ha⁻¹ nitrogen (156.02 g), while the lowest values were found on the control plot (135.74 g).

The thousand kernel weight value demonstrated an upward tendency, commencing at 40 kg ha⁻¹ nitrogen dose and attaining its peak at 240 kg ha⁻¹ nitrogen dose. Thereafter, a decline was discernible at the 260 kg ha⁻¹ nitrogen dose (Table 6).

Thousand kernel weight values for corn vary depend on to variety and growing conditions (Oktem & Oktem, 2020). It was explained that nitrogen fertilizers caused an increase in the leaf area of the plant during vegetative development and, as a result, the dry matter accumulation in the grain filling was high and, as a consequence, the thousand kernel weight increased with the application of nitrogen (Saracoglu & Oktem, 2021).

On the other hand, Aydın (1991) stated that the increase in nitrogen fertilizer doses increased the thousand grain weight, but the increase after 20 kg N da⁻¹ application was not much and made comments that supported our findings

Protein content of kernel (%)

The results of the variance analysis indicated that nitrogen applications had a statistically significant impact on the protein content of the kernel in 2022 and 2023 ($P \leq 0.01$ and $P \leq 0.5$, respectively). The protein content of the kernel was found to range from 14.48% to 12.52% on average of the two-years. However, the 280 kg ha⁻¹ and 320 kg ha⁻¹ treatments were included in the same statistical group.

As the dose of slow-release nitrogen fertilizer increased, protein content of kernel also increased. The protein content of the kernel is one of the significant quality parameter and it is influenced by multiple factors including genetic variation, climate and cultivation conditions (Oktem et al. 2010). Nitrogen plays an important role in the formation of a large number of organic compounds in plants, including amino acids, proteins and nucleic acids, which positively affects the grain protein ratio (Oktem & Oktem, 2005 and 2020). In this study, as the amount of N in the soil increased, the N uptake of the plant from the soil and the protein content of the grain increased. In accordance with the findings of Oktem et al. (2010), the presence of N deficiency in soil is associated with adverse effects on nitrogen metabolism, particularly with respect to protein synthesis. Dong et al. (2016) observed that the protein content of corn increased by 61.38-113% when fertilizers containing nitrification inhibitors were used. There are many studies reporting that the protein content increases with increasing nitrogen dose. (Patricio Soto et al., 2002; Keskin et al., 2005; Celebi et al., 2010; Oktem et. al., 2010).

Popping volume (cm³ g⁻¹)

The difference between the doses of slow-release nitrogen in terms of popping volume was found to be statistically significant ($P \leq 0.01$) in both years (Table 3). Popping volume ranged from 20.05 cm³ g⁻¹ to 28.63 cm³ g⁻¹ based on average of the two years. The highest popping volume value was observed with the application of 240 N kg ha⁻¹, while the lowest value was measured at control and application of 320 N kg ha⁻¹ (Table 7).

Applying heat to popcorn kernels initiates a series of events that lead to the expansion of water vapor within the kernel, thereby creating internal pressure. The kernels have a hardness characteristic of their physical composition, and the outer shell lacks the capacity for permeation. Concurrently, the starch present within the endosperm undergoes a transformation that is induced by the applied heat. Once a threshold pressure inside the kernel is attained, the external

shell undergoes rupture, under the effect of the pressure, the starch inside the kernel jumps out of the kernel, thereby forming the popcorn (Oktem & Oktem, 2020).

Table 7. Effect of slow-release nitrogen with NBPT inhibitor applications on popping volume and percentage of unpopped kernel of popcorn

Applications (kg ha ⁻¹)	Popping volume (cm ³ g ⁻¹)			Unpopped kernel (%)		
	2022	2023	Average	2022	2023	Average
Control	19.83 f	21.00 e	20.41 F †	11.30 b	10.58 c	10.94 C
40	22.17 e	21.73 de	21.95 E	10.80 b	9.16 d	9.98 D
80	24.27 d	23.87 cd	20.07 D	9.07 c	8.36 d	8.71 E
120	25.43 c	24.27 bc	24.85 CD	8.87 c	7.10 e	7.98 F
160	26.47 b	25.20 bc	25.83 BC	8.43 c	6.63 e	7.53 F
200	26.67 b	26.33 ab	26.50 B	7.33 d	4.84 f	6.09 G
240	28.83 a	28.43 a	28.63 A	6.27 d	3.70 g	4.98 H
280	26.20 bc	21.80 de	24.00 D	11.43 b	18.29 b	14.86 B
320	20.37 f	19.73 e	20.05 F	17.43 a	22.61 a	20.02 A
LSD	0.85	2.26	1.16	1.07	0.97	0.69

†: There is no statistically significant difference between the means in the same letter group at 0.05 level according to LSD test

The volume of popping is a significant quality criterion and high popping volume value is a desirable feature. It is of great importance for the commercial success of the product that the popcorn kernels are of a substantial size (Oktem & Kahramanoglu, 2021). The quality of the product is affected by many factors, including the weight of the kernel, the moisture content, growing conditions, climate and genotype (Oktem & Oktem, 2020). In this study, it was observed that the application of slow release nitrogen with NBPT positively affected the thousand grain weight (Table 6) and the explosion volume value (Table 7). The increase in grain size contributed to the increase in the explosion volume values. It has been reported that as the grain size increases, the explosion volume also increases (Soylu & Tekkanat 2007; Ozturk et al., 2016; Oktem & Kahramanoglu, 2021).

Percentage of unpopped kernel (%)

Based on the analysis of variance, slow-release nitrogen fertilizer applications were found to be statistically significant ($P \leq 0.01$) in terms of the percentage of unpopped kernels (Table 3). According to the average of two-year data, the highest percentage of unpopped kernels was obtained from the 320 kg ha⁻¹ N application, while the lowest value was observed in the 240 kg ha⁻¹ N application (Table 7). The presence of unpopped kernels is one of the quality-reducing characteristics of popcorn. Consequently, it is desirable to maintain a minimum level of unpopped kernels (Cihangir & Oktem, 2019). As the popping volume increases, the rate of percentages of unpopping kernel decreases. In the study, it was observed that the percentage of unpopped grains was the highest in 280 kg ha⁻¹ and 320 kg ha⁻¹ N applications. High nitrogen amount promotes vegetative growth of the plant. In this case, ripening of the grain is delayed and as a result, it is thought that high moisture content in the grain causes an increase in the rate of unpopped grains.

4. CONCLUSION

The application of slow-release nitrogen had a positive impact on grain yield and popcorn quality. Specifically, grain yield, ear length, thousand-kernel weight, the number of kernels per ear, and quality parameters such as protein content and popping volume increased with the application of slow-release nitrogen containing an NBPT inhibitor. As the nitrogen dose increased, grain yield and most tested parameters improved up to a threshold of 240 kg N ha⁻¹, beyond which a decline was observed. Regression analysis identified 240 kg N ha⁻¹ as the optimal slow-release nitrogen dose. The use of slow-release nitrogen fertilizers is recommended in regions with high evaporation and hot climates, such as the study area. Additionally, this study suggests that NBPT can be a promising additive to nitrogen fertilizers, as no adverse effects were observed with increasing levels of slow-release nitrogen applications.

ACKNOWLEDGEMENT

The Scientific Research Projects Coordination Unit of Harran University (HUBAP) provided financial support for the investigation (Project number: 22252). We would like to express our gratitude to the HUBAP council for their financial support in this endeavour.

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Identifying Loci Associated with Grain Quality Traits of Landraces and Modern Varieties of Spring Bread Wheat under Conditions of Western Siberia

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ARTICLE INFO

Research Article

Received: 1 October 2024

Accepted: 20 February, 2025

Published: 23 June 2025

Keywords:

Grain yield

NBPT

Popcorn

Popping volume

Slow release nitrogen

ABSTRACT

Landraces of wheat represent valuable genetic resources for enhancing the grain quality of modern wheat varieties. In this study, a panel comprising 94 landraces sourced from the VIR collection (Russia) and 142 wheat varieties from breeding programs of Russia and Kazakhstan was subjected to genome-wide association study (GWAS) to pinpoint genomic regions and identify candidate genes associated with grain quality traits in wheat breeding. Genotyping of the germplasm panel was conducted using the Affymetrix 25K platform, while phenotypic data for agronomic traits were collected over two growing seasons (2022 and 2023) in Western Siberia. Investigation of the genotypic structure revealed seven distinct clusters within the landraces and breeding varieties population, indicating a clear genetic differentiation among the landraces. Notably, the B genome of the landrace germplasm exhibited the highest proportion of SNP markers (41%). Based on experiment data among 294 MTAs seven unique loci were identified with a $-\log_{10}(p)$ value >6 in the landrace collection that had significant associations with grain protein content, grain gluten, strong gluten content, gluten index, and vitreousness (AX-158572632, wsnp_Ex_c10084_16572374, BobWhite_c31129_60, AX-158586137, AX-94949506, BobWhite_c46257_130, wsnp_JG_c5646_2148382). These markers were distributed on chromosomes A, 2B, 5B, 6B, and 6D with ranged of phenotypic variation 9–26%.

Citation: Shepelev, S., Pototskaya, I., Morgounov, A., Chursin, A., Esse, S., Koshkin, M., & Shamanin, V. (2025). Identifying loci associated with grain quality traits of landraces and modern varieties of spring bread wheat under conditions of Western Siberia. *Turkish Journal of Field Crops*, 30(1), 11-21. <https://doi.org/10.17557/tjfc.1559476>

1. INTRODUCTION

The reduction of genetic diversity in modern bread wheat breeding programs presents a challenge amidst climate change and evolving pests and pathogens (Kose et al., 2023). Primitive cultivars and landraces offer a rich source of genetic variability, cultivated over millennia under diverse environmental conditions with minimal inputs (Adhikari et al., 2022; Demirel et al., 2023). Despite their potential, the genotypic richness of this germplasm remains largely untapped in breeding efforts (Mitrofanova et al., 2009). Landraces from the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) harbor valuable genetic diversity for adapting to adverse environmental conditions and ensuring grain quality (Mitrofanova, 2012; Riaz et al., 2017; Dinglasan et al., 2019). The VIR collection of spring bread wheat comprises 15,048 accessions from 97 countries, representing genetically diverse landraces and breeding lines registered between 1920 and 1990 (Phan et al., 2018; Tyryshkin et al., 2022). The Kazakhstan-Siberia Spring Wheat Improvement Network (KASIB) was established in 2000 and involves 20 institutions from Russia and Kazakhstan. Over two decades, the network has conducted 12 joint experiments and evaluated over 500 spring bread wheat varieties (Gomez-Becerra et al., 2006; Morgounov et al., 2020). The core set of 142 spring bread wheat varieties (ON-KASIB) reflects the genetic diversity of the entire KASIB germplasm.

Genome-wide association studies (GWAS) offer a genetic tool to identify new markers associated with grain yield and quality by comparing wheat varieties from different breeding periods, including landraces. Understanding past selection processes and developing strategies for future breeding efforts are essential (Rasheed et al., 2016; Shvachko et al., 2024). Landraces can contribute to improving modern wheat varieties and countering genetic erosion, albeit with limited capacity to cope with spatially heterogeneous and temporally unpredictable environments (Jaradat, 2013). Numerous studies have demonstrated broader genetic diversity in landraces compared to commercial wheat varieties (Lopes et al., 2015; Pascual et al., 2020; Jambuthenne et al., 2022). At present, genomic selection and the discovery of untapped beneficial quantitative trait loci (QTL) play a significant role in incorporating genetic material from landraces into modern varieties through marker-assisted selection (MAS), pyramiding, or biotechnological tools, thereby enhancing their potential for deployment in breeding programs (Kroupin et al., 2019; Ma et al., 2020; Marone et al., 2021). Reif et al. (2005) investigated 253 CIMMYT or CIMMYT-related modern wheat varieties and landraces using 90 simple sequence repeat (SSR) markers distributed across the wheat genome. The study revealed numerous unique alleles in landraces absent in modern spring bread wheat varieties, representing valuable sources for broadening the genetic base of elite wheat breeding germplasm. Analysis of 687 wheat accessions, including 148 landraces and 539 cultivars mainly from China and Pakistan, through genotyping of 477 unique genome regions using a wheat 90K single nucleotide polymorphism array, identified signatures of selection associated with disease resistance, vernalization, quality, adaptability, and yield-related traits (Liu et al., 2019). Two core collections comprising 94 wheat landraces as sources of new alleles and 142 promising adapted wheat varieties from breeding programs in Russia and Kazakhstan, developed between 2000 and 2020, offer a broad spectrum of variation for searching useful agronomic traits in wheat breeding (Morgounov et al., 2024). Association mapping, conducted using a multi-locus GWAS-GLM panel on quality-related traits over two growing seasons, identified candidate genes for selection of target loci.

The objective of this study was to perform association mapping on landraces from the VIR collection and germplasm from the international KASIB program to detect genomic regions and identify candidate genes for enhancing grain quality in wheat varieties.

2. MATERIALS AND METHODS

Germplasm Panel

The germplasm panel comprised 94 accessions of wheat landraces sourced from various geographical locations (Supplementary Table 1). These studied landraces belonged to nine botanical varieties: *aureum* (Link) Mansf. (*albidum* Alef.), *graecum* (Koern.) Mansf., *lutescens* (Alef.) Mansf., *erythrospermum* (Koern.) Mansf., *ferrugineum* (Alef.) Mansf., *milturum* (Alef.) Mansf., *caesium* (Alef.) Mansf., *turcicum* (Koern.) Mansf., and *erythroleucum* (Koern.) Mansf. The collection of landraces from Russia encompassed diverse regions including Krasnodar Krai, the Volga region, the North-Western region, Western and Eastern Siberia, as well as the Far East. The collection of spring bread wheat from breeding programs of Russia and Kazakhstan (KASIB) consists of 142 accessions developed for the period from 2000 to 2020 (Supplementary Table 2).

Field and laboratory experimentation

The experiments were conducted over two growing seasons (2022 and 2023) and were planted in the field following black fallow. Accessions were distributed in plots measuring 1.4 m² each, spaced 0.15 m apart, with a sowing rate of 25 g of seeds per plot. In 2022 and 2023 there were four replications. Standard varieties included the early maturity variety Pamyati Azieva and the late maturity variety Element 22. Agronomic traits were recorded following standard agronomy

practices. Phenotypic evaluations of two core collections were conducted, covering 8 quality grain traits and the main yield components. Protein, gluten, ash content, and vitreousness of the tested accessions were analyzed using NIR-spectroscopy according to standard ISO 12099:2017(E). Wet gluten content and gluten index were evaluated using the Gluten Index Device-Y08, while SDS sedimentation volume was measured using the Sedimentation Device-15 (manufactured by YUCEBAS MACHINERY, Türkiye) according to GOST ISO 21415-2-2019 and GOST 34702-2020. The thousand kernel weight was determined as two parallels, 500 grains were counted and weighed with an accuracy of 0.01 g. The yield components were recorded following methodical instructions for exploring the wheat collection (Methodical instructions, 1985).

Experimental Sites and Weather

The experimental field's soil type is thin meadow chernozem, with an organic matter content ranging from 3.5% to 4%. The region experiences a sharply continental climate, with an average annual rainfall of approximately 300–380 mm and a sum of effective temperatures ranging from 2000°C to 2100°C. Factors limiting wheat grain yield and quality include insufficient rainfall, the threat of soil erosion by wind, and low phosphorus content. The average duration of the winter period is 164 days, with soil freezing to depths of 165–220 cm and snow cover varying between 20–25 cm. Growing seasons are characterized by average temperatures. In 2022, the rainfall for the growing season was 227 mm, with uneven distribution in May; and in 2023, it was 160 mm, with excess rainfall in August to early September.

Genotyping and Statistical Analysis

Genomic DNA was isolated from the leaves of 94 landraces and 142 spring bread wheat accessions using a protocol with modifications described by Delaporta et al. (1983) at a rate of 3 fold. Landrace and KASIB program accessions were genotyped using the Affymetrix 25K platform (Germany, www.traitgenetics.de). Markers with a minor allele frequency (MAF) lower than 5% and markers with not certain chromosome localization were excluded from the analysis. A total of 17,190 markers were including in GWAS. Marker-trait associations (MTAs), which was identified using the TASSEL v. 5.0 software with a method GLM (Bradbury et al., 2007) considered population structure (Q-matrix) and genetic kinship (K-matrix) using a Bayesian algorithm in the STRUCTURE v. 2.3.4 software (Pritchard et al., 2000). The number of subgroups (K) was calculated based on the highest value of ΔK , with K values ranging from 1 to 10, and 10 independent runs for each K value (Evanno et al., 2005) in the Structure Harvester web program (Earl & von Holdt, 2012). K-matrix was calculated using TASSEL v. 5.0 software. MTAs were considered for significance level of $-\log_{10}(p)$ value of >6 . The chromosomal localization of SNP markers was preserved within the T3/Wheat Association Mapping Panel, accessible at <https://wheat.triticeaetoolbox.org>; to hypothesize candidate genes utilizing the IWGSC RefSeq v. 1.0 reference genome. Conversion of SNP markers into locus-specific KASP-markers was determined as per <https://wheat.triticeaetoolbox.org> (Supplementary Table 3).

Results were analyzed using a two-way analysis of variance (ANOVA); the significance of mean differences was assessed using Tukey's test ($P < 0.05$) in SPSS (PASW) Statistics 20.0 («IBM», USA).

3. RESULTS AND DISCUSSION

Phenotypic characterization

Phenotyping of wheat landraces and varieties originating from diverse breeding programs for agronomic traits during two growing seasons showed that landraces and modern varieties differed above all on yield-related and grain quality traits (Table 1). The superiority of landraces over modern wheat varieties is evident in their higher levels of protein, gluten content, and grain vitreousness. These characteristics exhibit variations among different ecological groups: landraces from Russia displayed levels of 15.9–23.1%, 33.9–57.7%, and 57.9–67.8%, respectively; those from Kazakhstan showed levels of 15.1–20.7%, 31.7–50.7%, and 57.9–62.2%; and landraces from Central Asia demonstrated levels of 17.7–20.5%, 39.9–49.6%, and 59.1–61.7%.

Table 1. The yield-related and grain quality traits of landraces and program KASIB germplasm in 2022 and 2023.

Trait	Pamyati Azieva, St	Element 22, St	Russia	Landraces		KASIB
				Kazakhstan	Central Asia*	
Protein content, %	16.7	16.6	18.2 ^b	18.2 ^b	18.1 ^b	16.9 ^a
Gluten content, %	36.2	36.7	41.9 ^b	41.0 ^b	40.8 ^b	37.1 ^a
Ash content, %	1.82	1.81	1.81 ^c	1.77 ^a	1.82 ^c	1.78 ^a
Wet gluten content, %	46.2	48.1	47.0 ^d	44.5 ^b	44.9 ^b	41.4 ^a
Gluten index, %	86.0	70.1	75.1 ^a	79.2 ^b	79.9 ^b	95.5 ^d
Flour output, %	57.5	55.2	55.0 ^a	54.7 ^a	55.3 ^a	56.2 ^c
Vitreousness, %	56.8	56.7	57.9 ^b	57.9 ^b	59.1 ^c	56.4 ^a
Sedimentation volume, ml	61.3	40.0	33.1 ^a	32.9 ^a	33.3 ^a	39.7 ^d
No. spike grains, pcs.	29.5	38.0	24.6 ^a	24.6 ^a	23.2 ^a	30.9 ^d
Grain weight of spike, g	1.13	1.36	0.84 ^a	0.85 ^a	0.85 ^a	1.29 ^d
Thousand kernel weight, g	41.6	40.0	34.1 ^a	34.2 ^a	36.5 ^c	42.1 ^d
Yield, g/m ²	288	361	186 ^a	169 ^a	183 ^a	341 ^d

Note. Means with different letters within each column are significantly different ($p < 0.05$);

* Kyrgyzstan, Tajikistan, Uzbekistan

In comparison, the protein and gluten content of standard varieties ranged from 16.6–16.7% and 36.2–36.7%, respectively. Notably, modern wheat varieties exhibited reduced grain vitreousness at 56.4%, a key indicator associated with superior bread-making quality. However, landraces, particularly those from Central Asia, displayed a higher value of 59.1% for this trait (Table 1). Advancements in breeding techniques have led to improvements in grain technological characteristics, resulting in increased kernel size and yield in breeding wheat varieties. The superior bread-making quality of modern varieties was indirectly affirmed by SDS sedimentation (39.7 ml) and gluten index (95.5%), whereas landraces showed values of 32.9–33.3 ml and 75.1–79.9% for these traits, respectively. Modern varieties boasted a thousand kernel weight of 42.1 g, and flour output of 56.2%, whereas landraces exhibited indicators ranging from 34.1–36.5 g and 54.7–55.3%, respectively. The yield components of modern varieties differed from landraces: increased number of grains per spike, grain weight per spike (30.9 pcs. and 1.29 g). The grain yield of breeding varieties was also larger compared to that of landraces (341 g/m² vs. 169–186 g/m²).

Molecular Characterization

The differences in genomes polymorphism between landraces and modern varieties were detected during comparison of genotyping results of two collections (Figures 1).

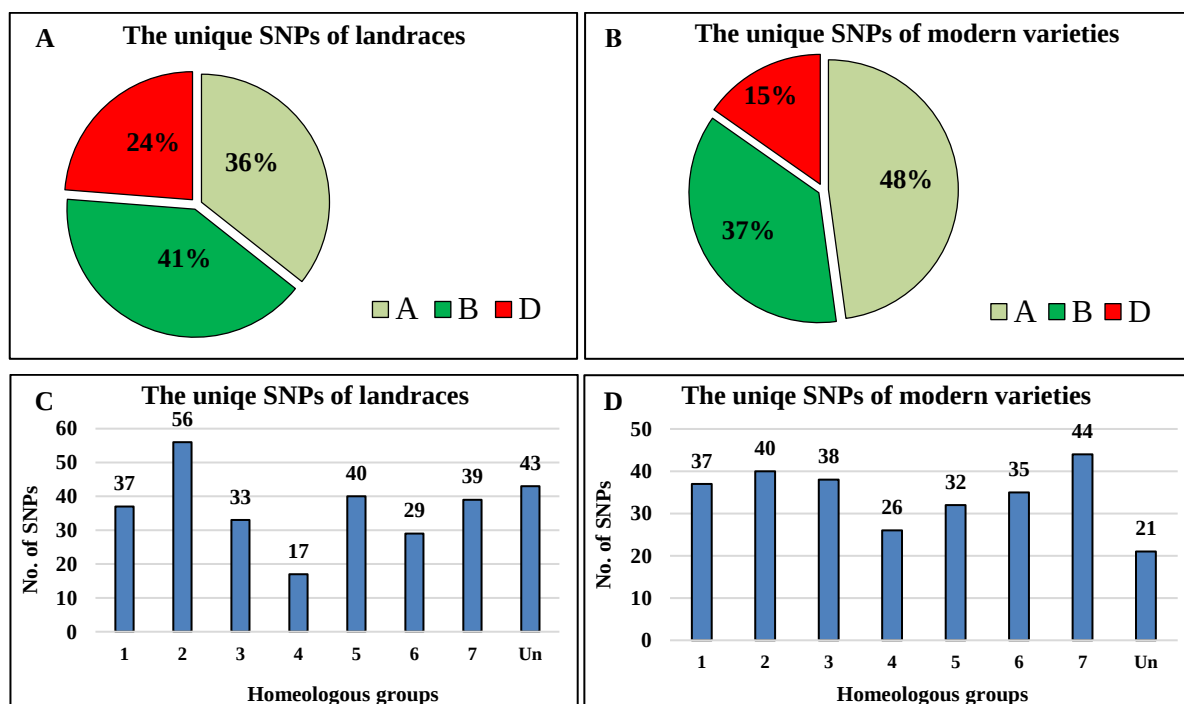


Figure 1. SNP loci distribution in the A, B, D genomes (sub-figures A, B), and homeologous groups of the germplasm of wheat landraces and modern varieties (sub-figures C, D); Note. Un – markers with unknown localization

In the landrace collection, a total of 294 SNPs were identified, distributed as follows: 36% in the A genome, 41% in the B genome, 24% in the D genome, and subsequently in the homeologous groups – 37 SNPs, 56 SNPs, 33 SNPs, 17 SNPs, 40 SNPs, 29 SNPs, and 39 SNPs, with 43 SNPs having an unknown localization. Conversely, in the collection of modern varieties, 273 SNPs were identified, with 48% in the A genome, 37% in the B genome, 15% in the D genome, and subsequently in the homeologous groups – 37 SNPs, 40 SNPs, 38 SNPs, 26 SNPs, 32 SNPs, 35 SNPs, and 44 SNPs, with 21 SNPs having an unknown localization. To dissect the genetic underpinnings influencing grain quality traits, we employed GWAS, unveiling a total of 294 markers in landrace accessions (36% SNPs within the A genome, 41% within the B genome, and 24% within the D genome). Similarly, within the KASIB program collection, 273 markers were identified (comprising 48% SNPs within the A genome, 37% within the B genome, and 15% within the D genome). Notably, the B genome of landrace germplasm exhibited the highest marker count (41%). Previous investigations, such as that conducted by Rahimi et al. (2019), highlighted a greater polymorphism within the B genome of both landraces and developed wheat varieties. Another study by Rufo et al. (2019) assessing genetic diversity across 354 bread wheat genotypes, including Mediterranean landraces and modern varieties, noted the lowest marker count within the D genome, with the B genome showcasing superior coverage. The linkage disequilibrium (LD) was investigated the distance between marker pairs 1,337,792 bp with an average density of 303 SNPs on one chromosome (Figure 2a). A population structure of varieties from both wheat collections was constructed using the STRUCTURE software, based on 17,190 SNP markers evenly distributed across all wheat chromosomes (Figure 2b). The landrace accessions clustered into four distinct groups labeled 1–4, including four varieties of program KASIB in fourth subcluster. An exception was the purple fifth subcluster, which encompassed 49% of the landraces included in the VIR collection between 1909 and 1948, along with 51% of modern wheat varieties from various breeding programs. Subclusters 6–7 were predominantly composed of germplasm from breeding programs in Russia and Kazakhstan, including one individual landrace accession (κ -34556, Kitayskaya).

Marker trait associations (MTAs) identified by GWAS.

Association mapping conducted on the landrace population found five SNP markers that were significantly ($p < 0.00001$) associated with protein, gluten content, and gluten index for two years of research: AX-15857 2632, Bobhite_c31129_60, AX-158586137, BobWhite_c46257_130, and wsnp_JG_c5646_2148382 (Table 2).

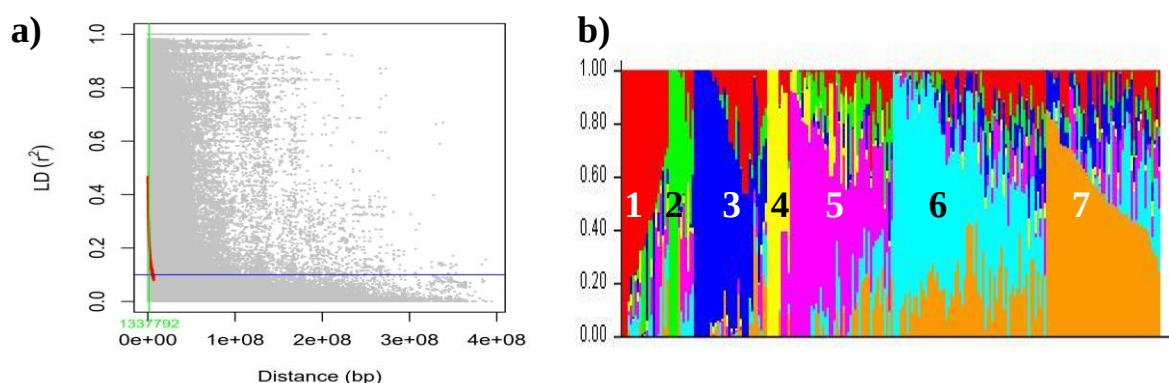


Figure 2. a) The rate of linkage disequilibrium (LD) decay based on 17,190 SNPs (p -value < 0.001); **b)** Population structure of landraces and KASIB germplasms using SNP marker system

Table 2. SNP markers with significant association identified in landraces genotypes.

Trait	Marker Name	Chr.	Position, Mb (RefSeq 1.0)	Target allele	Favorable season	P-value	R ² , %	Phenotypic effect
Protein content, %	BobWhite_c46257_130*	6B	514.675109	A/G	2022	5.19E-06	9	0.83
					2023	1.28E-07	11	0.43
	AX-15858 6137*	5B	599.619662	C	2022	2.55E-07	26	0.15
					2023	3.55E-06	21	0.36
Gluten content, %	BobWhite_c46257_130*	6B	514.675109	A/G	2022	5.14E-06	9	2.60
					2023	1.13E-07	11	1.44
	wsnp_JG_c5646_2148382*	6D	469.248518	C	2022	4.64E-06	22	0.30
					2023	4.64E-07	23	0.36
Strong gluten, %	Bobwhite_c31129_60*	2B	68.201577	C	2022	7.73E-11	15	0.71
					2023	1.42E-06	10	0.36
	AX-15857 2632*	2A	677.050366	G	2022	7.51E-09	11	13.7
					2023	4.37E-10	12	10.1
	wsnp_Ex_c10084_16572374	2A	692.934554	T	2023	4.45E-06	22	13.5
					2022	3.04E-12	14	18.7
Gluten index, %	BobWhite_c31129_60*	2B	68.201577	C	2022	3.36E-10	11	11.5
					2023	1.17E-08	10	11.6
	BobWhite_c46257_130*	6B	514.675109	G	2022	1.17E-08	10	11.6
					2023	8.39E-13	14	11.5
Vitreousness, %	AX-94949506	6A	610930266	A	2023	6.10E-06	19	3.76

Note. A/G – heterozygous alleles; *SNPs showing association during two growing seasons

At the same time, the presence of favorable allele of the marker BobWhite_c46257_130 increased the protein and gluten content but decreased the gluten index in landraces compared to modern varieties. In this study, the favorable allele of the marker wsnp_JG_c5646_2148382 (6D) was also associated with increasing of gluten content by 0.30–0.36%. In the genotypes of wheat landraces two SNP markers Bobwhite_c31129_60 and AX-158572632 associated with decreasing of strong gluten content and gluten index were identified. On the contrary, in 2023, the favorable allele of SNP wsnp_Ex_c10084_16572374 was detected in 27% of landrace accessions and increased the gluten index by 13.5%. For the vitreousness trait, in 2023 moderately significant association was discerned for the locus AX-94949506. This SNP was identified in genotypes of landraces Khivinka, Belokoloska, Khozyaystvennyi obrazets # 151, krasnaya, Sandyk, Rasa XXXIII 94 Bar-50.

In this study the extremely significant associations in genotypes of landraces were identified for seven loci localized on chromosomes 2A, 2B, 5B, 6B, and 6D with the range of phenotypic variation 9–26% (Table 2). There are currently no data of previous studies for markers AX-158572632 (chromosome 2A, region 677.05 Mb) and BobWhite_c46257_130 (chromosome 6B, region 514.67 Mb). Notably, these loci were present in 39% and 55% of landrace genotypes, respectively. According to date of Ruud and Lillemo (2018), SNP Bobwhite_c31129_60 linked to gene TraesCS2B02G107000, controlling resistance to *Septoria nodorum blotch*. Previously, the gene TraesCS2A02G443000 associated with duration of period from flowering to ripeness was identified in study of Alemu et al. (2021). SNP AX-158586137 located on chromosome 5B was associated with drought tolerance (Sallam et al., 2024). Christensen and Borgen (2023) investigated locus wsnp_JG_c5646_2148382 linked to gene *Bt9*, controlling resistance to common bunt. In our research, in B genome of landraces were detected SNPs showed a pleiotropic effect on two quality-related traits – BobWhite_c31129_60 (for strong gluten content and gluten index) and BobWhite_c46257_130 (for protein and gluten content). Since there is currently no information about the association of identified loci with quality-related traits, it can be assumed that these loci of wheat landraces are potential novel for breeding programs aimed at improving grain quality. Association mapping conducted on the landrace and KASIB varieties populations performed over two growing seasons, revealed 48 marker-trait associations (MTAs), of which 22 MTAs were significantly linked to protein, gluten, ash content in grain, gluten index, and flour output. Significant associations were identified on chromosomes 1A, 2B, 4A, 5D, 6A, and 7A (Table 3).

Table 3. SNP markers with significant association identified in landraces and modern varieties.

Trait	Marker Name	Chr.	Position, Mb (RefSeq 1.0)	Target allele	Favo- rable season	P-value	R ² , %	Phe- noty- pic effect
Protein content, %	TA005766-0499*	1A	11.895533	G	2022	2.97E-08	10	0.46
					2023	8.21E-12	15	1.55
	AX-94662379*	2B	74.250224	T	2022	1.42E-07	11	1.40
					2023	7.11E-13	17	1.90
	Excalibur_c30378_344*	4A	615.437273	G/T	2022	2.09E-08	13	1.44
					2023	7.84E-10	14	1.55
	BS00011469_51*	4A	621.848245	C	2022	1.29E-07	9	1.15
					2023	1.72E-08	10	1.27
	AX-94499635	4A	714.436088	A	2023	2.24E-09	13	1.61
	TG0131	5D	546.689237	T	2023	4.19E-08	10	1.03
	w SNP_JD_c4438_5567972	5D	546.689483	A	2023	1.56E-07	10	1.04
	AX-158551598	5D	546.824244	G	2023	3.3E-07	10	1.04
	AX-158586520*	5D	546.839205	C	2022	5.94E-07	10	1.15
					2023	1.23E-07	10	1.26
	BS00073116_51*	5D	546.864045	T	2022	6.21E-07	10	1.15
					2023	1.1E-07	10	1.27
	Excalibur_c42190_383*	5D	546.906373	A	2022	5.94E-07	10	1.15
					2023	1.23E-07	10	1.26
	Excalibur_c28592_173*	5D	546.910406	T	2022	5.94E-07	10	1.15
					2023	1.23E-07	10	1.26
	D_GDS7LZN01CBWNE_99	5D	546.700415	T	2023	3E-07	10	1.02
	CAP8_c145_89*	5D	547.273494	T	2022	6.67E-08	11	1.25
					2023	1.75E-08	11	1.35
	CAP7_rep_c12715_390*	5D	547.273595	C	2022	5.32E-08	11	1.25
					2023	2.38E-08	11	1.34
	w SNP_CAP11_c209_198671	5D	547.273762	T	2022	9.5E-08	11	1.23
					2023	4.55E-08	11	1.32
	AX-158586515*	5D	547.451565	T	2022	1E-07	11	1.24
					2023	2.66E-08	11	1.34
	AX-94626326*	5D	547.709624	A	2022	1.49E-07	11	1.19
					2023	3.12E-08	11	1.29
	Tdurum_contig75814_655*	6A	571.685447	C/T	2022	4.41E-07	10	1.17
					2023	4.71E-09	12	1.25
	AX-110992521	7A	27.107477	A/G	2023	1.45E-09	13	1.65
	AX-58590420*	7A	693.453814	G	2022	8.83E-10	14	1.04
					2023	8.42E-10	13	1.08
Gluten content, %	TA005766-0499	1A	11.895533	G	2023	3.9E-10	13	4.47
	AX-94662379	2B	74.250224	T	2023	9.49E-11	15	5.44
	Excalibur_c30378_344*	4A	615.437273	G/T	2022	2.97E-07	11	4.72
					2023	2.91E-08	13	4.43
	BS00011469_51*	4A	621.848245	C	2022	4.98E-08	10	4.07
					2023	6.45E-08	10	3.82
	AX-94499635	4A	714.436088	A	2023	5.85E-08	12	4.59
	TG0131*	5D	546.689237	T	2022	5.96E-07	9	3.11
					2023	2.07E-07	9	3.06
	w SNP_JD_c4438_5567972	5D	546.689483	A	2023	5.29E-07	10	3.11
	AX-158586520*	5D	546.839205	C	2022	2.13E-07	10	4.10
					2023	3.63E-07	10	3.81
	BS00073116_51*	5D	546.864045	T	2022	2.15E-07	11	4.07
					2023	4.58E-07	10	3.82
	Excalibur_c42190_383*	5D	546.906373	A	2022	2.13E-07	10	4.10
					2023	3.63E-07	10	3.81
	Excalibur_c28592_173*	5D	546.910406	T	2022	2.13E-07	10	4.10
					2023	3.63E-07	10	3.81
	CAP8_c145_89*	5D	547.273494	T	2022	2.26E-08	12	4.43
					2023	7.68E-08	11	4.05
	CAP7_rep_c12715_390	5D	547.273595	C	2022	1.88E-08	12	4.43
					2023	1.09E-07	11	4.01
	AX-158586515*	5D	547.451565	T	2022	4.13E-08	12	4.39
					2023	1.22E-07	11	4.01
	Tdurum_contig75814_655*	6A	571.685447	C/T	2022	2.17E-07	11	4.18
					2023	4.02E-09	13	5.00
	AX-110992521	7A	27.107477	A/G	2023	7.3E-09	13	5.00
	AX-158590420*	7A	693.453814	G	2022	2.97E-09	13	3.47
					2023	5.26E-09	13	3.21
	BS00011469_51	4A	621.848245	T	2023	1.04E-07	10	0.14
	TG0131	5D	546.689237	C	2023	9.58E-07	9	0.11
	BS00073116_51	5D	546.864045	G	2023	7.4E-07	10	0.14

Table 3. (continued)

Ash content, %	Excalibur_c42190_383	5D	546.906373	G	2023	4.44E-07	11	0.14
	Excalibur_c28592_173	5D	546.910406	C	2023	4.44E-07	11	0.14
	CAP8_c145_89	5D	547.273494	C	2023	6.94E-07	10	0.14
	CAP7_rep_c12715_390	5D	547.273595	T	2023	5.76E-07	10	0.14
	wsnp_CAP11_c209_198671	5D	547.273762	C	2023	3.52E-07	11	0.15
	AX-158586515	5D	547.451565	C	2023	5.4E-07	11	0.14
Flour output, %	AX-94626326	5D	547.709624	G	2023	3.88E-07	11	0.14
	AX-94662379	2B	74.250224	T	2022	2.43E-07	12	4.79
	AX-94499635	4A	714.436088	A	2022	2.36E-09	16	5.54
	AX-110992521	7A	27.107477	A/G	2022	3.47E-08	13	5.17
	TA005766-0499*	1A	11.895533	A	2022	2.83E-14	15	16.2
					2023	8.04E-18	18	13.0
Gluten index, %	Tdurum_contig41931_480*	1A	351.882499	A	2022	1.02E-16	25	19.8
					2023	1.56E-13	21	13.2
	AX-94662379*	2B	74250224	C	2022	1.43E-16	18	20.2
					2023	8.58E-20	21	15.7
	Excalibur_c30378_344*	4A	615.437273	G	2022	1.04E-14	17	18.7
					2023	1.61E-15	19	14.0
	BS00011469_51	4A	621.848245	T	2022	3.11E-07	7	11.0
	AX-94499635*	4A	714.436088	G	2022	3.15E-14	17	18.4
					2023	4.49E-19	22	14.9
	TG0131	5D	546.689237	C	2022	2.51E-07	7	9.27
	Tdurum_contig75814_655*	6A	571.685447	C	2022	5.26E-10	11	16.7
					2023	1.29E-10	12	4.65
	AX-110992521*	7A	27.107477	A	2022	2.47E-16	18	21.5
					2023	4.38E-18	20	16.4

Note. A/G, C/T, G/T – heterozygous alleles;

* SNPs showing association during two growing seasons

Fourteen markers were associated with high protein and gluten content: on chromosome 2B (AX-94662379), chromosome 4A (BS00011469_51, AX-94499635), chromosome 5D at 546.68–547.45 Mb (TG 0131, wsnp_JD_c4438_556 7972, AX-158586520, Excalibur_c42190_383, Excalibur_c28592_173, CAP8_c145_89, CAP7_rep_c12715_390, AX-158586515), chromosome 6A (Tdurum_contig75814_655), 7A chromosome (AX-110992521, AX-158590420). On average, the presence of favorable alleles of these SNPs increased the protein content by 1.0–1.4% and gluten content by 3–4% depending on a locus. However, favorable alleles of seven loci TA005766-0499 (1A), AX-94662379 (2B), BS00011469_51, AX-94499635 (4A), TG0131 (5D), Tdurum_contig75814_655 (6A) and AX-110992521 (7A) led to reduction of gluten index.

For the loci of chromosome 1A at the region 351.88 Mb (Tdurum_contig41931_480) and chromosome 4A at the region 615.43 (Excalibur_c30378_344), significant associations were found for two years of research on increasing of the gluten index by 13.2–18.8%. In 2022, an association with increased protein, gluten content, and flour output by 4.79–5.54% was found for three loci – AX-94662379 (2B), AX-94499635 (4A), AX-110992521 (7A).

As a result of the conducted studies, we detected the genomic region: 3 SNPs on chromosome 4A at 615.43–714.43 Mb and 12 SNPs on chromosome 5D at 546.68–547.70 Mb (RefSeq v. 1.0), containing a whole cluster of loci responsible for the concentration of protein and gluten (Table 3). The region on chromosome 4A demonstrated colocalization for both protein and gluten content was determined: candidate gene TraesCS4A02G341700 (621.84 Mb), which identified in study of Hu et al. (2020) like locus of resistance to Fusarium head blight; gene TraesCS4A01G330800 was previously described in study of Leonova and Ageeva (2022) to control the upper internode diameter; and new locus AX-94499635.

In our research, the protein and gluten content in landrace genotypes containing the heterozygous alleles G/T in Excalibur_c30378_344 (candidate gene TraesCS4A01G330800) was higher ($p < 0.0000002$) than that G/G haplotype of modern varieties, which was associated with high gluten index and excellent bread making quality. In this study the region of 5D chromosome was also characterized by colocalization for protein and gluten content. Numerous studies have confirmed the close relationship between these traits (Hao et al., 2022; Leonova et al., 2022). Nine candidate genes were annotated within at 1.02 Mb of 5D chromosome, of which six were previously described as candidate genes for resistance to Soil-borne wheat mosaic virus (Liu et al. 2020): TraesCS5D01G530600, TraesCS5D01G530700, TraesCS5D01G530900, TraesCS5D01G531100, TraesCS5D02G531200, TraesCS5D01G532100 genes. Among genes of this region, two were also linked to highly associated SNPs – TraesCS5D01G532400, TraesCS5D01G532800, and one new SNP (AX-158586520). The novel MTAs associated with grain protein content, grain gluten content, and gluten index were identified: Tdurum_contig41931_480 (1A), AX-158572632 (2A), AX-94499635 (4A), AX-158586137 (5B), AX-

158586520 (5D), AX-11099252, AX-158590420 (7A), which needs the evaluation of their effects and subsequent use in marker-assisted selection of wheat genotypes.

4. CONCLUSION

A comparative analysis between landraces and the promising wheat varieties from breeding programs of Russia and Kazakhstan for quality-related traits and genotypic difference showed the prospects of landraces as starting material for extending the genetic diversity of modern wheat varieties. Based on experiment data among 294 MTAs seven unique loci were identified with a $-\log_{10}(p)$ value >6 in the landrace collection that had significant associations with grain protein content, grain gluten, strong gluten content, gluten index, and vitreousness. 22 candidate genes in landraces and modern wheat varieties, and also seven new MTAs were identified, which can be used for improvement of the wheat grain quality in breeding programs. Genomic regions in chromosomes 4A and 5D were determined, demonstrating colocalization for protein and gluten content. The practical interest for breeding are landrace genes absent in the genotype of modern wheat varieties, the introgression of which allows preserving the gene pools of varieties lost in the twentieth century and extending the genetic diversity of wheat. Landraces as sources of valuable allelic variants of fourteen loci, associated with high protein and gluten content can be used for breeding to improve grain quality traits.

AUTHOR CONTRIBUTIONS

AM and VS conceptualized the idea for research. AC, SE and MK conducted the experiment and collected data. SS and IP collected reviews of literature and prepared the original draft of the manuscript. All the authors participated in the review and editing process to refine the paper before submission.

FUNDING

Field experiments were carried out with the support of the Ministry of Agriculture of the Russian Federation.

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Impact of Growing Conditions and Water Stress on Turmeric

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ARTICLE INFO

Research Article

Received: 4 October 2024

Accepted: 24 February 2025

Published: 23 June 2025

Keywords:

Curcumin

Flavonoid

Greenhouse

Rhizome

Substrate

Citation: Aydınşakir, K., Uysal Bayar, F., & Çınar, O. (2025). Impact of growing conditions and water stress on turmeric. *Turkish Journal of Field Crops*, 30(1), 22-34. <https://doi.org/10.17557/tjfc.1561441>

ABSTRACT

This study investigated the effects of three growing conditions and four water levels on turmeric yield and quality parameters (*Curcuma longa* L.) under a greenhouse condition in Bati Akdeniz Agricultural Research Institute, Türkiye, in the years 2021 and 2022. The experiment was designed as a split-block design, with the main factor being three growing conditions (GC₁: 25% perlite + 75% cocopeat, GC₂: 50% perlite + 50% peat, and GC₃: 75% peat + 25% zeolite). The sub factor was four irrigation water levels (IL₁: 100%, IL₂: 75%, IL₃: 50%, and IL₄: 25%) with three replications. Turmeric plants were irrigated based on daily solar radiation values reaching the greenhouse. It was found that irrigation levels affect plant height, tillers number, leaf width, leaf length, leaf area, rhizome weight, curcumin, total phenolic, and total flavonoid content. Fresh weight of rhizome for IL₁, IL₂, IL₃, and IL₄ treatments was measured as 426.9, 398.9, 308.6, and 253.1 g in 2021 and 608.0, 505.3, 380.0, and 219.7 g in 2022, respectively. It is concluded that decreasing irrigation levels has a positive effect on curcumin, total phenolic and flavonoid content, oil, ar-tumerone, alpha-tumerone, beta-tumerone, and alpha-zingiberene but had an adverse effect on rhizome weight, plant height, tillers number, and leaf area.

1. INTRODUCTION

Turmeric (*Curcuma longa* L.) is one of the world's most valuable and imperative spices. It is also commonly used as a coloring agent in the textile, food, and confectionery industries. In Indian traditional medicine, it is used as an anti-inflammatory, hepatoprotective, antitumor, and antiviral agent to heal wounds, prevent cancer, and relieve gastrointestinal and respiratory disorders (Nandakumar et al., 2022). It's cultivated throughout tropical Asian areas, particularly India, China, Indonesia, Jamaica, Peru, and Pakistan. Turmeric cultivation thrives both as a standalone crop and as an intercrop with coconut cultivation, particularly in regions with high potential like wet and middle zones. Traditionally cultivated turmeric has many local varieties and is known by village names (Salimath 2014; Nandakumar et al., 2022).

India has a unique position in the global spice market as the world leader in turmeric production, consumption, and exporting. It accounts for 78% of global production and 60% of global exports. In the year 2023, turmeric production was 1350000 tonnes, with an area and productivity of 369250 hectares. The global turmeric market size is forecast to be valued at USD 4 419.3 million. The growth pattern of the area, production, and productivity of turmeric over a period of time indicate the growing contribution of production to the area expansion and increased yield. The United States of America, United Kingdom, Australia, Saudi Arabia, and Canada are the major importers of turmeric (Sravani et al., 2023; Reddy, 2023). Market proximity is a significant factor in the competitive landscape of the global spice market. Türkiye proximity to the European market gives it a significant advantage over its Far Eastern competitors. Although there is no precise information on turmeric production in Türkiye, 28483 kg of turmeric was exported in 2023, generating an income of USD 113692 (EİB, 2023).

A substantial body of research has been conducted on the impact of climate change on agricultural products (Gonzalez-Zeas et al., 2014; Lionello et al., 2014; Chandio et al., 2020; Omar et al., 2021; Habib-ur-Rahman et al., 2022). In particular, researchers have highlighted the low yield of agricultural products, emphasizing the importance of creating new crop patterns. According to the projected climate change analysis in Türkiye, the air temperature is projected to increase by 2-5°C and precipitation is projected to decrease in some regions (Aydinsakir, 2023). In order to enhance and sustain agricultural productivity, it is vital to advance technologies and innovations that can mitigate environmentally and agriculturally sensitive climate fluctuations, as well as facilitate the development of novel cropping patterns. Plants are often exposed to adverse environmental conditions due to natural disasters and global climate change. The effects of environmental stress on plants can mainly reduce photosynthesis, chlorophyll content, water content, stomatal closure, and carbohydrate metabolism (Siddique et al., 2016; Sherin et al., 2022). Increased water stress due to climate change is one of the critical abiotic stresses causing most crop losses and can alter a plant at the morphological, anatomical, physiological, biochemical, and molecular levels (Seleiman et al., 2021). It also reduces photosynthetic efficiency by damaging the thylakoid membrane and reducing chlorophyll content (Song et al., 2010).

In Mediterranean countries such as Türkiye, where the climate is subtropical, adapting tropical plants could be considered a means of turning increasing temperatures into an opportunity. Once more, the necessity of controlled use of water resources, which is a consequence of global warming, has made it obligatory. Consequently, the cultivation of species that require a specific water consumption under controlled conditions will prevent the occurrence of excessive water consumption.

Swain et al. (2007) reported that irrigation is the greatest factor affecting crop growth, yield, and quality parameters among agronomic practices. Rathod et al. (2010) argued that soil moisture plays a vital role in turmeric cultivation. Producers often over-irrigate by increasing irrigation frequency and consequently the amount of water applied, which has a detrimental effect on applied nutrients, water, and soil properties. Venkatesha & Siddalingayya (2014) showed that upper soil moisture and nutrient uptake lead to greater growing factors as indicated by upper leaf area, plant height, and chlorophyll content at the top growing period and full dry matter at maturity, which could help in improving turmeric yield characteristics.

Some scientists informed effects of deficit irrigation water levels for turmeric. Numerous studies have demonstrated the positive impact of optimized irrigation regimes (Singte et al., 1997; Wiwatpinyo & Detpiratmongkol, 2008; Rathod et al., 2010; Mohamed et al., 2014; Kaur & Brar, 2016; Chitra et al., 2017; Ravindra et al., 2020; Tripathi et al., 2021; Santosh et al., 2021; Jayakumar et al., 2024), with weekly or controlled irrigation leading to enhanced fresh and dry rhizome weight, leaf width and height, leaf number, total carbohydrates, oil and curcumin content, number of leaves and tillers, height of the plant, leaf area, dry matter production and plant health.

The soilless culture systems, the most intensive production method in agriculture, are based on environmentally friendly technology, which can result in higher yields, even in areas with adverse growing conditions. Soilless systems can produce higher yields even in limited and unfavorable growing conditions. Water availability, nutrition, moisture, and soil aeration are key to plant growth in soilless culture systems (Tuzel et al., 2019). To avoid soil-borne bacterial diseases, turmeric production is moving towards soilless culture. The preferred substrates for turmeric production are those that provide good aeration and can retain moisture and nutrients (Kuehny et al., 2005). Anitha et al. (2022) argued that rhizome

rot and other soil-borne diseases can have a detrimental effect on traditional crops, reducing yield, curcumin content, and other quality indicators. The key to its effectiveness in soilless culture is the maintenance of an almost constant water profile in the root medium. This means that growing soilless can automatically increase yield and quality. Abdullah et al. (2018) evaluated those three different growing conditions (C: sand and cocopeat, B: sand and burned rice husk, M: cocopeat, burned rice husk and sand) on rhizomes of *Curcuma alismatifolia* and they reported that rhizome yield was not affected by growing conditions. However, turmeric plants cultivated in M produced a maximum number of marketable rhizomes m^{-2} (54.5), the highest number of marketable rhizomes clump⁻¹ (2.9), and a high number of storage roots rhizome⁻¹ (8.9).

A substantial body of research has been conducted on turmeric's cultivation and biochemical composition. Nevertheless, no study has been identified in which all subjects were discussed. Furthermore, this study represents the inaugural investigation of its kind in Türkiye. Several studies about turmeric have been conducted around the world. However, no research on how water stress and growing conditions affect turmeric has been found in Türkiye. Therefore, the present study aimed to (i) assess the impact of water stress on turmeric, (ii) determine the influence of different soilless cultivation systems, and (iii) analyze the total phenolic and flavonoid content, as well as oil components, of turmeric grown under greenhouse conditions.

2. MATERIAL AND METHODS

Research site and climate

The research was carried out in a plastic unheated greenhouse (36°56'North latitude, 30°53'East longitude, and an altitude of 28 m above sea level) at the Bati Akdeniz Agricultural Research Institute (BATEM) in Antalya, Türkiye, in the years 2021 and 2022. Figure 1 shows the greenhouse's relative humidity and average temperature during the study period. Average relative humidity and temperature range from 13.4°C to 32.9°C and 28.6% to 83.8% in 2021. In 2022, temperatures and relative humidity fluctuated between 11.1°C and 34.9°C and 31.6% and 78.0%, respectively.

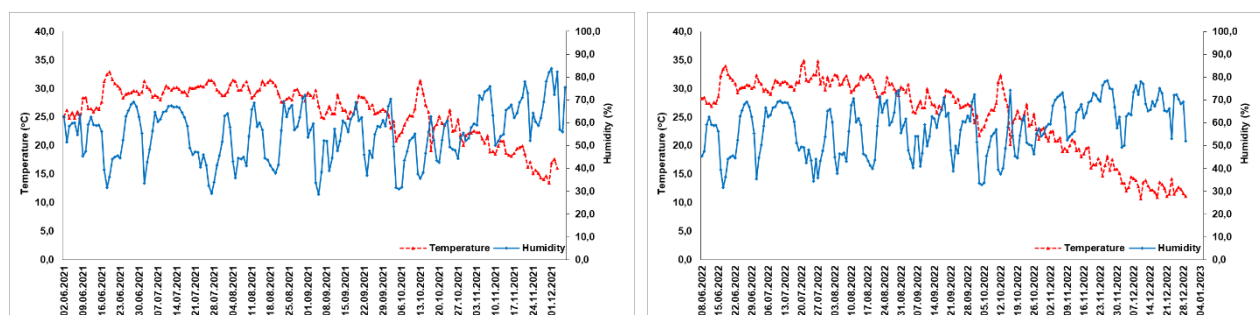


Figure 1. Average temperature and relative humidity measured inside greenhouse.

Treatments and statistical design

The study involved three growing conditions, each consisting of a volumetric mixture of cocopeat (C) and perlite (P) (GC₁: 25% C + 75% P), peat (Pe) and perlite (GC₂: 50% Pe + 50% P), and zeolite (Z) and peat (GC₃: 25% Z + 75% Pe). The four irrigation water amounts (IL₁: 100%, IL₂: 75%, IL₃: 50%, and IL₄: 25%) were also included. Table 1 provides an overview of the properties of the various substrates used in the study. The various growing conditions used in the main plot were arranged in a split block design, while the irrigation levels were arranged in subplot form. The design was a 4 × 3 split plot with three replications of each treatment.

Table 1. Some properties of different growing conditions used in study.

Properties	GC ₁	GC ₂	GC ₃
pH	5.50	6.10	6.20
Electrical Conductivity (micromhos cm ⁻¹)	940.00	445.00	340.00
Humidity (%)	14.60	10.30	18.50
Dry Matter (%)	85.40	89.70	81.50
Organic Matter (%)	29.20	78.80	66.80
Ash (%)	70.80	21.20	33.20
Total Nitrogen (%)	0.17	0.83	0.52
Carbon (%)	16.90	45.70	38.80
Carbon/Nitrogen	100.30	54.80	74.90
Total Iron (ppm)	929.00	844.00	1593.00
Total Manganese (ppm)	14.00	27.00	28.00
Total Zinc (ppm)	6.00	11.00	7.00
Total Copper (ppm)	0.00	8.00	6.00

GC₁: 25% Cocopeat + 75% Perlite, GC₂:50% Peat + 50% Perlite, and GC₃:25% Zeolite + 75% Perlite

Planting and growing conditions

Turmeric rhizomes obtained from genotype 99-5 in the gene pool of BATEM were used as plant material. Turmeric seedlings were implanted in 2.43 m³ polypropylene bags (12.0 m length × 0.45 m width × 0.45 m depth) with one row (0.45 m × 0.45 m spacing, 5 plants m⁻²) on the 2nd of June 2021 and on the 8th of June 2022 in the experiment. The distance between two adjacent polypropylene bags was set at 0.5 m. Three different types of growing conditions were placed in the polypropylene bags. There were 27 plants in each of the polypropylene bags. To collect the drainage water, the polypropylene bags were placed in a gutter.

Managing nutrients and irrigation

Hoagland and Arnon's (1950) nutrient solution was used to irrigate all treatments ensuring balanced nutrient levels. A nutrient solution tank (1,000 liters) and pump were installed at each irrigation level. Nitric acid was added to the solution in each irrigation tank to adjust the pH to between 5.5 and 6.0. All of the treatments were irrigated at the same time of day. The plants were irrigated using a drip irrigation system with a capacity of 1.6 L h⁻¹ and a pressure of 0.1 MPa. The solar radiation received in the greenhouse was used to determine the irrigation frequency. The solar radiation received was used to calculate the amount of water applied. A digital timer was used to schedule the irrigation automatically. The irrigation water application rate was determined by the radiation-based evapotranspiration method. For this purpose, the four irrigation rates of 100% (IL₁), 75% (IL₂), 50% (IL₃), and 25% (IL₄) times the standard rate were applied using a solar radiation device located in the greenhouse. The following equation (Guyot, 1998) was used to calculate the irrigation water amount (L).

$$I = \frac{R_i}{\lambda} \times A \quad (1)$$

Where I is the amount of irrigation water (L), R_i is the received solar radiation inside the greenhouse (MJ m⁻² day⁻¹), λ is the latent heat of water vaporization (MJ kg⁻¹), and A is the polypropylene bag area (m²).

The following equation was used to calculate the water consumption of the turmeric plant (L plant⁻¹).

$$WC = \frac{I-D}{PN} \quad (2)$$

where WC is the water consumption (L plant⁻¹), I is the amount of the irrigation water (L), D is the drainage water (L) and PN is the number of plants inside the polypropylene bags.

Harvest and measurements

Harvesting was done when the leaves turned yellow and started to dry 50%. On 6 December 2021 and 5 January 2023, 20 plants were harvested from each plot. Fresh rhizome weight was recorded after cleaning the rhizomes under running water. Dry weight was determined after drying in an oven at 40°C until a constant weight was achieved. Plant height and tiller number were measured at harvest for 20 selected plants per plot.

Analysis of total phenolic, flavonoid, and essential oil

The chemical analyses were conducted to determine the effects of water stress and growing media on key bioactive compounds in turmeric, which are linked to its nutritional and medicinal properties. The turmeric rhizomes were dried in an air-circulated oven (Venticell-404 Standard, MMM Group, Germany) at 40°C (7.272 m³ h⁻¹) until humidity reached

about 10% and ground before the extraction process. The grinding was carried out in a grinding machine (Retsch Grindomix, GM 200) at 10,000 rpm for 1 minute.

The total phenolic content of the samples was determined by Folin-Ciocalteu solution with some adjustments in the method proposed by Spanos & Wrolstad (1992). Accordingly, 900 μ L distilled water, 5 mL 0.1 N Folin-Ciocalteu solution, and 4 mL 7.5% sodium bicarbonate (Na_2CO_3) solution was added to 100 μ L sample extract, and the mixture was kept in the dark for 90 min. The absorbance of the mixture was measured in a spectrophotometer (Shimadzu UV 1800, Japan) at a wavelength of 765 nm. Total phenolic content was expressed as mg GAE (Gallic Acid Equivalent) g^{-1} dry matter.

The total flavonoid content of the sample extracts was determined spectrometrically by the method proposed by Zhishen et al. (1999). Accordingly, 4 mL of distilled water and 0.30 mL of 5% sodium nitrite (NaNO_2) solution were added to 1 mL of sample extract. After 5 min, 0.60 mL of 10% aluminum chloride (AlCl_3) solution and at the 6th min later 2 mL of 1 M sodium hydroxide (NaOH) solution were added to the mixture and the volume was completed to 10 mL with distilled water. Absorbance values were read in a spectrophotometer at 510 nm wavelength and the results were given as mg CE (Catechin Equivalent) g^{-1} dry matter.

For the determination of curcumin, the extraction process was carried out by revising the technique of Paulucci et al. (2013). Accordingly, 2.5 g of dried and ground turmeric powder was weighed and transferred to 50 mL falcon tubes. 15 mL of 70% ethanol-water mixture was added. After mixing in a vortex device, shaking was carried out in a temperature-controlled shaker at room temperature for 12 hours. The extracts, which were extracted and centrifuged, were first passed through 0.45 μ m membrane filters and transferred to amber vials by diluting at appropriate ratios in order to be injected into the device to be analyzed. The amount of curcumin was determined using gas chromatography (Agilent 1290) and mass spectrometry (6430 Triple Quadropole) (LC-MS/MS) on a Zorbax RRHD Eclipse Plus C18 column (3 μ m2.1 $\times$ 100mm) (Fischer et al., 2011). The first step was to prepare calibration solutions of curcumin. The parameters of the MS (polarity, fragmentation voltage, fragmentation ions, collision energies) were determined. Using the calibration solutions and MS parameters, the calibration curve of curcumin was constructed. Calibration curves were used for quantification.

The essential oils of turmeric used in the study were obtained using the hydro-distillation method in the Clevenger apparatus. Approximately 20 g of dry powder was added to 200 ml of distilled water and distillation was carried out for 2 hours and the essential oil ratio was calculated using the amount of essential oil obtained (Anonymous, 2011). To determine the constituents of the essential oils obtained, the essential oils were diluted 1:100 with hexane.

Analysis of the essential oil components was performed by GC/GC-MS (gas chromatography (Agilent 7890A) - mass detector (Agilent 5975C)) using a capillary column (HP Innowax Capillary; 60.0 m $\times$ 0.25 mm $\times$ 0.25 μ m). Helium gas was used as the carrier gas for analysis at a flow rate of 0.8 ml min^{-1} , and samples were injected into the instrument at a 40:1 split ratio in 1 μ L injection volume. The injector system temperature was maintained at 250°C. The column temperature program was programmed as 60°C (10 min), 60°C to 220°C at 4°C min^{-1} and 220°C (10 min). Using this temperature program, the total analysis time was 60 minutes. The scan range (m/z) for mass detection was 35-450 atomic mass units and the electron bombardment ionization was 70 eV. The results of the Wiley and Oil Adams libraries were used to identify the essential oil components. The percentages of the components obtained were determined using the FID detector and the components were identified using the MS detector (Uysal Bayar & Cinar, 2021).

Statistical analyses

36 experimental plots were used in a split block design with three growing media (main plot factor) and four irrigation levels (subplot factor), replicated three times for each treatment. During the experiment, the fresh and dry weight of the rhizome (g plant^{-1}), the plant height (cm), leaf length (cm), leaf width (cm), leaf area per plant (cm^2), the tillers number (per plant), the evapotranspiration (L plant^{-1}), the essential oil, phenolic and flavonoid content (%), and essential oil compounds were determined. SPSS Statistics Program (SPSS Inc., Chicago, IL, USA) was used for data analysis and LSD test was used to compare significant differences between averages ($p < 0.05$) (Dean et al., 2017).

3. RESULTS AND DISCUSSION

Plant water consumption

The daily average solar radiation and the plant water consumption of the turmeric are shown in Fig. 2. During the 2021 and 2022 growing seasons, solar radiation ranged from 1.98 to 12.00 $\text{MJ m}^{-2} \text{day}^{-1}$, and 0.18 to 17.97 $\text{MJ m}^{-2} \text{day}^{-1}$, respectively. The highest solar radiation was measured on the 14th of June 2021 and on the 16th of June 2022. Turmeric is considered to be a shade-loving plant. Therefore, excessive solar radiation in a region where latitude, climate, and weather patterns have a strong influence on the amount of sunlight, could limit the production of turmeric. Common signs of radiation stress in turmeric plants include leaf-tip burn and stunting, which have a direct impact on the rhizome yield.

Studies have shown that growing turmeric under shade or greenhouse improves leaf area, nutrient uptake, and photosynthetic rate, and reduces leaf temperature and transpiration, ultimately improving plant growth and yield (Hossain et al., 2009; Nair, 2019; Sharangi et al., 2022).

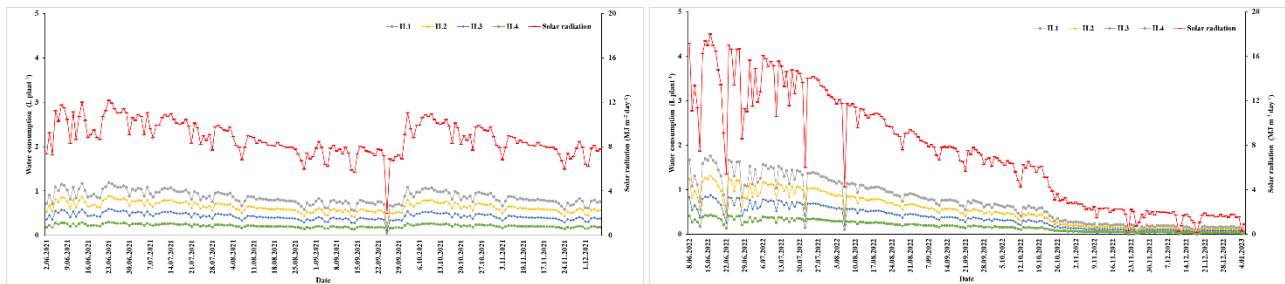


Figure 2. Daily solar radiation and cumulative water consumption.

Water consumption per plant ranged from 40.3 to 161.2 L (201 to 806 mm) in 2021 and from 63.2 to 253.0 L (316 to 1265 mm) in 2022. Rathod et al. (2010) and Sadarunnisa et al. (2010) showed improved turmeric growth and yield by using improved irrigation scheduling. Chitra et al. (2017) report that water is the major limiting factor of curcuma production, arguing that the timing and intensity of water stress during the growing period reduces photosynthesis and stunts growth, significantly reducing yield and yield parameters. Kaur & Brar (2016) reported that irrigation water applied between 515 and 936 mm was conducive to turmeric crop development, yielding the highest total and cured rhizome yields, the highest average mass for export-grade rhizomes and the lowest small rhizome production. Ravindra et al. (2020) measured that the irrigation water amount of turmeric varied between 1487 and 1835 mm in open field and surface irrigation conditions, while the irrigation water amount of turmeric varied between 498 and 706 mm in open field and drip irrigation conditions. Somdutt et al. (2022) determined the influence of deficit irrigation amounts (I_1 : 100% PE, I_2 : 80% PE, I_3 : 60% PE and I_4 : surface irrigated control plot) on turmeric and found that water consumption was 365.8, 322.6, 279.3 and 583.4 mm, respectively.

Effects of treatments on morphological parameters

The results with respect to yield and quality of turmeric, together with statistical analysis, are presented in Table 2. Considering the interactions, it was found that leaf width, were statistically different at $p < 0.05$ in 2021 whereas plant height and leaf length were statistically different at $p < 0.05$ and $p < 0.01$ in 2022. The average the plant height, tiller number, leaf length and width, and leaf area of turmeric plants under different growing conditions and irrigation levels and their variance analysis were given in Table 2. The different irrigation levels in both years influenced the plant height, tiller number, leaf length, and width significantly ($p < 0.01$). There is a significant effect of growing conditions ($p < 0.05$) and irrigation level ($p < 0.01$) on plant height in both experimental years, but growing conditions $\times$ irrigation level interaction was not significant in 2021. Plant heights determined under irrigation levels were 117.3, 104.8, 91.8, and 75.4 cm for IL_1 , IL_2 , IL_3 , and IL_4 , respectively in 2021 while 108.7, 89.8, 80.5, and 72.8 cm for IL_1 , IL_2 , IL_3 , and IL_4 , respectively in 2022 (Table 3). The highest plant height was achieved by the GC_1IL_1 treatment in 2021 and 2022, depending on the interaction between growing conditions and irrigation level. The highest plant height was achieved in the GC_1IL_1 treatment at 130.0 and 130.2 cm, while the lowest plant height was achieved in the GC_3IL_4 treatment at 73.7 and 56.0 cm in the study. With increasing water stress, plant height decreased. Plant height is one of the most important parameters affecting plant weight and is a good indicator of the effect of water stress on the plant. Water stress, an abiotic stress, causes an increase in root zone osmolality, which inhibits root uptake of water and plant nutrients (Sonneveld et al., 1999). Sadarunnisa et al. (2010) observed significantly higher plant height of turmeric on drip irrigation than on check-basin irrigation, leading to an increase of 91.7% in the benefit/cost ratio for the former compared to the latter. Mohamed et al. (2014) studied three treatments of irrigation spacing (weekly, bi-weekly, and tri-weekly) for *C. aromatica* and *C. domestica* and found that plant heights were 91.7, 88.3, and 60.3 cm for *C. aromatica* and 81.7, 66.7, and 46.7 cm for *C. domestica*, respectively, in Egypt. Tripathi et al. (2021) determined the plant height of turmeric (*Curcuma longa* L.) as 126.1, 128.8, 127.1, and 115.8 cm under 0.6, 0.9, 1.2 IW/CPE and rainfed conditions in West Bengal, India, respectively. On the other hand, Kaur & Brar (2016) reported the plant height of turmeric (*Curcuma longa* L.) as 29.6, 31.9, 33.4, and 35.4 cm under 0.6, 0.8, 1.0, and 1.2 IW/CPE in Ludhiana, India, respectively. Chitra et al. (2017) determined the seven different irrigation programs on *Curcuma longa* L. var. CO₂ and they measured plant height as 90.7, 93.3, 95.6, 84.1, 86.2, 79.3, and 93.4 cm for surface irrigation, 0.09 irrigation water/cumulative pan evaporation, drip once in a day at 80% PE, drip once in two days at 80% PE, drip once in a day at 60% PE, drip once in two days at 60% PE, drip once in a day at 40% PE, and drip once in two days at 40% PE in Coimbatore, India, respectively.

Table 2. Variance analysis results for yield and quality of turmeric.

Source of variation	df	2021														
		PH	TN	LW	LL	LA	FW	DW	C	TP	TF	EO	T	AT	BT	AZ
Replication	2	250.1	2.53	4.23	63.2	5311416	15257.2	203.3	0.03	0.65	3.78	0.03	40.4	19.3	20.46	3.96
Growing Conditions (GC)	2	1234.3*	2.03 ^{NS}	13.06**	110*	23002082**	150182**	10127.1**	0.77**	0.71 ^{NS}	3.41**	10.58**	33.3*	17*	0.92 ^{NS}	1.91 ^{NS}
Error (GC)	4	505.7	1.53	0.52	23.7	2150103	24065.4	356.7	0.05	0.54	0.04	0.1	3	2	0.67	0.17
Irrigation Level (IL)	3	2896.2**	4.69**	22.64**	174.7**	6392465 ^{NS}	58114.7*	2945**	0.83**	2.17**	1.95*	4.45**	36.3**	42.8**	8.95*	7.57**
GC × IL	6	323.4 ^{NS}	0.58 ^{NS}	4.79*	14.8 ^{NS}	1609496 ^{NS}	8669 ^{NS}	577.4 ^{NS}	0.08 ^{NS}	0.47 ^{NS}	0.14 ^{NS}	0.19 ^{NS}	6.8 ^{NS}	1.5 ^{NS}	0.89 ^{NS}	0.47 ^{NS}
Error (GC × IL)	18	918.9	1.93	7.84	62	5533046	38441	2022.8	0.28	0.84	1.25	2.12	18.2	12.8	4.57	2.23
Total	35															
Source of variation	df	2022														
		PH	TN	LW	LL	LA	FW	DW	C	TP	TF	EO	T	AT	BT	AZ
Replication	2	158	3.8	3.47	3.4	23112410	15906.1	1160.4	0.02	13.29	22.56	3.47	19.7	115.26	4.02	27.23
Growing Conditions (GC)	2	411.4*	2.93 ^{NS}	4.07 ^{NS}	24.4 ^{NS}	71258083 ^{NS}	110568.3*	6585.7**	0.13 ^{NS}	2.65 ^{NS}	4.27 ^{NS}	1.5 ^{NS}	20.52 ^{NS}	2.28 ^{NS}	1.52 ^{NS}	3.67 ^{NS}
Error (GC)	4	394.2	1.01	2.49	37.4	27744781	26261.6	1683.8	0.03	0.63	0.56	0.83	22.58	5.2	1.13	4.27
Irrigation Level (IL)	3	2150.2**	5.19**	30.58**	307.9**	329643804*	252260.9**	12334.3**	0.75**	2.63 ^{NS}	2.39*	5.7*	32.8*	15.8 ^{NS}	12.16**	8.81 ^{NS}
GC × IL	6	577.2*	0.28 ^{NS}	3.96 ^{NS}	63.5**	28803598 ^{NS}	42706.9 ^{NS}	2174.9 ^{NS}	0.03 ^{NS}	0.22 ^{NS}	0.2 ^{NS}	0.55 ^{NS}	7.67 ^{NS}	0.87 ^{NS}	1.67 ^{NS}	0.91 ^{NS}
Error (GC × IL)	18	742.9	2.04	8.27	88.8	85969007	80648.2	4251.7	0.17	2.56	3.78	1.98	18.54	18.15	3.65	6.52
Total	35															

PH: Plant height (cm), TN: Tillers number (number plant⁻¹), LW: Leaf width (cm), LL: Leaf length (cm), LA: Leaf area (cm²), FW: Fresh weight (g), DW: Dry weight (g), Curcumin (%), TP: Total phenolic (mg GAE g⁻¹), TF: Total flavonoid (mg CE g⁻¹), EO: Essential oil content (%), T: ar-tumerone (%), AT: alpha-tumerone (%), BT: beta-tumerone (%), AZ: alpha-zingiberene (%)

NS: not significant, *: significant at p < 0.05, **: significant at p < 0.01.

Table 3. Means of plant height, tillers number, leaf width, leaf length, and leaf area determined in different growing conditions and irrigation levels.

Treatment	Years									
	2021					2022				
	PH	TN	LW	LL	LA	PH	TN	LW	LL	LA
Growing Conditions (GC)	12.92*	NS	1.05**	4.3*	1427**	11.43*	NS	NS	NS	NS
GC ₁	105.0 a	3.3	6.0 a	42.2 a	6670.0 a	88.5 ab	2.7	11.1	30.7	13713.0
GC ₂	85.8 b	2.5	4.5 b	36.2 b	4067.6 b	81.8 b	2.0	10.3	33.4	10515.9
GC ₃	101.2 a	3.2	4.0 b	39.0 ab	4549.5 b	93.5 a	2.9	9.9	32.9	15300.0
Irrigation Level (IL)	14.91**	0.96**	1.22**	4.97**	NS	13.20**	0.91**	1.25**	3.96**	4492.2**
IL ₁	117.3 a	4.0 a	7.2 a	44.5 a	6305.1	108.7 a	3.4 a	12.2 a	38.9 a	20010.3 a
IL ₂	104.8 ab	2.9 b	4.4 b	40.4 b	4996.3	89.8 b	2.9 ab	11.5 a	34.4 a	15560.4 b
IL ₃	91.8 b	2.6 b	4.0 b	37.7 bc	4685.9	80.5 bc	2.1 bc	10.1 b	31.0 a	11306.8 bc
IL ₄	75.4 c	2.3 b	3.7 b	34.0 c	4395.5	72.8 c	1.7 b	8.0 c	25.0 b	5827.9 c
GC×IL	NS	NS	2.09*	NS	NS	22.87*	NS	NS	6.85**	NS
GC ₁ IL ₁	130.0	4.0	10.1 a	45.5	9133.6	130.2 a	3.5	13.5	43.8 a	21385.9
GC ₁ IL ₂	122.0	3.0	5.6 bc	44.8	6285.4	90.0 bc	3.5	12.9	32.2 bc	16492.3
GC ₁ IL ₃	90.0	3.0	4.3 c	42.7	5847.3	89.0 bc	2.0	9.7	23.7 d	11714.4
GC ₁ IL ₄	78.0	3.0	4.0 c	36.0	5413.7	87.8 bc	1.8	8.2	23.1 d	5259.2
GC ₂ IL ₁	94.3	3.7	7.0 b	40.3	4614.7	91.0 bc	2.7	12.0	35.6 b	14062.9
GC ₂ IL ₂	88.0	2.0	3.6 c	38.0	4154.4	87.2 bc	2.2	10.4	35.4 bc	10792.5
GC ₂ IL ₃	86.3	2.3	3.8 c	34.2	3914.3	74.5 cd	1.5	9.7	33.9 bc	9694.4
GC ₂ IL ₄	74.7	2.0	3.6 c	32.2	3587.0	74.7 cd	1.5	9.2	28.6 cd	7513.9
GC ₃ IL ₁	127.7	4.3	4.5 c	47.6	5167.1	104.8 b	3.9	11.0	37.3 ab	24582.1
GC ₃ IL ₂	104.3	2.0	4.0 c	38.4	4549.2	92.3 bc	3.2	11.2	35.7 b	19396.3
GC ₃ IL ₃	99.0	2.7	3.9 c	36.2	4296.1	78.0 cd	2.7	10.8	35.3 bc	12511.6
GC ₃ IL ₄	73.7	3.7	3.6 c	33.9	4185.8	56.0 d	1.8	6.7	23.4 d	4710.6

PH: Plant height (cm), TN: Tillers number (number plant⁻¹), LW: Leaf width (cm), LL: Leaf length (cm), LA: Leaf area (cm²)

GC₁: 25% Perlite + 75% Cocopeat, GC₂: 50% Peat + 50% Perlite, GC₃: 25% Zeolite + 75% Peat, IL₁: Irrigated at 100%, IL₂: Irrigated at 75%, IL₃: Irrigated at 50%, and IL₄: Irrigated at 25%.

NS: not significant, *: significant at p < 0.05, **: significant at p < 0.01.

*: Within each column, the levels containing the same letter form a group of means within which there are no statistically significant differences (95% confidence level).

As seen in the analysis of variance given in Table 2, only the effect of water amounts on tillers number was significant ($p < 0.01$). Tillers number was not influenced significantly by growing conditions and growing conditions $\times$ irrigation interaction. The highest tiller number (4.0) was determined in IL₁ irrigation treatments, whereas the lowest tiller number (2.3) was determined in IL₄ irrigation treatments in 2021 (Table 3). Similar results were obtained in 2022. The tiller number of turmeric consistently decreased with reduced irrigation amounts. Satyareddi & Angadi (2014) argued that appropriate quantities of water maintain the soil water balance in the effective root depth throughout the growing stage, resulting in high growth traits due to ideal cellular turgor, leading to cellular elongation and development of cellular walls, and thus maintaining acceptable soil physical conditions for plant growth. Aydınsakir et al. (2024) reported that reducing water amount adversely affects plant growth by reducing soil moisture content, which limits root water availability. Water stress also reduces plant growth through reduced photosynthetic rates and cell expansion. Gatabazi et al. (2019) argued that the primary marks of water stress generally lead to a reduction in cell growth, leading to decreased cell growth, especially the number of the tiller and leaf. Singte et al. (1997) measured maximum tillers/plant and turmeric yield with 100% evaporative replenishment than with 80% and 60% evaporative refill. Tripathi et al. (2021) stated that the tiller numbers varied between 2.1 and 2.5, while Kaur & Brar (2016) reported that the tiller numbers varied between 2.1 and 2.8. Chitra et al. (2017) found that the tiller number was 4.1 and 3.1 under drip once a day at 80% pan evaporation and drip once in two days at 40% pan evaporation conditions, respectively.

Leaf width, leaf length, and leaf area are the most critical morphological parameters directly associated with photosynthesis and increased crop yield and parameters. The effects of water levels, growing conditions, and water levels $\times$ growing conditions on leaf width were found statistically significant in 2021 (Table 2). On the other hand, the effects of water levels and water levels $\times$ growing conditions on leaf width were not statistically significant in 2022. The longest leaf width of 7.2 cm in 2021 and 12.2 cm in 2022 was obtained from the leaves of plants grown under the treatment of IL₁. The shortest leaf width of 3.7 cm in 2021 and 8.0 cm was obtained from the leaves of plants grown under the treatment of IL₄ (Table 3). In general, leaf width values increased as water stress decreased. The influences of deficit irrigation amount on leaf length were statistically significant at a 1% level. The longest leaf length of 44.5 cm in 2021 and 38.9 cm in 2022 was obtained from IL₁ treatment. The shortest leaf length of 34.0 cm in 2021 and 25.0 cm in 2022 was obtained from IL₄ treatment. As with the leaf width, leaf length values decreased as water stress increased. The values of plant height, leaf width and length, tillers number, and leaf area under the IL₁ treatment were higher than the IL₄ treatment by 52%, 85%, 64%, 41%, and 157%, respectively. Mohamed et al. (2014) argued that water stress decreased leaf width and they found leaf widths ranging from 12.7 to 18.3 cm for *C. aromatica* and 10.9 to 14.3 cm for *C. domestica*. Chungloo et al. (2024) found turmeric leaves to be the longest under well-watered conditions (17.1 cm) and significantly shorter under drought conditions (12.1 cm), with a 29% reduction in leaf length. Satyareddi & Angadi (2014) argued that compared to other irrigation methods evaluated (furrow irrigation: 4210 cm²plant⁻¹ and sprinkler irrigation: 4460 cm²plant⁻¹), drip irrigation applied at 50% available soil moisture depletion resulted in higher leaf area (4650 cm²plant⁻¹). Chitra et al. (2017) reported that the leaf area during the all-growing periods is an important aspect of turmeric, as it is closely related to the efficiency of photosynthesis, which is reflected in biomass production. They determined the leaf area as 3690, 3025, and 2357 cm² plant⁻¹ under drip irrigation daily at 80% pan evaporation, 60% pan evaporation, and 40% pan evaporation. The reason for the reduction in leaf area with water stress can be explained by the decrease in photosynthetic area due to the lack of adequate moisture in the root zone and the slow accumulation of photosynthetic substances. Findings are in confirmation with the results of Jirali (2001), Sadarunnisa et al. (2010), Deshmukh et al. (2009), and Rathod et al. (2010) in turmeric.

On the other hand, Beardsell et al. (1979) argued that adequate moisture in growing conditions is one of the most critical issues for crop growth and development. On the contrary, Abdullah et al. (2018) stated that the total rhizome yield of *Curcuma alismatifolia* was not affected by different growing conditions and they found that rhizome numbers per square meter as 76.2, 81.8, and 99.3 for cocopeat: sand, burnt rice husk: sand, and coco peat: burnt rice husk: sand, respectively. Gayathiri & Narendhiran (2020) studied the effects of 11 different growing conditions (GC₁: Coir pith + garden soil, GC₂: garden soil + farmyard manure, GC₃: garden soil + vermicompost, GC₄: neem cake + garden soil, GC₅: garden soil + coir pith+ farmyard manure, GC₆: garden soil + coir pith + vermicompost, GC₇: garden soil + coir pith + neem cake, GCT₈: garden soil + farmyard manure + vermicompost, GC₉: garden soil + farmyard manure + neem cake, GC₁₀: garden soil + vermicompost + neem cake, and GC₁₁: garden soil only) on turmeric minisetts in portray nursery. They found that the morphological features such as plant height (44.9 cm), leaf number (4.0), leaf length (20.0 cm), and leaf area (113.73 cm²) were measured in the highest GC₆ treatment. Vidanapathirana et al. (2022) determined the highest plant height from plants grown in cow dung: topsoil: sand (GC₁= 1: 1: 0.5) medium with 111.1 cm, while the lowest plant height was obtained from cow dung: topsoil (GC₂= 1:1) conditions with 97.8 cm. They also found fresh rhizome weight as 267.4 and 326.5 g plant⁻¹ under GC₁ and GC₂ growing conditions.

Effects of treatments on yield of rhizome, curcumin, total phenolic and flavonoid

Growing conditions $\times$ irrigation level interactions were found not to be statistically different ($p < 0.01$) in both years for dry weight, curcumin (%), total phenolic, and total flavonoids. However, irrigation levels were found to be statistically different in the study (Table 2). The fresh rhizome weight varied between 171.8-575.3 g plant⁻¹ in 2021 and 164.7-

826.7 g plant⁻¹ in 2022. The highest rhizome weight was determined in the GC₁IL₁, while the minimum was determined in the GC₃IL₄ in the study. On the other hand, the dry rhizome weight varied between 30.9-136.3 g plant⁻¹ in 2021 and 27.6-186.6 g plant⁻¹ in 2022 (Table 4). Similarly, the highest dry rhizome weight was obtained from the GC₁IL₁ treatment, while the lowest dry rhizome weight was obtained from the GC₃IL₄ treatment in the study. The rhizome weight was relatively lower due to the reduction in irrigation water. The weight of the rhizome in 2022 is higher than in 2021 in the study. Similar findings were reported by Rathod et al. (2010), Mohamed et al. (2014), Anandaraj et al. (2014), Kaur & Brar (2016), Sandeep et al. (2017), Ravindra et al. (2020), Tripathi et al. (2021), and Somdutt et al. (2022), and who observed an increase in rhizome yield with higher irrigation levels in regions with different ecologies around the world.

Table 4. Means of fresh weight, dry weight, curcumin, total phenolic, and total flavonoid parameters determined in different growing conditions and irrigation levels.

Treatment	Years									
	2021					2022				
	FW	DW	C	TP	TF	FW	DW	C	TP	TF
Growing Conditions (GC)	114.02*	20.41**	0.21**	NS	0.58**	119.00*	27.46**	NS	NS	NS
GC ₁	469.1 a	100.5 a	1.69 b	32.14	28.82 a	521.4 a	112.9 a	1.71	30.72	30.34
GC ₂	249.6 b	45.9 b	2.15 a	34.70	32.14 a	329.7 b	66.6 b	1.57	32.16	34.40
GC ₃	321.9 b	56.0 b	2.11 a	29.84	21.78 b	433.7 ab	83.3 b	1.50	23.48	22.62
Irrigation Level (IL)	131.66*	23.57**	0.24**	0.45**	0.67*	137.41**	31.71**	0.23**	NS	6.34*
IL ₁	426.9 a	85.8 a	1.65 b	26.30 b	22.20 b	608.0 a	126.9 a	1.18 b	21.90	23.14 b
IL ₂	398.9 a	74.4 a	1.84 b	30.22 b	26.15 b	505.3 ab	103.8 ab	1.65 a	27.36	27.80 b
IL ₃	308.6 ab	66.5 ab	2.12 a	35.45 a	28.44 ab	380.0 b	79.5 b	1.71 a	31.22	29.92 ab
IL ₄	253.1 b	43.1 b	2.34 a	37.00 a	33.42 a	219.7 c	40.3 c	1.84 a	34.50	35.66 a
GC×IL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
GC ₁ IL ₁	575.3	136.3	1.23	21.20	19.93	826.7	186.6	1.28	21.32	20.40
GC ₁ IL ₂	544.8	103.5	1.40	34.12	28.34	657.3	138.6	1.67	28.50	29.24
GC ₁ IL ₃	403.8	102.5	2.03	35.61	30.92	369.3	79.0	1.90	36.41	33.72
GC ₁ IL ₄	352.5	59.6	2.11	37.60	35.84	232.3	47.3	1.99	36.92	38.04
GC ₂ IL ₁	294.7	52.1	1.79	31.73	28.77	443.3	88.5	1.11	24.30	29.92
GC ₂ IL ₂	248.4	49.1	2.16	33.74	31.24	313.3	66.9	1.63	31.43	34.16
GC ₂ IL ₃	220.3	43.6	2.23	36.35	31.86	300.0	65.2	1.75	33.32	33.84
GC ₂ IL ₄	235.0	38.7	2.42	37.13	36.70	262.0	45.9	1.79	39.30	39.70
GC ₃ IL ₁	410.7	69.0	1.91	25.92	18.00	554.0	105.5	1.14	20.20	19.10
GC ₃ IL ₂	403.5	70.7	1.95	22.74	18.75	545.3	106.0	1.65	21.96	20.14
GC ₃ IL ₃	301.5	53.3	2.10	34.32	22.44	470.7	94.2	1.48	24.18	22.35
GC ₃ IL ₄	171.8	30.9	2.48	36.34	27.68	164.7	27.6	1.75	27.24	29.02

FW: Fresh weight (g), DW: Dry weight (g), Curcumin (%), TP: Total phenolic (mg GAE g⁻¹), TF: Total flavonoid (mg CE g⁻¹)

GC₁: 25% Perlite + 75% Cocopeat, GC₂: 50% Peat + 50% Perlite, GC₃: 25% Zeolite + 75% Peat, IL₁: Irrigated at 100%, IL₂: Irrigated at 75%, IL₃: Irrigated at 50%, and IL₄: Irrigated at 25%.

NS: not significant, *: significant at $p < 0.05$, **: significant at $p < 0.01$.

*: Within each column, the levels containing the same letter form a group of means within which there are no statistically significant differences (95% confidence level).

As shown in Table 2, in the first year of the experiment, there was a significant difference ($p < 0.01$) between the irrigation treatments. While curcumin varied between 1.65 % and 2.34 % in the first year of the study, in the second year the variation was between 1.18 % and 1.84 %. Depending on the irrigation treatments, the highest curcumin was obtained from the IL₄ irrigation treatment in 2021 and 2022 (Table 4). In the present study, water stress induced an increase in curcumin content. Raven et al. (2005) reported that abiotic stress can have similar effects on accumulating secondary metabolites. Prolonged water stress reduces key physiological traits and switches on defense mechanisms, resulting in higher production of secondary metabolites, including curcuminoids in our study. The highest curcumin amount was obtained as 2.48% from GC₃IL₄ treatment in the first year and 1.99% from GC₁IL₄ treatment in the second year, while the lowest curcumin was 1.23% from GC₁IL₁ treatment in the first year and 1.11% from GC₂IL₁ treatment in the second year. It was detected that as the water stress decreased, the curcumin amount increased. There is a dilemma regarding the change in curcumin content, as some studies reported that curcumin content increased with increasing water stress. In contrast, others showed that curcumin content decreased with decreasing water stress. Mohamed et al. (2014) claimed that curcumin in dry rhizomes increased with weekly irrigation compared with bi-weekly and tri-weekly irrigation. Chintakovid et al. (2022) found that under full irrigation conditions in the greenhouse, curcumin content decreased by 35.0% compared to water stress conditions. On the contrary, Chungloo et al. (2024) found that the curcumin content of turmeric under well-watered conditions was the minimum (9.60 mg g⁻¹ DW), which was significantly increased by 31.0% when exposed to water-deficit conditions (12.52 mg g⁻¹ DW). Further research and validation under different water stress conditions is required to provide definitive data on the curcumin content of turmeric plants.

The content of total phenolics and total flavonoids obtained from the three growing conditions and four irrigation amounts used in the experiment, and the results of the analysis of variance of these yields, are presented in Table 2. For

total phenolics and total flavonoids in 2021 and 2022, it was noticed that the interactions were not statistically different. The total phenolic changed between 21.20-37.60 mg GAE g⁻¹ dry weight in the first year and 20.20-39.30 mg GAE g⁻¹ dry weight in the second year of the research while the total flavonoid changed between 18.00-36.70 mg CE g⁻¹ dry weight in 2021 and 19.10-39.70 mg CE g⁻¹ dry weight in 2022. The highest total phenolic was obtained as 37.00 mg GAE g⁻¹ dry weight and 34.50 mg GAE g⁻¹ dry weight from IL₄ treatment in 2021 and 2022 while the lowest total phenolic was 26.30 mg GAE g⁻¹ dry weight and 21.90 mg GAE g⁻¹ dry weight from IL₁ treatment in 2021 and 2022 in the study. In 2021 and 2022, the highest total phenolics were found in the IL₄ irrigation level, depending on the irrigation level (Table 4). On the other hand, the highest total phenolics were obtained from GC₂ in the first and second years of the study. Albergharia et al. (2020) reported that the widely accepted notion of a general increase in phenolic compounds in response to water shortage is incorrect, stating that, contrary to this notion, this complex mechanism is different in each plant species. Therefore, further studies are needed to fully explain the role of different irrigation levels and different growing conditions on the total phenolic content of turmeric.

Essential oil and components

The influence of different water amounts on the essential oil, ar-tumerone, alpha-tumerone, beta-tumerone, and alpha-zingiberene in turmeric is given in Table 5. The effect of irrigation water amounts was significant ($P < 0.01$) for essential oil, ar-tumerone, alpha-tumerone, beta-tumerone, and alpha-zingiberene in 2021 and 2022 (Table 2). Essential oil, ar-tumerone, alpha-tumerone, beta-tumerone and alpha-zingiberene content increased significantly when irrigation levels were reduced. It was found that growing conditions were not statistically different for beta-tumerone and alpha-zingiberene content in 2021 and 2022. The highest essential oil (5.94%-6.83%), ar-tumerone (51.5%-51.3%), alpha-tumerone (24.4%-19.7%), beta-tumerone (6.59%-6.65%), and alpha-zingiberene (5.12%-6.72%) content was obtained from IL₄ irrigation treatment in both of study years (Table 5). Essential oil compounds can either decrease or show no change in concentration when exposed to water stress (Albergharia et al., 2020), so the widely held belief that there is a widespread increase in essential oil compounds in response to water stress is mostly incorrect. Additionally, Jiang & Huang (2001) and Weidner et al. (2009) found either a decrease or an increase in the levels of oil compounds in response to water stress. Furthermore, the systematic review of the effect of water stress on the content of oil compounds of medicinal plants by Albergharia et al. (2020) found no widespread increase in oil compounds as response to water stress. Mohamed et al. (2014) reported that water stress leads to biochemical perturbations and may alter the way plants behave with respect to primary and secondary metabolite biosynthesis, that fats are essential for cell functioning, and that the plasma membrane may be the primary site of water stress damage. Chungloo et al. (2024) found that the bisdemethoxycurcumin and demethoxycurcumin content of turmeric plants under well-watered conditions was the minimum (5.23 and 5.43 mg g⁻¹ DW), which was significantly increased when exposed to water-deficit conditions (7.16-7.38 mg g⁻¹ DW). Results obtained from the current study recommend that increased water amount can limit specific components to increase secondary metabolites in confirmation with the findings of Battaieb et al. (2010), Gatabazi et al. (2022), El Sherif et al. (2022), Khatatb et al. (2023) and Aydınsakir et al. (2024).

Table 5. Means of essential oil compounds determined in different growing conditions and irrigation levels.

Treatment	Years									
	2021					2022				
	EO	T	AT	BT	AZ	EO	T	AT	BT	AZ
Growing Conditions (GC)	0.31**	2.11*	1.60*	NS	NS	NS	NS	NS	NS	NS
GC ₁	4.56 b	47.2 b	22.7 a	5.43	3.58	5.66	50.1	18.6	5.33	6.17
GC ₂	6.31 a	50.3 a	21.1 ab	5.35	4.32	6.33	51.0	18.2	5.32	5.14
GC ₃	4.85 b	49.9 a	20.3 b	4.92	4.22	5.79	48.4	17.7	4.71	6.02
Irrigation Level (IL)	0.35**	2.44**	1.85**	1.57*	0.73**	1.19*	3.85*	NS	1.25**	NS
IL ₁	4.34 c	46.7 c	19.3 b	4.30 b	2.92 c	4.94 b	47.1 b	16.9	3.93 c	4.50
IL ₂	5.04 b	48.9 bc	20.7 b	4.73 b	3.84 b	5.72 ab	49.9 ab	17.2	4.61 bc	5.51
IL ₃	5.63 a	49.4 ab	21.1 b	5.32 ab	4.28 b	6.20 a	51.1 a	18.7	5.28 b	6.36
IL ₄	5.94 a	51.5 a	24.4 a	6.59 a	5.12 a	6.83 a	51.3 a	19.7	6.65 a	6.72
GC×IL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
GC ₁ IL ₁	3.87	42.8	20.7	5.09	2.37	5.08	45.0	17.6	3.84	5.07
GC ₁ IL ₂	4.34	47.6	21.1	5.03	3.49	5.59	50.9	18.2	4.64	6.26
GC ₁ IL ₃	4.58	48.3	22.7	5.38	4.10	5.89	51.7	19.1	5.17	6.41
GC ₁ IL ₄	5.43	50.2	26.1	6.23	4.38	6.07	53.0	19.5	7.67	6.92
GC ₂ IL ₁	5.15	48.8	19.6	3.88	3.40	4.83	49.4	17.0	3.74	3.07
GC ₂ IL ₂	6.15	50.5	20.9	4.86	3.71	6.22	50.6	17.3	4.59	4.85
GC ₂ IL ₃	6.94	50.8	20.4	5.98	4.23	6.50	53.1	18.5	5.70	6.22
GC ₂ IL ₄	7.00	50.9	23.6	6.70	5.93	7.76	50.7	19.9	7.25	6.42
GC ₃ IL ₁	4.01	48.4	17.4	3.92	2.99	4.92	46.9	16.0	4.21	5.36
GC ₃ IL ₂	4.63	48.7	20.2	4.28	4.33	5.35	48.2	16.3	4.60	5.43
GC ₃ IL ₃	5.37	49.2	20.1	4.62	4.51	6.23	49.0	18.6	4.98	6.45
GC ₃ IL ₄	5.39	53.5	23.5	6.85	5.07	6.67	49.5	19.9	5.05	6.82

EO: Essential oil content (%), T: ar-tumerone (%), AT: alpha-tumerone (%), BT: beta-tumerone (%), AZ: alpha-zingiberene (%)

GC₁: 25% Perlite t + 75% Cocopeat, GC₂:50% Peat + 50% Perlite, GC₃:25% Zeolite + 75% Peat, IL₁: Irrigated at 100%, IL₂: Irrigated at 75%, IL₃: Irrigated at 50%, and IL₄: Irrigated at 25%.

NS: not significant, *: significant at $p < 0.05$, **: significant at $p < 0.01$.

^a : Within each column, the levels containing the same letter form a group of means within which there are no statistically significant differences (95% confidence level).

4. CONCLUSIONS

Several scientists have studied the water stress of turmeric in different countries. However, there is a lack of research on analyzing irrigation levels and growing conditions of turmeric crops in Türkiye. The present research determined the effects of irrigation water amounts and different growing conditions in turmeric cultivated in the greenhouse on yield, yield parameters, and essential oil. The data showed that deficit irrigation treatments significantly decreased rhizome dry and fresh weight, plant height, leaf length and width, tillers number, leaf area, and essential oil, but increased curcumin, total phenolic, total flavonoid, and essential oil compounds as compared to control irrigation treatments. In the research, the maximum rhizome fresh and dry weight was obtained from IL₁ treatments. Compared with the IL₁ water level, the mean curcumin content increases were 19, 26, and 34% and the total phenolic content increases were 16, 27, and 32% for IL₂, IL₃, and IL₄ treatments, respectively. Growing conditions GC₁ (covering 25% perlite + 75% cocopeat) indicated good crop growth and increased the fresh rhizome yield up to 73% and 33% compared to GC₂ (covering 50% peat + 50% perlite) and GC₃ (covering 25% zeolite + 75% peat). The combination of GC₁IL₁, also improved plant height, rhizome weight, and leaf area of turmeric. In conclusion, it can be argued that although water stress is an advantage for producing curcumin, essential oil, total phenolic compounds, and total flavonoids, it is a disadvantage for the yield of rhizomes and the morphological parameters. The data obtained from the current study can be used to select phenotypic traits for the development of drought-tolerant turmeric plants for breeders or to help producers achieve a higher yield per unit area under water stress conditions.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support from the General Directorate of Agricultural Research and Policies under Project No: TAGEM/TBAD/B/20/A07/P06/5300.

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Improving of Simple Selection Index to Determine Oat (*Avena sativa* L.) Genotypes for Dual Purpose Production

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ARTICLE INFO

Research Article

Received: 18 November 2024

Accepted: 26 March 2025

Published: 23 June 2025

Keywords:

Dry hay yield

Dual purposed

Grain yield

Oat genotypes

Selection index value

Yield related traits

ABSTRACT

Selection indices might be crucial to develop multipurpose genotypes in most environments for oat production. This study aimed to improve simple selection index value to determine oat genotypes for dual purpose production. The research was carried out in Kahramanmaraş conditions for two consecutive years between 2020-21 and 2021-22. In the research, a total number of 28 oat genotypes (10 landraces and 18 commercial cultivars) were used as plant materials and, plant height (PH), panicle numbers m-2 (PN m-2), grain number per panicle (GNP), thousand-kernel weight (TKW), harvest Index (HI), dry hay yield (DHY) and grain yield (GY) traits were investigated. Based on grain yield and dry hay yield, formula of selection index value for dual purposed oat genotypes was determined; SI (Selection Index) = AGYG (Average Grain Yield of Each Genotype) – AGYE (Average Grain Yield of Genotypes by the Experiments)/AGYE + ADHYG (Average Dry Hay Yield of Each Genotype)-ADHYE (Average Dry Hay Yield by Experiments)/ADHYE. Total selection index value was also obtained by collecting of selection index values belong to other investigated traits and dual purposed selection index values of oat genotypes.

According to the results, Kahraman with 0.784 value had the highest selection index, which can be used to determine the dual purposed oat genotypes. This genotype was also followed by Kırklar (0.355), TL227 (0.629) and TL341 (0.579) oat genotypes. These oat genotypes with higher selection index values may be suggested for both DHY and GY. Total selection index vales also pointed out same oat genotypes for the experiment region. The results also showed that formula of this simple selection index value may be applied for other cereals such as corn, wheat, barley sorghum etc. and as well as the different traits.

Citation: Dokuyucu, T., Başkonuş, T., & Dumlupınar, Z. (2025). Improving of simple selection index to determine Oat (*Avena sativa* L.) genotypes for dual purpose production. *Turkish Journal of Field Crops*, 30(1), 35-42. <https://doi.org/10.17557/tjfc.1587314>

1. INTRODUCTION

Oat (*Avena sativa* L.) as a cool season cereal crop is grown for human food and livestock feed grown annually both in autumn and spring through the world (Hocaoglu, 2022). Oat breeders aim to improve oat genotypes suitable for growing as dual- purposed crop (Iannucci et al., 2011). In literature, dual purposed crops defined as “grain harvest after grazing or cutting” (Suttie and Reynolds, 2004). However, in recent years, cereals (oat, barley and triticale) used as dual purposed crops produced limited biomass due to climate change and irregular precipitation all over the world, as well as low grain yield after grazing or cutting. Thus, there are too limited environments that dual purposed oat grown worldwide. Fewer grain and fodder (biomass) yields are obtained in most environments due to low temperature or inadequate precipitation after cutting or grazing. Francia et al. (2006) reported significant reduces in oat grain yield after grazing. In addition to this, activity of grazing or cutting of dual purposed oat is very limited due to wet soils during winter conditions in larger areas. Oat is grown for grain or fodder crop as a pure stand as well as accompanied plant with the most common annual forage legumes in most environments of the world. Therefore, selection of oat genotypes with higher grain and hay yield as a pure stand in many of environments is crucial. Cereals are crucial for animal feed with high dry matter contents (Basaran et al. 2017). Oat breeders have also put effort to improve oat genotypes with higher grain yield and hay yield without lodging. In practice, oat growers can harvest oat plants for hay product in 3/4 part of oat field, then leave 1/4 parts of oat in field to harvest for grain, or vice versa. Thus, we suggest using dual purposed oat varieties with higher grain and hay yield in the same season in oat production systems. Adapting of dual purposed oat varieties may provide an option to oat growers to decide harvesting for grain or hay in same season based on the environmental conditions such as drought in generative period and market prices. Determination of oat genotypes with higher grain yield and hay yield is being gradually important by oat breeders. Thus, this research suggests a selection index value to determine dual purposed oat genotypes with higher grain and hay yield in oat production areas.

Bio-agronomic characterization carried out using appropriate statistical methods continues to be a useful tool for the initial description and classification of oat collections, as it allows plant breeders to identify and select valuable genetic resources for direct use by farmers, in breeding programs, or for planning efficient germplasm conservation and use (Achleitner et al. 2008; Rezai and Frey, 1990). In literature, harvest index (HI) which is ratio of grain weight to total plant weight, was improved by Beaven in 1914 as called migration coefficient (Donald and Hamplin, 1976). “The migration coefficient” concept was largely ignored until 1962, in 1962, Donald suggested term “harvest index” and recommended it as an important reference to assess progress in germplasm development towards improved yield potential. In recent years, there has been substantial attention given to harvest indices of crops and association of HI with grain yield (Sinclair, 1998). Leaf area index was the other important index to evaluate the crops as vegetative growth (Fang et al., 2019). Golden ratio was also another mathematical model applied to plants (Zhang et al., 2014). Harmonization ratios of post to pre-anthesis durations of wheat genotypes by thermal times to investigate relationships between harmonization ratios and grain yield, phenological periods by the thermal times (Akkaya et al., 2006). As seen in literature, improved index values are used efficiently in breeding programs of cereal plants.

Some researchers used to evaluate the traits by principal component analysis and coefficient of correlations. Iannucci et al. (2011) reported that the genetic variation in the germplasm for agronomic characteristics can be subjected to multivariate analysis (principal component analysis (PCA) and cluster analysis) to establish the relationships among various accessions. However, these models frequently don't give any chance to evaluate the performance of oat genotypes for grain yield and hay yields simultaneously. In many researches related with oat genotypes, determining oat genotypes with higher grain yield and hay yield is not easy. There is no available formula to evaluate the performance of oat genotypes for both grain yield and hay yield at the same season in literature. Oat breeders need a simple formula giving certain results to determine the oat genotypes with higher grain and hay yield. For these reasons, this research aimed to improve a simple selection index or formula to evaluate dual purposed oat genotypes for higher grain and hay yields, at the same season.

2. MATERIALS AND METHODS

This research was carried out in Kahramanmaraş, Türkiye, for two years, between 2020 and 2022. Results of soil analysis and climate data were given in Table 1 and 2, respectively. The information about oat genotypes used in the study is also given in Table 3.

The soil of the experiment sites had a clay loam structure in both years and had moderate organic matter, unsaline, neutral in pH, and high phosphorus (Table 1).

Table 1. Some physical and chemical properties of experiment soils.

Properties	2020	2021
Saturation (%)	57.4	57.6
pH	6.5	7.0
Salt (%)	0.1	0.1
CaCO ₃ (%)	0.3	5.2
Organic matter (%)	2.8	3.8
K (mg kg ⁻¹)	235	129.0
P (mg kg ⁻¹)	16.2	18.7

Table 2. Climate data of experiment years in Kahramanmaraş location of Türkiye

Months	Rainfall (mm)			Temperature (°C)			Relative Humidity (%)	
	2020-2021	2021- 2022	1930-2023	2020-2021	2021- 2022	1930-2023	2020-2021	2021- 2022
November	55.4	15.80	78	12.4	13.8	11.9	65.9	63.0
December	56.8	120.8	130.6	8.2	7.5	6.7	74.7	69.8
January	211.6	128	124	7.0	4.5	4.8	70.2	71.4
February	29.6	70.6	112.2	9.3	8.7	6.2	59.8	69.3
March	129.2	99.8	95.1	10.4	7.1	10.4	61.2	62.1
April	16.8	9	73	16.6	18.2	15.1	57.5	45.7
May	13.2	28	38.8	23.5	20.4	20.1	43.3	50.7
June	0	4	8.6	25.5	26.2	24.9	49.0	50.8
Total	512.6	476	660.3					
Average				14.1	13.3	12.5	60.2	60.3

In the experiments, 28 oat genotypes were sown in a randomized complete block designs with four replications in the both years. Oat seeds (450 seeds m⁻²) were sown in plots with 5 m long in 6 rows with 0.20 m apart. Sowing dates were on 18 October 2020 in the first year and 22 October 2021 in the second year. Fertilizers were supplied as the pure 69 kg ha⁻¹ P₂O₅ and 27 kg N ha⁻¹ with 150 kg ha⁻¹ di-ammonium- phosphate in first year, 50 kg ha⁻¹ P₂O₅ and 50 kg N ha⁻¹ with 20-20 compose fertilizer (250 kg ha⁻¹) in the second year, at sowing time. Top dress was supplied as 56 kg N ha⁻¹ with 215 kg ha⁻¹ Ammonium Nitrate (26%) at jointing stage in both years. Herbicide (Fullamin 50 LC) was used for broad leaf weed control in the first year on 08 March 2020 at the tillering stage and in the second year on 06 April 2021 at the starting of jointing stage.

Table 3. Oat genotypes used in the experiments

No	Cultivar Name	Origin	Featured Traits	Registration Date
1	Kirkklar	Türkiye	Higher Grain Yield (GY) and thousand-kernel weight (GW)	2014
2	Kahraman	Türkiye	Higher GY and Biomass Yield (BY), stem thickness	2014
3	Sebat	Türkiye	Medium plant height (PH)	2011
4	Yeniceri	Türkiye	Higher Yield and TKW	2013
5	Sari	Türkiye	Higher Panicle weight and TKW	2014
6	Arslanbey	Türkiye	Early maturity, higher stability,	2017
7	Amasya	Türkiye	Local variety	Landrace
8	Ankara 84	Türkiye	Registered local variety	1975
9	2-3	Türkiye	Registered local variety	1963
10	Checota	USA	Medium Earliness, medium resistance to winter	1986
11	Faikbey	Türkiye	Resistance to lodging, higher PH	2004
12	Seydisehir	Türkiye	Resistance to lodging, higher PH	2004
13	Yesilkoy-330	Türkiye	Registered local variety	1975
14	Dirilis	Türkiye	Higher BY, higher stem thickness	2017
15	TL 38	Türkiye	Genetic Resources Nursey	Landrace
16	TL42	Türkiye	Genetic Resources Nursey	Landrace
17	TL137	Türkiye	Genetic Resources Nursey	Landrace
18	TL 218	Türkiye	Genetic Resources Nursey	Landrace
19	TL 227	Türkiye	Genetic Resources Nursey	Landrace
20	TL 242	Türkiye	Genetic Resources Nursey	Landrace
21	TL 330	Türkiye	Genetic Resources Nursey	Landrace
22	TL 341	Türkiye	Genetic Resources Nursey	Landrace
23	TL 374	Türkiye	Genetic Resources of Nursey	Landrace
24	Yazir	Türkiye	Early maturity	2021
25	Kazan	Türkiye	High hay yield	2020
26	Otag	Türkiye	High hay yield	2020
27	Katmerli	Türkiye	Early maturity	2020
28	Yagiz	Türkiye	Non-lodging high grain and hay yield	2024 production permitted

In the experiments, traits such as plant height (PH), panicle number per square meter (PN m⁻²), grain number per panicle (GNP), thousand-kernel weight (TKW), harvest index (HI), dry hay yield (DHY) and grain yield (GY) were investigated by the methods used by Nawaz et al. (2004), Assefa (2006), Gautam et al. (2006); Dumlupinar et al. (2017), Ceri and Acar (2019).

The data obtained from two-year trial results was subjected to variance analysis and means were compared using LSD test (JMP 15.1 SAS Institute Inc., 2020).

Formula of selection index for dual purposed oat genotypes,

$$\text{Selection Index (SI)} = \frac{\text{AGYG} - \text{AGYE}}{\text{AGYE}} + \frac{\text{ADHYG} - \text{ADHYE}}{\text{ADHYE}}$$

where,

AGYG: Average Grain Yield of Each Genotype

AGYE: Average Grain Yield of Genotypes by the Experiments

ADHYG: Average Dry Hay Yield of Each Genotype

ADHYE: Average Dry Hay Yield of Genotypes by Experiments

Selection index was calculated for the investigated traits as following;

For Dry High Yield (ADHYG-ADHYE)/ADHYE;

For Plant Height (APHG-APHE)/APHE;

For Panicle Number per m² (APNG-APNE)/APNE;

For Grain Number per Panicle (AGNPG-AGNPE)/AGNPE;

For Thousand Kernel Weight (ATKWG-ATKWE)/ATKWE;

For Harvest Index (AHIG-AHIE)/AHIE;

This formula was improved by modified land equivalent ratio (LER) formula, which evaluate the performances of crop varieties in mixed and sole production systems (Dariush et al., 2006). Selection index formula will give us an index value about the performance of oat genotypes having higher or less grain and dry hay yield compared to the average grain and dry hay yield of experiments. Genotypes having higher GY and DHY compared to average GY and DHY of experiments will have higher selection index values, genotypes with less GY and DHY compared to average of experiments will have lesser values on selection index. Thus, selection index value for two important traits for GY and DHY of genotypes will be applied successfully for selection of dual purposed oat genotypes. This formula can be applied for the other cereals and traits. For example, this formula may be easily applied for selection of corn varieties for high GY and silage yield.

Total selection index was also calculated as the sum of all index values of dual purpose and the other traits in the experiment.

3. RESULTS AND DISCUSSION

According to the results, years were found significantly different for all investigated traits except TKW, while genotypes and G×Y interaction varied substantially for all characteristics except DHY (Table 4). The mean values of the investigated traits are shown in Table 5 and calculated selection index values is given in Table 6.

Table 4. Degrees of freedom, and mean square values of investigated traits for oat genotypes.

	DF	DHY	GY(MS)	PH	PN m ⁻²	GNP	TKW	HI
Years	1	7.2477**	313543.68**	1199.09**	663394.34**	2339704.0**	8.66 ^{ns}	7782.15**
Genotypes	27	0.0914 ^{ns}	9436.31**	512.64**	24084.27**	101847.8**	90.2543**	102.7484*
G×Y	27	0.0783 ^{ns}	2856.15*	261.66**	27681.49**	46810.98**	7.47882*	177.60648**
Error	110	0.0653	1407.4	94.59	6662.6	10414.7	4.6915	51.615
CV %		2.44	16.11	9.44	20.91	14.44	7.95	51.61

**p<0.01, *p<0.05, ^{ns}: not significant

According to the results, dry hay yields of genotypes were changed between 13060- 8190 kg ha⁻¹ with TL218 and Kazan genotypes, respectively. TL218 oat genotype was followed by Sebat, Yeniceri, Sari, Amasya, Ankara 84, Checota, Faikbey, Yagiz, TL42, TL227, TL242, TL341, TL374 and Dirilis oat genotypes for DHY (Table 5). Dry hay yield among the years was significantly different due to climate differences, especially rainfall and temperature. Previous works also pointed out oat genotypes with different DHY. Ceri and Acar (2019), reported 6140 to 9940 kg ha⁻¹ dry hay yield. Kılınç and Gokkus (2022) reported dry hay yield varied between 530 to 2030 kg ha⁻¹ in oat genotypes. GY of oat genotypes were changed between 3152 kg ha⁻¹ and 1713 kg ha⁻¹ with Kahraman and Otag oat genotypes, respectively. Kahraman and TL227 oat genotypes were the same group for GY (Table 5). A significant variation among the years due to climate changes especially for the rainfall and temperature was detected for grain yield of oat genotypes. As an average, oat genotypes had GY with 1955 kg ha⁻¹ and 2774 kg ha⁻¹ in the first and second years, respectively. In previous works, researcher reported that GY between 4950 and 2360 kg ha⁻¹ by Dumlupinar et al. (2012), between 8600-3350 kg ha⁻¹ by Sabanduzen and Akcura (2017), 1150 and 710 kg ha⁻¹ by Sonkaya (2019), between 6142- 4026 kg ha⁻¹ by Duzme (2020), between 6229-3537 kg ha⁻¹ by Yasar (2021). These differences between the results were due to change of environmental conditions in years and genotypic variance. Oat genotypes differently responded to the experiment location with different climate and topographical conditions, causing significantly different results for PH, PN m⁻², GNP, TKW and HI (Table 4) which is also in line with previous works. Ercan et al. (2016) reported that grain yield varying among the oat genotypes 2360-4950 kg ha⁻¹. Halil and Uzun (2019) reported that average grain yield oat genotypes were found 4548 kg ha⁻¹. Yasar (2021) reported that grain yield varied between 3537-6229 kg ha⁻¹ in oat genotypes. Caliskan and Koc (2019) reported that plant height varying among the oat genotypes were 135- 175 cm. Hocaoglu (2020) reported plant height varied between 98-163 cm in oat genotypes. Ataman (2022), reported plant height varied between 42-84.1 cm. Altuner and Ulker (2019) reported that panicle number 340 PN m⁻² as the lowest while 380 PN m⁻² the highest. Kececioğlu et al. (2021) reported that panicle number varying among the oat genotypes 502-630 PN m⁻². Ercan et al. (2016) reported that grain numbers per panicle among the oat genotypes 47-93 grains per panicle. Yilmaz and Sonkaya (2020) reported that grain number per panicle varying among the oat genotypes 28 to 46 grains per panicle, Kahraman et al. (2021) reported that grain number per panicle among the oat genotypes 64- 143 grains. Ercan et al (2016) reported that thousand-kernel weight varying among the oat genotypes 25-51 g. Dumlupinar et al (2017) reported that thousand-kernel weight varied 16.32- 42.62g in oat genotypes. Duzme (2020) reported that harvest index of oat genotypes was found between 7.2 to 36.5%. Ataman (2022) reported that harvest index varied between 36.3-63%.

Table 5. Average values of two years for investigated traits of oat genotypes.

	Genotypes	DHY (kg ha ⁻¹)	GY (kg ha ⁻¹)	PH (cm)	PN m ⁻²	GNP	TKW (g)	HI (%)
1	Kirklar	9640 B-E	2869 BC	103.7 B-F	287.5 KL	705.3 EFG	33 AB	29.87 BC
2	Kahraman	9870B-E	3152 A	102.2 C-G	354.1 H-J	681.16 G-J	29.6 C-F	31.77 AB
3	Sebat	10530 A-E	2256 GJI	103.5 B-F	268.3 L	816.5 B-E	23.6 I-M	26.67 FG
4	Yeniceri	10650 A-E	2683 D	97.2 D-H	371.6 F-I	837.16 ABC	23.3 KLM	23.63 H-K
5	Sari	10440 A-E	2314 FGHI	1106.8 A-E	517.5 A	735.6 C-G	27.7 EFG	23.92 H-K
6	Arslanbey	9510 B-E	2617 DE	89.9 HIJ	411.6 C-F	729.1 C-G	32.1 BC	25.37 F-I
7	Amasya	12270 AB	2241 HI	113.5 AB	442.5 B-D	678.3 G-J	25.8 G-J	29.14 C-E
8	Ankara 84	11640 A-D	2421 FG	105.8 A-E	458.3 B	714.3 D-G	23.3 J-M	27.13 D-F
9	Apak 2-3	8990 CDE	2710 E-I	104.8 B-E	432.5 B-D	573.5 JKL	29.4 DEF	19.10 MN
10	Checota	12210 AB	2356 FGH	112.2 ABC	355 H-J	820.3 A-E	29.8 CDE	24.35 G-J
11	Faikbey	11670 A-D	2642 DE	111.4 ABC	315.8 JK	823.3 A-D	24.8 H-K	27.32 D-F
12	Seydisehir	9820 B-E	2681 D	112.0 ABC	332.5 I-K	806.6 C-F	23.3 KLM	22.17 JK
13	Yeskoy-330	10070 B-E	2177 IJ	109.4 ABC	417.5 B-E	665.6 G-K	23.3 KLM	22.14 JK
14	Dirilis	10520 A-E	2021 JKL	85.1 IJ	451.6 BC	698.6 FGH	26.8 GH	25.72 F-H
15	TL 38	9120 CDE	2357 FGHI	104.9 BDE	361.6 G-I	803 C-F	24 I-L	33.55 A
16	TL42	10690 A-E	2013 JKL	104.7 BDE	310 J-L	933.5 A	24.1 I-L	19.52 LM
17	TL137	10040 B-E	2474 EF	104 BDEF	445.8 B-D	579.6 I-L	31.7 BCD	24.63 G-I
18	TL 218	13060 A	2216 HI	108.7 ABC	332.5 I-K	747.16 C-G	32 BC	27.44 C-F
19	TL 227	11830 ABC	3006 AB	110 ABC	401.6 D-G	587 H-L	34.7 A	23.83 H-K
20	TL 242	10810 A-E	2264 GJI	109.2 ABC	427.5 B-E	678.3 G-J	25. H-K	21.62 KL
21	TL 330	9480 B-E	2430 FG	107.5 A-D	440 B-D	529.6 L	28.1 EFG	29.26 CD
22	TL 341	11420 A-D	2741 CD	109.7 ABC	430 B-E	735.6 C-G	31.2 BCD	25.52 F-H
23	TL 374	12350 AB	1760 M	116.1 A	386.6 E-H	691.6 F-I	24.1 I-L	16.74 N
24	Yazir	9280 CDE	1886 LM	93.3 F-J	400.8 D-G	770.6 C-G	21.2 M	23.40 H-K
25	Kazan	8190 E	2210 HI	82.3 J	444.1 B-D	559.16 L	33.4 AB	26.75 E-G
26	Otag	8850 DE	1713 M	91.1 G-J	267.5 L	924 AB	24.3 I-L	21.76 KL
27	Katmerli	9130 CDE	1992 KL	86.1 HIJ	433.3 B-D	360.6 M	21.8 LM	22.94 I-K
28	Yagiz	10520 A-E	2021 JKL	85.1 IJ	451.6 BC	698.6 FGH	26.8 GH	25.72-H

According to the selection index values calculated for DHY of oat genotypes, TL227 (0.346), TL341 (0.318) and Kahraman (0.290) oat genotypes had positive and the highest selection index values for DHY. In addition to these Faikbey (0.235) and Kirklar (0.215) oat genotypes also had positive selection index values for DHY (Table 6). This means that oat genotypes with positive and higher selection index values had higher DHY than the average DHY of experiments. Especially, oat genotypes (TL227 and TL341) with positive and higher selection index values were suitable for experiment location, may also be suggested for the higher DHY.

According to the results, selection index values of oat genotypes for GY, Kahraman (0.494) and Kirklar (0.440) oat genotypes had higher selection index values, and these genotypes were followed by TL227 (0.283), TL341 (0.261) and Sari (0.254) (Table 6). These cultivars had higher GY than the average GY of experiments. Especially Kahraman and Kirklar genotypes for GY with higher selection index values may be suggested for experiment location.

Table 6. Calculated selection index values for the oat genotypes by the investigated traits

Genotypes	DHY (kg ha ⁻¹)	GY (kg ha ⁻¹)	Dual purposed SI	PH (cm)	PN m ⁻²	GNP	TKW (g)	HI (%)	Total SI
Sari	0.062	0.254	0.316	0.049	0.303	-0.004	-0.007	-0.067	0.590
Seydisehir	0.187	0.169	0.356	0.248	-0.014	0.143	-0.012	0.017	0.738
Sebat	-0.027	-0.055	-0.082	-0.080	-0.358	0.059	-0.153	0.016	-0.598
Ankara 84	0.183	0.098	0.281	0.098	0.197	0.081	-0.102	0.104	0.659
Apak 2-3	-0.275	-0.309	-0.584	-0.190	0.013	-0.279	-0.007	-0.333	-1.380
Checota	0.199	0.046	0.245	0.062	-0.094	0.157	0.059	-0.035	0.394
Yeniceri	0.090	0.078	0.168	0.009	0.086	0.295	-0.011	0.076	0.623
Kirklar	0.215	0.440	0.655	0.159	-0.050	0.234	0.420	0.403	1.821
Amasya	0.187	-0.001	0.186	-0.004	0.081	-0.045	-0.106	0.108	0.220
Kahraman	0.290	0.494	0.784	0.269	0.240	0.320	0.451	0.598	2.662
Faikbey	0.235	0.175	0.410	0.217	-0.073	0.306	0.026	0.205	1.091
Arslanbey	-0.046	0.100	0.054	-0.081	0.161	0.197	0.282	0.117	0.730
Yesilkoy	-0.245	-0.205	-0.450	0.014	-0.010	-0.225	-0.057	-0.197	-0.925
Dirilis	-0.005	-0.320	-0.325	-0.493	0.011	-0.148	-0.164	-0.120	-1.239
TL38	-0.131	0.015	-0.116	-0.022	-0.093	0.106	-0.140	0.316	0.051
TL42	-0.131	-0.047	-0.178	-0.122	-0.354	0.134	-0.265	-0.307	-1.092
TL137	-0.060	0.113	0.053	0.069	0.188	-0.142	0.206	0.027	0.401
TL218	0.037	-0.059	-0.022	-0.024	-0.211	-0.005	0.151	0.303	0.192
TL227	0.346	0.283	0.629	0.324	0.299	0.114	0.541	0.220	2.127
TL242	-0.136	-0.034	-0.170	-0.135	0.052	-0.118	-0.121	-0.181	-0.673
TL330	-0.119	-0.092	-0.211	0.112	0.154	-0.278	0.057	0.193	0.027
TL341	0.318	0.261	0.579	0.242	0.206	0.200	0.302	0.175	1.704
TL374	-0.132	-0.180	-0.312	-0.225	-0.265	-0.159	-0.373	-0.588	-1.922
Yazir	-0.264	-0.382	-0.646	-0.278	-0.175	-0.111	-0.426	-0.270	-1.906
Kazan	-0.198	-0.249	-0.447	-0.296	0.072	-0.316	0.159	0.001	-0.827
Otag	-0.476	-0.513	-0.989	-0.370	-0.590	0.032	-0.386	-0.408	-2.711
Katmerli	-0.192	-0.338	-0.530	-0.387	-0.044	-0.644	-0.355	-0.240	-2.200
Yagiz	0.076	0.055	0.131	0.069	0.216	0.067	0.055	0.200	0.738

Sum of selection indices of DHY and GY a new selection index value for dual purposed oat genotypes. These selection index values will give us information about dual purposed oat genotypes having higher DHY and GY over the average of experiments and experiment region. According to the results, oat genotype, Kahraman with 0.784 selection index value had the highest selection index, which will be used to determine dual purposed oat genotypes. This genotype was also followed by Kahraman (0.784), Kirklar (0.655), TL227 (0.629) and TL341 (0.579) oat genotypes (Table 6). These oat genotypes with higher selection index may be suggested for experiment location for both DHY and GY. Oat genotypes with selection index values below zero are unsuitable for dual purposes for DHY and GY in experiment location. We can understand that Kahraman, Kirklar and TL227 genotypes with positive and higher selection index values had higher DHY and GY than the other oat genotypes. These genotypes were suitable for dual purposed production in experiment region and might be registered.

Selection index values may also be calculated for the other traits related to GY and DHY. Selection index values of plant height were positive and higher for TL227 (0.324), Kahraman (0.269), Seydisehir (0.248) and TL341 (0.242) genotypes. Otag (-0.989), Yazir (-0.646) and Katmerli (-0.530) oat genotypes had the negative and less selection index values for PH. This situation was due to those cultivars with negative and the less selection index values were improved for Konya region where winter conditions are harsher than Kahramanmaras experiment site. Therefore, growth of these oat cultivars with negative and the less selection index values were slower and unsuitable for experiment location.

Selection index values of Sari (0.303), TL227 (0.299), Kahraman (0.240) and TL341 (0.206) oat genotypes for PN m² were positive and higher values than the other genotypes. Otag (-0.590) Sebat (-0.358) and TL42 (-0.354) oat genotypes had negative and the lowest selection index values in the experiment.

Calculated selection index values for grain numbers per panicle (GNP) were the positive and higher for Kahraman (0.320), Faikbey (0.306), Yeniceri (0.295), Kirkclar (0.234) and TL341 (0.200) genotypes. It was negative and lowest for Katmerli (-0.644) and Kazan (-0.316) genotypes.

While TL227 (0.541), Kahraman (0.451), Kirkclar (0.420) and TL341 (0.302) oat genotypes had positive and higher selection index values for TKW, Yazir (-0.426), Otag (-0.386), TL374 (-0.373) and Katmerli (-0.355) oat genotypes had negative and lowest selection index values for TKW.

Selection index values of HI for Kahraman (0.598), Kirkclar (0.403), TL38 (0.316) and TL218 (0.303) oat genotypes were positive and higher than the other genotypes. TL374 (-0.588) and Otag (-0.488) oat genotypes had negative and less selection index values for HI.

As mentioned above, selection index values may be calculated for each yield related traits such as PH, PN m⁻², GNP and TKW, and HI. These values of yield related traits may provide information about oat genotypes. In addition, all these selection index values may be collected with dual purposed selection index values and another selection index called total selection index value might be obtained. These total selection index values may give us information about oat genotypes that best for all the investigated traits.

According to the total selection index values, Kahraman oat genotype with (2.662) had the highest value followed by TL227 (2.127), Kirkclar (1.821) and TL341 (1.704) oat genotypes.

It is concluded that commercial cultivars Kahraman and Kirkclar may be suggested for GY, DHY and related traits for experiment location. In addition, oat genotypes TL227 and TL341 may be evaluated to register for DHY and GY as well as yield related traits for experiment location. Oat genotypes with negative and lower total selection index values were not suitable for experiment region and oat genotypes with lower but positive total selection index values may also be evaluated for the other locations.

4. CONCLUSION

The results show that calculated selection index value for oat genotypes will give us an information about the both GY and DHY of dual purposed oat genotypes which will be the best for any region. Thus, GY and DHY of oat genotypes would be evaluated together, and chosen of oat genotypes with the highest GY and DHY for dual purposed production. According to the results of dual purposed and total selection index values, commercial oat cultivars Kahraman and Kirkclar were determined suitable for dual purposed oat production. TL227 and TL341 oat genotypes were suggested to register for experiment region. This selection index values may be calculated for the other cereals such as corn, wheat, barley, rye, sorghum etc. This selection index value will help us to select the cereal varieties with higher GY and silage or dry hay yields, or the other yield related traits. For example, corn varieties with higher GY and silage yields may be selected by the selection index values for dual purposed. This selection index value may be easily applied for the other cereals.

ACKNOWLEDGEMENT

This study was a project that was financed by the Scientific Research Projects Coordination Unit of Kahramanmaraş Sutcu İmam University, Project No: 2021/7-8D. This research was also part of the PhD thesis of Tugba BASKONUS, supported by Council of Higher Education as a fellowship on Sustainable Agriculture in 100/2000 program.

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Identification of Morphological Agronomic and Quality Characteristics of Hungarian Vetch (*Vicia pannonica* Crantz.) Mutants

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ARTICLE INFO

Research Article

Received: 5 November 2024

Accepted: 11 March 2025

Published: 23 June 2025

Keywords:

Breeding

Gamma ray

Hungarian vetch

Mutant lines

Mutations

Citation: Efe, B., Ünal, S., Mıntaş, H., Yeler, E. E., & Nalbant, M. (2025). Identification of morphological agronomic and quality characteristics of Hungarian vetch (*Vicia pannonica* Crantz.) mutants. *Turkish Journal of Field Crops*, 30(1), 43-54. <https://doi.org/10.17557/tjfc.1579717>

ABSTRACT

Gamma-ray irradiation of seeds is an effective method for creating genetic variation in plant breeding. This study aimed to evaluate the morphological, agronomic, and quality characteristics of Hungarian vetch (*Vicia pannonica* Crantz.) mutant lines obtained through gamma radiation. The experiment was carried out in Golbaşı-Ankara during the 2019 and 2020 growing seasons according to Randomized Complete Block Design with four replications involving eleven mutant lines and two control cultivars. The mutant lines exhibited a range of outcomes, including both positive and negative effects. However, no significant alterations were observed in the quality characteristics. The TB1007 mutant line demonstrated superior yield and quality, whereas the TB1002 and TB1005 mutant lines only performed well in terms of yield. The highest hay yields were obtained from TB1007 and TB1002, with 42.50 and 41.93 kg ha⁻¹, respectively, followed by the cv. Tarm Beyazı-98 and TB1005 mutant, with 39.14 and 39.01 kg ha⁻¹, respectively, in 2019. The TB1007 mutant line had the highest digestible protein content, with a content of 16.4% in 2019 and 16.7% in 2020, averaging at 16.5%. These lines are suitable for breeding programs that, especially studies involving crosses.

1. INTRODUCTION

The *Vicia* genus, which belongs to the Fabaceae family, includes nearly 200 species, many of which are winter annual legumes commonly referred to as vetches (Renzi et al., 2020; Acikgoz, 2021). Vetches are valuable the forage crops of economic importance (Zhang and Mosjidis, 1998) and are also known for improving soil fertility (Acikgoz, 2021). These crops in dry regions with continental climates, where their high production potential makes them an excellent choice for forage (Uzun et al., 2004, Sahin-Demirbag et al., 2008, Erkovan, 2022).

Hungarian vetch (*Vicia pannonica* Crantz.) is commonly cultivated as a winter crop due to its high forage yield potential and resistance to both cold and drought. Its cultivation has been expanding in semi-arid regions, particularly in Türkiye. A diploid annual legume ($2n=2x=12$) Hungarian vetch is found across Southern and Central Europe, Anatolia, North-West Africa, and Caucasia (Naydenova and Aleksieva, 2014; Acikgoz, 2021). This species adapts well to the continental climate, and is planted in autumn, even in areas with harsh winters, such as Central and Eastern Anatolia (Acikgoz, 2021). Hungarian vetch is mainly grown for hay production, though its seeds are also valuable in poultry feed. In Türkiye's semi-arid regions, forage yields of Hungarian vetch range from 7.5 to 35 tons per hectare, while hay yields range from 2 to 7 tons per hectare (Unal et al., 2011; Erdogan et al., 2016). Yield varies greatly depending on environmental conditions and genotypes. The wide genetic diversity of genotypes is a key element for an effective and efficient breeding programme focusing on yield-related traits (Ergun et al., 2023). However, many forage crops, including vetches, suffer from limited genetic diversity, restricting breeding efforts (De la Rosa et al., Acikgoz, 2021).

Mutation breeding is a key method in plant breeding for enhancing genetic resources (Chaudhary et al. 2019; Shi et al., 2020; Sarsu et al. 2021). Mutations, sudden changes in a plant's genetic structure, can be induced using physical or chemical mutagens (Karim et al., 2008; Acikgoz, 2021). Plant breeding programs utilize both direct and indirect mutation methods to generate new traits, which can then be used for further crossbreeding and genetic improvement (Sagel et al., 1994; Sehirali and Ozgen, 2007; Sabancı and Ayrancı, 2015; Cimen et al., 2019). However, only five mutant cultivars have been released in fodder crops to date, which is relatively few (Acikgoz, 2021). Notable examples of vetch cultivars developed through mutation breeding include the common vetch cultivars Nechinovskaya 84 and Nikian, which were made using direct mutation breeding, and the Topleza variety, developed through the use of a mutant line in crossing. Additionally, two vetch lines, VSMP 04 and Mutant Line No. 1, have shown improvements in grain yield. (Micke, 1993; Artık and Pekşen, 2006; Aleksieva and Naidevova, 2012; Efe and Unal, 2017; Mikić et al., 2019).

Mutation breeding of Hungarian vetch began in 2010 at the Field Crops Central Research Institute, to increase genetic variation. The test material, Tarm Beyazi-98, was exposed to various doses of Gamma radiation. Plants with desirable traits were selected from the M_2 to M_4 generations, and by the M_5 generation, line selection and seed multiplication had begun. In 2019 and 2020, yield trials were conducted with 11 mutant lines and two control cultivars.

The primary objectives of this research were to (1) investigate the morphological, agronomical, and forage quality traits of the mutant lines under semi-arid conditions, (2) compare the mutant lines to control cultivars in order to assess the effects of the mutations, and (3) provide detailed data on the development of genetic diversity, particularly focusing on yield and quality traits.

2. MATERIAL AND METHOD

Material

Eleven mutant lines and two control cultivars (cv. Kansur and cv. Tarm Beyazi-98) were used as plant material in the study (Table 1).

Table 1. The mutant lines and control cultivars of Hungarian vetch

Number	Lines Cultivars	Doses (Gy)	Mutant lines
1	Tarm Beyazi-98	60	TB603
2	Tarm Beyazi-98	60	TB605
3	Tarm Beyazi-98	80	TB802
4	Tarm Beyazi-98	80	TB805
5	Tarm Beyazi-98	80	TB806
6	Tarm Beyazi-98	100	TB1002
7	Tarm Beyazi-98	100	TB1005
8	Tarm Beyazi-98	100	TB1006
9	Tarm Beyazi-98	100	TB1007
10	Tarm Beyazi-98	100	TB1008
11	Tarm Beyazi-98	100	TB1009
12	Kansur	-	Control
13	Tarm Beyazi-98	-	Control

The soil condition at experimental area was slightly alkaline and poor in organic matter while the levels of P₂O₅, K₂O, and CaCO₃ were relatively high.

Rainfall in spring and autumn is necessary for germination, emergence, growth, and development of plants in Hungarian vetch cultivation in the Central Anatolian region. Rainfall in the experimental years was lower than the long-term average (Table 2) (Anonymous, 2021). 2020 also received more precipitation than the first year, especially higher amounts in April, May and June. Temperature values were lower than the long-term average in the first year and higher in the second year. Relative humidity was below the long-term average in both years.

Table 2. Climatic data of experimental field (TSMS, 2021)

Mounths	Precipitation (mm)			Temperature (°C)			Relative moisture (%)		
	2019	2020	LT*	2019	2020	LT	2019	2020	LT
January	36.2	17.4	32.5	-0.8	-2.2	-1.4	83.9	74.8	88.2
February	36.4	33.2	22.7	2.2	0.5	1.0	75.0	70.9	80.9
March	20.6	14.2	40.3	5.0	5.6	5.1	59.2	60.7	71.2
April	23.4	39.0	24.5	7.9	8.2	9.6	61.1	54.8	63.6
May	3.8	40.6	47.5	15.1	13.3	14.3	55.4	55.9	62.6
June	15.0	74.3	38.1	18.7	17.3	18.5	58.7	53.7	58.9
July	7.2	0.6	9.5	17.8	23.1	22.2	54.3	42.3	46.1
August	9.2	0.0	8.1	20.8	22.7	22.5	47.1	37.5	45.0
September	4.2	11.6	18.5	16.3	20.9	18.0	47.2	47.8	49.5
October	9.2	20.0	33.0	12.0	15.6	12.0	54.6	54.3	64.6
November	35.2	2.4	15.6	6.7	4.5	5.6	64.4	77.8	74.9
December	21.4	10.2	28.1	0.9	4.0	0.8	83.6	86.7	85.8
Total/Average	221.80	263.50	318.40	10.22	11.13	10.68	62.04	59.77	65.94

Method

Hungarian vetch cultivar, Tarm Beyazi-98, was performed for Cobalt-60 (Co60) gamma irradiation at different doses (40, 60, 80, 100, 120, and 140 Gy) (105 seeds for each dose) in 2010 at the Türkiye Atomic Energy Authority (TAEK), Ankara Nuclear Research and Training Centre (ANAEM). M1 plants were grown in bulk. In the following generations (M2, M3, M4), observations were made on the mutant plants and the plants with the desired characteristics were carried to the next generation. When M5 generation was achieved, line selection and seed multiplication were started. However, as lines with the desired performance could not be obtained with 40, 120 and 140 gamma ray doses, these lines are not included in the present study. During the 2016-2018 growing seasons, sufficient seed of the mutant lines was produced to conduct the field yield trial. At this stage, the performance of the mutant lines was determined to be used in the further breeding process. In 2019 and 2020 growing seasons, yield trials were conducted in the research field of the Field Crops Central Research Institute, Ankara.

This research was carried out in a Randomized Comlepte Block Design with four replications in the field of Field Crops Central Research Institute Gölbaşı-İkizce Agricultural Research and Application Field in dry conditions in 2019 and 2020 growing seasons. The seeds of the mutant lines and the check cultivars were sown by hand in plots consisting of 6 rows with a row spacing of 25 cm. The length and width of the plots were fixed at 5 m and 1.5 m respectively. A distance of 2 m between blocks and 0.5 m between plots was left. At the same time as planting, DAP fertiliser was applied at a rate of 100 kg per hectare.

Morphological and agronomic data were collected between 15 May - 21 June 2019 and 21 May - 2 July 2020. The following morphological and agronomical traits were determined: natural plant height habitus (NPH) (cm), plant height (PH) (cm), stem diameter (SD) (mm), stem number (SN), pods per plant (PPP) (number), seeds per pod (SPP) (number) and 1000 seed weight (SW) (g), forage yield (FY) (kg ha⁻¹), hay yield (HY) (kg ha⁻¹), biological yield (BY) (kg ha⁻¹), grain yield (GY) (kg ha⁻¹) and harvest index (HI) (%). The quality traits of dry matter (DM) (%), digestible dry matter (DDM) (%), crude protein (CP) (%), digestible crude protein (DCP) (%), Acid Detergent Fibre (ADF) (%), Neutral Detergent Fibre (NDF) (%) and Relative Feed Values (RFV) (%) were identified through NIRS (Forage calibration package Lhay5fe.eqa). The significance levels of the differences between the examined traits of the mutant lines and check cultivars were determined using two-year combined analysis of variance (ANOVA) and then the genotypes were grouped according to Student's t multiple comparison tests (LSD) at 5% probability. Hierarchical cluster analysis (HCA) was performed to determine the similarities between the mutant lines and the control cultivars. Correlation-based Principal Component Analysis (PCA) was also used to examine the relationships between characters and to identify the dominant genotypes with respect to these characters. HCA and PCA were carried out using Jump 11 statistical software.

3. RESULTS AND DISCUSSIONS

Morphological traits

The natural plant height habitus, plant height, stem diameter, stem number, pods per plant, number of seeds per pod and 1000 seed weight (ANOVA) results of mutant lines and check cultivars are given in Table 3. Combined ANOVA results showed that there weren't significant differences among study genotypes for morphological traits in Table 3. Year effects seemed significant for stem number ($P<0.01$) and 1000 seed weight ($P<0.01$). No significant differences appeared on year x genotype interactions for traits.

Table 3. The combined variance analysis results (mean squares) of natural plant height habitus, plant height, stem diameter, stem number, pods per plant, number of seeds per pod and 1000 seed weight

Source	DF	Natural plant height habitus (cm)	Plant height (cm)	Stem diameter (mm)	Stem number	Pods per plant (number)	Seeds per pod (number)	1000 seed weight (g)
Years	1	38.02	206.13	0.08	43.16**	61.85	6.01	1599.96**
Error 1	3	65.41	48.15	0.30	0.32	26.28	0.73	2.54
Genotypes	12	30.35	41.52	0.06	0.21	24.42	0.37	18.02
Years x Genotypes	12	29.13	39.82	0.05	0.30	18.67	0.51	13.69
Combined error	72	31.37	36.25	0.05	0.25	13.25	0.51	18.68

** Significant at 1%; DF Degrees of freedom

Table 4. Natural plant height habitus, plant height, stem diameter and stem number of the standard varieties and mutant lines in 2019, 2020 and two-years averages

Genotypes	Natural plant height habitus (cm)			Plant height (cm)			Stem diameter (mm)			Stem number		
	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.
TB603	36.3	37.9 bc	37.1	41.8	42.1 b	42.0	2.3	2.4	2.3	3.7	3.0	3.3
TB605	38.7	37.2 bc	38.0	43.2	41.9 b	42.6	2.2	2.2	2.2	3.8	2.6	3.2
TB802	38.4	36.0 bc	37.7	44.1	40.8 b	42.4	2.1	2.3	2.2	3.7	2.4	3.0
TB805	41.7	36.9 bc	39.3	47.7	39.8 bc	43.8	2.7	2.3	2.5	3.8	2.5	3.1
TB806	36.3	36.9 bc	36.6	41.7	40.2 bc	40.9	2.3	2.4	2.3	4.0	2.6	3.3
TB1002	39.8	36.2 bc	38.0	45.5	40.7 b	43.1	2.2	2.2	2.2	3.9	2.2	3.0
TB1005	40.6	39.8 b	40.2	45.9	42.9 b	44.4	2.2	2.3	2.3	3.8	2.6	3.2
TB1006	39.1	32.1 c	35.6	44.4	33.2 c	38.8	2.1	2.3	2.2	3.4	2.3	2.8
TB1007	41.2	36.9 bc	39.1	48.9	42.7 b	45.8	2.2	2.3	2.2	4.4	2.2	3.3
TB1008	38.9	47.0 a	42.9	44.4	51.4 a	47.9	2.4	2.4	2.4	4.1	2.8	3.4
TB1009	38.5	37.8 bc	38.2	43.3	44.3 ab	43.8	2.1	2.3	2.2	3.3	2.7	3.0
Kansur	34.9	36.6 bc	35.7	41.8	40.2 bc	41.0	2.2	2.3	2.3	3.8	2.5	3.1
Tarm Beyazi-98	39.5	35.9 bc	37.7	44.6	40.5 b	42.6	2.3	2.3	2.3	3.9	2.5	3.2
Mean	38.8	37.6	38.2	44.4	41.6	43.0	2.2	2.3	2.3	3.8	2.5	3.1
LSD _(0.05)	9.3	6.5	5.6	10.0	7.1	6.0	0.4	0.2	0.2	0.8	0.7	0.5
CV. (%)	16.8	12.0	14.7	15.6	11.9	14.0	13.0	6.8	10.3	14.3	18.4	15.9

LSD Least significant differences; CV (%) Coefficient of variation

When the natural plant height habitus (NPH) is examined, the highest and lowest values were obtained from the genotypes TB1008 (42.9 cm) and TB1006 (35.6 cm), respectively (Table 4). NPH in Hungarian vetch was reported to change between 35.7-54.0 cm (Aggunlu, 1999). The data in present study are in agreement with the researcher. The highest plant height (PH) was obtained from line TB1008 (47.9 cm), followed by mutant line TB1007 (45.8 cm). The lowest PH was measured by the line numbered TB806 (40.9 cm) (Table 4). PH results are also similar to the findings of Sayar et al. (2012); Eviz (2016); Hashalıcı et al. (2017) and Tankus (2020). It was observed that the mutation had no effect on stem diameter (SD). SD varied between 2.2 and 2.5 mm (Table 4). The data of the present study are consistent with the findings of different researchers such as Sayar (2014); Efe and Unal (2017). It has been determined that the mutagen does not significantly affect stem number (SN), but it does affect plant height (PH). The stem count ranged from 2.8 to 3.4 (Table 4). These findings are consistent with previous studies conducted by Sayar (2014); Efe and Unal (2017).

Table 5. Number of pods per plant, number of seeds per pod and 1000 seed weight of the standard of varieties and mutant lines in 2019, 2020 and two-year averages

Genotypes	Pods per plant (number)			Seeds per pod (number)			1000 seed weight (g)		
	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.
TB603	13.6	13.1	13.4	4.7	5.1	4.9	46.0	55.0	50.5
TB605	11.5	15.7	13.6	4.4	5.2	4.8	46.8	53.0	49.9
TB802	12.8	15.8	14.3	4.9	5.0	4.9	51.6	54.7	53.1
TB805	13.2	20.0	16.6	4.9	5.2	5.0	48.5	55.9	52.2
TB806	14.8	15.6	15.2	4.5	5.3	4.9	48.9	53.5	51.2
TB1002	14.3	16.9	15.6	4.3	5.1	4.7	49.6	53.9	51.7
TB1005	17.9	15.4	16.6	4.8	4.5	4.6	45.8	55.3	50.6
TB1006	15.2	14.2	14.7	4.9	4.7	4.8	44.2	54.2	49.2
TB1007	15.5	18.0	16.7	4.8	4.6	4.7	44.9	55.5	50.2
TB1008	12.2	17.1	14.6	4.5	5.2	4.8	43.9	54.6	49.3
TB1009	12.9	14.4	13.7	4.4	5.3	4.8	43.8	54.9	49.3
Kansur	13.4	9.1	11.3	4.6	6.1	5.4	44.3	52.3	48.3
Tarm Beyazi-98	10.7	12.8	11.7	4.2	4.7	4.5	44.3	51.8	48.0
Mean	13.7	15.2	14.4	4.6	5.1	4.8	46.4	54.2	50.3
LSD _(0.05)	5.0	5.4	3.6	1.0	1.1	0.7	6.7	5.6	4.3
CV. (%)	25.5	24.9	25.2	15.1	14.6	14.8	10.1	7.2	8.6

LSD Least significant differences; CV (%) Coefficient of variation

The mutagen effect was higher in PPP than in other traits and higher variation occurred for this trait. Therefore, coefficient of variation was found to be high in PPP. According to the average results, the lowest, highest and average PPP were 11.3, 16.7, and 14.4 respectively. The number of SPP ranged from 4.5 to 5.4, with an average of 4.8. SW changed from 48.0 to 53.1 g and the average value had 50.3 g. When Tarm Beyazi-98 had 11.7 PPP, mutant lines were found between 13.4 and 16.7. The same situation was observed in SW. While SW in Tarm Beyazi-98 was 48 g, this value reached up to 53.1 g in mutant lines. The increase in these two traits in mutant lines naturally led to increased seed yield. When PPP was evaluated TB1007 (16.7 number) gave the highest number of pods. This was followed by line TB1005 (16.6 number). The lowest PPP was obtained from the control cultivar Kansur (11.3 number) (Table 5). In a study of Diyarbakır conditions, PPP was determined to vary between 15.6 and 29.5 (Seydosoglu, 2014). PPP values we obtained are in agreement with the researcher. SPP and SW values were 4.5-5.4 number and 48.0-53.1 g respectively. In the previous study SPP was counted as 2.8-5.7 number (Efe and Unal, 2017) and 2.5-3.2 number (Seydosoglu, 2014). SW values were also detected to change from 29.9 to 46.6 g in the past study (Seydosoglu, 2014).

It is widely acknowledged in the scientific literature that the effects of mutation applications vary considerably according to the species, variety, and line of the plant in question. Furthermore, these effects can be either positive or negative in terms of the plant's physiological, morphological, and agricultural characteristics (Aleksieva ve Naidevova, 2012; Efe ve Unal, 2017; Ogutcen et al., 2018). The results of this study indicate that the impact of gamma-ray treatment on morphological and agricultural characteristics in mutant lines is variable. This finding is consistent with other studies in this area.

Agronomic traits

The results of the variance analysis for forage and hay yields, biological yield, grain yield and harvest index in the genotypes are presented in Table 6. As indicated in Table 6, significant differences were identified between the genotypes for biological yield and harvest index at the $p < 0.01$ level. A significant difference ($P < 0.01/P < 0.05$) was observed for all traits in the years x interactions category. There was no significant difference between years at observed traits in Table 6. However, year values are given to show similarities. Evaluations and comments were made based on general average values. While there was no difference in forage yields between genotypes in 2020, there were significant differences in 2019 data, so they were grouped. Furthermore, genotypes responded differently to years, so the year x genotype interaction was significant.

Table 6. The combined variance analysis results (mean squares) of forage and hay yields, biological yield, grain yield and harvest index

Source	DF	Forage yield (kg ha ⁻¹)	Hay yield (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	Harvest index (%)
Years	1	2970.01	2294.11	24.94	5.41	15.70
Error 1	3	3142.24	250.51	105.64	7.51	22.68
Genotypes	12	939.48	78.02	67.32**	2.67	70.05**
Years x Genotypes	12	1165.30*	85.60*	86.77**	4.37**	115.40**
Combined error	72	519.42	43.61	25.70	1.52	19.15

* Significant at 5%; ** Significant at 1%; DF: Degrees of freedom

Table 7. Forage and hay yields of the standard varieties and mutant lines in 2019, 2020 and two-year averages

Genotypes	Forage yield (kg ha ⁻¹)			Hay yield (kg ha ⁻¹)		
	2019	2020	Ave.	2019	2020	Ave.
TB603	100.87 bcd	98.29	99.58	32.48 abc	24.57	28.53
TB605	101.87 bcd	79.58	90.73	31.46 bc	19.90	25.68
TB802	90.71 cd	98.63	94.67	27.12 c	24.66	25.89
TB805	116.67 abcd	76.08	96.38	34.15 abc	19.02	26.59
TB806	91.47cd	90.00	90.73	30.84 bc	22.50	26.67
TB1002	131.13 ab	95.61	113.37	41.93 a	23.90	32.92
TB1005	129.53ab	105.39	117.46	39.01 ab	26.35	32.68
TB1006	111.60 abcd	79.72	95.66	34.74 abc	19.93	27.33
TB1007	140.60 a	95.83	118.22	42.50 a	23.96	33.23
TB1008	100.13 bcd	125.96	113.05	31.54 bc	31.49	31.51
TB1009	87.33 d	110.39	98.86	27.12 c	27.60	27.36
Kansur	92.53 cd	105.50	99.02	29.36 bc	26.38	27.87
Tarm Beyazi-98	121.60 abc	116.11	118.86	39.14 ab	29.03	34.08
Mean	108.93	98.24	103.58	33.95	24.56	29.26
LSD. (0.05)	31.20	34.11	22.72	10.33	8.53	6.58
CV. (%)	19.97	24.21	22.00	21.2	24.21	22.57

LSD Least significant differences; CV (%) Coefficient of variation

According to the results of the combined analysis of the Tarm Beyazi-98 had the highest forage yields (FY) (118.86 kg ha⁻¹) and the TB605 and TB806 was the lowest FY (90.73 kg ha⁻¹) (Table 7). FYs were reported as 122.7-233.6 kg ha⁻¹ in Sayar et al. (2012), 100.39-173.39 kg ha⁻¹ in Tankus (2020), and 116.07-260.0 kg ha⁻¹ in Hashalıcı et al. (2017). As the present study results were smaller than the data of Sayar et al. (2012), but they were similar to the values of Tankus (2020) and Hashalıcı et al. (2017). The combined analysis of Tarm Beyazi-98 produced the highest hay yields (HY) (34.08 kg ha⁻¹) while TB605 had the lowest HY (25.68 kg ha⁻¹) (Table 7). These research results correspond with the findings of Sayar et al. (2012), Eviz (2016), Hashalıcı et al. (2017), Tankus (2020), Ertekin et al. (2020).

Table 8. Biological yield, grain yield and harvest index of the standard varieties and mutant lines in 2019, 2020 and two-year averages

Genotype	Biological yield (kg ha ⁻¹)			Grain yield (kg ha ⁻¹)			Harvest index (%)		
	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.
1 (TB603)	24.03 abcd	20.42 de	22.23 c	3.72 de	5.20	4.46	16.1 cd	26.0 a	21.0 b
2 (TB605)	24.22abcd	26.13 cde	25.18 abc	4.59 abcde	4.68	4.63	18.9 cd	17.7 cd	18.3 bc
3 (TB802)	22.77 cd	20.96 cde	21.86 c	4.17 cde	4.31	4.24	18.9 cd	20.4 bc	19.6 b
4 (TB805)	22.37 cd	27.17 abcd	24.77 abc	3.81 de	4.72	4.26	17.4 cd	17.1 cd	17.3 bc
5 (TB806)	20.87 d	22.95 cde	21.91 c	5.59 abc	3.00	4.29	27.2 ab	13.6 d	20.5 b
6 (TB1002)	18.47 d	24.75 cde	21.61 c	6.29 a	5.35	5.82	34.0 a	21.1 abc	27.6 a
7 (TB1005)	29.13 abc	18.77 e	23.95 bc	5.07 abcd	4.87	4.97	16.8 cd	25.4 a	21.1 b
8 (TB1006)	28.89 abc	28.20 abc	28.54 ab	6.19 ab	4.95	5.57	21.6 bc	18.0 cd	19.8 b
9 (TB1007)	30.60 a	26.69 bcd	28.64 ab	5.04 abcd	5.30	5.17	17.0 cd	19.8 bc	18.4 bc
10 (TB1008)	29.91 ab	21.27 cde	25.59 abc	3.83 de	5.20	4.51	13.1 d	24.5 ab	18.8 bc
11 (TB1009)	24.84 abcd	34.73 a	29.79 a	4.57 bcde	6.73	5.65	18.8 cd	19.4 c	19.1 bc
12 (Kansur)	23.27 bcd	33.82 ab	28.54 ab	3.17 e	5.51	4.34	13.4 d	16.4 cd	14.9 c
13 (Tarm Beyazi-98)	21.87 d	28.12 abc	24.99 abc	3.30 e	5.43	4.36	15.4 cd	19.2 c	17.3 bc
Mean	24.71	25.69	25.20	4.56	5.02	4.79	19.1	19.9	19.5
LSD. (0.05)	6.91	7.61	5.05	1.71	1.83	1.23	7.37	4.95	4.36
CV. (%)	19.50	20.66	20.12	26.10	25.42	25.76	26.87	17.33	22.43

LSD Least significant differences; CV (%) Coefficient of variation

According to the average biological yields (BY) the TB1002 and TB1009 had the lowest and highest averages at 21.61 and 29.79 kg ha⁻¹ respectively (Table 8). In a previous study it was found as 19.69 – 39.07 kg ha⁻¹ (Cebeci, 2017). Furthermore some study results were reported that BY in different Hungarian vetch varieties were found to be 18.8-26.4 kg ha⁻¹ (Ucar, 2022) and 27.20 kg ha⁻¹ (Koç, 2020). The findings of these studies align with each other. Based on the two-year averages the TB802 and TB1002 had the lowest and highest grain yields (GY) at 4.24 and 5.82 kg ha⁻¹ respectively (Table 8). Similar to this study Koç (2020) calculated GY of Hungarian vetch to be 5.32 kg ha⁻¹. A comparison of the cultivar (Tarm Beyazi-98) with GY of the lines (TB1002, TB1009, TB1006, TB1007 and TB1005) revealed that the lines exhibited higher yields, ranging from 13.99 to 33.49%. It is thought that the high GY can be explained by mutations impacting PPP and SW (Sattler et al., 2014; Shi et al., 2020). This increase in GY clearly shows

the positive effect of mutation application (Fang et al., 2012). The averages changed from 14.9% (Kansur) to 27.6% (TB1002) in harvest index (HI) (Table 8). In a study comparable to this one Koç (2020) determined that HI for Hungarian vetch was 19.58%.

No significant differences were observed between the years for the five aforementioned traits. The genotypes exhibited disparate responses to varying rainfall and temperature levels in both years, resulting in notable alterations in their yearly rankings. Consequently, the genotype-by-year interaction was found to be statistically significant at the 1 or 5% level.

Quality traits

Variance analysis results of dry matter, digestible dry matter, crude protein, digestible protein, acid detergent fiber, neutral detergent fiber and relative feed values for genotypes are given in Table 9. When Table 9 was observed there was no significant difference between the genotypes, years and year x genotype interaction in terms of these four traits.

Table 9. The combined variance analysis results (mean squares) of dry matter, digestible dry matter, crude protein, digestible protein, acid detergent fiber, neutral detergent fiber and relative feed values

Source	D F	Dry Matter (%)	Digestible Dry Matter (%)	Crude Protein (%)	Digestible Protein (%)	Acid Detergent Fiber (%)	Neutral Detergent Fiber (%)	Relativ e Feed Values
Years	1	0.17	2.10	0.83	0.35	3.46	32.05	288.21
Error 1	3	0.07	1.09	0.82	0.28	1.80	0.06	6.92
Genotypes	12	0.09	1.38	1.18	0.55	2.27	6.11	66.10
Years x Genotypes	12	0.04	1.16	1.08	0.48	1.92	9.41	85.95
Combined error	72	0.14	1.72	0.64	0.32	2.84	8.62	90.20

DF: Degrees of freedom

Table 10. Dry matter, digestible dry matter, crude protein and digestible protein values of the standard varieties and mutant lines in 2019, 2020 and two-year averages

Genotypes	Dry Matter (%)			Digestible Dry Matter (%)			Crude Protein (%)			Digestible Protein (%)		
	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.
1 (TB603)	88.6	88.8	88.7	62.1	62.3	62.2	24.1	22.0	23.1	17.1	15.7	16.4
2 (TB605)	89.2	88.9	89.0	62.2	62.9	62.5	22.6	21.7	22.2	16.1	15.5	15.8
3 (TB802)	88.9	88.7	88.8	62.1	60.6	61.3	22.5	21.4	21.9	16.0	15.2	15.6
4 (TB805)	88.8	88.7	88.7	60.8	62.5	61.7	21.3	21.9	21.6	15.1	15.6	15.4
5 (TB806)	88.7	88.7	88.7	61.7	60.8	61.2	22.6	22.8	22.7	16.0	15.9	15.9
6 (TB1002)	89.3	88.9	89.1	60.9	61.7	61.3	21.6	22.0	21.8	15.3	15.6	15.4
7 (TB1005)	88.9	88.8	88.9	61.8	61.0	61.4	22.8	21.1	21.9	16.1	15.0	15.6
8 (TB1006)	88.8	88.6	88.7	62.0	61.7	61.9	22.0	22.7	22.3	15.7	16.0	15.8
9 (TB1007)	89.0	88.9	88.9	62.4	63.8	63.1	23.1	23.4	23.3	16.4	16.7	16.5
10 (TB1008)	88.8	88.9	88.9	60.0	62.0	61.0	22.0	22.3	22.2	15.5	15.8	15.7
11 (TB1009)	88.7	88.7	88.7	60.8	62.2	61.5	22.7	21.9	22.3	15.9	15.6	15.7
12 (Kansur)	89.0	89.1	89.1	61.7	62.5	62.1	23.0	22.3	22.6	16.2	15.8	16.0
13 (Tarm Beyazi-98)	89.1	88.7	88.9	61.9	61.7	61.8	22.4	24.0	23.2	15.9	16.8	16.4
Mean	88.9	88.8	88.8	61.6	62.0	61.8	22.5	22.3	22.4	15.9	15.8	15.9
LSD _(0.05)	1.1	0.4	0.5	3.0	2.7	1.9	2.0	1.5	1.2	1.4	1.0	0.8
CV _(%)	0.6	0.2	0.4	2.3	2.0	2.1	4.0	3.0	3.6	4.2	2.9	3.6

LSD Least significant differences; CV (%) Coefficient of variation

The two-year average results indicated that dry matter (DM) content exhibited a range of values between 88.7 and 89.0%, with an average of 88.8%. The amount of digestible dry matter (DDM) changed in a range of 61.0 and 63.1% in the present study (Table 10). Furthermore, the literature indicates that DDM ratios range from 59.43 to 63.21% (Bingöl et al., 2007), 59.10 to 68.69% (Aksoy and Nursoy, 2010), 62.4% (Cacan and Yılmaz, 2015), and 56.0 to 65.5% (Bayar and Cacan, 2019). In this study crude protein (CP) rates ranged from 21.6 to 23.3% (Table 10). In addition in the literature CP rates were measured at 15.10-21.35% (Elvan, 2019); 23.1-26.8% (Bayar and Cacan, 2019); 17.66-24.89% (Guzelogulları and Albayrak, 2012); 22.0-27.0% (Eviz, 2016). Except for Elvan's (2019) earlier experiments support our findings. The concept of digestibility pertains to the capacity of feed materials for utilisation by livestock. A higher degree of digestibility indicates that a greater proportion of the ingested feed content can be processed by the digestive tract (Sari et al., 2018). Digestible protein (DP) ratio is lower than CP ratio. Consequently, DP ratio exhibited fluctuations between 15.4% and 16.5% in the present study (Table 10).

Table 11. Acid detergent fiber, neutral detergent fiber and relative feed values of the standard varieties and mutant lines in 2019, 2020 and two-year averages

Genotypes	Acid Detergent Fiber (%)			Neutral Detergent Fiber (%)			Relative Feed Values		
	2019	2020	Ave.	2019	2020	Ave.	2019	2020	Ave.
1 (TB603)	34.4	34.1	34.3	51.1	45.9	48.5	114.8	126.5	120.7
2 (TB605)	34.2	33.4	33.8	47.0	47.0	47.0	123.3	124.3	123.8
3 (TB802)	34.5	36.3	35.4	45.5	49.6	47.5	127.0	113.9	120.5
4 (TB805)	36.0	33.9	34.9	52.4	44.9	48.7	108.1	129.7	118.9
5 (TB806)	34.9	36.1	35.5	46.7	47.7	47.2	122.9	118.6	120.7
6 (TB1002)	36.0	34.9	35.4	49.8	46.8	48.3	113.8	122.9	118.3
7 (TB1005)	34.7	35.8	35.3	47.0	47.2	47.1	122.4	120.2	121.3
8 (TB1006)	34.5	34.9	34.7	47.6	47.6	47.6	121.2	120.6	120.9
9 (TB1007)	34.0	32.3	33.1	45.0	42.4	43.7	129.2	139.8	134.5
10 (TB1008)	37.1	34.5	35.8	49.3	44.8	47.1	113.9	129.0	121.4
11 (TB1009)	36.1	34.3	35.2	47.8	45.7	46.7	118.7	126.6	122.6
12 (Kansur)	34.9	33.9	34.4	46.1	47.2	46.6	125.1	123.8	124.4
13 (Tarm Beyazi-98)	34.7	34.9	34.8	47.5	45.5	46.5	121.3	127.1	124.2
Mean	35.1	34.6	34.8	47.9	46.3	47.1	120.1	124.8	122.5
LSD _(0.05)	3.9	3.4	2.5	7.4	5.2	4.3	22.5	18.7	13.9
CV. (%)	5.1	4.6	4.8	7.1	5.1	6.2	8.6	6.9	7.8

LSD Least significant differences; CV (%) Coefficient of variation

The two-year average revealed that acid detergent fiber (ADF), neutral detergent fiber (NDF) and relative feed values (RFV) ranged from 33.1 to 35.5%, 46.5 to 48.7%, 8.3 to 134.5 respectively (Table 11). ADF ratios were found to be between 34.1% and 37.3% (Kusvuran et al., 2014), 34.1% (Cacan and Yilmaz, 2015), 30.01%-37.14% (Hashalıcı et al., 2017), 30.0%-42.3% (Bayar and Cacan, 2019), and 32.19%-39.87% (Elvan, 2019). Furthermore NDF ratios were identified at 38.3-45.0% (Kara, 2013), 40.7% (Cacan and Yilmaz, 2015), 39.05-46.79% (Hashalıcı et al., 2017), 37.2-7.5% (Bayar and Cacan, 2019), 45.87-54.46% (Elvan, 2019). RFV were determined to be 106-180 (Aksoy and Nursoy, 2010), 143.2 (Cacan and Yilmaz, 2015), 118.8 (Yilmaz et al., 2015), 112.0-163.8 (Bayar and Cacan, 2019), and 98.77 and 125.45 (Elvan, 2019).

Feeds are classified according to quality standards as either prime, 1st, 2nd, 3rd, 4th or 5th quality. The properties of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), digestible dry matter (DDM) and relative feed value (RFV) can be employed to categorise feed according to its quality requirements (Cacan et al., 2015; Elvan, 2019). For this reason, the following parameters were determined in terms of forage quality: DM, DDM, CP, digestible protein (DP), ADF, NDF and RFV. The results of the current study are in agreement with those of previous trials (Hashalıcı et al., 2017; Bayar and Cacan, 2019; Ertekin et al., 2020). The results of the quality criteria analysis indicated that, while there was no significant difference between the genotypes in terms of mutagen applications, the TB1007 line demonstrated first-class feed value. The remaining genotypes exhibited a second-class quality of feed.

Hierarchical cluster analysis (HCA)

It is of significant importance to be aware of the similarities in agro-morphological traits associated with high forage yield in order to facilitate the development of effective breeding programs. The efficacy of breeding programs during the initial breeding period is contingent upon the accurate phenotype selection of numerous individual plants within the nursery plot.

Upon further examination, it becomes evident that there are two main groups (Figure 1). The groups are categorised according to the quantity of FY and HY. The group A is divided into two subgroups (A1 and A2) based on the observed differences in NPH, PH, and SN traits. Similarly, the group B is also classified into two subgroups (B1 and B2) due to the presence of distinct NPH, PH, SN, and SD traits. Upon analysis of the six traits in question, it was observed that there was a close relationship between NPH and PH, as well as between FY and HY. The TB805 line differed from other lines in terms of SD. The TB1006 line exhibited notable differences in PH and SN compared to other genotypes.

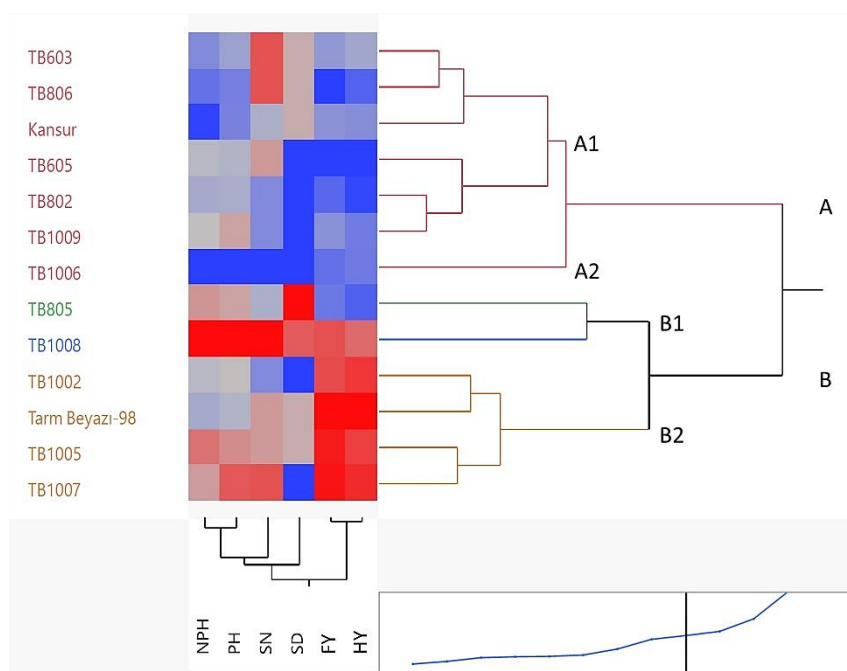


Figure 1. Dendrogram as determined by cluster analysis according to agro- morphological values and quality traits of genotypes

Principal component analysis (PCA)

Principal Component Analysis (PCA) was employed to ascertain the degree of correlation between traits and genotypes in the present study, as depicted on the biplot graph. The analysis also aimed to quantify the contribution of these traits and genotypes to the overall variation observed in the study data set (Figure 2).

Figure 2 presents the PCA results concerning the averages of the measured traits and genotypes. The mean value was calculated over two years. PCA1 and PCA2 collectively accounted for 57.1% and 23.3% of the total variation, respectively. When added together, they account for 80.4% of the total variation.

The examined traits were classified into six groups (Figure 2). The first group included NPH and PH, while the sixth included FY and HY. The TB1005 mutant line was located opposite other mutant lines such as TB605 and TB802. The TB1007, TB1005 and TB1002 mutant lines exhibited the highest data for both -FY and HY traits. The lowest yields were observed in the TB605, TB802, TB806 and TB1009 mutant lines. Additionally, the mutant line TB1006 exhibited the lowest SN.

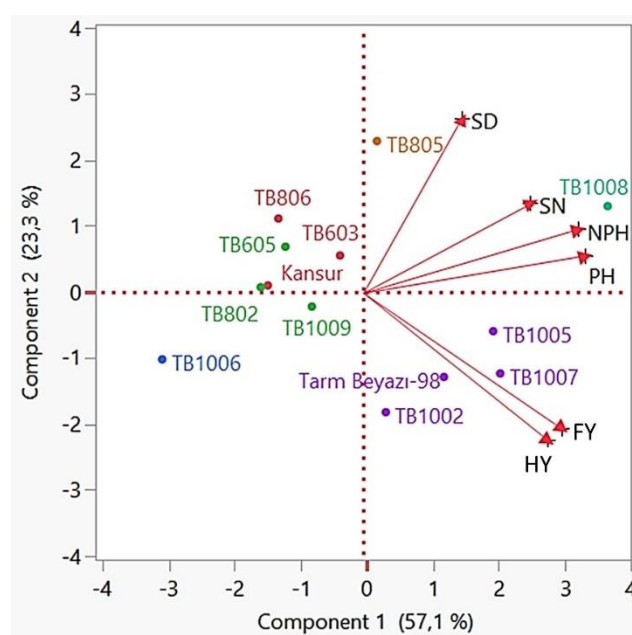


Figure 2. Biplot of principal component analysis on observed traits

4. CONCLUSIONS

A total of 13 line/variety sets, comprising 11 mutant lines and two control varieties, were subjected to an evaluation process to study the effects of mutations on morphological, agronomic and quality traits. Evaluation of the mutant lines revealed a range of effects on the traits examined, including both positive and negative results. A negative effect in the TB1006 mutant and a positive effect in the TB1008 mutant was observed in plant height. This has broadened the genetic base of Hungarian vetch. Nevertheless, the quality criteria remained largely unaltered. While the TB1002 and TB1005 mutant lines exhibited a yield advantage, the TB1007 mutant line demonstrated a yield and quality advantage. These three mutant lines are recommended for use in breeding programs, particularly in future crossing studies.

FUNDING

We would like to express our gratitude to the General Directorate of Agricultural Research and Policies for their invaluable support in the conduct of this research project, which is registered under the following number: TAGEM/TBAD/16/A03/P01/004.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest associated with this study.

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Effect of Vermicompost and Biochar of Pruning Waste on Soil Properties and Faba Bean (*Vicia faba* L.) Yield under Calcareous Soil

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ARTICLE INFO

Research Article

Received: 7 May 2024

Accepted: 1 April 2025

Published: 23 June 2025

Keywords:

Actinomycetes

Organic carbon

Pruning waste

Seed yield

Soil amendments

ABSTRACT

A two-year field study was conducted to compare the effect of biochar and vermicompost of olive tree pruning waste together with chemical fertilizer on microbial population and soil chemical status and faba bean yield in a calcareous soil in Tarem city, Iran. The treatments included wood biochar (BIC) at two levels of 5 (BIC₅) and 10 (BIC₁₀) t ha⁻¹; wood vermicompost (VCM) at two levels of 5 (VCM₅) and 10 (VCM₁₀) t ha⁻¹; 50% (NPK₅₀) and 100% (NPK₁₀₀) recommended chemical fertilizer; along with a control. The results showed that the highest population of total bacteria was obtained from BIC₁₀ (297.8×10^6 CFU/g dry soil), actinomycetes from VCM₁₀ (99.5×10^5 CFU/g dry soil), and fungi from NPK₅₀ (104.5×10^3 CFU/g dry soil). In both years, vermicompost treatments reduced soil pH by 1.6-9.2% compared to control, but biochars showed the highest pH and EC. Organic treatments, especially VCM₁₀ and BIC₁₀, increased the soil organic carbon (OC) compared to the control (by 12.9-35.4%) and NPK (by 57.2-79.1%). Ammonium (N-NH₄⁺) and nitrate (N-NO₃⁻) nitrogen of soil decreased in line with increasing the use of biochar (BIC₁₀) and vermicompost (VCM₁₀), but increasing NPK, from NPK₅₀ to NPK₁₀₀, increased soil N-NH₄⁺ and N-NO₃⁻ by 21.3% and 10.7%, respectively. In both years, the highest number of pods (45.9 and 57.8 in the first and second year, respectively), number of seeds (187.3 and 240.2 in the first and second year, respectively) and seed yield (1.997 and 2.502 t ha⁻¹ in first and second year, respectively) were obtained from soils treated with VCM₁₀. In addition, the highest amount of N seeds (5.579%), K (0.839%) and Fe (66.3 mg kg⁻¹) was observed in BIC₁₀ and the highest amount of P seeds (0.519%) was observed in VCM₁₀. Totally, organic amendments were superior to chemical fertilizers in terms of improving soil fertility and seed yield.

Citation: Namaki, M. H., Ansari, M. H., & Akhgari, H. (2025). Effect of vermicompost and biochar of pruning waste on soil properties and faba bean (*Vicia faba* L.) yield under calcareous soil. *Turkish Journal of Field Crops*, 30(1), 55-66. <https://doi.org/10.17557/tjfc.1480172>

1. INTRODUCTION

Faba bean is an important leguminous crop cultivated in the northern and northwest regions of Iran. The seeds of this plant serve as edible components that can partially substitute for meat and dairy products in human nutrition, owing to their high content of complex carbohydrates, dietary fiber, and proteins, which exceed 30% on a dry weight basis (Ruisi et al., 2017). Nevertheless, the average dry seed yields in Iran (2120 kg ha^{-1}) and specifically in the Tarom region (1160 kg ha^{-1}) (Ministry of Agriculture-Jihad in Iran Yearbook, 2022), remain considerably low, falling short of the global average of 8600 kg ha^{-1} (FAOSTAT, 2022). Lower yield of faba bean in Iran can be attributed to insufficient rainfall and poor soil fertility (Mesgaran et al., 2017). In the Tarom region, most of the cultivated land consists of calcareous soil (Mesgaran et al., 2017; Shiri and Farbodi, 2022). Such soils often lead to nutrient deficiencies in various plants. The elevated pH (>7) and the presence of CaCO_3 in calcareous soils frequently result in the limitation of essential nutrients, including N, P, S, Zn, Fe, Cu, Mn, and B, for plant uptake (Aboukila et al., 2018). Research by Bezabeh et al. (2021), El-Husseiny et al. (2021), and Gad et al. (2017) has documented the reduced yield of faba bean in calcareous soils compared to normal soils. Houassine et al. (2019) identified that the diminished yield of faba bean in calcareous soils is linked to inadequate root development and the plant's limited capacity to absorb nutrients, especially N, P, and Fe. Bezabeh et al. (2021), also concluded that the high pH in calcareous soil results in decreased solubility of micronutrients such as Cu, Mn, Fe, and Zn, adversely affecting faba bean growth.

In addition to showing soil health and quality, organic matter content is a good indicator of soil fertility, resulting from the interaction of physical, chemical, and biological processes. Organic matter improves soil porosity and permeability by enhancing the granulation conditions. However, soil organic matter content cannot be easily enhanced significantly, or its high levels cannot be easily preserved. Various methods have been suggested to increase soil organic matter content, including application of organic and biological fertilizers (Chen et al., 2018; Alan et al., 2024).

Among organic fertilizers, vermicompost has a proven superiority in maintaining soil fertility in the long run (Guridi et al., 2017). Soil amendment by vermicompost is an ecological management program and a strategy to value plant and animal residues (Baghbani-Arani et al., 2017). Vermicompost production, as an environmentally friendly technology, is a semi-aerobic process performed by a certain group of earthworms (*Eisenia fetida*) with the help of some soil-borne microorganisms, especially bacteria and actinomyces (Mahanta et al., 2012; Lim et al., 2016). This process alleviates environmental issues related to manure (the entrance of an excessive amount of heavy metals and pathogens, the loss of soil nutrients by leaching, and the emission of hydrogen sulfide (H_2S), ammonia (NH_3), and other toxic gases). Vermicompost has also been reported to take a shorter time for biological conversion and has high contents of minerals, hormones, enzymes, humic acid, and different available nutrients (El-Haddad et al., 2014; Lim et al., 2016). Vermicompost contains nutrients in readily accessible forms for plants, such as nitrates, exchangeable phosphorous (P), potassium (K), iron (Fe), copper (Cu), and soluble magnesium (Mg) (Soobhany et al., 2017). Guridi et al. (2017) reported that vermicompost contains biologically active compounds, such as plant growth regulators, and is highly capable of preserving soil fertility. If it is incorporated with agricultural nutrient management, crop production costs can be reduced remarkably (Martinez-Balmori et al., 2013).

One another way to increase soil organic matter using crop residues is pyrolysis (Ding et al., 2016). Pyrolysis is the process of biomass decomposition by heat which produces biochar when it happens in anaerobic or almost anaerobic conditions. Biochar is, indeed, the char produced from crop biomass and residues. In other words, biochar is a solid residue produced by the thermal analysis of biomass in a closed container under limited oxygen conditions and medium temperatures ($<700^\circ\text{C}$) (El-Naggar et al., 2021). Biochar is a porous compound with a high specific area and potential for nutrient recycling, soil ventilation, economic profit, and waste system management. It is a long-term factor in economical and reliable carbon sequestration (El-Naggar et al., 2021). Other beneficial effects of biochar application in agricultural lands are the increase in soil organic matter, the long-term carbon storage in soil, the improvement of water retention, the increase in cation exchange capacity, interaction with soil nutrient cycle by moderating soil pH, the increase in soil fertility, and the reduction of nutrient leaching (Shi et al., 2023; Senay and Tepecik, 2024). Chen et al. (2021) showed that biochar application can increase pH in tropical soils. Like its other properties, biochar pH depends on raw material type, production temperature, and production duration. So far, only a few studies have addressed the effect of biochar on the pH of calcareous soil. Van Zwieten et al. (2010) applied several types of biochar in calcareous soils and reported that only one increased soil pH (the initial pH of 7.67). Biochar effectively contributes to releasing chemical elements, including various metal ions (Baiaamonte et al., 2019; Tepecik et al., 2024). It also results in the absorption of anion nutrients, like phosphate ions, although the mechanism of these processes is not fully known (Abhishek et al., 2022). However, most research on biochar and vermicompost has been conducted in acidic soils and hot areas, and research has been rare on calcareous soils. Given the high volume of waste produced by the pruning of olive shrubs in Tarom County in Zanjan, Iran, their processing into vermicompost and biochar is a chance to improve soil fertility for its long-term use. Therefore, the present research aimed to shed light on the effect of vermicompost and biochar produced from olive shrub residues on some soil properties and nutrient content, as well as faba bean yield, in calcareous soil in field conditions.

2. MATERIALS AND METHOD

Study site and experimental design

The research was conducted as a field study in a research farm in Tarom County, Zanjan (Long. 48°30' E., Lat. 37°21' N., Alt. 214 m from sea level) in 2021 and 2022. The mean temperature and total annual precipitation were 17.5 °C and 379 mm. Figure 1 presents temperature, precipitation, and sunny hours during the plant growth period. During the growth period of the plant, the mean temperature in the first year was 11.8°C and in the second year was 13.1°C. Total precipitation in the first and second years was 242 and 257 mm, respectively. Also, the mean sunny hours in the first and second year were 224 and 211 h, respectively (Figure 1). Before the experiments, the soil was sampled from a 0-30 cm depth to analyze its physical and chemical characteristics. The results are reported in Table 1. The results of soil analysis showed that the soil texture is clay loam, pH in the first and second years is 8.11 and 7.86, respectively. Electrical conductivity and organic carbon in the first and second years is 1.16 and 0.89 dS m⁻¹, as well 0.32 and 0.25%, respectively (Table 2).

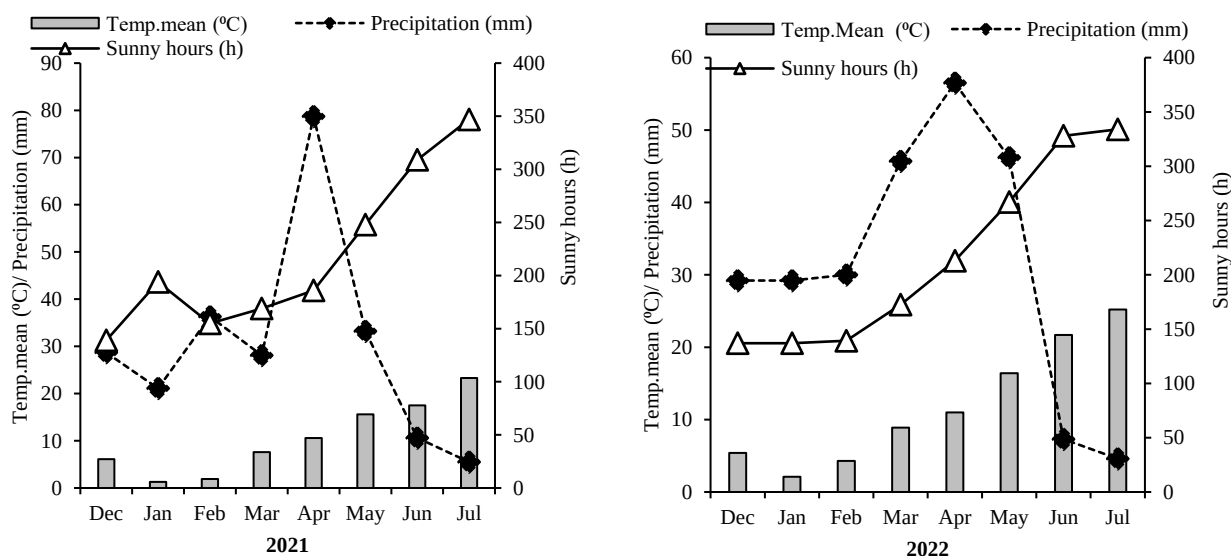


Figure 1. Mean temperature, total precipitation and sunny hours per month during plant growth (Abbar Weather Station)

Table 1. Some physical and chemical properties of the studied soil

Year	Texture	Silt	Sand	Clay	Organic Carbon	Calcium carbonate Equivalent	pH	Electrical conductivity (dS m ⁻¹)	Cation exchange capacity (cmol _c kg ⁻¹)
		(%)							
2021	Clay loam	21	52	27	0.32	23.5	8.11	1.16	19.6
2022		23	46	31	0.25	25.3	7.86	0.89	21.7

Table 2. Characteristics of organic materials used in the experiment

Chemical characteristics	Unit	Biochar	Vermicompost
pH	–	8.10 ± 0.02	7.37 ± 0.00
EC	(dS m ⁻¹)	5.23 ± 0.30	1.31 ± 0.10
Ash	(%)	32.30 ± 1.57	18.90 ± 1.70
C	(%)	64.10 ± 2.10	27.50 ± 0.77
N	(%)	0.86 ± 0.00	1.41 ± 0.04
C:N	–	109.40 ± 5.70	26.50 ± 1.34
P	(g kg ⁻¹)	1.91 ± 0.20	1.03 ± 0.30
K	(g kg ⁻¹)	8.66 ± 0.40	3.19 ± 0.20
Fe	(mg kg ⁻¹)	320.30 ± 10.60	1046.70 ± 19.80

The research was conducted in a randomized complete block design with three replications. The treatments included wood biochar (BIC) at two levels of 5 and 10 t ha⁻¹ (BIC₅ and BIC₁₀, respectively), vermicompost (VCM) at two levels

of 5 and 10 t ha⁻¹ (VCM₅ and VCM₁₀, respectively), chemical fertilizer (NPK) at two levels of half and full rate recommended by the soil analysis (NPK₅₀ and NPK₁₀₀ respectively), and a control.

Preparation of biochar and vermicompost

A large number of pruned branches were collected from orchards in Tarom County to prepare biochar. One- and two-year-old branches were preferred. The pruning waste of the olive shrubs was chopped into 20-30 mm pieces, washed with distilled water, and heated in an oven at 75°C for two days. Then, an electrical furnace was modified to provide anaerobic conditions for pyrolysis. To this end, two cylindrical reactors made of steel with a diameter of 20 cm and height of 40 cm were designed and fabricated by the Azarkhakab Company and were placed inside the electrical furnace for pyrolysis. The reactors had an inlet for gas and an outlet for the gas and liquid phases produced. The lid had a heat insulator to minimize heat loss. The pyrolysis process was conducted at 350°C at a rate of 3°C/min. The samples were kept at the target temperature for 180 minutes, and then the furnace was gradually cooled down to 100°C or lower through heat exchange with the environment at a rate of 2°C/min (Kim et al., 2016).

The olive pruning waste was chopped to 20-30 mm pieces and put in a 3-m³ vermireactor containing a stable and very active population of *Eisenia Andrei*. Some manure was added to the waste to increase the earthworms' activity. The earthworm population density was 250 g/kg in the upper layers. The moisture in the vermireactor was kept at 75-80%. The sample was collected from the reactor's last layer (after 60 days of earthworm processing) once the manure was processed by the earthworms (Lazcano et al., 2008). The vermicomposting process changed the pruning waste's physical and chemical features significantly. The vermicompost was dark in color, had a good appearance, and was converted into a homogenous compound after the earthworms' activity. The physical and chemical properties of vermicompost and biochar are reported in Table 2.

Agronomic operations

The experimental plots were composed of six sowing lines, each 5 m long, with an on-row spacing of 20 cm, inter-row spacing of 40 cm, and density of 80,000 plants ha⁻¹. The faba bean (cv. 'Barakat') seeds were sown by hand at a depth of nearly 5 cm on 6 December. They were then watered by sprinklers. Since the research aimed to compare organic treatments with chemical fertilization and control, only Zn was added to the soil of all treatments. In addition, 150 kg ha⁻¹ N as urea (in three allocations), 100 kg ha⁻¹ phosphate as superphosphate triple, and 100 kg ha⁻¹ K as sulfate potassium were applied in the chemical NPK treatment. The weeds were controlled mechanically (hand weeding).

After 60 days, the soil samples were air-dried and their pH, EC, organic C, mineral N, K, P and Fe that were available were measured by conventional methods. Soil organic carbon content was determined by fresh oxidation using potassium dichromate and thick sulfuric acid (Walkley & Black, 1934), mineral N (nitrate and ammonium) was determined by 2M potassium chloride (Mulaney, 1996), absorbable P was measured by the Olsen method with 0.5N sodium bicarbonate (Olsen, 1954) using spectrophotometry. Absorbable K of ammonium acetate was estimated by flame photometry (Champam & Pratt, 1982). EC and pH were determined in a soil: water ratio of 1:5 (Hesse, 1971). The soil samples' Fe, Mn, Cu, and Zn were determined by the extraction method with DTPA and the reading of an atomic absorption device (Lehmann, 2007).

Soil microbial populations

To count the soil microbial populations, samples were taken 40, 80, and 120 days after the planting. Two 10-g soil samples from the root area, from each plot, were separately put in 250-mL Erlenmeyer flasks containing 25 mL of sterilized distilled water (Aneja, 2007). The flasks were shaken rotationally for 20 minutes. Then, they were kept still for 15 seconds for the coarser particles to precipitate. Serial dilutions up to 10⁶ were done by repeatedly adding 1 mL of soil suspension to 9 mL of sterilized distilled water. All determined microorganisms estimated using the optimum dilution for each group were total bacterial count × 1/10⁶ according to Allen et al. (1950), total actinomycetes count × 10⁵ according to Jensen (1930), and total fungal count × 10³ according to Martin (1950).

Plant sampling

To measure the fresh yield of the faba beans, after the pods reached their maximum size and weight (when the pods were green), the pods of five plants were harvested. The samples were taken from the middle rows after eliminating about 0.5 from both ends to avoid the marginal effect. At the physiological maturity stage, 2 m² were harvested and 10 plants were randomly selected to calculate biological and dry seed yields. Total dry weight was estimated on the whole plants (shoot plus pods) oven-dried at 75°C for 72 hours. The concentrations of different elements in the faba bean seeds were determined by grinding them and sampling 15 g. The N content was measured by the Kjeldahl method (Kjeldahl 2300; FOSS, Hoganas, Sweden), the P content by the vanadomolybdo-phosphoric colorimetric method (Jackson, 2005), and the Fe contents by atomic absorption spectrometry (Jones, 1972).

It should be noted that treatments in the second year were used as in the first year and all the works done in the first year was repeated in the second year (Except for the soil microbial populations measured only in the second year).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using SAS 9.1 software. When the F-test revealed the significance of the treatments, the means were separately compared using the LSMEANS method with least significant difference (LSD) adjustment at $P = 0.05$.

3. RESULTS AND DISCUSSION

Soil microbial population

Table 3 shows that the microbial populations counted in the soil (bacteria, actinomycetes, and fungi on days 40, 80, and 120) responded to the treatments, differing from the control. The difference widened over time from day 40 to day 120. Increasing the rate of organic fertilizers and applying NPK increased the number of soil bacteria compared to the control over time. The highest number of bacteria was related to the treatment of BIC₁₀ ($228, 318, \text{ and } 346 \times 10^6$ CFU/g dry soil on days 40, 80, and 120, respectively). The number of actinomycetes also increased in the treated soils from day 40 to day 120, but it was $24.65, 23.29, \text{ and } 17.27 \times 10^5$ CFU/g dry soil on days 40, 80, and 120 in the untreated soils, respectively. The maximum actinomycete population was recorded in VCM₁₀ ($76.45, 108.3, \text{ and } 113.8 \times 10^5$ CFU/g dry soil on days 40, 80, and 120, respectively). The second highest was related to BIC₁₀ (Table 3). However, the fungal population responded to the treatments differently from the bacterial and actinomycete populations over time. The total number of fungi decreased versus the control in all treatments (except for NPK0.5). In addition, the fungal population in VCM₁₀ was $60, 48, \text{ and } 40 \times 10^3$ CFU/g dry soil on days 40, 80, and 120, respectively. They were $49, 37, \text{ and } 51 \times 10^3$ CFU/g dry soil in BIC₁₀ and $57, 72, \text{ and } 36 \times 10^3$ CFU/g dry soil in NPK₁₀₀. Overall, the highest mean fungal population was obtained from the soil treated with NPK₅₀ (104×10^3 CFU/g dry soil) and the control (101×10^3 CFU/g dry soil) (Table 3). The increased population of bacteria and actinomycetes can be attributed to the positive effect of vermicompost and biochar by creating a beneficial substrate for the activity of soil microorganisms. This activity increases over time with the rise of air temperature (Elad et al., 2011; Zhang et al., 2022). It has been reported that biochar has significant effects on rhizosphere bacteria, which can be effective in improving soil fertility (Ding et al., 2016; Mohamed et al., 2017). The application of vermicompost, also, increased the soil's bacterial population, but its positive effect on the population of actinomycetes was more substantial than that of the biochar and NPK, which can be related to the highest solution nutrients in these treatments. It seems that vermicompost provides a suitable substrate for increasing the population of actinomycetes mainly by improving the physical and structural conditions of the soil, preserving soil moisture, and increasing cation exchange capacity (CEC) (Mosavi-Azandehi et al., 2023). Vermicompost is replete with microbial populations, especially bacteria and actinomycetes, which play a key role in supplying the cycle of some nutrients. In addition, organic matter undergoes peculiar biochemical interactions in earthworms' digestion system so that the waste of the earthworms can be absorbed by the plants to supply their nutrient requirements (Mosavi-Azandehi et al., 2023).

Table 3: Effect of experimental treatments on the microbial community of soil by (CFU/g dry soil) at different periods (40, 80, and 120 days after planting)

	Bacteria $\times 10^6$ CFU/g dry soil				Actinomycetes $\times 10^5$ CFU/g dry soil				Fungi $\times 10^3$ CFU/g dry soil			
	40	80	120	mean	40	80	120	mean	40	80	120	mean
Control	33.3f	40.2d	34.3e	35.9e	24.65d	23.29f	17.27f	21.73f	108.1a	99.29b	102.5a	101.4ab
NPK ₅₀	74.1e	112.6c	169.7d	118.8d	33.17c	42.94c	52.03e	42.71e	108.7a	118.3a	86.56b	104.5a
NPK ₁₀₀	93.7de	129.9c	240c	154.6c	41.46bc	52.14c	74.18d	55.89d	57.14de	72.93d	36.69d	55.58d
BICO ₅	170.9b	295.8a	329.6a	265.5b	54.98b	67.25c	73.16d	65.09c	98.15b	89.1c	100.4a	95.91b
BIC ₁₀	228.7a	318.5a	346.2a	297.8a	62.82ab	76.57b	86.11c	75.09b	49.64e	37.94f	51.57c	46.38e
VCM ₅	99.1d	111.1c	181.9d	130.7cd	52.03b	78.61b	101.44b	77.36b	88.95c	74.86d	89.08b	84.29c
VCM ₁₀	127.8c	244.4b	284.4b	261.5b	76.45a	108.37a	113.83a	99.51a	60.89d	48.62e	40.1d	49.87de
S.O.V												
S		63318**				3244**				458.5**		
S(R)		7652				244				1405		
T		6764**				915**				6119**		
S $\times$ T		3512**				280**				374**		
E		306				23.7				25.1		

Similar letter(s) in each column shows insignificance of the difference based on the LSD test at the $P < 0.05$ level.

**Significant at the $P \leq 0.01$ level; * significant at the $P \leq 0.05$ level; ns no significant difference.

S: Sampling time, R: Replication, T: Treatments, E: Error, CV: Coefficient of Variation.

Soil status

The results revealed that pH, EC, and OC were influenced by the treatments in both years (Table 4). The comparison of means showed that the application of vermicompost (VCM5 and VCM10) reduced soil pH by, on average, 6.1% in the

first year and 9.25 in the second year compared to the control, whereas the biochar at both levels had the highest pH and EC in both years. The organic treatments, especially VCM10 and BIC10, increased soil OC versus the control and NPK by 12.9-35.4% and 57.2-79.1%, respectively. The highest soil OC in both years was obtained from VCM10 (4.16% in the first year and 4.28% in the second year) (Table 5).

Table 4. Variance analysis for the effect of the experimental treatments on chemical properties of the soil in both years

S.O.V	Mean Square (MS)									
	pH	EC (dS m ⁻¹)	N-NH ₄ ⁺	N-NO ₃ ⁻¹	Olsen P	K	Fe	Mn	Cu	OC (%)
			(mg kg ⁻¹)							
Y	2.11 ^{ns}	0.011 ^{ns}	50.3 ^{ns}	21.6 ^{ns}	21.5 ^{ns}	55510 ^{ns}	0.708 ^{ns}	4.67 ^{ns}	0.28 ^{ns}	0.26 ^{ns}
Y(R)	1.67	0.093	252	106	332	863381	3.44	38.4	0.157	1.37
T	0.25 ^{**}	0.81 ^{**}	58.6 [*]	128 ^{**}	129 ^{**}	934920 ^{**}	3.14 ^{**}	18.9 ^{**}	0.59 ^{**}	7.29 ^{**}
T × Y	0.0192 ^{**}	0.56 ^{**}	13.5 ^{ns}	5.6 ^{ns}	225 ^{**}	1157485 ^{**}	4.31 ^{**}	35.2 ^{**}	0.79 ^{**}	20.8 ^{**}
E	0.0045	0.031	2.87	15.1	19.2	75476	0.343	2.16	0.102	0.299
CV (%)	0.832	34.1	4.19	14.8	16.7	20.6	12.37	12.01	11.1	19.2

**Significant at the $P \leq 0.01$ level; * significant at the $P \leq 0.05$ level; ns no significant difference.

Y: Year, R: Replication, T: Treatments, E: Error.

Table 5. The means comparison for the effect of the experimental treatments on pH, EC, OC, and Olsen phosphorus of the soil in both years

Treatments	pH			EC (dS m ⁻¹)			OC (%)			Olsen P (mg kg ⁻¹)		
	Y1	Y2	Mean	Y1	Y2	Mean	Y1	Y2	Mean	Y1	Y2	Mean
Control	8.284b	7.891c	8.087c	0.8144b	0.248c	0.531bc	0.536c	0.827d	0.675d	29.2b	15.53c	22.36c
NPK ₅₀	8.327b	7.841c	8.078c	1.307a	0.312c	0.809a	0.754c	0.662d	0.705d	42.28a	23.18bc	27.73ab
NPK ₁₀₀	8.315b	7.780d	8.053c	0.283c	0.365bc	0.324cd	0.818c	0.508d	0.655d	18.95d	20.98bc	19.97c
BIC ₅	8.595a	8.072b	8.333b	0.308c	0.771b	0.539b	4.610b	4.364b	4.485ab	21.94cd	27.64b	24.79bc
BIC ₁₀	8.706a	8.304a	8.505a	0.3855c	1.237a	0.811a	6.034a	5.712a	5.37a	22.16cd	40.02a	32.99a
VCM ₅	8.082c	7.841c	7.941d	0.296c	0.279c	0.287d	3.570b	3.038c	3.13c	25.52bc	22.76bc	24.14bc
VCM ₁₀	8.22bc	7.659e	7.961d	0.298c	0.284c	0.291d	4.162b	4.281b	4.22b	27.97bc	27.88b	27.92ab

Similar letter(s) in each column show insignificance of the difference based on the LSD test at the $P < 0.05$ level.

Biochar can reduce or increase soil pH. This is related to the evolution of organic matter added to the soil and its decomposition level. Furthermore, biochar can increase soil CEC (El-Naggar et al., 2021), showing the buffer capacity of the soil. Soil CEC may increase with biochar application, so soil capacity to preserve nutrients increases. However, the effect of biochar on soil pH in calcareous soils is weaker than the effect of vermicompost, which is related to the buffer capacity of the soil due to its calcium carbon content (Abhishek et al., 2022). In both measurement years, pH was lower in both vermicompost levels, which is related to the acidic pH of the vermicompost on the one hand and the stimulation of microorganisms and the resulting increase in CO₂ production and the decrease in pH on the other (Baghbani-Arani et al., 2017). These results indicate the positive effect of vermicompost in reducing pH in calcareous soils and subsequently, the improved availability of lowly mobile elements, e.g., P, in these soils (Sarma et al., 2018; Yuksel et al., 2024). The increase in EC is seemingly associated with the minerals of biochar and the effect of biochar on soil compounds and the release of dissolved nutrients. Biochar contains some nutrients, such as K, Ca, and Mg (Tepecik et al., 2022; Hammadi & Alwan, 2023). When biochar is applied to the soil, the K, Fe, and Ca ions of the soil solution may increase, which may be a reason for the increased EC of the soil (Mohamed et al., 2017; Hammadi & Alwan, 2023).

The soil ammonium (N-NH₄⁺) and nitrate (N-NO₃⁻¹) content was influenced by the organic treatments (Table 4). As the rate of biochar (BIC10) and vermicompost (VCM10) was increased, the N-NH₄⁺ and N-NO₃⁻¹ content of the soil decreased, but the increase in the application of the chemical fertilizers from NPK50 to NPK100 increased soil N-NH₄⁺ and N-NO₃⁻¹ contents by 21.3 and 10.7%, respectively. In addition, NPK100 was related to the highest N-NH₄⁺ content (51.4 mg kg⁻¹) and N-NO₃⁻¹ content (33.5 mg kg⁻¹), and BIC10 was related to the lowest ones (Figure 2). The C/N ratio plays a critical role in the N content during the decomposition of the organic matter added to the soil. The increase in soil C content often increases the C/N ratio and reduces N mineralization. The application of biochar to the farmlands in a tropical region reportedly increased N availability (Steiner et al., 2007). The decline in N availability may be related to biochar's high C/N ratio, and consequently, its higher potential for converting N to its organic form and the uptake of N-NH₄⁺ to biochar, which, in turn, reduces the potential of N leaching and increases its fixation for higher fertility of the surface soil over time (Steiner et al., 2007).

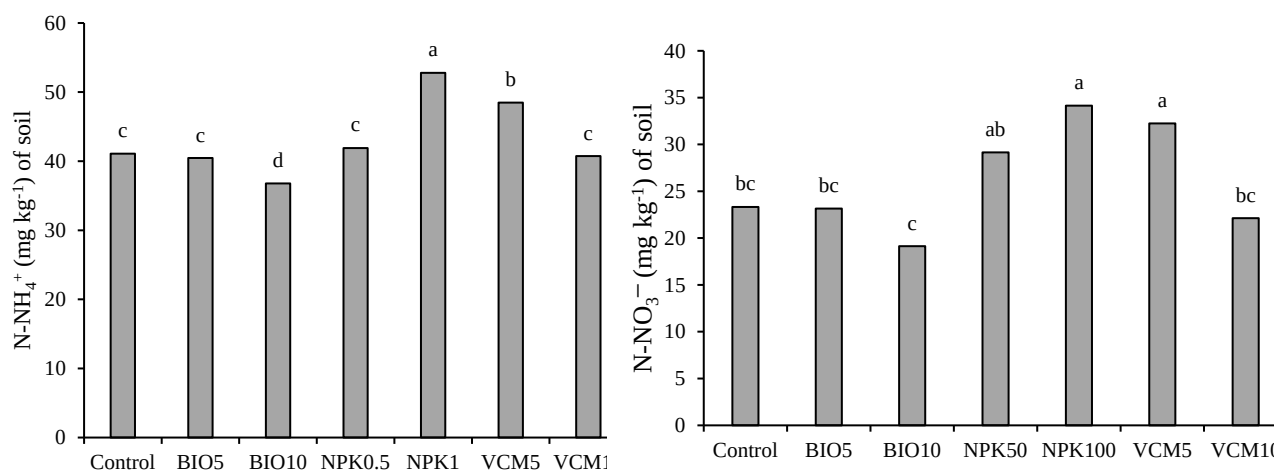


Figure 2. The means comparison for the main effect of the experimental treatments on N-NH₄⁺ and N-NO₃⁻ of the soil

The treatments influenced P, K, Fe, Mn, and Cu in both years (Table 4). Based on the results, the soils treated with the biochar exhibited the highest P, K, and Mn contents but the lowest Fe and Cu contents, whereas the soils treated with vermicompost had the highest Fe and Cu contents. It should be noted that the increase in the application of biochar (BIC10) and vermicompost (VCM10) reduced Fe and Cu. In addition, the variations in the nutrients of the NPK-treated soils differed from those of the soils treated with biochar or vermicompost and were closer to the control (Table 6). Nelissen et al. (2015) reported that the application of biochar in sandy and loamy soils improved available P significantly. Wu et al. (2022) stated the application of rice straw biochar increased available P significantly by 13.8% versus the no-biochar treatment. Abhishek et al. (2022) reported that biochar could increase P availability by adsorption and also through increasing soil CEC. In a study on the effect of the application of seed residue biochar on the alkaline elements of the soil, Wu et al. (2022) revealed that it increased K availability but reduced exchangeable Ca, Mn, and Na. Nelissen et al. (2015) found that the biochar of walnut shells increased soil Mn over a 67-day experimental period. Baiamonte et al. (2019) applied compost and biochar produced from it to Mediterranean (sandy and non-calcareous) soil and revealed that Zn and Cu content decreased significantly in the biochar treatment than in the compost treatment. Chintala et al. (2014) reported that applying biochar to the soil influenced the availability of nutrient ions by influencing ion exchange capacity and microbial activities. The formation of functional groups and absorption spots on the biochar surface also increases soil capacity in creating complexes with metal ions and their uptake (Zhang et al., 2022).

Table 6. The means comparison for the effect of the experimental treatments on K, Fe, Mn, and Cu of the soil in both years

Treatments	K (mg kg ⁻¹)			Fe (mg kg ⁻¹)			Mn (mg kg ⁻¹)			Cu (mg kg ⁻¹)		
	Y1	Y2	Mean	Y1	Y2	Mean	Y1	Y2	Mean	Y1	Y2	Mean
Control	1724b	691d	1207bc	4.049b	6.246a	5.148ab	15.67ab	9.18c	12.43bc	0.839bc	1.218a	1.029a
NPK ₅₀	2530a	1090b-d	1810a	3.017c	4.836b-d	3.927c	17.11a	9.46c	13.29b	0.806c	0.8441c	0.825c
NPK ₁₀₀	862c	1393bc	1128bc	6.128a	4.026-d	5.077ab	9.79c	13.55ab	11.67bc	1.162a	0.804cd	0.983ab
BIC ₅	1018c	1632b	1325b	5.579 a	3.83de	4.706b	9.901c	14.84a	12.37bc	1.016a-c	0.795cd	0.906bc
BIC ₁₀	1471b	2395a	1933a	4.253 b	2.856e	3.55c	14.31b	16.20a	15.25a	0.849bc	0.761d	0.804c
VCM ₅	943c	844cd	893c	5.572a	5.34ab	5.458a	10.14c	8.052c	9.59d	1.068ab	1.021b	1.044a
VCM ₁₀	1027c	1021cd	1024bc	5.453 a	5.093bc	5.273ab	11.102c	11.06bc	11.08cd	1.052ab	0.9877b	1.020ab

Similar letter(s) in each column show insignificance of the difference based on the LSD test at the P < 0.05 level.

Agronomic traits

The number of pods, the number of seeds, plant weight, and seed yield were affected by the interaction of treatment and year (Table 7). Most treated plants had higher pod number (17.3-49.4% in the first and 11.6-43.8% in the second year) and seed number (26.2-53.1% in the first and 19.4-45.2% in the second year) than the control plants, although the difference was insignificant between some treatments and the control. In both years, the soils treated with VCM10 were related to the highest number of pods (45.9 and 57.8 pods m⁻² in the first and second years, respectively) and seeds (187.3 and 240.2 seeds m⁻² in the first and second years, respectively), and BIC10 and VCM5 were ranked lower (Table 8). According to Chinthapalli et al. (2015), organic fertilizers are eventually responsible for increasing plant biomass. Since vermicompost and biochar contain some macro and micronutrients, they increase flower buds by expanding the photosynthesizing area (Eo and Park, 2019; Alan et al., 2024). The increase in the flower number finally results in an increase in pod production, but if the plant is not capable enough to persistently mobilize materials to flowers, most flowers will fall and consequently, pod formation and number will decrease (Nafees et al., 2023). Compared to the control, the treatments increased pod yield by 4.53-20.3% in the first and 5.3-35.5% in the second year and seed yield by 10.4-

39.6% in the first and 11.7-45.1% in the second year. Furthermore, the treatment of VCM10 produced the highest seed yield in both years (1.997 and 2.502 t ha⁻¹ in the first and second years, respectively). But, it significantly differed from VCM5 (1.607 t ha⁻¹), BIC10 (1.823 t ha⁻¹), and BIC5 (1.653 t ha⁻¹) in the first and only from BIC10 (2.053 t ha⁻¹) in the second year (Table 8).

Table 7. Variance analysis for the effect of the experimental treatments on agronomic traits in both years

S.O.V	Mean Square (MS)								
	Pod numbers m ⁻²	Seed numbers m ⁻²	Pod yield	Seed yield	Biological yield	N	P	K	Fe
				(t ha ⁻¹)			In seed (%)		
Y	1.92ns	143ns	378860**	1060**	433ns	21.3**	0.154**	0.0011ns	943**
R(Y)	10.7	13.9	658	114	235	0.194	0.00061	0.0015	85.7
T	217**	6558.**	72930*	250*	33969**	3.82*	0.023**	0.173**	509**
T × Y	52.5**	635*	95737**	209*	1326*	6.04**	0.0059ns	0.156**	398**
E	7.88	201.9	20190	75.4	496.2	1.506	0.0054	0.0245	52.3
CV (%)	8.29	10.12	16.3	8.7	14.81	31.6	17.4	31.7	13.3

**Significant at the $P \leq 0.01$ level; * significant at the $P \leq 0.05$ level; ns no significant difference.

Y: Year, R: Replication, T: Treatments, E: Error.

Table 8. The mean comparison of experimental treatments effect on pod number, seed number and yield of pod, seed and biological of faba bean in both years

Treatments	Pod numbers m ⁻²			Seed numbers m ⁻²			Pod yield (t ha ⁻¹)			Seed yield (t ha ⁻¹)			Biological yield (t ha ⁻¹)		
	Y1	Y2	Mean	Y1	Y2	Mean	Y1	Y2	Mean	Y1	Y2	Mean	Y1	Y2	Mean
Control	23.0e	32.4c	13.7c	90.4e	125c	108c	9.70b	10.60c	10.15d	1.208c	1.418c	1.310d	3.56f	2.55d	2.94e
NPK ₅₀	29.0cd	37.2bc	16.8bc	123d	144c	134b	10.02b	11.05c	10.54cd	1.345b	1.603bc	1.475cd	3.23f	3.14cd	3.08de
NPK ₁₀₀	32.0c	33.6c	18.81bc	130cd	149c	139b	10.49ab	10.89c	10.70cd	1.540ab	1.667bc	1.605b-d	5.34d	3.76c	4.55cd
BIC ₅	27.8d	36.3bc	17.46bc	123d	156bc	139b	10.16ab	14.35bc	12.26bc	1.653ab	1.633bc	1.64b-d	3.91e	5.63b	4.77bc
BIC ₁₀	40.0ab	45.4b	19.95b	167ab	183b	175a	12.01ab	15.82ab	13.92ab	1.823a	2.053ab	1.935ab	6.22b	6.11ab	6.18ab
VCM ₅	38.0b	40.8bc	21.83ab	146bc	151c	148b	10.96ab	12.74bc	11.86cd	1.607ab	1.883bc	1.745bc	5.72c	6.51ab	6.11ab
VCM ₁₀	45.9a	57.8a	27.07a	187a	240a	187a	12.20a	19.26a	15.74a	1.997a	2.502a	2.250a	7.26a	6.95a	7.106a

Similar letter(s) in each column show insignificance of the difference based on the LSD test at the $P < 0.05$ level.

Although the NPK100 treatment had higher pod and seed yield than the control, it was less effective than the organic fertilizers. In addition, organic fertilizers significantly provide optimal growth and nutrition conditions for plants (Sheikh et al., 2022). Organic fertilizers improve plant growth and development, thereby increasing their yields, by supplying macro and micronutrients required by the plant, improving the soil's physical, chemical, and biological features, increasing its water retention capacity, contributing to the proper expansion of the plant's root system by improving soil structure and porosity (El Nahhas et al., 2021; Cukurcalioglu et al., 2023), synthesizing plant hormones by bacteria, and enhancing mineral uptake and translocation (Nafees et al., 2023). These are the positive effects of biochar application on the soil, leading to the plant's growth improvement by improving soil conditions, including aeration, and enhancing water and nutrient availability in the soil. The increase in N increases protoplasm and cell division, resulting in an increase in cell size and leaf area and finally the improvement of the vegetative growth by enhancing the photosynthesis activity (Saikia et al., 2010). The stem dry weight increased significantly in the culture medium containing biochar. Kim et al. (2016) stated that the highest corn dry weight was obtained from the treatment of 5% biochar.

The N, P, K, and Fe contents were affected by the interaction of the treatment and year (Table 7). The comparison of the seed N content of different treatments revealed that although the seeds of the plants treated with biochar contained the highest amount of N in both years (6.74 and 4.40% in the first and second years, respectively), the seeds of the plants treated with vermicompost and NPK100 had lower N than the control (Table 9). Unlike the seed N content, its P content was the highest in the vermicompost treatments. The biochar treatments were superior to the control in this respect, but not significantly (Table 9). The application of biochar positively influenced plant growth, maybe due to the supply of N and other nutrients required by the plant at balanced levels. However, biochar had a more positive effect on the plant traits at higher levels (Hammadi et al., 2023), which can be ascribed to the increased stability of water in soil particulates and the higher level of P in the soils mixed with biochar. The positive effect of biochar mainly depends on the capability of biochar to retain soil nutrients owing to its high specific area. This, in turn, increases soil EC (El Nahhas et al., 2021).

Table 9. The mean comparison of experimental treatments effect on N, P, K and Fe content of seed of faba bean in both years

Treatment	Seed N			Seed P			Seed K			Seed Fe		
	Y1	Y2	Mean	Y1	Y2	Mean	Y1	Y2	Mean	Y1	Y2	Mean
Control	5.35a-c	3.666ab	4.514b	0.3206b	0.4936ab	0.407ab	0.476b	0.573b	0.525b	71.67ab	44.55e	58.11ab
NPK ₅₀	3.96cd	3.446ab	3.701c	0.298b	0.426b	0.362b	0.503b	0.376b	0.440b	53.27b	41.65f	47.46bc
NPK ₁₀₀	2.78d	2.64b	2.71de	0.339b	0.404b	0.372b	0.510b	0.46b	0.485b	55.21b	41.65f	48.43bc
BIC ₅	6.30ab	3.95a	5.133ab	0.317b	0.5096ab	0.413ab	0.453b	0.356b	0.405b	75.06a	54.24b	64.65a
BIC ₁₀	6.74a	4.40a	5.579a	0.3783ab	0.403b	0.391ab	0.796a	0.881a	0.839a	67.8ab	64.89a	66.35a
VCM ₅	2.56d	2.56b	2.513e	0.455a	0.49ab	0.473ab	0.363b	0.476b	0.422b	30.02c	53.27c	41.65c
VCM ₁₀	4.47b-d	2.05b	3.264cd	0.4036ab	0.634a	0.519a	0.316b	0.34b	0.328b	60.0ab	46.49d	53.25a-c

Similar letter(s) in each column show insignificance of the difference based on the LSD test at the $P < 0.05$ level.

In terms of the seed K content, too, the biochar treatments significantly outperformed the control (0.796 and 0.505% higher in the first and 0.881 and 0.573% higher in the second year), but other treatments did not differ from the control significantly. In addition, except for the biochar treatments, the chemical fertilizer treatments had higher K content than the control, but not significantly (Table 9). Other researchers have also supported the increase in soil K in the presence of biochar. Since the soils treated with biochar had the highest P content, it was expected that the seeds of the plants in these soils had the highest P content, but the seeds of those treated with VCM10 had higher P content in both years (Table 9). On the contrary, the highest Fe content in the soil and seeds was recorded by the treatments of vermicompost and biochar, respectively. In this regard, BIC10 had the highest N and K content in both seeds and soil. This can be attributed to a set of intricate factors influencing nutrient uptake from the soil by the plant, including soil pH and EC, as well as the method and volume of nutrient uptake and plant root secretions (Sheikh et al., 2022; Alan et al., 2024). Of course, the amount of seed N and K and soil in BIC10 was higher than the other treatments. This finding can be attributed to a set of complex factors influencing the absorption of elements in the soil by the plant, so that the value of soil pH and EC, as well as the method and volume of element absorption and plant root secretions have always been discussed (Rekha et al., 2023; Yuksel et al., 2024). Finally, we conclude that the concentration of N, P, K and Fe in faba bean seeds indicates their content in soils under different treatments. These results are consistent with Rivelli and Libutti (2023) who demonstrated that the application of organic amendments such as biochar and vermicompost from hardwood chips increased plant nutrient availability. Song et al. (2022) reported that applying biochar and vermicompost significantly contributed to the increase of plant nutrients in poor soils. This can be explained by the ability of these organic amendments to increase the availability of soil nutrients and their content in faba bean plants.

4. CONCLUSION

The obtained results showed that the conversion of the shoots obtained from pruning olive trees into biochar and vermicompost can lead to the production of environmentally friendly organic fertilizers, which had a positive effect on the fertility of poor limestone soil, as it significantly increased the population of bacteria and actinomycetes in the soil and caused changes in some soil characteristics such as OC, pH, and EC, and also increased the usability of some macro- and micro-elements, compared to NPKs and control. Of course, soils treated with biochars showed the highest amount of P, K, and Mn and the lowest amount of Fe and Cu, while vermicompost soils showed the highest amount of Fe and Cu. In addition, the application of 10 t ha⁻¹ was better than 5 t ha⁻¹, so that in most of the measured traits, the application of 10 t/ha of biochar and vermicompost was superior to 5 t ha⁻¹. The amount of nitrate and ammonium nitrogen in the soil also decreased significantly in line with the increase in the amount of biochar and vermicompost. In both years, the highest number of pods, number of seeds and seed yield were obtained from soils treated with VCM10, and BIO10 and VCM5 were ranked lower. In addition, the highest amount of N, K and Fe seeds was obtained from BIO10, but the highest amount of P seeds was observed in VCM10. Based on the obtained results, organic amendments were superior to chemical fertilizers in improving soil fertility and seed yield. To obtain higher seed yield in similar calcareous soils, the use of vermicompost, especially 10 t ha⁻¹, is superior to biochar. These results showed that organic fertilizers have the potential to produce healthier and safer plants with higher yields than chemical fertilizers. The use of organic fertilizers in the sustainable production of agricultural products and the management of soil nutrients has gained attention and popularity. The present study revealed that using biochar and vermicompost is a better option for the sustainability of faba bean production, which can guarantee food security and safety.

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Forage Yield of Foxtail Millet (*Setaria italica* (L.) P. Beauvois) and Its Some Quality and Nutritional Values of Fodder and Silages Obtained from Different Morphological Parts and Silages with Alfalfa (*Medicago sativa* L.)

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ARTICLE INFO

Research Article

Received: 8 October 2024

Accepted: 31 January 2025

Published: 23 June 2025

Keywords:

Foxtail millet

Hay

Nutritive value

Silage, forage quality

ABSTRACT

The research was carried out in the 2022 and 2023 summer growing seasons in Muğla province, which is under the influence of the Coastal Mediterranean Climate, according to the randomized complete block design with three replications. The 2022 and 2023 summer growing seasons to determine the forage yield of foxtail millet and some of its nutritional values of its fodder and silages, obtained from different morphological parts and in combination with alfalfa. The fresh fodder yield ($t\ ha^{-1}$), dry fodder yield ($t\ ha^{-1}$), crude ash ratio (%) crude protein ratio (%), dry matter ratio (%), ADF (%), NDF (%), digestible dry matter (DDM, %), dry matter intake (DMI, %), total digestible nutrients (TDN, %), net energy-lactation (Nel, $Mcal\ lb^{-1}$), net energy-maintenance (NEm, $Mcal\ lb^{-1}$), net energy-gain (Neg, $Mcal\ lb^{-1}$), relative feed value (RFV, %), pH, fleig score were determined from dry fodder, silage and foxtail millet-alfalfa silages. The characteristics which were examined in the study determined statistically significant at $P<0.01$. Fresh fodder yield and dry fodder yields were varied between 14.50-20.95 $t\ ha^{-1}$ and 4.71-7.31 $t\ ha^{-1}$ respectively. The highest crude protein ratio (16.76%), dry matter ratio (42.06%), crude ash ratio (7.83%), DDM (67.95%), DMI (2.71%), TDN (65.42%), NEL (0.68 $Mcal\ lb^{-1}$), NEm (0.73 $Mcal\ lb^{-1}$) NEg (0.40 $Mcal\ lb^{-1}$), RFV (142.92%) and the lowest ADF (26.89%) and NDF (44.23%) were determined from spike of foxtail millets's dry fodder. The highest crude protein (14.82%) was determined in silage from leaf, the lowest ADF (38.80%) and NDF (54.76%), the highest TDN (51.73%), DDM (58.67%), DMI (2.20%), Nel (0.52 $Mcal\ lb^{-1}$), NEm (0.55 $Mcal\ lb^{-1}$) and RFV (100.49%) were determined in silage from spike, the highest dry matter ratio (92.61%) was determined in stem, the lowest pH (4.56, 4.93 and 5.92) and the highest fleig score (207.06, 192.53 and 186.49) were determined in whole plant, leaf and spike of foxtail millet silages. The highest crude protein (22.92%), dry matter ratio (93.13%), TDN (60.15%), DDM (64.62%), DMI (2.48%), Nel (0.62 $Mcal\ lb^{-1}$), NEm (0.66 $Mcal\ lb^{-1}$) and RFV (124.42), the lowest ADF and NDF (31.71% and (48.33%) were determined in %100 alfalfa silage. The highest fleig score (215.27) was determined in 75% Seteria + 25% Alfalfa silage. It can be concluded that; the foxtail millet hay is seen to be sufficient on its own when evaluated in terms of some forage quality aspects and nutritional value. Foxtail millet can be ensiled into reasonable silage, but the quality can be improved with the addition of alfalfa or different legumes or other protein sources.

Citation: Demiroğlu Topçu, G., Boyacı, S., Tenikecier, H. S., & Ateş, E. (2025). Forage yield of foxtail millet (*Setaria italica* (L.) P. Beauvois) and its some quality and nutritional values of fodder and silages obtained from different morphological parts and silages with alfalfa (*Medicago sativa* L.). *Turkish Journal of Field Crops*, 30(1), 67-75. <https://doi.org/10.17557/tjfc.1563362>

1. INTRODUCTION

The foundation of a sustainable livestock forage system is built upon perennial legumes and grasses, which are integrated within crop rotation systems as well as natural hayfields and pastures (Kosolapov et al., 2015). Beyond natural pastures and rangelands, another significant source of roughage is forage crops cultivated on agricultural land. This source plays a critical role, particularly in meeting the demand for roughage when grazing on pasture and rangeland areas is not feasible during the winter, warm-season crops are cultivated to produce hay and silage to ensure the nutritional requirements of livestock are met. Supplementary feed sources play a crucial role in enhancing the productivity of cattle, swine, and poultry. These additional nutritional inputs are crucial for meeting the animals' elevated energy and nutrient requirements, thereby optimizing growth rates, reproduction, and overall performance in livestock production systems (Kosolapov et al., 2014).

Summer annual grasses hold significant value for livestock producers due to their rapid growth, high forage yield, and potential to provide excellent forage quality when harvested at the optimal growth stage. Key species among these grasses include *Sorghum* spp., *Sorghum* × *drummondii* (sudangrass), and several millets such as pearl millet (*Pennisetum glaucum*), browntop millet (*Urochloa ramosa*), foxtail millet (*Setaria italica* (L.) P. Beauvois), and Japanese millet (*Echinochloa esculenta*). Notably, German millet and Hungarian millet are recognized varieties of foxtail millet (*Setaria italica* (L.) P. Beauvois) (Baltensperger, 2001).

Foxtail millet (*Setaria italica* (L.) P. Beauvois), a member of the genus *Setaria* within the *Panicaceae* tribe, comprises approximately 125 species, primarily distributed in tropical Africa, with a smaller presence in temperate regions (Genckan, 1983). This species is an annual, warm-season grass, with its natural range in China, and is considered one of the oldest cultivated crops globally (Skerman & Riveros, 1990). The grains of *S. italica* (L.) P. Beauvois have been utilized as a staple food in China, Egypt, and India for centuries (Ghimire et al., 2019). In Eastern European countries, the grains are processed into porridge, bread, and used in beer production. Approximately 85% of foxtail millet grain production is allocated for human consumption, while 6% is used as poultry feed. Moreover, the species is cultivated for hay and silage production (Hatipoglu & Tukul, 2009). Foxtail millet, in particular, is well-adapted to drought conditions due to its rapid growth rate and high adaptability to tropical climates. Additionally, it offers a high protein content and is characterized by its leafy and multi-stemmed structure, which allows regrowth after grazing or cutting. Regarding biomass production, foxtail millet can achieve yields comparable to maize (*Zea mays* L.), requiring approximately 60% of the water needed by corn. These summer annual grasses are versatile and can be utilized for grazing, hay, silage, or green chop production. For ensiling purposes, millet should be harvested at the mid-dough stage for optimal preservation and quality (Hamilton et al., 1978).

The study aimed to determine the forage yield of foxtail millet and some quality and nutritional values of fodder and silages obtained from different morphological parts and silages with alfalfa (*Medicago sativa* L.).

2. MATERIALS AND METHODS

The research was conducted at Karabaglar neighborhood, Mentese District, Mugla, Türkiye, with three replications in randomized complete block design, in 2022 and 2023 summer growing season. The soil properties of the study were given in Table 1.

Table 1. Soil properties of the study area¹.

Parameters	
pH	7.50
EC (ds/m)	0.260
Lime (%)	12.71
Organic Matter (%)	2.18
Total Nitrogen (%)	0.112
Phosphorus (P, mg kg ⁻¹)	41.40
Potassium (K, mg kg ⁻¹)	120.30
Calcium (Ca, mg kg ⁻¹)	4172.20
Magnesium (Mg, mg kg ⁻¹)	240.80
Iron (Fe mg kg ⁻¹)	14.73
Copper (Cu, mg kg ⁻¹)	2.10
Zinc (Zn, mg kg ⁻¹)	2.61
Manganese (Mn, mg kg ⁻¹)	1.75

¹ The analyses were performed in Ege University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition Laboratory.

Table 2. Climatic conditions of the study area¹

Months	Total Precipitation (mm)			Mean Temperature (°C)			Relative Humidity (%)		
	2022	2023	Long-Term ²	2022	2023	Long-Term ²	2022	2023	Long-Term ²
May	9.90	136.50	51.40	19.10	16.20	17.70	53.90	74.70	62.30
June	42.40	23.20	36.70	23.20	21.60	22.80	34.50	66.50	52.40
July	-	2.00	14.10	28.00	28.60	26.40	40.40	34.10	45.80
August	9.40	-	12.70	26.10	27.30	26.30	38.40	50.50	47.80
Mean	-	-	-	24.10	23.40	23.30	41.80	56.40	52.10
Total	61.70	161.70	114.90	-	-	-	-	-	-

¹The data retrieved from Turkish State, Meteorological Service. ²Long-Term covers the period between 1927-2021.

Some meteorological data of the experimental area during the vegetation period are presented in Table 2. The study area has a Mediterranean climate, with environmental and soil characteristics suitable for drought-resistant crops such as millet. During the summer, temperatures typically exceed 30°C, which aligns well with the optimal growth range of millet (25–30°C). In the summer, temperatures usually exceed 30°C, which is quite suitable for the optimal growth temperatures of millet, which are 25–30°C. The total precipitation of the experimental area was 61.7 mm in 2022 and 161.7 mm in 2023, mean temperature of 24.1 °C in 2022 and 23.4 °C in 2023, 41.8% in 2022 and 56.4% in 2023 relative humidity and between May and August of the study years. The total precipitation, mean temperature and relative humidity 52.1% in long term of the experimental area was 114.9 mm and 23.3 °C between May and September of the study years respectively.

Foxtail millet population obtained from Karabağlar neighborhood, Menteşe District, Muğla, Türkiye, was used as seed material. Seeds were sown in 8 rows with 5 m long plots by hand with 0.60 m inter-row space at 5.0 kg ha⁻¹ seeding rate on 10.05.2022 and 10.06.2023. The second year's sowing was delayed due to meteorological and soil conditions. Fertilization was done in the experimental plots as 250 kg N, 100 kg P₂O₅ and 100 kg K₂O per hectare. Half of the nitrogen and all of the phosphorus and potassium were applied to the base at planting, and the other half of the nitrogen was applied between the rows when the plants reached 20–25 cm in height. It has been observed that when regular irrigation is done every week, the plant grows an average of 40 cm in height. Before the plant starts to produce spikes (after about the 7th week), it has been observed that when 250 kg ha⁻¹ of urea fertilizer is given with irrigation water, the growth in spike length increases. The last irrigation is done when the spikes turn about 50% ripening. The foxtail millet plots were harvested at milk dough ripening stage. The alfalfa samples were obtained from Ege University, Faculty of Agriculture, Field Crops Department Experimental Area, Izmir, Türkiye, at 10% flowering stage for silage mixtures. After the harvest of the foxtail millet, plants were separated to spike, leaf, stem and whole-plant to determine fresh fodder yield (t ha⁻¹), dry fodder yield (t ha⁻¹), crude protein (CP) ratio (%), dry matter (DM) ratio (%), crude ash (CA) ratio (%), ADF (%), NDF (%), digestible dry matter (DDM, %), dry matter intake (DMI, %), total digestible nutrients (TDN, %), net energy-lactation (Nel, Mcal lb⁻¹), net energy-maintenance (NEm, Mcal lb⁻¹), net energy-gain (Neg, Mcal lb⁻¹), relative feed value (RFV, %), pH, fleig score from dry fodder, silage and foxtail millet-alfalfa silages. Plants were harvested (foxtail millet: at full-ripening stage; alfalfa: at 10% flowering stage) by hand by cutting at soil level and left to wither for 2 hours. Withered plants were chopped to about 1–2 cm in size and the chopped materials were thoroughly mixed to attain homogeneity (Demiroğlu Topçu & Kahya, 2023). Different mixture ratios (foxtail millet%-alfalfa %; 100-0, 75-25, 50-50, 25-75, 0-100; on the basis of fresh weight) were used for preparing the silage in four replications with a randomized plot design. Samples without additives of each foxtail millet-alfalfa silage mixture, standalone foxtail millet and different parts of foxtail millet (500±20 g) were placed in separate vacuum bags (thickness 110 microns or more), and after 99.9% of the air was removed by vacuum, bags were glued and closed (Johnson et al., 2005). The bagged silage samples were stored in a dark and cool environment at 24±4°C for 60 days. Oven drying for feed analysis at temperatures above 60°C can result in, heat damaged protein and elevated fiber and lignin values (Reed & Van Soest, 1984). For this reason, samples (both fresh fodder and silage) were dried at 60 °C for 48 hours followed by storage for a further day at room temperature, ground to small (≤1 mm) pieces and used for the analyses (Ates & Tenikecier, 2022a; Tenikecier & Ates, 2022). The samples were analyzed for N using procedures of the Association of Official Analytical Chemists (AOAC, 2019). Crude protein (CP) ratio (%) of the samples were calculated by multiplying N contents by a coefficient of 6.25. They dried in a forced-air oven at 60°C to a constant weight to determine the dry matter (DM) ratio (%) (Pereira et al., 2019). Crude ash (CA) ratio (%), acid detergent fiber (ADF, %), neutral detergent fiber (NDF, %) contents were determined by Weende and Van Soest methods (AOAC, 2019; Van Soest et al., 1991). The digestible dry matter (DDM, %), dry matter intake (DMI, %), relative feed value (RFV, %), total digestible nutrients (TDN, %), net energy for lactation (Nel, Mcal lb⁻¹), net energy for maintenance (NEm, Mcal lb⁻¹), and net energy for gain (Neg, Mcal lb⁻¹) were calculated using established equations for forage evaluation (Schroeder, 1994). The pH of the silages was measured using a pH meter. Fleig score was calculated using the formula suggested by Kılıç (1986).

Statistical analysis was performed using the TARIST (Acikgoz et al., 2004) statistical software package, and Fisher's Least Significant Difference (LSD) test was used for post hoc comparisons (Duzgunes et al., 1987), implemented with MSTAT-C software.

3. RESULTS AND DISCUSSION

Forage Yield

Fresh and dry fodder yields were varied between 14.50-20.95 t ha⁻¹ and 4.71-7.31 t ha⁻¹ respectively (Figure 1.). Dagtekin et al. (2020) determined herbage yield between 279.8-662.6 g plant⁻¹ and hay yield between 84.6-145.3 g plant⁻¹ respectively. Mohajer et al. (2011) determined hay yield between 4.80-11.80 t ha⁻¹ and herbage yield between 19.30-37.00 t ha⁻¹. Mohajer et al. (2013) determined fresh yield between 22.83-34.00 t ha⁻¹ and dry matter yield between 6.23-8.23 t ha⁻¹. Olak & Tan (2016) determined hay yield between 2.47-6.98 t ha⁻¹. Nematpour et al. (2020) determined hay yield 5.43 t ha⁻¹. Lu et al. (2024) determined the total weight at flowering stage 16.00 g plant⁻¹. The fresh and dry fodder yields were determined lower than those researchers.

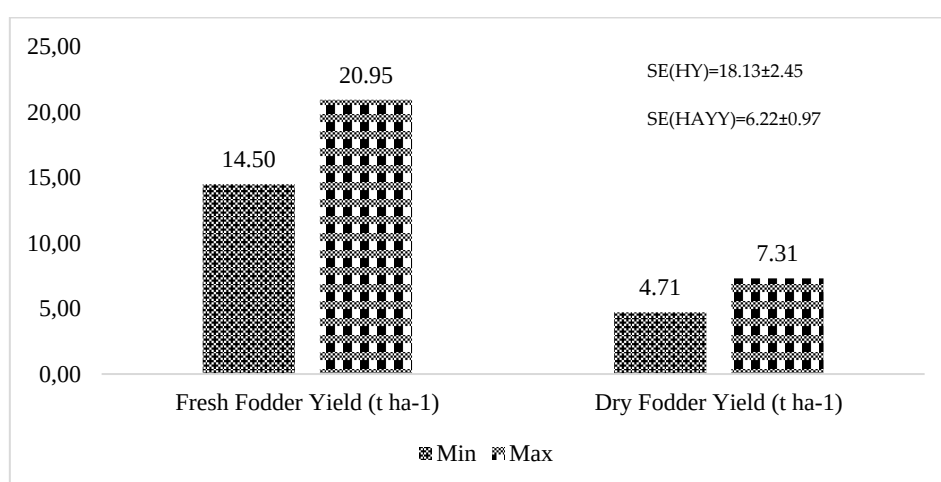


Figure 1. Fresh and dry fodder yield of foxtail millet.

Nutritional and Quality Properties of Different Morphological Parts and The Whole Plant of Foxtail Millet

The results of some nutritional and quality traits of dried different morphological parts and the whole plant of foxtail millet are given in Table 3. Crude protein ratio (%), dry matter ratio (%), crude ash ratio (%), ADF (%), NDF (%), DDM (%), DMI (%), TDN (%), NEL (Mcal lb⁻¹), NEm (Mcal lb⁻¹), NEg (Mcal lb⁻¹) and RFV (%) means of dried some morphological parts and the whole plant of foxtail millet were determined statistically significant at P<0.01.

Table 3. Some nutritional and quality aspects of dried different morphological parts and the whole plant of foxtail millet.

Characteristics	Spike	Leaf	Stem	Whole plant	Mean	LSD [‡]
Crude Protein Ratio (%)	16.76a	12.40c	9.65d	15.18b	13.50	0.993**
Dry Matter Ratio (%)	42.06a	25.69d	38.45b	34.23c	35.11	3.502**
Crude Ash Ratio (%)	5.34b	4.15c	2.63d	7.83a	4.99	0.747**
ADF (%)	26.89c	36.90b	47.33a	36.77b	36.97	2.754**
NDF (%)	44.23c	57.03b	67.57a	60.46b	57.35	3.593**
DDM (%)	67.95a	60.16b	52.03c	60.26b	60.10	2.142**
DMI (%)	2.71a	2.11b	1.78c	1.99b	2.15	0.138**
TDN (%)	65.42a	53.92b	41.92c	54.07b	53.83	3.164**
Nel (Mcal lb ⁻¹)	0.68a	0.55b	0.41c	0.55b	0.55	0.043**
NEm (Mcal lb ⁻¹)	0.73a	0.58b	0.42c	0.58b	0.58	0.041**
NEg (Mcal lb ⁻¹)	0.40a	0.25b	0.09c	0.25b	0.25	0.042**
RFV (%)	142.92a	98.16b	71.59d	92.74c	101.35	4.362**

a-d: The difference between groups containing different letters in the same row is statistically significant, [‡]: **, P<0.01

The highest crude protein (16.76%), dry matter ratio (42.06%), DDM (67.95%), DMI (2.71%), TDN (65.42%), NEL (0.68 Mcal lb⁻¹), NEm (0.73 Mcal lb⁻¹) NEg (0.40 Mcal lb⁻¹), RFV (142.92%) and the lowest ADF (26.89%) and NDF (44.23%) were determined from spike of foxtail millet respectively. The highest crude ash (7.83%) was determined from foxtail millet whole-plant (Table 3). Arbabi & Ghoorchi (2008) determined dry matter 303 g kg⁻¹, crude protein 105.3 g kg⁻¹, ADF 334.1 g kg⁻¹, NDF 547.2 g kg⁻¹, DDM 629 g kg⁻¹ and TDN 613.1 g kg⁻¹ respectively. Mohajer et al. (2011) determined crude protein ratio 5.3-11.3%, digestibility 56.2-69.7, ADF 26.2-35.3% respectively. Dastenal et al. (2012) determined ADF between 31.2-32.0 %. Mohajer et al., (2013) determined crude protein between 7.80-11.66%, crude fiber between 39.48-39.67%, ADF between 29.3-31.29% and ash between 7.49-18% respectively. Heuzé et al. (2015) determined crude protein ratio between 8.3-12.5%, ADF 33.4-43.8 %, NDF 48.4- 72.0 % respectively. Olak & Tan (2016) determined crude protein ratio between 11.97-14.04 %, ADF 34.95-39.33, NDF 56.13-64.71 respectively. Peiretti & Tassone (2016) determined dry matter 299 g kg⁻¹, crude ash 110 g kg⁻¹, crude protein 55.3 g kg⁻¹, ADF 421 g kg⁻¹ and NDF 674 g kg⁻¹ respectively. Dagtekin et al. (2020) determined crude protein ratio 10.3-15.6%, ADF 39.8-49.8, NDF 74.9-85.6 respectively. The results of the study were determined similar to mentioned above researchers. Rohweder et al. (1978) determined the limit values of quality standards according to crude protein, ADF and NDF ratios of forages. They report that the relative feed value is considered 83-100 when the ADF and NDF ratios are between 39-41% and 61-65% respectively, and if the RFV is greater than 151, the feed is the best quality for grass hays. The RFV values of the spike and leaf of foxtail millet and whole plant shows enough potential for good forage quality.

Nutritional and Quality Characteristics of Silage from Different Morphological Parts and Whole Plant of Foxtail Millet

The results of some nutritional and quality characteristics of silage from different morphological parts and the whole plant of foxtail millet are given in Table 4. Crude protein ratio (%), dry matter ratio (%), ADF (%), NDF (%), DDM (%), DMI (%), TDN (%), NEL (Mcal lb⁻¹), NEm (Mcal lb⁻¹), pH and Fleig score and RFV (%) means of silages from different morphological parts and the whole plant of foxtail millet were determined statistically significant at P<0.01.

Table 4. Some nutritional and quality aspects of silage from some parts of foxtail millet

Characteristics	Spike	Leaf	Stem	Whole-Plant	Mean	LSD [‡]
Crude Protein Ratio (%)	11.62c	14.82a	12.78b	13.28b	13.12	1.12**
Dry Matter Ratio (%)	92.48b	92.30c	92.61a	92.23c	92.40	0.10**
ADF (%)	38.80c	48.40b	48.55b	53.09a	47.21	4.48**
NDF (%)	54.67b	66.50a	64.92a	63.77a	62.46	7.01**
TDN (%)	51.73a	40.69b	40.52b	35.29c	42.06	5.15**
DDM (%)	58.67a	51.20b	51.08b	47.54c	52.12	3.49**
DMI (%)	2.20a	1.80b	1.85b	1.88b	1.93	0.26**
NEL (Mcal lb ⁻¹)	0.52a	0.40b	0.39b	0.34b	0.41	0.06**
NEm (Mcal lb ⁻¹)	0.55a	0.41b	0.40b	0.33c	0.42	0.07**
pH	5.09b	4.93b	6.27a	4.56b	5.21	1.00**
Fleig Score	186.49a	192.53a	139.29b	207.06a	181.34	39.87**
RFV (%)	100.49a	71.62b	73.25b	69.36b	78.68	17.29**

a-c: The difference between groups containing different letters in the same row is statistically significant, [‡]: **: P<0.01

The highest crude protein (14.82%) was determined in silage from leaf, the lowest ADF (38.80%) and NDF (54.76%), the highest TDN (51.73%), DDM (58.67%), DMI (2.20%), NEL (0.52 Mcal lb⁻¹), NEm (0.55 Mcal lb⁻¹) and RFV (100.49%) were determined in silage from spike, the highest dry matter ratio (92.61%) was determined in stem, the lowest pH (4.56, 4.93 and 5.92) and the highest Fleig score (207.06, 192.53 and 186.49) were determined in silages from whole plant, leaf and spike of foxtail millet (Table 4.). Arbabi & Ghoorchi (2008) determined pH 4.88, crude protein 108.00 g kg⁻¹, ADF 495.80 g kg⁻¹, NDF 612.60 g kg⁻¹, DDM 609.70 g kg⁻¹ and TDN 579.80 g kg⁻¹ respectively. Peiretti & Tassone (2016) determined dry matter 470 g kg⁻¹, crude protein 52.6 g kg⁻¹, ADF 407 g kg⁻¹, NDF 644 g kg⁻¹, pH 4.76 respectively. Nematpour et al. (2020) determined silage dry matter content 318 g kg⁻¹, crude protein 143 g kg⁻¹, ADF 308 g kg⁻¹, NDF 584 g kg⁻¹, pH 4.65, DDM 649 g kg⁻¹, NEL 1.49 Mcal kg⁻¹ and RFV 104 g kg⁻¹ respectively. The results of the study were determined similar to those researchers. Karaer et al. (2024) determined pH of the sorghum × Sudan grass hybrid silages between 4.40-4.79. Silage quality is classified based on Fleig scores as follows: very good for Fleig scores of 85–100; good for Fleig scores of 60–84; moderate for Fleig scores of 40–59; satisfactory for Fleig scores of 20–39; worthless for Fleig scores of <20 (Kilic, 1986). When the Fleig score of the silage from different part of foxtail millet examined, it can be declared that the foxtail millet can be ensiled appropriately. The RFV were determined between 69.36%-100.49% in silage from different parts of foxtail millet. When the RFV which were declared by Rohweder et al. (1978) for grass hays, enough potential for good forage quality was determined in the RFV of the silage from spike (100.19%) of foxtail millet.

Nutritional and Quality Aspects of Foxtail Millet+Alfalfa Silages

The results of some forage and nutritional quality of foxtail millet+alfalfa silages are given in Table 5.

Table 5. Some nutritional and quality aspects of foxtail millet+alfalfa silages

Characteristics	100% Foxtail Millet	100% Alfalfa	75% Foxtail Millet + 25% Alfalfa	50% Foxtail Millet + 50% Alfalfa	25% Foxtail Millet + 75% Alfalfa	Mean	LSD [‡]
Crude Protein Ratio (%)	13.28d	22.92a	14.70c	15.53c	17.75b	16.84	1.33**
Dry Matter Ratio (%)	92.23b	93.13a	91.87b	91.89b	92.20b	92.26	0.81**
ADF (%)	53.09a	31.17b	53.64a	53.78a	53.32a	49.00	2.82**
NDF (%)	63.77b	48.33c	66.70a	65.50ab	63.45b	61.55	2.50**
TDN (%)	35.29b	60.51a	34.67b	34.50b	35.04b	40.00	3.24**
DDM (%)	47.54b	64.62a	47.12b	47.00b	47.36b	50.73	2.20**
DMI (%)	1.88b	2.48a	1.80b	1.83b	1.89b	1.98	0.10**
NEI (Mcal lb ⁻¹)	0.34b	0.62a	0.33b	0.33b	0.34b	0.39	0.03**
NEm (Mcal lb ⁻¹)	0.33b	0.66a	0.33b	0.32b	0.33b	0.39	0.04**
pH	4.56ab	4.73a	4.34c	4.43bc	4.76a	4.56	0.20**
Fleig Score	207.06c	202.19d	215.27a	211.57b	199.01e	207.02	2.10**
RFV (%)	69.36b	124.42a	65.72b	66.76b	69.45b	79.14	4.48**

a-d: The difference between groups containing different letters in the same row is statistically significant, [‡]: **, P<0.01

Crude protein ratio (%), dry matter ratio (%), ADF (%), NDF (%), DDM (%), DMI (%), TDN (%), NEI (Mcal lb⁻¹), NEm (Mcal lb⁻¹), pH and fleig score and RFV (%) means of foxtail millet+alfalfa silages were determined statistically significant at P<0.01. The highest crude protein (22.92%), dry matter ratio (93.13%), TDN (60.15%), DDM (64.62%), DMI (2.48%), Nel (0.62 Mcal lb⁻¹), NEm (0.66 Mcal lb⁻¹) and RFV (124.42%), the lowest ADF and NDF (31.71% and 48.33%) were determined in %100 alfalfa silage. The highest Fleig score (215.27) was determined in 75% Seteria + 25% alfalfa silage (Table 5.). Ozturk et al. (2006) determined DM between 20.44-28.78%, CP 7.93-19.25%, NDF between 43.27-45.43, ADF between 23.90-34.56%, pH 3.13-5.43 and Fleig score between 13.67-108.89 of alfalfa+maize silages at different ratios (100, 70:30, 55:45, 40:60, 25:75). Zhang et al. (2017) determined the CP between 87.30-172 g kg⁻¹, NDF between 449-459 g kg⁻¹ and ADF between 247-257 g kg⁻¹ of alfalfa+maize silages at different ratios (100, 80:20, 60:40, 40:60, 20:80). The results were determined similar to Ozturk et al. (2006), Arbabi & Ghoorchi (2008), Peiretti & Tassone (2016), Zhang et al. (2017) and Nematpour et al. (2020). When the pH values were examined, the pH value observed in the inoculant-free alfalfa silage was surprising despite its high protein content. However, a literature review revealed that similar values for alfalfa, fodder pea (*Pisum arvense* L.) and Persian clover (*Trifolium resupinatum* L.) were also reported by Adiyaman et al. (2014), Tao et al. (2017), Ates & Tenikecier (2022b) and Tenikecier & Ates (2024). The Fleig scores of the foxtail millet+alfalfa silages were determined similar to Karaer et al. (2024) and Kılıç (1986). According to Fleig score of the foxtail millet+alfalfa silages, it can be concluded that the silage from foxtail millet and alfalfa has appropriate ensiling capacity. Rohweder et al. (1978) determined the limit values of quality standards according to crude protein, ADF and NDF ratios of forages. They report that the relative feed value is considered 100 when the ADF and NDF ratios are 41% and 53% respectively, and if the RFV is greater than 151, the feed is the best quality in alfalfa. According to RFV of the foxtail millet silage and silage mixture with alfalfa has enough forage quality.

A 500 kg beef cattle (*Bos taurus* L.) with superior milking ability, nursing a calf during the first 3 to 4 months postpartum, requires a minimum of 28.6 Mcal of digestible energy (NRC, 2001). These energy demands can be met with approximately 11.8 kg of DM intake per day from forage sources (Essig, 1985). Daily digestible DM intake has a stronger correlation with total DM intake than with DM digestibility. In most forage crops, cell wall constituents typically make up 55-85% of the DM content, and these components are influenced by numerous factors (Tenikecier & Ates, 2018). DM serves as a crucial indicator of the quantity of nutrients available to the animal in a given feed source, directly impacting nutritional efficiency and feed quality. To digest cellulose and hemicellulose, which are classified as water-insoluble carbohydrates found in plant cell walls, ruminant animals break down the cell wall through physical mastication during rumination, followed by slow fermentation facilitated by cellulolytic bacteria in the rumen. Feed intake is influenced by the high fiber content in roughages, as these fibers occupy space in the rumen for extended periods, slowing digestion. The ADF content in forage plants is a key indicator of feed quality; thus, knowing the ADF ratio is essential prior to ration formulation. Additionally, the NDF value of forage grasses provides insight into the bulkiness of the grass. A higher NDF value indicates that the forage has a larger volume, which can affect feed intake and digestion dynamics (Atalay & Ates, 2020). The nutrition quality of the forages shows considerable differences depending on different places and times (Stodart et al., 1975). Olak & Tan (2016) identified foxtail millet as a warm-season species. Consequently, these plants demonstrate rapid growth and development due to their high photosynthetic efficiency. However, this characteristic can

negatively impact their nutrient composition and feed value. Warm-season species typically have lower crude protein content and higher concentrations of structural fibers such as ADF and NDF, which can reduce their overall nutritional quality as forage. It is thought that the differences between the study results and previous foxtail millet studies are due to ecological conditions, cultivation techniques and harvest time.

4. CONCLUSIONS

It can be concluded that foxtail millet hay is adequate on its own in terms of certain forage quality parameters and nutritional value. However, our research found that adding alfalfa to foxtail millet silage influenced both the crude protein content—crucial for animal nutrition—and the Fleig score, a key indicator of silage quality. Overall, while foxtail millet can be ensiled into reasonably good-quality silage by itself, its quality can be further enhanced with the addition of alfalfa, other legumes, or alternative protein sources.

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Effects of Irrigation Level, Plant Density and Nitrogen Doses on Grain Yield and Yield Parameters of Sweet Sorghum (*Sorghum bicolor* L. Moench var. *saccharatum*)

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ARTICLE INFO

Research Article

Received: 3 March 2025

Accepted: 1 May 2025

Published: 23 June 2025

Keywords:

Density

Irrigation level

Nitrogen

Sweet sorghum

ABSTRACT

Experiments were carried out at Bursa Uludağ University Faculty of Agriculture Application and Research Centre in 2022 and 2023 summer seasons to evaluate the effects of irrigation level, plant density and nitrogen dose on grain yield and yield parameters of sweet sorghum [*Sorghum bicolor* (L.) Moench var. *saccharatum*]. Each field trail included thirty-two treatments represent the combinations among four irrigation levels, two planting densities and four nitrogen fertilization doses. A split-split plot design with three replications was used in both seasons, where the four deficit irrigation levels (0%, 33%, 66% and 100% of crop evapotranspiration (ET_c)) were arranged in the main plots, while two planting densities (142857 and 285714 plants ha⁻¹) were randomly allocated in the sub plots and the four nitrogen doses (0, 70, 140 and 210 kg ha⁻¹) were randomly distributed in the sub-sub plots. The results showed that the three factors and their interactions on grain yield and yield components were significant. Grain yield increased with increasing irrigation level, plant density, nitrogen doses and their interactions in both years. In the study, maximum grain yield was obtained with all N doses at I₃ in 2022, while it was obtained with N₃ in I₁, I₂ and I₃ in 2023. Grain yield increased with increasing plant density in 2022, it did not interact with nitrogen doses, but they interacted in 2023 and the highest grain yield was determined at N₃ interacting with P₂. In the plant density and irrigation level interactions, the maximum grain yield was obtained from the interaction of D₂ and I₃ in the rainy year 2022, while it was obtained from the interaction of D₂ and I₂ in the dry year 2023.

Citation: Kostereli, G., & Turgut, I. (2025). Effects of irrigation level, plant density and nitrogen doses on grain yield and yield parameters of sweet sorghum (*Sorghum bicolor* L. Moench var. *saccharatum*). *Turkish Journal of Field Crops*, 30(1), 76-87. <https://doi.org/10.17557/tjfc.1649968>

1. INTRODUCTION

Today, it is known that global climate change has a profound effect on plant production. In addition to climate change, factors such as decreased soil fertility, temperature fluctuations, unseasonal rainfall, and increased CO₂ concentration are the most critical threats to open-air factory agriculture (Cai et al. 2011). Sweet Sorghum, one of the C4 crops, has adapted to a wide range of climate and soil conditions. It is a short-term crop that matures earlier at higher temperatures. It is known not only as a "high-energy crop" due to its high photosynthesis rate but also as the "camel among crops" due to its drought resistance. It can be grown in soils ranging from heavy clay to light sand. It responds effectively to available growth factors such as water stress, planting density and nitrogen fertilization. (Taha et al. 1999).

Sorghum is an important source of food and fodder, especially in rainfed regions, and its resilience to harsh environmental conditions makes it the fifth most widely grown cereal worldwide after wheat, rice, maize and barley (FAOSTAT, 2023; Aydinsakir et al. 2021). Grain sorghum, which is grown as a main or second crop in the Mediterranean region of Turkey, stands out for its resistance to stress conditions such as high temperature, drought, salinity and waterlogging, while studies in Adana show that it requires less fertilizer and is more tolerant to environmental stresses compared to maize (İnal et al. 2020). Thanks to its strong root system, sorghum can yield even under arid and saline conditions (Tari et al. 2013) and offers higher yield and quality with appropriate fertilization and irrigation practices (Islam et al. 2012; Khelil et al. 2013). Furthermore, agronomic factors such as plant density and irrigation management play a decisive role in yield, and it has been reported that grain yield can be significantly increased by increasing plant density (Selim, 1995; Wang et al. 2024; Schatz et al. 1990).

Yield and quality of plants are affected by environmental factors as well as genetic traits. In order to reduce the effect of environmental factors, it is necessary to determine the appropriate plant density, irrigation level and nitrogen dose of sorghum varieties as genetic material suitable for different soil structures and ecological conditions. Many studies have been carried out to investigate the responses of sorghum to different irrigation and nitrogen doses and plant density on yield and yield components. On the other hand, the number of studies on the effects of these three factors on sorghum yield and yield components is limited.

This study investigated the effects of plant densities, irrigation levels and nitrogen doses on grain yield and yield parameters of sweet sorghum.

2. MATERIALS AND METHODS

Experiment design and location

Experiments were conducted at Bursa Uludağ University Faculty of Agriculture Application and Research Centre with the geographical location of 40° 13' 47.22" N, 28° 51' 25.22" W and elevation of 100 m above mean sea level during summer seasons in both 2022 and 2023 to study the effect of deficit irrigation, planting density and nitrogen fertilization on yield and some yield parameters of sweet sorghum (*Sorghum bicolor* (L.) Moench var. *saccharatum*). Each field trail included thirty-two treatments represent the combinations among four irrigation levels, two planting densities and four nitrogen fertilization doses. Field experiments were conducted in a randomized split-split block design with three replications in both seasons, where the four irrigation levels were arranged in the main plots, while plant populations were randomly allocated in the sub plots and the four nitrogen doses were randomly distributed in the sub-sub plots. Main plots were set as 11.20 m x 11.00 m, sub plots as 11.20 m x 5.00 m rows and sub-sub plots as 2.80 m x 5.00 m rows. Gulseker, a sweet sorghum variety developed at Bursa Uludağ University Faculty of Agriculture, Department of Field Crops, was used as a genetic material, four irrigation levels (% Etc) (I): 0% (I₀), 33% (I₁), 66% (I₂) and 100% (I₃) of crop evapotranspiration (ET_c), four nitrogen doses (kg ha⁻¹) (N): 0 (N₀), 70 (N₁), 140 (N₂) and 210 (N₃) and two plant populations (plants ha⁻¹) (D): 142857 (D₁) and 285714 (D₂) plants ha⁻¹ (sowing spacing; 0.10 x 0.70 m and 0.05 x 0.70 m, respectively). In previous studies, plant densities were obtained with row spacings of 0.10 x 0.70 m and 0.05 x 0.70 m (Turgut et al. 2005). In this context, it was decided to investigate the above two plant densities.

Soil and climate characteristics of the trial sites

Soil physical and chemical characteristics are shown in Table 1.

Table 1. Soil properties of experimental area

2022		2023	
Properties (mg kg ⁻¹)	Quantities	Properties (mg kg ⁻¹)	Quantities
Texture	Clay	Texture	Clay
pH	7.61	pH	6.63
EC (mS cm ⁻¹)	0.041	EC (mS cm ⁻¹)	0.035
Lime (% CaCO ₃)	4.79	Lime (% CaCO ₃)	1.58
Organic matter (%)	2.66	Organic matter (%)	1.66
Total nitrogen (N) (%)	0.13	Total nitrogen (N) (%)	0.083
Available phosphorus (P) (mg kg ⁻¹)	6.7	Available phosphorus (P) (mg kg ⁻¹)	3.31
Potassium (K)	178.09	Potassium (K)	64.82

Soil samples were collected before sowing through the experimental site to determine in both years. As seen in Table 1, the soil structure of Bursa Uludağ University Agricultural Research and Application Center, where the experiment was established, is rich in lime and clay values, and has a heavy texture (Katkat et al. 1985). There is no salinity problem. Na⁺ 0.26 me 100g⁻¹, K⁺ 0.92 me 100g⁻¹, Mg⁺⁺ 10.20 me 100g⁻¹, Ca⁺⁺ 30.42 me 100g⁻¹ (Aksoy et al. 2001). It is rich in potassium and phosphorus, and poor in organic matter content.

Table 2. Climatic data in the growing season

Average Temperature (°C)				Average Humidity (%)			Monthly Precipitation (mm)		
Months	2022	2023	Long Term Average (1995-2023)	2022	2023	Long Term Average (1995-2023)	2022	2023	Long Term Average
June	22.3	24.3	22.2	71.3	63.0	70.1	129.2	0.0	56.7
July	24.0	26.1	24.7	61.5	5.8	63.5	2.1	0.0	12.6
August	25.3	27.1	25.2	69.6	60.4	64.5	40.3	0.0	15.4
September	20.7	22.6	21.5	65.2	62.9	66.5	39.2	0.0	36.7
October	15.8	18.2	16.1	71.3	70.5	74.4	11.7	7.3	52.2
Total	-	-	-	-	-	-	222.5	7.3	173.6
Average	21.6	23.7	21.9	67.8	52.5	67.8	-	-	-

In the first year (2022), a total precipitation of 222.5 mm was determined during the sweet sorghum vegetation period (June-October). In 2023, a total of 7.3 mm precipitation occurred. More precipitation occurred in the first year of the experiment compared to the second year. Among the trial years, it was observed that the temperature value was warmer in 2023 than in 2022. When the rainfall amounts of the trial years are compared, it is seen that a significant amount of rainfall was received in 2022, while 2023 was a drier year.

Cultural practices

Sowing was done by pneumatic planter as 0.05 x 0.70 m in the third week of June in both years. P₂O₅ of 150 kg ha⁻¹ was applied on 5cm rows side by pneumatic planter with planting in the all-experimental area. The trial area was hoed by hand twice to remove weeds and aerate the soil. Planting densities were achieved as follows; 285714 (D₂) plants ha⁻¹, resulting from 0.05 x 0.70 m planting type and 142857 (D₁) plants ha⁻¹, were thinned to be 0.10 x 0.70 m planting type. Nitrogen doses were applied by hand as urea (46% N) and ammonium sulfate (21% N) in two equal doses on 10 cm the rows side and covered; after thinning (20 days after planting) and 35 days later. Four different irrigation levels were applied by drip irrigation according to experiments were carried out in dry conditions. Irrigation treatments investigated are provided in Table 3.

Crop evapotranspiration (ET_c, mm) was calculated according to:

$$ET_c = E_o \times K_c \text{ (1)}$$

where E_o is the evaporation of standard Class-A pan and K_c is the crop coefficient as suggested by Doorenbos and Pruitt (1977). The K_c values used during the growing season were those proposed in FAO-56 (Allen et al. 1998) for sweet sorghum (0.30 for the initial stage, 0.30–1.20 for the crop development stage, 1.20 for the mid-season stage, and 1.05 for the late-season stage of crop development). According to the results of the water analysis, the quality of the irrigation water used in the research was determined as C₂S₁. At the beginning of field trial, sprinkler irrigation method was used for germinating and emerging seeds. Afterward, the calculated amount of irrigation water was applied at weekly intervals, and the water was used to the plots via a drip irrigation system. The laterals were placed 0.1 m next to the plant rows. The dripper lines had inline compensating emitter pressure, and the discharge rate of the emitters was 1.6 L ha⁻¹ at an operating pressure of 1 bar. The emitter spacing was chosen as 0.30 m based on the soil characteristics.

Table 3. Irrigation treatments

Treatments Description	
I ₃	100% ET _c restoration during the whole growing season
I ₂	66% ET _c restoration during the whole growing season
I ₁	33% ET _c restoration during the whole growing season
I ₀	0% ET _c restoration during the whole growing season

Harvest was done by hand in first week on November in both years.

Data collection

Each plot contained four rows; each row 5.00 m long. Data were collected from the two center rows in four rows for every parcels.

During the physiological ripening period (136 days after sowing) in harvest, the following traits were determined:

Grain weight per bunch (g bunch⁻¹); the bunches obtained from each plant were weighed, the grain weight in the collected bunch (g bunch⁻¹) was weighed and determined by converting it to 14% moisture.

Thousand kernel weight (g 1000 grains⁻¹); 4 randomly counted 100 grains of grains obtained from the clusters of each matured plant in each plot were weighed and calculated by proportion (g 1000 grains⁻¹) and converted to 14% moisture.

Grain yield of sweet sorghum (kg ha⁻¹); the grains obtained from the clusters of each mature plant in each plot were weighed to determine the grain weight in the plot (g plot⁻¹) and converted to 14% moisture. The yield per hectare (kg ha⁻¹) was calculated taking into account the plot area.

Statistical analysis

The data from the trials were statistically analyzed according to the split-plot experimental design with 3 replications in randomized blocks. The effects of irrigations, plant populations, nitrogen doses and their interactions were evaluated using F-test at 0.05 and 0.01 probability levels. F-protected least significant difference (LSD) was calculated according to Steel and Torrie (1980) at 0.05 probability level. Analyses of variance (ANOVA) for all data were analyzed with JMP 9.0.2 software to determine the significance of treatments and their interactions.

3. RESULTS AND DISCUSSION

The results of the variance analysis of the data we obtained in our study conducted in 2022 and 2023 are given in Table 4. The ANOVA results show the effects of three main factors: irrigation levels, plant densities and nitrogen doses were significantly different at 1% probability level on grain weight per panicle in both seasons. Also, the effects of three interactions which were irrigation levels × planting densities, irrigation levels × nitrogen doses, and irrigation levels × planting densities × nitrogen doses were significantly different at 1% probability level on grain weight per panicle in 2022 and 2023. The effects of plant densities × nitrogen doses interactions were not significantly different in 2022 but significantly different at 1% probability level on grain weight per panicle in 2023. The effects of three main factors which were irrigation levels, plant densities, nitrogen doses and effects of four interactions which were irrigation levels × planting densities, irrigation levels × nitrogen doses, plant densities × nitrogen doses and irrigation levels × planting densities × nitrogen doses were significantly different at 1% probability level on 1000 grain weight in both of season. According to the results, the effects of two main factors, irrigation levels and plant densities levels, were significantly

different at 1% probability level on grain yield of sweet sorghum in both seasons. However, effects of nitrogen doses were significantly different at 5% probability level in 2022 and they were significantly different at 1% probability level in 2023.

Table 4. The results of the variance analysis for both years

Year	Source of Variation	df	Mean Square		
			Grain weight of bunch	Thousand kernel weight	Grain Yield
2022	Blocks	2	6.282 ^{ns}	0.007 ^{ns}	4673.660 ^{ns}
	I	3	1629.630**	33.795**	775879.000**
	D	1	3106.510**	6.678**	7334329.000**
	N	3	75.169**	3.772**	14784.300*
	I×D	3	166.680**	17.552**	123610.000**
	I×N	9	75.210**	4.017**	30632.380**
	D×N	3	1.498 ^{ns}	6.556**	6768.380 ^{ns}
	I×D×N	9	90.223**	2.683**	53622.500**
	Error	48	4.471	0.463	4.144.000
	CV (%)		4	2	6
2023	Blocks	2	6.455 ^{ns}	0.194 ^{ns}	1073.580 ^{ns}
	I	3	4447.150**	50.183**	2415015.000**
	D	1	258.070**	0.631 ^{ns}	8152148.000**
	N	3	381.668**	3.360**	169383.000**
	I×D	3	312.336**	4.963**	471353.000**
	I×N	9	195.067**	8.118**	103670.000**
	D×N	3	433.649**	3.025**	182469.000**
	I×D×N	9	47.192**	5.222**	61449.500**
	Error	48	7.088	0.470	2.754.000
	CV (%)		6	2	5

I, D, N; Irrigation Level; Plant Density; Nitrogen Dose respectively.
*, **: significant difference at probability level of 5% and 1% respectively.

Grain weight per panicle (g)

Graphs of average values for irrigation levels, plant densities and nitrogen doses in the 1st and 2nd years of the study are shown in Fig.1 and Fig.2. From these figures, it is seen that the grain weight per panicle increases significantly as the irrigation levels increase in 2022. In contrast, there is a significant increase in the applications irrigated according to I₀ in 2023, but there is no significant difference between the I₁, I₂ and I₃ applications. Alderfasi et al, (2016); and Salem, (2015) stated that the increase in irrigation level led to an increase in grain weight per panicle and their results were similar to our study. In both years of the study, the grain weight per panicle decreased significantly with the increase in plant density. Many studies have reported that increasing plant density decreased grain weight per panicle (Zhou et al, 2022; Sahu et al, 2018; Alderfasi et al, 2016; Salem, 2015; Zand et al, 2014; Thakur et al, 2009). In the study, the grain weight per panicle generally increased in parallel with the increase in nitrogen doses, but there was an exception in 2022 with a decrease in N₂ and in 2023 with no significant increase in N₁ dose compared to N₀ (Fig.1 and Fig.2). Salem, (2015); Amiri et al, (2014); Thakur et al, (2009) reported that increasing nitrogen dose increased grain weight per panicle.

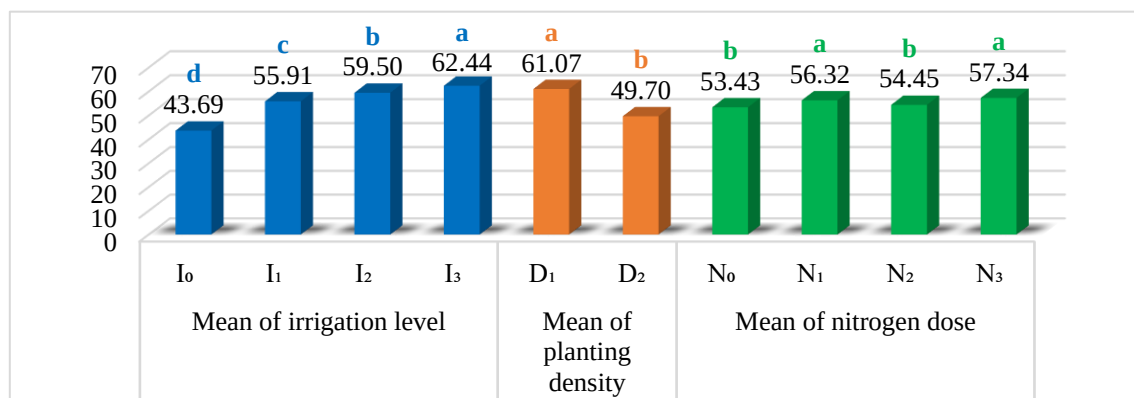


Figure 1. Effect of irrigation levels, planting densities and nitrogen doses on grain weight(g) per panicle in 2022

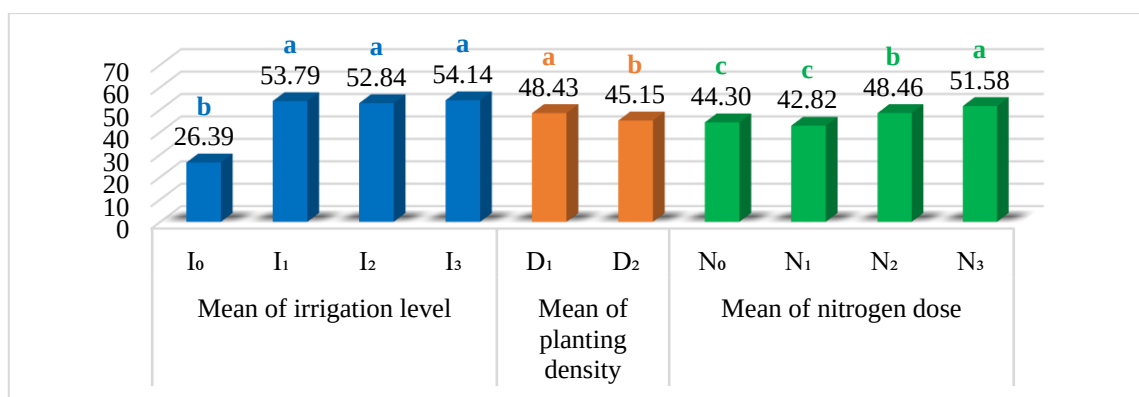


Figure 2. Effect of irrigation levels, planting densities and nitrogen doses on grain weight(g) per panicle in 2023

Table 5 shows the pairwise interactions of irrigation levels, plant densities and nitrogen doses for grain weight per panicle. The irrigation level $\times$ nitrogen dose interaction, which was significant in both years, revealed that the grain weight per panicle increased as the irrigation level increased at each nitrogen dose. However, the highest grain weight per panicle was determined in 2022 at I₂ and I₃ irrigation levels at N₁ nitrogen dose and I₃ irrigation level at N₃ nitrogen dose, while the highest values in 2023 were obtained from I₂ and I₃ irrigation levels at N₂ nitrogen dose and from I₂ irrigation level at N₃ nitrogen dose. Salem (2015) revealed in his study that increasing nitrogen dose with increasing irrigation level increased grain yield per panicle. The significance of the planting density $\times$ nitrogen dose interaction was not exhibited a stable attitude over the years. According to this interaction, which was significant in 2023, the highest grain weight per panicle was obtained from the D₁ plant density at the N₂ nitrogen dose, while there was no significant difference between the plant densities at the N₃ nitrogen dose. Salem, (2015); Turgut et al, (2005) found that increasing nitrogen dose at low plant density increased grain weight per panicle and obtained similar results with our study. The Irrigation level $\times$ planting density interaction, which was significant in 2022, revealed that the grain weight per panicle increased with increasing irrigation levels from I₀ to I₃ at the D₁ planting density, but increased up to the I₂ irrigation level at the D₂ planting density and did not change at the I₃ irrigation level. In 2023, the highest grain weight per panicle was obtained from I₃ irrigation level at D₁ planting density, while it was determined from I₁ irrigation level at D₂ planting density (Table 5). Alderfasi et al, (2016); Salem, (2015) found that increasing irrigation level at low plant density increased grain weight per panicle.

Table 5. Effects of I $\times$ N, D $\times$ N and D $\times$ I interactions on grain weight per panicle (g)

Irrigation level × Nitrogen dose interaction									
2022					2023				
	N ₀	N ₁	N ₂	N ₃		N ₀	N ₁	N ₂	N ₃
I ₀	46.45 ^f	40.62 ^h	43.32 ^g	44.38 ^{f g}	I ₀	25.45 ^h	26.50 ^h	21.10 ⁱ	32.50 ^g
I ₁	51.18 ^e	53.83 ^d	58.25 ^{b c}	60.37 ^b	I ₁	54.25 ^c	52.57 ^{c d}	50.40 ^{d e}	57.95 ^b
I ₂	56.70 ^c	65.05 ^a	56.23 ^{c d}	60.00 ^b	I ₂	48.08 ^e	41.32 ^f	60.18 ^{a b}	61.78 ^a
I ₃	59.37 ^b	65.78 ^a	60.02 ^b	64.60 ^a	I ₃	49.40 ^e	50.90 ^{d e}	62.17 ^a	54.08 ^c
Planting density × Nitrogen dose interaction									
2022					2023				
	N ₀	N ₁	N ₂	N ₃		N ₀	N ₁	N ₂	N ₃
D ₁	59.34	61.99	59.80	63.16	D ₁	46.45 ^c	39.78 ^e	55.60 ^a	51.89 ^b
D ₂	47.51	50.65	49.11	51.52	D ₂	42.14 ^d	45.87 ^c	41.33 ^{d e}	51.27 ^b
Planting density × Irrigation level interaction									
2022					2023				
	I ₀	I ₁	I ₂	I ₃		I ₀	I ₁	I ₂	I ₃
D ₁	47.99 ^d	63.27 ^b	62.21 ^b	70.83 ^a	D ₁	28.65 ^c	51.07 ^{c d}	53.84 ^{b c}	60.16 ^a
D ₂	39.39 ^e	48.55 ^d	56.78 ^c	54.06 ^c	D ₂	24.13 ^e	56.52 ^b	51.84 ^c	48.12 ^d

1000 grain weight (g)

Two-year average values of 1000 grain weight are given in Fig. 3 and Fig. 4. Differences were observed between the averages obtained due to differences in the climate data of the two years. While 222.5 mm of precipitation was received in the 2022 trial season, 7.3 mm of precipitation was received in the 2023 trial season. For this reason, when the 1000 grain weight averages were compared, it was determined that 1000 grain weight increased proportionally as irrigation levels increased in 2022. While the highest 1000 grain weight was obtained from the I₃ irrigation level, a significant increase was detected between the I₀ irrigation level and other irrigation levels in 2023. Also, the highest 1000 grain weight was obtained from the I₁, I₂ and I₃ irrigation levels. The results obtained in other studies also revealed that increasing irrigation level increased 1000 grain weight (Alderfasi et al, 2016; Abadi et al, 2014). The effects of plant densities on 1000 grain weight were found to be significant in 2022 and the highest average was obtained at low plant

density D_1 , but the effects of plant densities on 1000 grain weight were not found to be significant in 2023. Zhou et al, (2022); Sahu et al, (2018); Alderfasi et al, (2016); Soleymani et al, (2011) found that increasing plant density decreased 1000 grain weight. In our study, it is thought that the fact that the year 2023 was both arid and hotter caused no difference between the effects of plant densities. The study showed a significant effect on nitrogen doses per 1000 grain weight in both years. However, N_0 , N_1 and N_2 nitrogen doses were in the same group and gave the highest 1000 grain weight in 2022, while N_1 , N_2 and N_3 nitrogen doses were in the same group and gave the highest 1000 grain weight in 2023 (Fig.3 and Fig.4). Many other studies revealed that increasing nitrogen dose increased 1000 grain weight (Zhou et al, 2022; Shahrajabian et al, (2021); Abadi et al, (2014); Amiri et al, 2014; Zand et al, (2014); Soleymani et al, 2011). In our research, it is thought that the soil characteristics of the experimental site in 2022 biased the effects of nitrogen doses on 1000 grain weight.

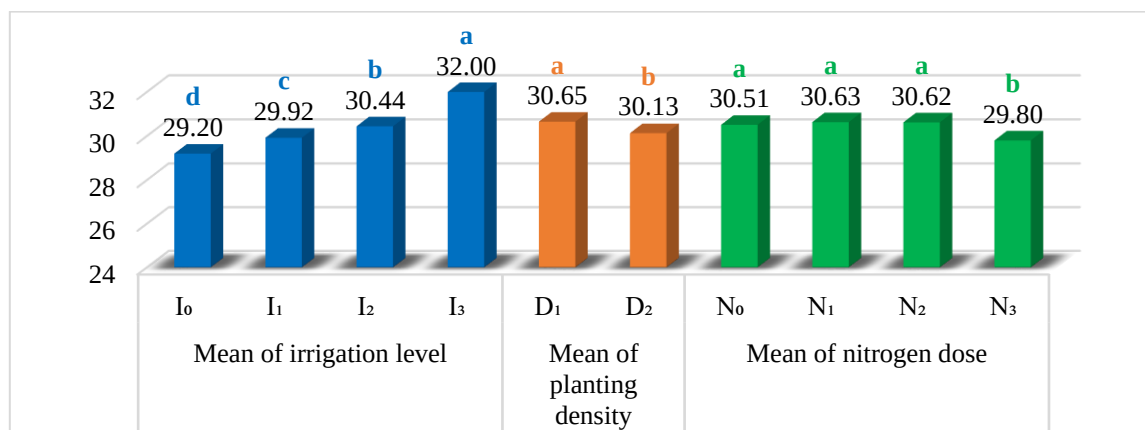


Figure 3. Effect of irrigation levels, planting densities and nitrogen doses on 1000 grain weight (g) in 2022

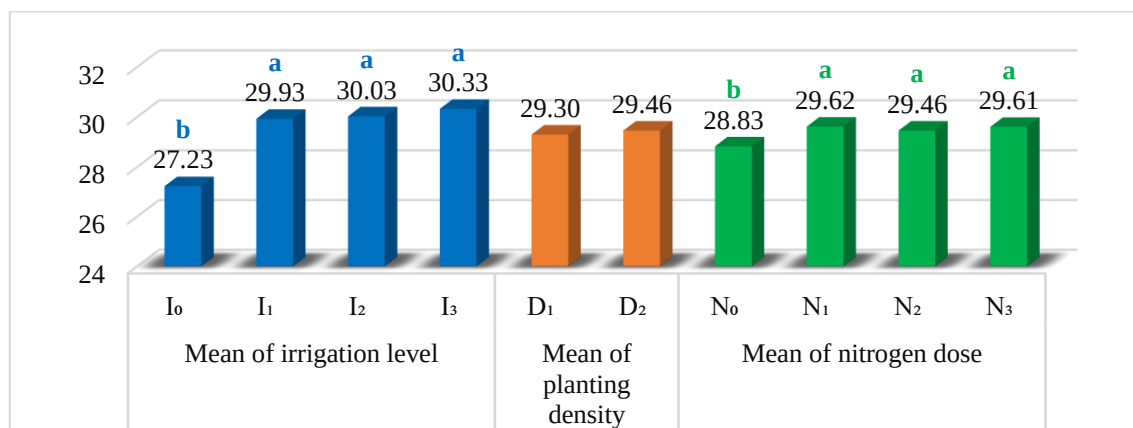


Figure 4. Effect of irrigation levels, planting densities and nitrogen doses on 1000 grain weight (g) in 2023

Binary interactions of irrigation levels, plant density and nitrogen doses on 1000 grain weight are shown in Table 6. Irrigation levels $\times$ nitrogen doses interactions were found to be significant in both years, and it was determined that as irrigation levels increased at each nitrogen dose, 1000 grain weight increased. The highest 1000 grain weight in 2022 was obtained from the interaction of I_2 irrigation level and N_0 nitrogen dose and the interaction of I_3 irrigation level and N_0 , N_1 , N_2 , N_3 irrigation levels. In the 2023 season with a dry climate, the highest 1000 grain weight was determined by the interactions of I_1 irrigation level and N_1 , N_3 nitrogen doses, the interactions of I_2 irrigation level and N_2 , N_3 nitrogen dose, and the interactions of I_3 irrigation level and N_0 , N_1 , N_2 nitrogen doses. The effects of plant density $\times$ nitrogen dose interactions on 1000 grain weight were found to be significant in both years. According to this interaction, no significant increase in 1000 grain weight was detected despite the increasing plant density at other nitrogen doses except N_0 nitrogen dose in both years, but in 2022, N_0 nitrogen dose interacted with D_1 plant density and the highest 1000 grain weight was obtained, and in 2023, the highest 1000 grain weight was observed in D_2 plant density due to the N_0 nitrogen dose interaction and each obtained from N_1 , N_2 , N_3 nitrogen dose interactions at two plant densities. Zand et al, (2014); Soleymani et al, (2011) found that 1000 grain weight increased with increasing nitrogen dose at low plant density. The effects of plant density $\times$ irrigation level interactions on 1000 grain weight were found to be significant in both years. In 2022, as the irrigation level increased from I_0 to I_3 in both plant densities, 1000 grain weights increased, and the highest

1000 grain weight was determined in D₁ and D₂ plant densities at I₃ irrigation level. In the dry season of 2023, irrigation level and plant densities interacted bidirectionally. Plant densities increased 1000 grain weight from I₀ to I₁ when irrigation applications started. Still, there was no significant increase between I₁, I₂ and I₃ irrigation levels, and the interactions of increasing plant density with I₁, I₂, I₃ irrigation levels increased 1000 grain weight except I₀ irrigation level. The highest 1000 grain weight was determined in the interactions of D₂ plant density and I₁, I₂, I₃ irrigation level in 2023. In another study, it was determined that increasing the irrigation level at low plant density will increase 1000 grain weight (Alderfasi et al, 2016).

Table 6. Effects of I×N, D×N and D×I interactions on 1000 grain weight (g)

Irrigation level × Nitrogen dose interaction									
2022					2023				
	N ₀	N ₁	N ₂	N ₃		N ₀	N ₁	N ₂	N ₃
I ₀	29.64 ^c	28.80 ^{d e}	30.18 ^c	28.20 ^e	I ₀	27.36 ^e	27.86 ^{c d}	27.36 ^d	27.86 ^{c d}
I ₁	29.45 ^{c d}	30.05 ^c	30.06 ^c	30.13 ^c	I ₁	29.39 ^b	30.87 ^a	28.60 ^{b c}	30.84 ^a
I ₂	31.46 ^{ab}	31.45 ^b	30.06 ^c	28.78 ^{d e}	I ₂	29.19 ^b	28.89 ^b	31.09 ^a	30.97 ^a
I ₃	31.50 ^{ab}	32.24 ^a	32.17 ^{ab}	32.10 ^{ab}	I ₃	30.92 ^a	30.86 ^a	30.78 ^a	28.77 ^b
Planting density × Nitrogen dose interaction									
2022					2023				
	N ₀	N ₁	N ₂	N ₃		N ₀	N ₁	N ₂	N ₃
D ₁	31.53 ^a	30.82 ^b	30.47 ^b	29.80 ^c	D ₁	28.25 ^b	29.64 ^a	29.71 ^a	29.59 ^a
D ₂	29.49 ^c	30.45 ^b	30.77 ^b	29.80 ^c	D ₂	29.41 ^a	29.61 ^a	29.20 ^a	29.63 ^a
Planting density × Irrigation level interaction									
2022					2023				
	I ₀	I ₁	I ₂	I ₃		I ₀	I ₁	I ₂	I ₃
D ₁	28.86 ^c	31.45 ^b	30.49 ^c	31.83 ^{ab}	D ₁	27.80 ^c	29.64 ^b	29.88 ^b	29.87 ^b
D ₂	29.55 ^d	28.40 ^c	30.38 ^c	32.17 ^a	D ₂	26.65 ^d	30.21 ^{ab}	30.18 ^{ab}	30.80 ^a

Grain Yield of Sweet Sorghum (kg ha⁻¹)

The effects of four different nitrogen doses at four different irrigation levels with different plant densities on grain yield in sweet sorghum are shown in Figures 5 and 6. According to the figures, with the increasing irrigation level in 2022, grain yield increased significantly and the highest grain yield was obtained from I₃ irrigation level. On the other hand, there was a significant increase in applications irrigated according to I₀, and the highest grain yield was obtained from I₁ irrigation level in 2023. Many researchers have found that grain yield increases with increasing irrigation level (Wang et al, 2024; Alderfasi et al, 2016; Salem, (2015); Abadi et al, (2014). Additionally, grain yield increased significantly as the plant density increased in both years of the study. Zhou et al, (2022); Sahu et al, (2018); Alderfasi et al, (2016); Salem, (2015); Soleymani et al, (2011); Thakur et al, (2009); Turgut et al, (2005) determined that increasing plant density increases grain yield. On the other hand, Shahrajabian et al, (2021) reported that grain yield decreased as plant density increased, but the minimum plant density applied in the study was 250000 plants ha⁻¹, which is equivalent to the maximum plant density in our study. In the study, grain yield increased significantly as the applied nitrogen doses increased in both years, and the highest grain yield was obtained from N₃ nitrogen dose application (Figure 7 and Figure 8). Many studies supported our results and indicated that increasing nitrogen dose increased grain yield (Zhou et al, 2022; Salem, 2015; Abadi et al, 2014; Amiri et al, 2014; Thakur et al, 2009). In addition, Shahrajabian et al, (2021) determined that increasing nitrogen dose up to 160 kg ha⁻¹ increased grain yield, while Turgut et al, (2005) determined that increasing nitrogen dose up to 150 kg ha⁻¹ increased grain yield. On the other hand, Soleymani et al, (2011) concluded that grain yield decreased as nitrogen dose increased.

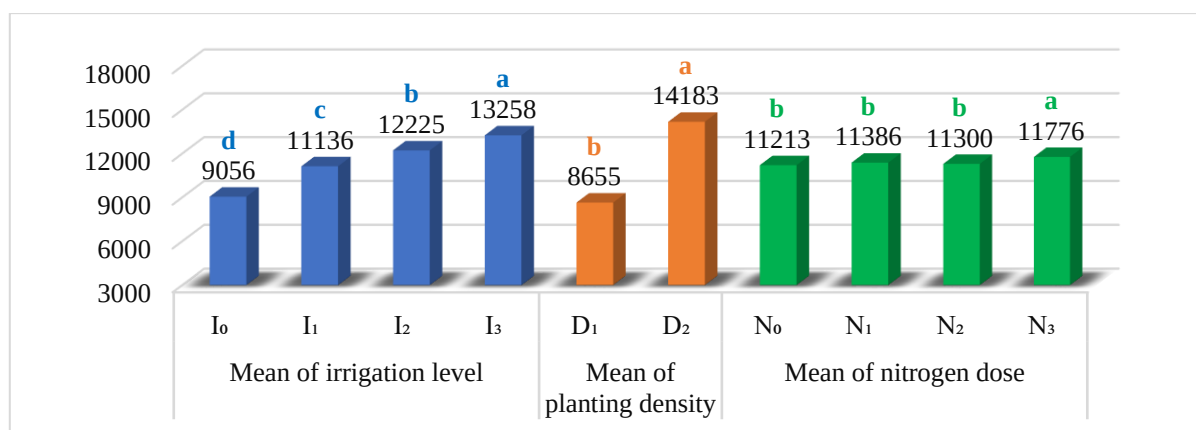


Figure 5. Effect of irrigation levels, planting densities and nitrogen doses on grain yield (kg ha⁻¹) in 2022

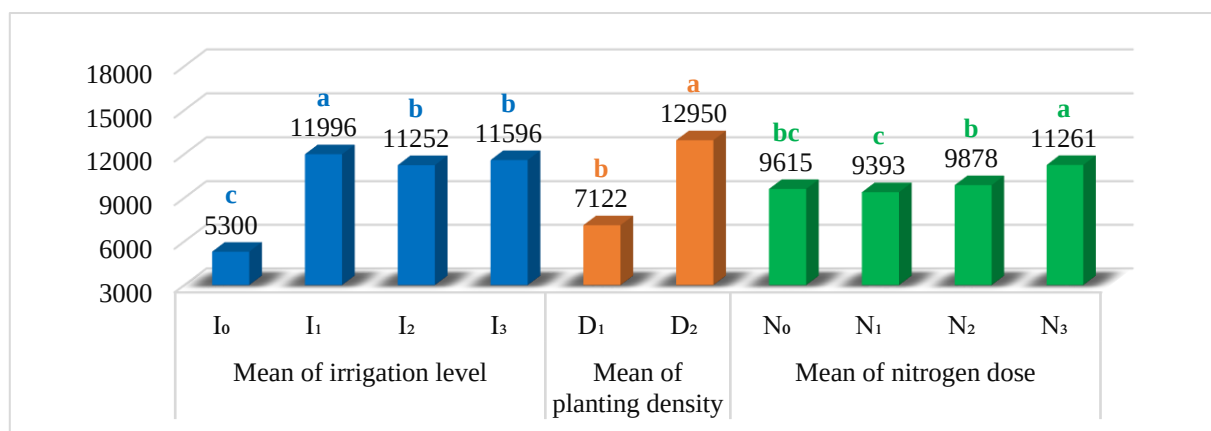


Figure 6. Effect of irrigation levels, planting densities and nitrogen doses on grain yield (kg ha⁻¹) in 2023

The effects of the pairwise interactions of irrigation levels, plant densities and nitrogen doses on grain yield are given in Table 7. The irrigation level $\times$ nitrogen dose interaction, which was important in both years, showed that grain yield increased as the irrigation level increased at each nitrogen dose. However, while the highest grain yield was obtained with I₃ irrigation level at all nitrogen doses in 2022, the highest grain yield in 2023 was determined at N₃ nitrogen dose at I₁, I₂ and I₃ irrigation levels. Wang et al, (2024) reported that grain yield decreased with increasing nitrogen dose at low irrigation level, but grain yield increased with increasing nitrogen dose at normal irrigation level. In another study, Salem (2015) stated that increasing the nitrogen dose with increasing irrigation level increased grain yield. When the average yields of plant density $\times$ nitrogen dose interactions were evaluated, although the increase in plant density made a significant increase on grain yield at all nitrogen doses in both years, plant density $\times$ nitrogen dose interactions did not show a significant effect on grain yield in 2022, while as the nitrogen dose increased at high plant density in 2023, grain yield also increased and the highest grain yield was detected in the interaction of D₂ plant density and N₃ nitrogen dose. In different studies, it was observed that increasing nitrogen doses at high plant density increased grain yield (Salem, 2015; Zand et al, 2014; Turgut et al, 2005). On the other hand, Shahrajabian et al, (2021) revealed that increasing nitrogen doses at low plant density increased grain yield, but the lowest plant density in their study was equivalent to the highest plant density in our study. When the grain yield averages of plant density $\times$ irrigation level interactions were compared, a two-way increase in grain yield was detected in both years of the study. While high D₂ plant density increased grain yield at every irrigation level, it was determined that grain yield increased as irrigation level increased in both plant densities. While the highest grain yield in 2022 was obtained from the interaction of D₂ plant density and I₃ irrigation level, in 2023 it was obtained from the interaction of D₂ plant density and I₁ irrigation level. Alderfasi et al, (2016); Salem, (2015) reported that increasing nitrogen doses at high plant density increased grain yield.

Table 7. Effects of I×N, D×N and D×I interactions on grain yield (kg/ha)

Irrigation level×Nitrogen dose interaction									
2022					2023				
	N ₀	N ₁	N ₂	N ₃		N ₀	N ₁	N ₂	N ₃
I ₀	9912 ^h	8148 ^j	8815 ^{i j}	9348 ^{hi}	I ₀	5864 ⁱ	5905 ⁱ	4114 ^j	5316 ⁱ
I ₁	9967 ^h	11288 ^{fg}	11019 ^g	12270 ^{dc}	I ₁	12515 ^{b c}	11734 ^d	10595 ^{e f}	13141 ^a
I ₂	12001 ^{ef}	12770 ^{bcd}	11707 ^{efg}	12424 ^{cde}	I ₂	9897 ^g	9113 ^h	12516 ^{b c}	13483 ^a
I ₃	12974 ^{abcd}	133378 ^{ab}	13661 ^a	13061 ^{abc}	I ₃	10186 ^{fg}	10820 ^e	12276 ^{c d}	13104 ^{ab}
Planting density×Nitrogen dose interaction									
2022					2023				
	N ₀	N ₁	N ₂	N ₃		N ₀	N ₁	N ₂	N ₃
D ₁	8343	8856	8543	8876	D ₁	7187 ^c	5682 ^f	7942 ^d	7678 ^d
D ₂	14083	13915	14058	14675	D ₂	12044 ^c	13104 ^b	11809 ^c	14845 ^a
Planting density×Irrigation level interaction									
2022					2023				
	I ₀	I ₁	I ₂	I ₃		I ₀	I ₁	I ₂	I ₃
D ₁	6856 ^g	9039 ^f	8740 ^f	9984 ^c	D ₁	4366 ^f	7839 ^d	7691 ^d	8593 ^c
D ₂	11255 ^d	13233 ^c	15711 ^b	16532 ^a	D ₂	6234 ^c	16154 ^a	14813 ^b	14600 ^b

4. CONCLUSION

The effects of irrigation levels, plant density and nitrogen doses on grain yield and yield components of sweet sorghum, a highly adaptable C₄ crop that matures early under high temperatures under changing climatic conditions were investigated. The results revealed that as the irrigation level increased from 0% to 100% of crop evapotranspiration (ET_c), grain weight per panicle, 1000 grain weight and grain yield also increased. As the plant density increased from 142857 plants ha⁻¹ to 285714 plants ha⁻¹, grain weight per panicle and 1000 grain weight decreased, while grain yield increased significantly. As the nitrogen dose increased from 0 kg ha⁻¹ to 210 kg ha⁻¹, grain weight per panicle, 1000 grain weight and grain yield increased in both trial years. In the study, maximum grain yield was obtained with 0 kg ha⁻¹, 70 kg ha⁻¹, 140 kg ha⁻¹ and 210 kg ha⁻¹ nitrogen doses at 100% of crop evapotranspiration (ET_c) irrigation level in 2022 with rainy climatic conditions, while it was obtained with 210 kg ha⁻¹ nitrogen dose in irrigated treatments (33%, 66% and 100% of crop evapotranspiration (ET_c)) in 2023 with arid climatic conditions. When the plant density and nitrogen dose interactions were compared, although grain yield increased with increasing plant density in 2022, it did not interact with nitrogen doses, but plant density and nitrogen doses interacted in 2023 and the highest grain yield was determined at 210 kg ha⁻¹ nitrogen dose interacting with 285714 plants ha⁻¹ plant density. In the plant density and irrigation level interactions, the maximum grain yield was obtained from the interaction of 285714 plants ha⁻¹ plant density and 100% of crop evapotranspiration (ET_c) irrigation level in the rainy year 2022, while it was obtained from the interaction of 285714 plants ha⁻¹ plant density and 33% of crop evapotranspiration (ET_c) irrigation level in the dry year 2023. According to the results obtained from our study, a plant density of 285714 plants ha⁻¹, N dose of 210 kg ha⁻¹ and 100% irrigation level are recommended to obtain high grain yield in semi-humid climatic conditions for sweet sorghum production. The climatic conditions of both study years were different, with significant differences in rainfall and temperatures. In 2022, the small difference between the effects of nitrogen doses is thought to be due to the fact that the rainfall washed away the applied nitrogen and reduced the effect of nitrogen. In 2023, when there was almost no rainfall and the air temperature was about 2 °C warmer, it was observed that some plants in the experimental area did not fill the grains. Therefore, the responses of some traits to the treatments differed. The research should be carried out in different locations to further support the literature and the most appropriate treatments under different climatic conditions.

ACKNOWLEDGEMENT

First of all, I would like to thank my esteemed Professors, Prof. Dr. İlhan TURGUT, Prof. Dr. Abdurrahim Tanju GÖKSOY and Prof. Dr. Hayrettin KUSÇU, who designed this study and supported us at every point, Uludağ University, Faculty of Agriculture, Department of Field Crops, and my family and friends who were with me at every stage of the study.

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Molecular Marker Aided Selection of Restorer of Fertility (Rf/rf) Alleles to Develop Maintainer Lines in Sunflower

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ARTICLE INFO

Research Article

Received: 3 December 2024

Accepted: 21 April 2025

Published: 23 June 2025

Keywords:

CMS

Maintainer

MAS

Restorer

SCAR

Sunflower

ABSTRACT

Sunflower (*Helianthus annuus* L.), one of the main oil crops in the world, is also increasingly used for its ornamental value. Marker-assisted selection (MAS) helps identify desired traits through molecular markers linked to specific genes, speeding up the breeding process. In sunflower breeding, dominant and codominant markers are used to detect Rf1/rf1 alleles for the selection of plants carrying maintainer and/or restorer (Rf) nuclei. In a breeding program, the status of Rf/rf alleles in the nucleus of the maintainer (B) lines cannot be ascertained without a test-cross due to the presence of fertile cytoplasm. The aim of this study was to test the utilization of the SCAR markers linked to Rf/rf alleles in sunflower to test if the markers can be integrated into a breeding program to develop maintainer lines. The plant materials included restorer and maintainer lines, as well as backcross and test-cross populations segregating for Rf/rf alleles. The markers (PPR621.5) have proven to be valuable for MAS in sunflower breeding. The Rf/rf specific dominant markers were used with the atp9 internal control marker to differentiate PCR failure from the absence of an allele. The markers were also phenotypically confirmed using advanced lines, BC₁F₁, BC₂F₁, and BC₁F₂, as well as test-cross populations. The application of SCAR markers in developing new restorer and maintainer lines can enable efficient determination of Rf/rf allele status, thereby eliminating the need for test crosses in maintainer nuclei and significantly enhancing breeding efficiency. Detection of Rf/rf alleles by molecular markers in maintainer populations with fertile cytoplasm may help reduce cost and breeding time to develop maintainer lines.

Citation: Zailasheva, A., Sancar, B., Zonon, M., Karabeniz, A., Pakashtica, V., Sever Mutlu, S., & Mutlu, N. (2025). Molecular marker aided selection of restorer of fertility (Rf/rf) alleles to develop maintainer lines in sunflower. *Turkish Journal of Field Crops*, 30(1), 88-97. <https://doi.org/10.17557/tjfc.1595579>

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the world's fourth most important oil crop, primarily cultivated in temperate and semi-arid regions. In addition to its primary purpose for human consumption, sunflower oil has many applications and can be utilized as a supplement in the chemical and pharmaceutical industries. Sunflower originated in North America and was probably first introduced to Europe through Spain in the 16th century as a garden ornamental flower and gradually moved eastward and northward in the European continent reaching Russia in the 18th century (Azarin et al. 2023). Sunflower as oil crop and ornamental use is mainly hybrids developed via use of male sterility.

The techniques of cytoplasmic male sterility (CMS) and fertility restoration (Rf) have been extensively utilized for hybrid seed production in sunflower, and in some other major field crops. Leclercq (1969) discovered CMS in sunflower through an interspecific hybridization between a wild sunflower species, *Helianthus petiolaris*, and a cultivated sunflower, *H. annuus*. Although multiple types of CMS have been identified, the current production of commercial hybrids utilizes the male-sterile cytoplasm (PET1) discovered by Leclercq (1969). Numerous PET1 fertility restoration lines have been created, and one to four dominant fertility restoration genes have been identified (Serieys, 1996). Rf2 is present in nearly all cultivated sunflower lines (Reddy and Thammiraju 1977, Leclercq and Philippon 1984, Miller and Fick 1997). Two dominant nuclear alleles, Rf1 and Rf2, are required to restore male fertility in most cultivated sunflower lines.

Sunflower is the second largest commodity grown from hybrid seed after maize (Dimitrijevic and Horn, 2018). Commercial hybrid seed production relies on cytoplasmic male sterility (CMS) and male fertility restorer (RF) lines. Fertility restorer genes are nuclear-based and tend to surmount the F1 generation's cytoplasmic male sterility. A high grain filling percentage requires a satisfactory fertility restoration. Rauf (2019) reviewed a selection of the more than 70 cytoplasmic male sterility sources developed for sunflower. These CMS sources can only be tapped using appropriate restorer genes. Hybrid sunflower breeding generally relies on the Rf1 gene obtained from the T66006-2-1-B line (Kinman, 1970). Diversification of cytoplasmic and fertility restorer genes is an essential breeding goal to reduce genetic susceptibility to diseases and pathogens. Talukder et al. (2019) have identified a variety of fertility restorer genes (Rf1–Rf7, Rf-PEF1) compatible with diverse CMS sources. A selection of the validated markers used to identify various Rf genes, and their corresponding linkage group is presented in Table 2. Molecular markers are used to identify and differentiate the alleles. These markers are particularly important for plant breeding and genetic studies (Akkose Baytar et al. 2021). Marker-assisted selection (MAS) allows for rapid and efficient selection of plants carrying Rf genes. These markers save time and cost in seed production, ensure that genetic purity is maintained, and prevent contamination in hybrid seed production. The use of molecular markers to determine whether Rf1 and Rf2 genes are homozygous or heterozygous in sunflower maintainer lines is important in plant breeding and genetic studies. These markers aim to detect the presence or absence of cytoplasmic male sterility (CMS) fertility restorer genes. It is a critical step in maintainer line development that would nullify the need for cumbersome test crossing.

The study by Horn et al. (2003) showed fine mapping of the Rf1 gene. Anashchenko and Duka (1985, 1986) identified Rf genes from different sources of fertility restoration. Liu et al. (2003) performed fine mapping of the fertility-restoring Rf1 gene for cytoplasmic male sterility in cotton (*Gossypium hirsutum* L.). Similarly, Wu et al. (2014) developed an InDel marker linked to the Rf1 gene in cotton and evaluated its use in breeding programs.

The development and use of these markers contribute to the acceleration of genetic studies in sunflowers and other crops and the establishment of more effective breeding programs. The aim of this study was to evaluate the utility of the molecular markers to identify plants carrying restorer of fertility alleles at homozygote or heterozygous (RfRf, rfrf, or Rfrf) states in segregating maintainer populations in sunflower breeding.

2. MATERIALS AND METHODS

Plant Material

The plant materials were grown at Akdeniz University, Antalya, Türkiye, during the spring and fall growing seasons in 2023 and 2024.

A homozygous restorer line resistant to herbicide sulfonylurea (SU) obtained from Osterras seed company (Antalya, Türkiye), and was crossed with a commercial male-sterile ornamental sunflower hybrid, Sunrich Orange F₁ (SOF₁). Resulting F₁s were backcrossed both to SOF₁ and to Predovik, a commonly used public maintainer line. The BC₁F₁ population of SOF₁ segregated 1:1 for both male sterility/fertility and SU herbicide resistance. Fertile and SU resistant BC₁F₁ plants were backcrossed to SOF₁ once more to create the BC₂F₁ population; the

same plants were also used as male parents and backcrossed to the F_1 s originating from the Sunrich Orange F_1 x Peredovik cross. As expected, the resulting BC_2F_1 population of SOF_1 segregated 1:1 for both male sterility/fertility and SU herbicide resistance, while the BC_1F_1 population derived from Peredovik segregated for SU herbicide resistance (1:1) and was all fertile due to Peredovik fertile cytoplasm. The SU-resistant BC_1F_1 -Peredovik plants were selfed to generate the BC_1F_2 population. In the BC_1F_1 and BC_2F_1 populations of SOF_1 , the Rf allele was expected to segregate 1:1 (Rfrf:rfrf) in line with the observation of male fertile/male sterile phenotypes. In the Peredovik BC_1F_1 population, Rf/rf allele was expected to segregate 1:1 (Rfrf:rfrf) as well in the BC_1F_2 population; the ratio was 3:1 (Rf:rfrf) for Rfrf plants, and all rfrf for the other half of the population.

Test cross populations: Two different test cross populations were created. The first one was heterozygous fertile BC_1F_1 plants from the SOF_1 population that was crossed with a CMS line to generate a population segregating 1:1 for male sterility/fertility due to Rfrf / rfrf genotypes in the nucleus. The second one was the same heterozygous fertile BC_1F_1 plants from the SOF_1 population that were crossed with the maintainer line Peredovik, which is expected to segregate 1:1 for Rfrf:rfrf allele in the nucleus, but all plants were expected to be fertile due to the Peredovik cytoplasm.

Table 1. The pedigree, generations, and properties of plant materials used in the study.

Population Genotype	Pedigree	Generation	Properties
Restorer	Osterras	Advance line	Sulfonylurea (SU) resistant
Maintainer (Peredovik)	Public	Advance line	Maintainer
Sunrich Orange F_1 (SOF_1)	Commercial	Hybrid	Male-sterile (CMS) ornamental hybrid
F_1	SOF_1 x Restorer	F_1 s	SU resistant-sterile cytoplasm, fertile hybrid
F_1 s x Peredovik	F_1 x Peredovik	F_1 p	Maintainer (fertile) cytoplasm
F_1 s x SOF_1	F_1 s x SOF_1	BC_1F_1 s	SU resistance and male fertility segregate (1:1), sterile cytoplasm
BC_1F_1 s (fertile +SU) x F_1 p	BC_1F_1 s x F_1 p	BC_1F_1 p	SU resistance (1:1), restorer of fertility in nucleus 3:1 (Rf:rfrf)
BC_1F_1 s (fertile +SU) x SOF_1	BC_1F_1 s x SOF_1	BC_2F_1 s	Sterile cytoplasm, 1:1 segregation for male sterility and SU resistance
BC_1F_2 p	BC_1F_1 p x BC_1F_1 p	BC_1F_2 p	Fertile cytoplasm, restorer of fertility in nucleus 3:1 (Rf:rfrf) for half, and all rfrf for the other half
Test-cross	BC_1F_1 p (Rfrf) x CMS	BC_1F_1	Sterile cytoplasm, segregated 1:1 for fertility/sterility
Test-cross	BC_1F_1 s (rfrf) x CMS	BC_1F_1	Sterile cytoplasm, all sterile,

DNA Extraction

Genomic DNA was isolated according to the standard Cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). The youngest leaf materials were incubated with 15 mL extraction buffer (100 mM of Tris/HCl pH 8.0, 1.4 M of NaCl, 20 mM EDTA, 2% CTAB, 1% Na₂S₂O₃) at 65°C for 1 hour. After chloroform–isoamyl alcohol (24:1) solution was added to each tube and the samples were obtained by centrifugation. Approximately 300 µL of the supernatant was transferred into new tubes, and 300 µL of isopropanol was added to each sample. After mixing, the tubes were stored overnight at –20 °C. The samples were centrifuged for 10 min at 14,000 × g and washed with 70% ethanol. After adding 100 µL of sterile pure water, the tubes were stored at 4 °C for short-term and at –20 °C for long-term use.

Polymerase Chain Reaction

SCAR markers used for analysis, relevant primers, expected sizes of PCR amplification products, and literature are presented in Table 2.

Table 2. Sequences of SCAR primers, lengths of PCR products, and related literature

Marker	Primer sequence (5'-3')	Expected size of the SCAR marker and allele (Rf/rf)	Reference
67N04_F1a	TGCAAGATAGGCGACTGAG GGCTCATCTCCAATTA	170 (Rf)	Horn et al. 2019
67N04_F2b	TGAGGGCTCATCTCCAGCTG	155 (rf)	Horn et al. 2019
67N04_R	GGCTGCCATTAGTGAAGGAG	Common (Rf/rf)	
PPR621.5 F1	CAGTAATCTCCACATGAACATTG	164 (rf)	Horn et al. 2019
PPR621.5 F2	CAATAATCTCCACATGAACATTC	164 (Rf)	Horn et al. 2019
PPR621.5 Rev	CCGGATTGTGTTCCGATTAG	Common (164 Rf/rf)	
HRG02 F	AAA CGT GGG AGA GAG GTG G	738 (Rf)	Horn et al. 2003
HRG02 R	AAA CGT GGG CTG AAG AAC TA		
atp9_for	GGTGCAAATCAATAGGGGCCG	474 (internal control)	Accession X51895
atp9_rev	ACCGAATGAATGCGTCACAAGG		

For the Rf1/rf1 restorer gene, 67N04 is a codominant SCAR marker. One of the three primers is common to both alleles of Rf/rf, one is specific to the Rf allele, and the other to the rf allele. If the triple primer set creates only a 170 bp long band, it indicates that the plant is RfRf homozygous restorer, if only a 155 bp long fragment is present, then it is rfrf homozygous maintainer, and a heterozygous genotype yields both fragments.

Again, two different dominant markers designed from a gene developed for Rf1 and shown as a candidate gene for Rf1; the primer combination PPR621.5_F1/PPR621.5_Rev creates a 164 bp long band specific to the rf (maintainer) allele only, while the primer combination PPR621.5_F2/PPR621.5_Rev creates a 164 bp long band specific to the Rf (restorer) allele only. Dominant markers are used in combination with the atp9 primers as an internal PCR control.

The HRG02 dominant SCAR marker was developed specifically for the Rf1 restorer allele by Horn et al. (2003). This dominant SCAR marker is utilized in conjunction with the atp9 marker as well to figure out if the absence of the expected 738 bp long marker band is due to a PCR failure or to a homozygous rfrf genotype.

For the PCR reaction, 2 µl of DNA was mixed with 13 µl of master mix. The master mix contained 1.5 µl dNTP (5 mM), 4.7 µl ddH₂O, 1.5 µl 10x PCR buffer with MgCl₂, 1.8 µl forward primer (10 mM) and 1.8 µl reverse primer (10 mM), and 0.2 µl Taq polymerase. PCR was performed on a thermal cycler according to the program denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 45 seconds, 45 seconds annealing depending on the primer combination, 30 seconds polymerization at 72°C. The PCR program was finalized with elongation at 72°C for 6 minutes. SCAR markers were separated in a 1.5% agarose gel electrophoresis and ethidium bromide and subsequent photography in a UV light system.

Data Analysis

In this study, the Chi-square (χ^2) test was used to test deviation between observed and expected phenotypic ratios. Observed (O) and expected (E) frequencies were calculated, and the Chi-square value was determined with the formula. The p-value was calculated using the degrees of freedom and Chi-square distribution table, and statistical significance was evaluated (Hartl and Cochrane, 2005; NCBI, 2010).

3. RESULTS

67N04 marker

PCR screening was performed with 67N04_F1a/Rev and 67N04_F2b/Rev primers on 32 different sunflower plant samples representing maintainer, CMS, and Restorer x CMS hybrids. With the codominant marker (67N04_F1a/Rev) developed for the Rf1 (Rf/rf) restorer gene, a 170 bp long Rf homozygous restorer band was obtained in hybrids, 17 to 23, having a heterozygous (Rfrf) genotype. The homozygous rfrf nucleus genotype was evident on the maintainer and CMS samples with a 155 bp long rf allele fragment (Figure 1).

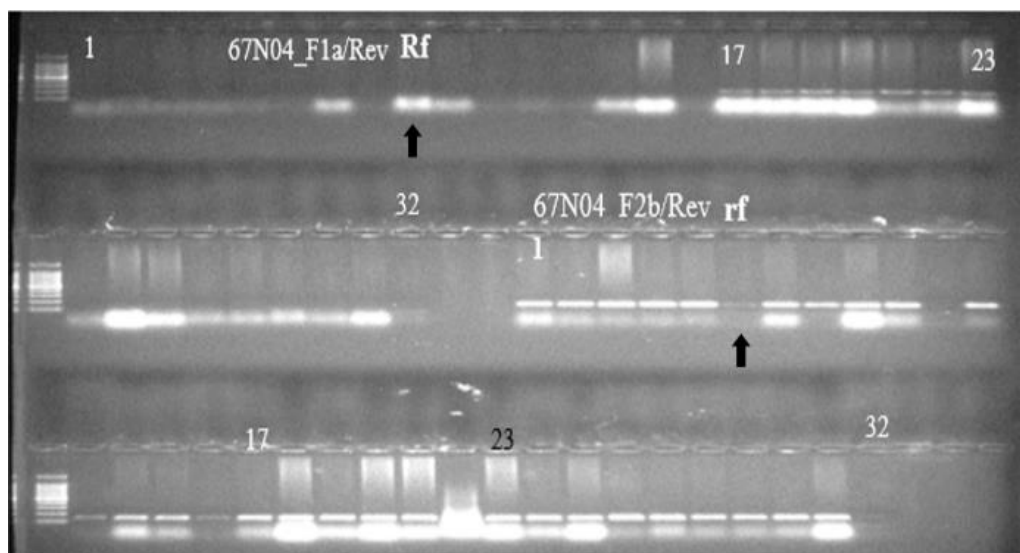


Figure 1. Primers 67N04_F1a/Rev (170 bp (Rf)) and 67N04_F2b/Rev (155 bp (rf)). 1-16 and 24-32 maintainer and CMS lines, respectively; 17-23 hybrids (restorer x CMS).

PPR621.5 marker

Primer screening was performed on the same 32 plant samples using PPR621.5_F1/Rev and PPR621.5_F2/Rev primers. As expected, the rf allele-specific primer combination PPR621.5_F1/Rev yielded 164 bp long fragments on maintainer, CMS, and hybrid plant samples. Rf1 specific primer combination (PPR621.5_F2/Rev) yielded the expected 164 bp long allele-specific band on the hybrids (Figure 2).

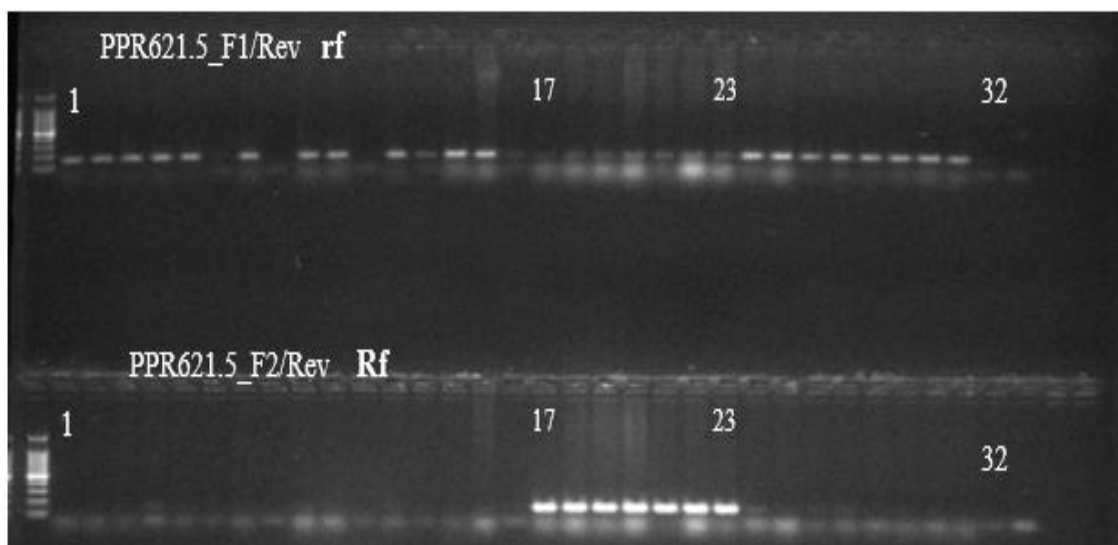


Figure 2. Marker PPR621.5_F1/Rev for the maintainer lines. Marker PPR621.5_F2/Rev for the restorer lines. 1-16 and 24-32 maintainer and CMS lines; 17-23 hybrids (restorer x CMS).

Because the marker is dominant (allele specific), atp9 internal PCR control primers were also added into the reactions while using PPR621.5_F2/Rev primers. In a segregating maintainer population (BC₁F₂p in Table 1) of 105 plant samples, the expected 164 bp for the Rf1 allele and the 474 bp long atp9 fragments were formed, where 72 plants showed the presence of the Rf1 allele (RfRf+Rfrf) while 33 plants had homozygous (rfrf) maintainer nuclei (Figure 3).

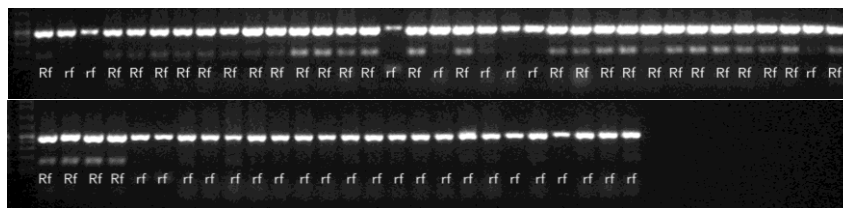


Figure 3. Dominant Rf1 specific PPR621.5_F2/Rev marker and atp9 internal PCR control in an F₂ segregating maintainer population (BC₁F₂p).

The primer PPR621.5_F2/Rev and atp9 were also tested in a BC₁F₁ population (BC₁F₁p in Table 1) consisting of 70 plants segregating for Rf/rf alleles in a fertile cytoplasm. We further confirmed the Rf1 allele-specific dominant marker, where 45 plants yielded the 164 bp Rf1 allele (Rf1rf1) while the rest lacked that allele (rf1rf1) (Figure 4).

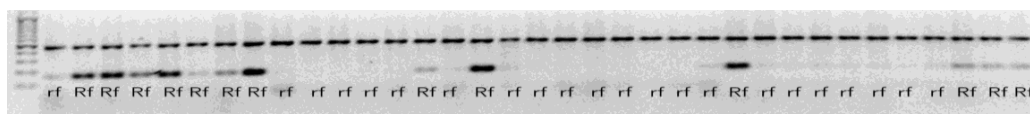


Figure 4. Marker PPR621.5_F2/Rev for Rf allele (164 bp) for the restorer lines and internal control marker atp9 (474 bp).

The 288 plants made up of segregating maintainer populations at BC₂F₁ and BC₁F₂ were genotyped using the marker. Maintainer lines homozygous for rf1rf1 in the nucleus were determined. PPR621.5 F2/Rev marker was also used for Rfs allele detection along with the atp9 primers (Figure 5). The marker showed expected segregation ratios in both populations, 1:1 and 3:1, respectively.

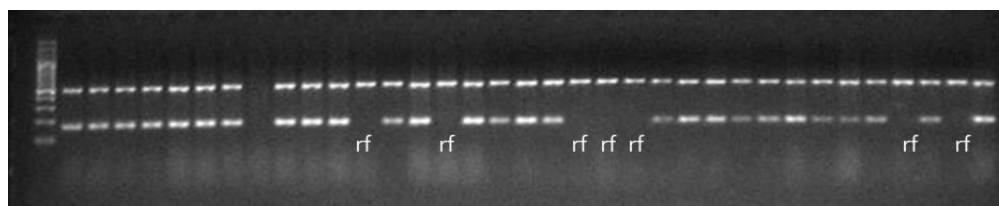


Figure 5. Marker PPR621.5_F2/Rev and atp9 for the Rf1 allele. The plant material consisted of both BC₁F₂ and BC₂F₁ maintainer populations.

In order to test the result of marker screening, a test cross was carried out. A few of the 76 plants either homozygous (rfrf) or heterozygous (Rfrf) with maintainer cytoplasm at the BC₁F₁ population, segregating for the Rf/rf allele, were crossed with a CMS line. The Rf allele-specific SCAR marker PPR621.5_F2/Rev indicated that 42 plants were homozygous (rfrf) and 34 heterozygous (Rfrf) for the restorer of fertility alleles in their nuclei (Figure 6).

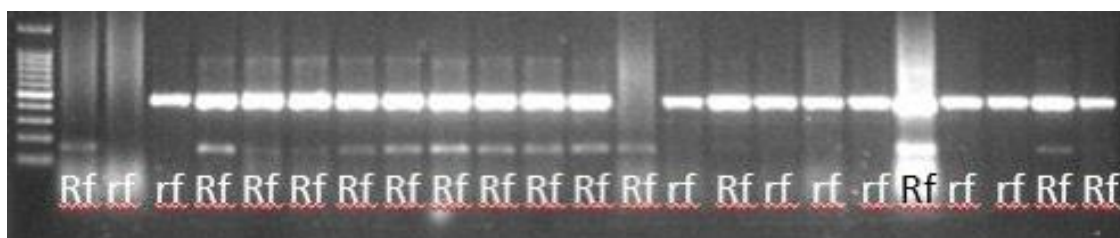


Figure 6. Marker PPR621.5_F2/Rev for the Rf allele segregating 1:1 in A BC₁F₁-B population of 76 samples.

The heterozygous (Rfrf) fertile BC₁F₁p plants were used as donor and crossed to a CMS line to create test-cross populations. The 16 plants obtained from the test cross (Rfrf x rfrf) were planted for phenotyping where 8 of them were fertile and 8 sterile (Figure 7).



Figure 7. Testcross result of BC₁F₁p line with heterozygous (Rrfr) nucleus was crossed to a CMS line to create the test-cross population where fertility/sterility segregated 1:1.

The homozygous (rfrf) fertile BC₁F₁p plants were used as donor and crossed to a CMS line to create test-cross populations. The 30 plants obtained from the test cross (rfrf-B x rfrf-A) were also planted, where all 30 showed complete sterility (Figure 8).



Figure 8. The BC₁F₁p line with homozygous (rfrf) nucleus was crossed to a CMS line to create the test-cross population, where all plants were sterile.

HRG02 marker

The dominant SCAR marker HRG02 linked to the Rf1 allele was also used in combination with primers for the atp9 as an internal control. The screening was performed on 32 plant samples consisting of maintainers, CMS, and hybrids (Restorer x CMS) with primers HRG02 F/R/atp9. The HRG02 F/R amplified a 738 bp band only in hybrid (17-23) samples (Figure 9).

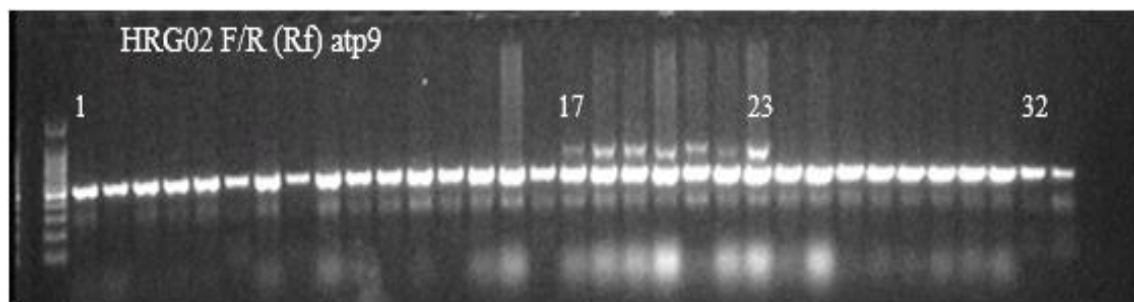


Figure 9. Duplex PCR reaction with the marker HRG02 and atp9, using maintainer (1-16), hybrids (17-23), and CMS (24-33) plant samples.

In Figure 10, the 8 BC₁F₁s plants exhibited a genotypic segregation ratio of 1:1, with 50% fertile and 50% sterile individuals, as expected. These plants were analyzed using the markers HRG02/Rev (Rf), PPR621.5 F1/Rev (rf), and PPR621.5 F2/Rev (Rf) with atp9 internal control primers. Upon examination of the marker results, it was determined that the outcomes of HRG02 were consistent with those of PPR621.5 F1 and PPR621.5 F2 markers.

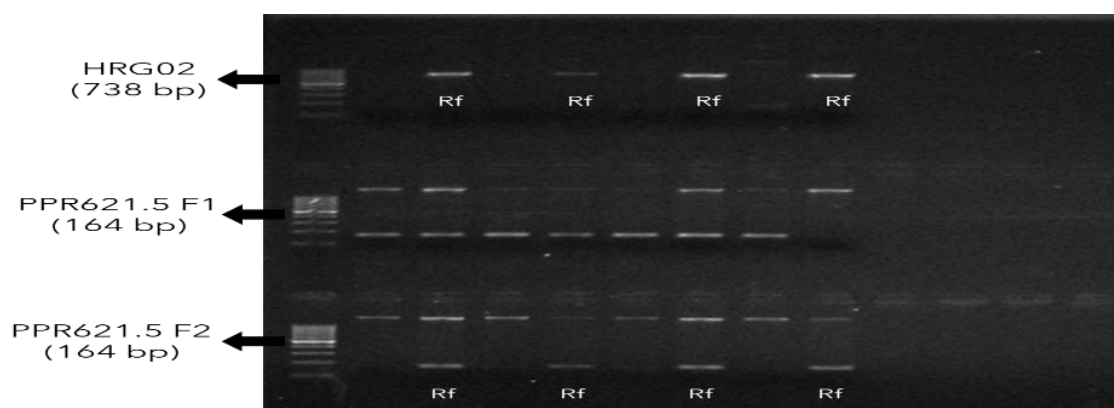


Figure 10. Duplex PCR reaction with the marker HRG02, PPR621.5 F1, PPR621.5 F2, and atp9, using BC₁F₁s plant samples segregating for Rf allele.

4. DISCUSSION

The tightly linked or allele-specific SCAR markers are highly effective for utilization in MAS. In this study, SCAR markers were exploited to detect the Rf/rf (restorer of fertility) allele at CMS, maintainer, and restorer populations. We tested six markers linked to the Rf/rf alleles to determine and confirm the reliable markers. All six developed SCAR markers enabled discrimination between restorer lines, maintainer lines, and CMS lines. However, PPR621.5 F2/Rev primers with atp9 internal control primers can be successfully and reliably used for selection of Rf/rf alleles at various backgrounds.

The presence of Rf genotypes was examined in 26 perennial and 5 annual sunflower species using SCAR markers by Markin et al. (2017). Their analysis of annual sunflower species using HRG01 and HRG02 markers produced ambiguous findings. They stated that all five of the species under study had HRG01 PCR products (about 450 bp). HRG02 amplification products (about 740 bp) were observed only in *H. annuus* and *H. petiolaris*. HRG02 marker was detected in 50% of the *H. annuus* (441236, 441245) samples and in 25% of the *H. annuus* (441183) and *H. petiolaris* (440560) samples (Markin et al. 2017). In contrast to the findings of annual species research, only 12 out of 26 species showed HRG01 amplification, but the marker HRG02 (about 740 bp) was identified in all perennial species examined. Therefore, the HRG02 marker detected in perennial species can be considered a highly informative identifier of the Rf1 gene.

Figures 3, 4, 5, 6, 9, and 10 demonstrate that duplex PCR reactions worked very well and therefore atp9 represents a reliable internal control. The markers HRG02 and HRG01 developed by Horn et al. (2003) have proven to be almost universally usable in hybrids between restorer and maintainer lines.

The 67N04 primer, developed as a codominant SCAR marker for the Rf1/rf1 restorer gene in the study by Horn et al. (2019), is highly valuable as it enables the identification of heterozygous individuals. Additionally, in

the same study, two SCAR markers (PPR621.5R and PPR621.5M) were successfully developed to identify restorer and maintainer lines in PCR reactions. These markers serve as effective tools to distinguish between the cytoplasmic restoration and maintenance pools of PET1.

Four markers were developed in Reddemann and Horn (2018) study: HRO_ATP9-PET2, which differentiates CMS PET2 from normal, fertile cytoplasm; HRO_PET1, which is specific for CMS PET1 and indicates that orfH522 is absent in CMS PET2; HRO_ATP1-PET1, which detects CMS PET1 in conjunction with the internal atp1 control; and HRO_ATP1, which serves as an internal PCR control that is present in all cytoplasms. Application of these four diagnostic markers allowed for a clear differentiation between CMS PET1, CMS PET2, and the fertile cytoplasm in sunflower. Results for CMS PET2 indicated that the molecular mechanisms behind PET2 male sterility in sunflower depend on recombination events involving the atp9 gene, which leads to two new open reading frames, orf288 and orf231. Furthermore, no PCR signal for orfH522 was visible in CMS PET2, clearly differentiating it from the CMS PET1 mechanism.

In the study by Babych et al. (2021), the SCAR marker HRG01 linked to the Rf1 gene met the criteria for identifying fertility restoration in the desired material. The use of molecular markers, particularly the SCAR marker HRG01 linked to the Rf1 gene, was preferred for selecting maintainers and fertility restorers in diverse early-generation populations.

5. CONCLUSION

In this study, sunflower plant materials that belong to CMS, maintainer or restorer populations, segregating for the Rf/rf alleles, were tested using the Rf/rf specific co-dominant and dominant SCAR markers. Using plant populations at various backgrounds and generations, we showed that markers offer a reliable selection tool for Rf/rf alleles in breeding populations. The PPR621.5 F2 and PPR621.5Rev markers in particular represent valuable tools for MAS in sunflower breeding. The development of new restorer and maintainer lines for sunflower breeding with SCAR markers can now be carried out much more efficiently, and there will be no need for test crossing to understand the status of Rf/rf alleles in the nucleus of maintainer lines. This finding, as also shown in Figure 10, suggests that PPR621.5 F1, PPR621.5 F2, and HRG02 markers can be reliably used for the detection of Rf1/rf1 alleles.

ACKNOWLEDGEMENT

This study was supported by TUBITAK - KBAG under project number 122R004.

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Performance of Oat under Different Sowing Densities and Nitrogen Levels

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ARTICLE INFO

Research Article

Received: 10 February 2025

Accepted: 12 May 2025

Published: 23 June 2025

Keywords:

Avena

Fertilizer

Grain yield

Sowing density

β-glucan

Citation: Karakuzu, T., & Erbaş Köse, Ö. D. (2025). Performance of oat under different sowing densities and nitrogen levels. *Turkish Journal of Field Crops*, 30(1), 98-109. <https://doi.org/10.17557/tjfc.1636732>

ABSTRACT

Oats have historically been utilized as both animal feed and human food and continue to play a significant role in these capacities today. Understanding the extent to which agricultural practices influence the yield and quality of oat cultivars is of great importance to breeders. The aim of this study is to determine the effects of different sowing densities and nitrogen applications on the grain yield, yield components, and some quality traits of oats. In the study, various traits of oats were examined, including plant height, panicle length, number of spikelets per panicle, number of grains per panicle, grain yield, thousand-grain weight, groat percentage, ash content, protein content, starch content, β-glucan content, fat content, acid detergent fiber, and neutral detergent fiber. This study was conducted over two years during the 2019-2020 and 2020-2021 growing seasons in Bilecik, Türkiye. The experiments were established using a split-plot design, where the main plots were assigned nitrogen doses (0, 40, 80, 120, and 160 kg N ha⁻¹) and the subplots were assigned sowing densities (150, 300, 450, and 600 seeds m⁻²), with three replications. The highest grain yield was obtained at sowing densities of 300 and 450 seeds m⁻² as 4.45 t ha⁻¹ and 4.56 t ha⁻¹, respectively. Since there was no statistically significant difference in yield between these densities, a sowing density of 300 seeds m⁻² can be recommended on oat. Additionally, the highest grain yield was achieved with a nitrogen dose of 80 kg per hectare. Based on the results of this study, the combination of nitrogen dose 80 kg and sowing density 450 seeds m⁻² showed the optimal performance in terms of yield and quality. In conclusion, grain yield and quality traits in oats have shown significant differences based on the years, applied nitrogen doses, and sowing density practices.

1. INTRODUCTION

Oat belongs to the *Avena* genus of the *Poaceae* family and is classified as diploid, tetraploid, or hexaploid based on its chromosome number. Cultivated oat falls into the hexaploid group with a chromosome count of $2n=42$. Türkiye, situated within various global genetic centres, serves as a significant genetic centre for oat. It is reported that the country boasts a rich diversity of oat forms and varieties, with the origins of cultivated *Avena sativa* and *Avena byzantina* tracing back to Anatolia.

Oat ranks sixth in global cereal production, following wheat, corn, rice, barley, and sorghum (FAO, 2022). In recent years, oat production has declined in many countries due to the reduced use of oats as feed. Additionally, this decline in production has been related to higher monetary returns per hectare from other crops such as corn, soybeans, and wheat (Cınar, 2023). Furthermore, factors such as sensitivity to cold and drought, grain shedding, lodging, and asynchronous maturation also constrain oat cultivation (Mut et al., 2021a). Oat, with a history dating back two millennia, has primarily been used in animal feeding. However, in recent years, oats have become increasingly popular in human nutrition due to recognizing their benefits in food and health domains. Oat is nutritionally superior to other cereal grains (Rauf et al., 2019) and is rich in certain antioxidants and anti-carcinogenic compounds, making them highly beneficial for health (Michels et al., 2020). To achieve high grain yield and quality per unit area in oat, it is essential not only to select productive varieties resilient to biotic and abiotic stress factors suitable for the region's ecological conditions but also to improve cultivation techniques. Yield is influenced by the interaction of genetic factors, environmental conditions, and cultivation practices (Erbaş Köse, 2022).

These cultivation techniques, which affect plant yield and quality, encompass various operations from field preparation to harvest. Among these, fertilization and sowing density, which hold significant economic importance for growers, are paramount. Nitrogen is one of the most essential nutrients for oats, directly influencing plant growth, development, and yield potential. Nitrogen fertilization increases the protein content of oat grain, contributing significantly to their nutritional value. Furthermore, nitrogen applications enhance the biological productivity of oat, leading to improvements in yield components (Leghari et al., 2016). However, excessive nitrogen use can have negative effects, such as lodging, excessive leaf development, and increased susceptibility to diseases (Mut et al., 2021a). Therefore, the proper dosage and timing of nitrogen fertilization are crucial for achieving optimal yield and quality in oats. The effectiveness of nitrogen fertilization is influenced by environmental factors, sowing density, and genetic traits, highlighting the need for application strategies tailored to specific ecosystems (Ju et al., 2022). Providing the necessary nutrients in appropriate amounts and timing throughout the plant's growth period is crucial for achieving high yields. Among these nutrients, nitrogen stands out as the most essential substance in cultivation, serving as a building block for proteins, enzymes, vitamins, and hormones in plants (Leghari et al., 2016). In our country, due to insufficiently informed fertilizer use practices, there is a prevalent misconception that excessive nitrogen application can lead to higher yields. However, improper and excessive use of nitrogen by growers can be economically detrimental and unsuitable for plants. In cereals, nitrogen excess can promote lodging, while nitrogen deficiency can result in weak stem and leaf development, slowed growth, early flowering, and reduced yield and product quality (Farhan et al., 2024).

Sowing density is another factor that affects plant growth and development as well as fertilization. It holds significant economic importance for growers, as optimal plant density is vital for achieving higher grain yield per unit area than varieties under specific ecological conditions. In oats, traits such as panicle number per unit area, panicle length, grain count per panicle, and grain yield per panicle impact grain yield per unit area (Mut et al., 2018a) and these traits vary depending on sowing density (Altuner and Ulker, 2019). Sowing density is influenced by genotype, sowing time, seed size, and ecological conditions. Excessive seed use increases costs and can lead to yield losses.

To achieve high yield and good quality, it is essential to implement the most suitable cultivation techniques specific to the region where the crop is grown. Among these agricultural practices, sowing density and nitrogen fertilization are critical in oat cultivation.

The aim of this study is to assess the effects of different sowing densities and nitrogen fertilizer doses on grain yield, yield components, and some quality traits of oat.

2. MATERIALS AND METHODS

Plant materials and field experiments

The field experiment was conducted during the 2019-2020 and 2020-2021 growing seasons at the Agricultural Application and Research Center of Bilecik Seyh Edebali University of Bilecik. The study area is located between 30° 10' North latitude and 40° 11' East longitude, with an elevation of 500 meters above sea level. Kahraman oat cultivar, which was developed by the Thrace Agricultural Research Institute, was used as material in the experiment. This

cultivar was chosen because it is one of the widely cultivated oat cultivars in Türkiye and has shown good adaptability to diverse agro-ecological conditions. Its widespread use and stable performance across regions make it a suitable candidate for evaluating agronomic responses to nitrogen application and sowing density.

Sowing dates were 2 November 2019 and 12 November 2020, respectively. The experiments were established using a Split-Plot Design with three replications. This study implemented four sowing densities (150, 300, 450, 600 seeds m^{-2}) and five nitrogen doses (0, 40, 80, 120, 160 $kg\ ha^{-1}\ N$). The experiments were designed with nitrogen doses assigned to the main plots and sowing densities to the subplots. Each plot was 4 meters long, with a row spacing of 20 cm and consisted of 6 rows. Sowing was carried out by hand. Along with the sowing, all plots were fertilized with 160 kg of Triple Super Phosphate fertilizer (43-44% P_2O_5) per hectare. Potassium fertilization was not applied based on the results of soil analysis. The experimental fields were previously cultivated with wheat in the preceding season. The soil analysis indicated that the potassium levels were sufficient. The adequate presence of potassium in the soil ensures that plants can naturally obtain this nutrient from the soil. Half of the nitrogen fertilizer was applied at sowing using Ammonium Sulfate (21% N), while the other half was applied during the tillering stage using Urea (46% N). Weed control was managed during the tillering stage. Harvesting was done with a sickle on July 15, 2020, for the first year, and on July 21, 2021, for the second year. All the plots were hand harvested. One row on each side of the plots and a 50 cm section from the plot ends were excluded. The harvested material was left to dry in the plots. In order to facilitate threshing with the plot threshing machine, the plants were left to dry under sunlight for one to two weeks. Then threshed using a threshing machine.

Climate and soil characteristics of the experimental area

Figure 1 presents the climate data for the 2019-2020 and 2020-2021 growing seasons in Bilecik province, obtained from the Turkish State Meteorological Service (Bilecik Meteorological Directorate). The average temperature during both the 2019-2020 and 2020-2021 growing seasons was recorded at 11.6 °C. While the total rainfall was 482.6 mm during the 2019-2020 growing season, it decreased to 436.1 mm in the 2020-2021 seasons. Relative humidity levels were recorded as 63.0% and 60.3% for the 2019-2020 and 2020-2021 growing seasons, respectively (Figure 1). Based on the soil analysis results, the soil at the experimental site was classified as clayey-loamy (40%), with a slightly alkaline pH of 7.78, moderately calcareous (6.84%), mildly saline (0.45%), rich in phosphorus (22.16 $kg\ ha^{-1}$), high in potassium (66.90 $kg\ ha^{-1}$), and having a moderate organic matter content of 2.26%.

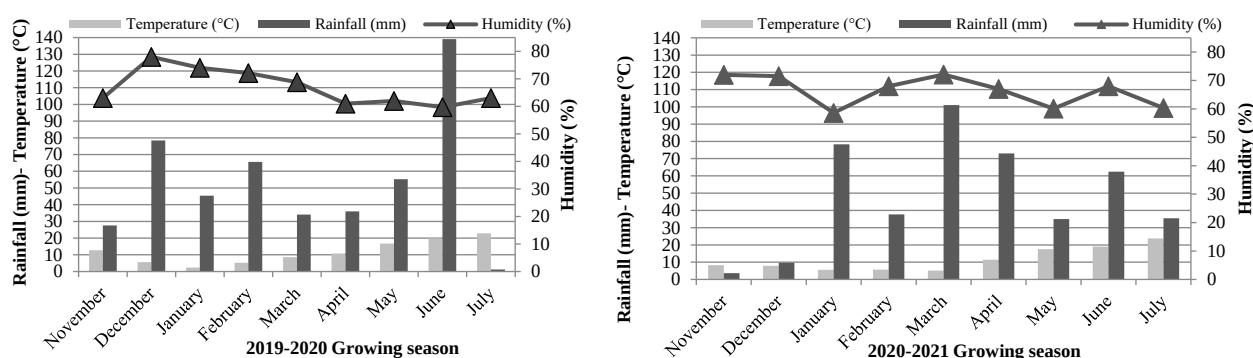


Figure 1. Meteorological conditions in the experimental areas during the 2019-2020 and 2020-2021. (In the 2019-2020 growing season, the total rainfall, average temperature, and humidity were 482.6 mm, 11.6°C, and 67.9%, respectively. In the 2020-2021 season, these values were 436.1 mm, 11.6°C, and 66.4%, respectively) (The data was obtained from Bilecik Regional Directorate of Meteorology)

Grain yield and some yield components, along with physical and chemical analyses

In this study, plant height (cm), panicle length (cm), the number of spikelet per panicle, and the number of grains per panicle were measured in 10 plants. Grain yield (GY) was calculated by converting the plot yield, determined by weighing the grains after harvest and threshing, into hectares and expressed in tons. Plant height (PH) was measured from the soil surface to the tip of the panicle. Panicle length (PL) was determined by measuring the distance in cm from the first node on the main stem to the tip of the uppermost spikelet. The number of spikelet per panicle (SPP) was recorded by counting all spikelet on each panicle. Similarly, the number of grains per panicle (KPP) was determined by counting all grains on each panicle. The thousand-grain weight (TGW) was determined by counting four sets of 100 seeds each using a seed counting device (Chopin Technologies-Numigral, France), averaging the results, and then multiplying by 10. Groat percentage (GP) was calculated by manually removing the hulls from a 20-gram sample of grains, weighing the dehulled grains, and then expressing this weight as a percentage of the total sample weight.

Oat samples designated for chemical analysis were cleaned of foreign materials and ground using a hammer mill to pass through a 0.5 mm sieve. Samples were stored in a refrigerator at +4 °C and analyzed within three months after each harvest. Ash content (AC) was determined according to AACC 08-01.01, crude protein content (PC) according to AACC 46-30.01, β -glucan content (β C) according to AACC Method 32-23.0.1 and starch content (SC) according to AACC 76-33.01 methods (AACC, 2020). Fat content (FC) was determined using the Soxhlet method (Welch, 1977), while acid detergent fiber (ADF) and neutral detergent fiber contents (NDF) were determined using the method described by Van Soest et al. (1991) with an ANKOM 220 Fiber Analyzer (ANKOM, model A2001, Macedon, NY, USA).

Statistical analysis

A normality test was applied to the data of the traits, and traits that did not exhibit a normal distribution were transformed before being subjected to variance analysis. Variance analysis for all studied characteristics was conducted using the Split-Plot Design with the MSTAT-C statistical package, based on combined data across years. Differences among the means were assessed using the Duncan's multiple comparison test.

3. RESULTS AND DISCUSSION

The total rainfall at the research site was higher in the first year (482.6 mm) compared to the second year (436.1 mm). While there were no significant differences in average temperature and humidity between the years, these values varied significantly across months (Figure 1). The combined variance analysis results of the study with four sowing densities and five different nitrogen doses are presented in Table 1.

Table 1. The mean of squares and their significance found as a result of variance analysis of the data belonging to the examined features for the combined years

Source of variation	SD	PH	PL	SPP	KPP	GY	TGW	GP
Year (Y)	1	4871.3**	43.0**	1118.7	3641.9**	16.89**	61.6**	1232.8**
Replication (R)	4	9.2	5.8	14.7	17.5	4.77	15.3	64.0
Nitrogen doses (ND)	4	188.9**	5.7	435.3**	1872.2**	25.34**	6.1	16.5
Y $\times$ ND	4	59.7	1.9	126.4**	670.2*	1.69*	14.6*	15.9
Error ₁	16	31.3	2.6	13.0	14.4	0.68	3.8	7.4
Sowing density	3	80.3**	5.1**	134.4**	583.3**	7.06**	98.3**	39.2**
Y $\times$ SD	3	8.2	2.9*	8.1	62.5*	4.72**	11.0*	7.6
ND $\times$ SD	12	20.2	2.8**	30.0**	51.8**	3.64**	9.7**	12.3
Y $\times$ ND $\times$ SD	12	20.4	1.2	28.0**	44.3*	3.14**	2.8	15.1
Error ₂	60	12.8	1.1	6.7	20.2	0.95	3.4	12.0
Coefficient of Variation (%)		3.4	5.1	6.6	8.6	7.0	4.2	4.3

Source of variation	SD	AC	PC	SC	β G	FC	ADF	NDF
Year (Y)	1	15.130**	41.37**	5307.0**	66.77**	0.18	354.1**	1104.0**
Replication (R)	4	0.007	0.26	4.9	0.02	0.04	2.4	15.6
Nitrogen doses (ND)	4	0.008	3.93**	18.6*	0.36**	0.13	2.6	4.0
Y $\times$ ND	4	0.059**	1.37**	25.8**	0.21*	0.34	2.7	6.3
Error ₁	16	0.004	0.23	5.1	0.05	0.20	0.9	3.6
Sowing density	3	0.202**	0.05*	138.7**	0.89**	0.49**	23.5	8.4
Y $\times$ SD	3	0.001	2.93**	9.0	0.01	0.76**	0.8	4.0
ND $\times$ SD	12	0.008	0.28	3.1	0.04	0.15	1.0	4.7
Y $\times$ ND $\times$ SD	12	0.011	0.45*	4.8	0.03	0.19	0.7	6.8
Error ₂	60	0.006	0.22	7.9	0.08	0.12	0.6	3.3
Coefficient of Variation (%)		3.65	3.16	5.7	6.11	6.86	6.1	5.6

**Significant at $P < 0.01$, *Significant at $P < 0.05$, ND: Nitrogen doses, SD: Sowing density, PH: Plant height (cm), PL: Panicle length (cm), SPP: Number of spikelet per panicle, KPP: Number of kernel per panicle, GY: Grain yield ($t\ ha^{-1}$), TGW: Thousand-grain weight (g), GP: Groat percentage (%), AC: Ash content (%), PC: Protein content (%), SC: Starch content (%), β C: β -glucan content (%), ADF: Acid detergent fiber (%), NDF: Neutral detergent fiber (%)

Statistically significant differences were found between the years in terms of traits studied, excluding SSP and FC. Significant differences were identified among nitrogen doses for the traits plant height, number of spikelet per panicle, number of grains per panicle, grain yield, protein content, starch content, and β -glucan. Regarding sowing densities,

significant differences were observed for all traits studied except NDF and NDF value (Table 2 and Table 3). The nitrogen dose (ND) $\times$ sowing density (SD) interactions are presented in Table 4 and Table 5. It was determined that the ND $\times$ SD interaction was not significant for quality traits (Table 5).

Table 2. The mean values of grain yield and yield components for sowing density and nitrogen doses in the combined years

	PH	PL	SPP	KPP	GY	TGW	GP
Sowing density							
150	104.26 c	21.09 a	33.95 a	54.94 a	4.20 c	42.11 b	78.49 b
300	106.01 bc	20.59 ab	33.03 a	54.45 a	4.45ab	44.47 a	78.84 ab
450	106.66 ab	20.46 b	33.13 a	54.42 a	4.56 a	41.46 b	79.57 ab
600	108.21 a	20.09 b	29.22 b	45.80 b	4.36 b	40.14 c	81.07 a
Nitrogen dose							
N ₀	102.16 c	19.74	27.73 c	41.24 d	4.16 c	41.82	79.72
N ₄₀	105.58 b	20.86	28.48 c	45.06 c	4.64 b	42.94	79.83
N ₈₀	106.57 ab	20.65	36.32 a	60.33 a	4.83 a	41.87	79.50
N ₁₂₀	109.89 a	20.55	36.88 a	60.42 a	4.25 c	41.80	80.69
N ₁₆₀	107.22 ab	20.98	32.27 b	54.94 b	4.08 c	41.78	78.71
Year							
2019-2020	112.66 a	21.16 a	35.39	57.91 a	4.51 a	42.76 a	82.70 a
2020-2021	99.91 b	19.96 b	29.28	46.89 b	4.27 b	41.33 b	76.29 b

The values followed by common letters at each column are not significant at $P>0.05$ level of probability using the Duncan test.

Plant height

Plant height was higher in the first year (112.66 cm) than in the second year (99.91 cm). This discrepancy is attributed to the higher rainfall in the first year (482.6 mm) than in the second year (436.1 mm) (Figure 1). The tallest plant height was determined at a seeding density of 600 seed m² (108.21 cm), while the shortest plant height was recorded at a seeding density of 150 seed m² (104.26 cm). In terms of nitrogen doses, the tallest plant height was achieved with the N₁₂₀ treatment (109.89 cm), whereas the shortest height was observed in the N₀ treatment (102.16 cm) (Table 2). Plant height is influenced by various environmental factors including water availability, temperature, nutrient levels, soil properties, sunlight, and genetic factors (Buerstmayr et al., 2007). Furthermore, our findings indicate that plant height increased with higher nitrogen doses and sowing densities. This relationship may be explained by increased plant competition at higher sowing densities and enhanced vegetative growth due to nitrogen fertilization. Altuner and Ulker (2019) reported in their study on oat varieties that agricultural practices, such as fertilization and sowing density, influence plant height.

Table 3. The mean values of quality traits for sowing density and nitrogen doses in the combined years

	AC	PC	SC	β G	FC	ADF	NDF
Sowing density							
150	2.05 d	15.26 a	49.04 a	4.32 c	4.97 ab	14.19	32.74
300	2.10 c	15.02 ab	47.66 a	4.44 bc	4.78 b	13.65	32.68
450	2.16 b	14.79 b	46.16 b	4.51 b	4.95 ab	12.64	32.11
600	2.24 a	14.54b	44.02 c	4.73 a	5.10 a	12.28	31.61
nitrogen dose							
N ₀	2.15	14.45 c	47.08 a	4.32 c	4.99	13.46	32.89
N ₄₀	2.11	14.76 b	47.88 a	4.48 b	5.02	13.18	31.99
N ₈₀	2.15	14.95 b	46.59 ab	4.53 ab	4.83	13.45	32.27
N ₁₂₀	2.14	15.55 a	46.62 ab	4.66 a	4.97	12.65	31.85
N ₁₆₀	2.14	14.81 b	45.45 b	4.50 b	4.94	13.23	32.43
Year							
2019-2020	1.78 b	14.32 b	50.87 a	3.75 b	4.91	14.91 a	35.32 a
2020-2021	2.49 a	15.49 a	42.57 b	5.24 a	4.99	11.47 b	29.25 b

The values followed by common letters at each column are not significant at $P>0.05$ level of probability using the Duncan test.

Panicle length

The panicle length was 21.16 cm in the first year, while it decreased to 19.96 cm in the second year. No statistically significant differences were observed in panicle length among the nitrogen doses. Jaipal and Shekhawat (2016) demonstrated that genetic factors play an important role in determining panicle length. This finding suggests that while environmental factors, such as nitrogen fertilization, can influence plant growth, genetic variation is a key determinant of this trait. The study highlights the importance of genetic diversity in shaping panicle length, emphasizing that it is a primary factor in its expression, independent of external inputs such as fertilizers. The longest panicle length of 21.09 cm was obtained at a sowing density of 150 seed m², while the shortest of 20.09 cm was at 600 seed m². Regarding panicle length, sowing densities of 150 and 300 seed m² were statistically classified in the same group. In the ND × SD interaction, the longest panicle length of 22.02 cm was achieved with a sowing density of 150 seed m² and an application of 80 kg of nitrogen per hectare. The shortest panicle length of 19.03 cm was observed at the same sowing density with no nitrogen applied (N₀). Although panicle length does not directly affect yield, it is an indirect factor (Table 4). Kaziu et al. (2019) reported that cultivars with longer panicles tend to have higher grain yields, with panicle lengths ranging from 26.0 to 37.0 cm. Mut et al. (2021b) conducted a study on 255 oat genotypes, revealing that panicle length (ranging from 21.27 to 37.70 cm) is influenced by both genotype and year.

Table 4. The mean data for the sowing density and nitrogen dose interactions of grain yield and yield components over the combined years

ND	SD	PH	PL	SPP	KPP	GY	TGW	GP
N ₀	150	97.29	19.03e	27.57e	42.93ef	4.11fgh	42.22bcd	76.38
	300	102.56	20.11b-e	28.87de	44.35ef	4.12fgh	43.16bcd	76.78
	450	101.89	20.18b-e	28.13de	41.44fg	4.24e-h	40.84d-g	80.55
	600	106.89	19.65de	26.37e	36.26g	4.16 fgh	40.07fg	81.19
N ₄₀	150	104.67	20.96a-d	29.30de	46.19ef	4.20fgh	41.27c-g	78.67
	300	105.72	21.03a-d	29.40de	45.36ef	4.61cde	46.45a	80.57
	450	104.72	20.23b-e	29.30de	48.13de	4.95abc	42.27c-f	77.96
	600	107.22	21.22abc	25.90e	40.58fg	4.80a-d	41.79c-g	82.16
N ₈₀	150	105.39	22.02a	39.27a	59.35ab	4.51def	40.29rf	78.68
	300	104.22	20.28b-e	34.97b	61.02ab	4.68bcd	44.88ab	79.26
	450	110.00	20.31b-e	34.33b	64.13a	5.09a	42.63b-e	78.23
	600	106.67	20.00cde	36.70ab	56.82bc	5.04ab	39.69g	81.84
N ₁₂₀	150	108.56	21.57ab	39.33a	64.40a	4.02gh	43.41bc	81.41
	300	110.28	20.85a-d	37.23ab	62.95a	4.39d-g	42.85bcd	79.12
	450	109.89	20.20b-e	39.87a	61.66ab	4.62cde	41.42c-g	81.29
	600	110.84	19.58de	31.07cd	52.68cd	3.98gh	39.52g	80.95
N ₁₆₀	150	105.39	21.87a	34.30b	61.81ab	4.15fgh	42.36cc-f	77.33
	300	107.28	20.67a-d	34.70b	58.58ab	4.45def	45.00ab	78.45
	450	106.78	21.37abc	34.00bc	56.73bc	3.91h	40.14fg	79.85
	600	109.45	20.02cde	26.07e	42.63ef	3.83h	39.64g	79.22

The values followed by common letters at each column are not significant at P>0.05 level of probability using the Duncan test.

Number of spikelet per panicle

In the first year, the number of spikelets per panicle was significantly higher at 35.39 no, compared to 29.28 no in the second year. The lowest number of spikelets per panicle was observed with the N₀ treatment (27.73 no), while the highest was with the N₁₂₀ dose (36.88 no). Statistical analysis indicated that the N₈₀ and N₁₂₀ nitrogen doses were in the same group regarding spikelet numbers per panicle. The highest number of spikelets per panicle, 33.95 no, was obtained at a sowing density of 150 seeds m², while the lowest, 29.22 no, was at 600 seeds m². For spikelet numbers, the sowing densities of 150, 300, and 450 seed per m² were statistically grouped together (Table 2). In the ND × SD interaction, the maximum number of spikelets per panicle of 39.87 no, was achieved with N₁₂₀ dose and a sowing density of 450 seed per m² (Table 4). In oats, the number of spikelets per panicle is one of the important selection criteria for improving grain yield. Doehlert et al. (2001) reported that the number of spikelets per panicle is influenced by both variety and environmental factors. Mut et al. (2021b) found that in their two-year study with 255 oat genotypes, the number of spikelets per panicle varied depending on the year and genotype. In our study, it is thought that the high number of spikelets per panicle in the first year was due to the greater total rainfall during the growing season of that year. However, in the experimental field, the first year experienced higher winter rainfall, while spring rainfall was lower. This could influence flowering, as winter rainfall supports early vegetative growth, whereas spring rainfall is crucial for reproductive development. Therefore, the higher spikelet number observed in the first year may be attributed to

favorable winter conditions, while the limited spring rainfall could have impacted flowering and yield development (Buerstmayr et al., 2007).

Table 5. The mean data for the sowing density and nitrogen dose interactions of quality traits over the combined years

ND	SD	AC	PC	SC	βG	FC	ADF	NDF
N ₀	150	2.04	14.31	50.08	4.24	4.93	14.96	33.48
	300	2.06	14.49	48.75	4.26	4.74	14.20	33.50
	450	2.21	14.59	46.23	4.34	5.11	12.54	31.38
	600	2.29	14.41	43.25	4.44	5.18	12.10	30.72
N ₄₀	150	2.04	14.99	49.51	4.36	4.91	14.14	32.41
	300	2.06	14.92	49.26	4.45	4.92	13.20	32.14
	450	2.11	14.63	47.77	4.44	5.10	12.41	32.08
	600	2.23	14.51	44.98	4.68	5.15	12.96	31.34
N ₈₀	150	2.02	15.53	49.09	4.30	4.82	14.53	34.30
	300	2.15	14.98	47.27	4.45	4.67	14.14	32.81
	450	2.19	14.85	46.15	4.56	4.73	13.00	33.45
	600	2.25	14.43	43.87	4.83	5.10	12.15	31.02
N ₁₂₀	150	2.08	16.02	48.38	4.31	4.98	13.32	31.51
	300	2.15	15.90	47.28	4.63	5.04	12.84	32.06
	450	2.15	15.23	45.62	4.70	4.80	12.40	31.68
	600	2.20	15.06	45.22	5.00	5.08	12.03	32.15
N ₁₆₀	150	2.08	15.47	48.16	4.38	5.21	14.01	32.03
	300	2.10	14.83	45.80	4.43	4.55	13.89	32.88
	450	2.14	14.65	45.06	4.49	5.01	12.84	31.98
	600	2.25	14.30	42.80	4.69	4.98	12.16	32.85

The values followed by common letters at each column are not significant at $P>0.05$ level of probability using the Duncan test.

Number of kernel per panicle

The number of kernels per panicle was 57.91 in the first year, while it decreased to 46.89 in the second year. The number of seeds per panicle ranged from a minimum of 41.24 under the N₀ treatment to a maximum of 60.42 under the N₁₂₀ dose. In terms of the number of kernels per panicle, the 80 kg and 120 kg nitrogen doses per hectare were statistically in the same group. The number of kernels per panicle was highest at 54.94 with a sowing density of 150 seeds m², and lowest at 45.80 seeds with a sowing density of 600 seeds m². Regarding the number of kernels per panicle, the sowing densities of 150, 300, and 450 seeds m² were statistically in the same group (Table 2). In the ND × SD interaction, the number of kernels per panicle was highest at 64.40 seeds with the N₁₂₀ dose and a sowing density of 150 seeds m², and lowest at 36.26 seeds with the N₀ treatment and a sowing density of 600 seeds m² (Table 4). Among the factors influencing yield in oats, the number of kernels per panicle is one of the most critical criteria. It is known that this trait has a key role in indirect selection in breeding programs. Kaziu et al. (2019) highlighted that the number of kernels per panicle is a vital criterion for oat production and that it is affected by different years and varieties. Our study also found significant differences between years, with these variations attributed to changes in environmental conditions. The number of kernels per panicle increased up to a nitrogen dose of 120 kg per hectare and then decreased (Table 2). Up to a certain level, nitrogen promotes vegetative growth and reproductive development, enhancing processes such as photosynthesis and nutrient allocation to seed formation. This leads to an increase in the number of kernels per panicle. Excess nitrogen often promotes vegetative growth, diverting nutrition resources away from reproductive structures, such as seeds. Altuner and Ulker (2019) reported that nitrogen doses did not affect the number of kernels per panicle in oat varieties, whereas Pecio and Bichoński (2010) observed that the number of kernels per panicle increased with higher nitrogen doses.

Grain yield

The grain yield was 4.51 t ha⁻¹ in the first year, while it decreased to 4.27 t ha⁻¹ in the second year. The lowest grain yield was obtained with the N₁₆₀ nitrogen dose at 4.08 tons ha⁻¹, while the highest was with the N₈₀ nitrogen dose at 4.83 t ha⁻¹. The highest grain yield was achieved at 4.56 t ha⁻¹ with a sowing density of 450 seeds per m², and the lowest at 4.20 t ha⁻¹ with a sowing density of 150 seeds per m². In terms of grain yield, the sowing densities of 300 and 450 seeds per m² were statistically in the same group. In the ND × SD interaction, the highest grain yield was 5.09 t ha⁻¹ with the N₈₀ dose and a sowing density of 450 seeds per m², while the lowest was 3.83 t ha⁻¹ with the N₁₆₀ nitrogen dose and a sowing density of 600 seeds per m² (Table 2). Grain yield, one of the most complex inherited agronomic trait, is influenced by numerous factors. Studies have shown that grain yield is affected by genetic differences (Kahraman et al., 2021), environmental factors (Kebede et al., 2023), both genetic differences and environmental factors (Buerstmayr et

al., 2007), as well as agricultural practices (Mut et al., 2021a; Altuner and Ulker, 2019). In our study, it is believed that the variations in grain yield across years are primarily due to environmental factors, especially total rainfall during the growing season. The second year experienced a higher amount of rainfall during the spring. As a result, the higher total winter rainfall in the first year likely contributed more significantly to tillering and vegetative growth, providing a more substantial water supply. This suggests that winter precipitation plays a critical role in supporting early plant development, which is essential for optimal growth and productivity in cereals such as oats. In their study on oats, Pecio and Bichoński (2010) reported that grain yield varied based on nitrogen fertilizer doses. May et al. (2020) conducted a study to determine the effects of nitrogen dose and fungicide applications on oat yield and quality traits. They tested eight different nitrogen doses ranging from 0 to 140 kg per hectare and reported that grain yield increased up to the highest nitrogen rate. Our study found that grain yield increased by applying an 80 kg nitrogen dose per hectare, but subsequently declined with higher doses (Table 2). Moderate nitrogen levels enhance photosynthetic capacity and biomass accumulation, thereby supporting grain filling. However, higher doses may lead to excessive vegetative growth, causing nutrients to be allocated to leaves and stems rather than to grains. In other words, in this study, excessive nitrogen is thought to negatively affect flowering and grain filling. A high sowing density in oats does not always result in increased grain yield due to the potential reduction in the number of grains per panicle and individual grain weight. Excessive tillering or sowing density can lead to a decrease in yield components (Ju et al., 2020). For oats, specific environmental factors, such as the timing and amount of rainfall, play a significant role in determining how sowing density affects yield. These factors are critical for optimizing growth, as winter rainfall often enhances early vegetative growth, while spring rains are essential for reproductive development.

Thousand-grain weight and Groat percentage

It was determined that the thousand-grain weight was higher in the first year (42.76 g) compared to the second year (41.33 g). Nitrogen doses did not have an effect on the thousand-grain weight. The highest thousand-grain weight of 44.47 g was obtained at a sowing density of 300 seeds per m², while the lowest, 40.14 g, was observed at 600 seeds per m². In the ND × SD interaction, the thousand-grain weight was highest at 46.45 g with the N₄₀ dose at a sowing density of 300 seeds per m², and lowest at 39.52 g with the N₁₂₀ dose at a sowing density of 600 seeds per m² (Table 4). It was observed that the groat percentage was higher in the first year (82.70%) compared to the second year (76.26%). Nitrogen doses did not affect the groat percentage. In the study by Peltonen-Sainio (1997), it was reported that while nitrogen applications improved overall yield and other yield components, no significant change was observed in the groat percentage. The highest groat percentages of 81.07% was achieved at a sowing density of 600 seeds per m², while the lowest, 78.49%, was recorded at a sowing density of 150 seeds per m² (Table 2). Thousand-grain weight and groat percentage, which define grain size and nutritional value and are critical physical quality criteria, are considered varietal traits. However, these characteristics can vary significantly depending on the year and climatic factors (Mut et al., 2018b). In our study, the total rainfall in the first year was greater than that in the second year (Figure 1). Consequently, it was determined that the grains developed better in the first year, and these characteristics were higher compared to the second year (Table 2). Kaziu et al. (2019) reported that thousand-grain weight varies based on genetic factors, agro-ecological conditions, and agricultural practices, and identified it as a key factor for in defining high production potential in varieties. Mohr et al. (2003) found that thousand-grain weight decreases with increasing nitrogen doses, whereas Maral et al. (2013) reported that it increases with higher nitrogen doses. In contrast, Altuner and Ulker (2019) observed that thousand-grain weight was unaffected by nitrogen doses. Groat percentage represents the economic yields for millers and the digestible portion of the grain for animal husbandry. Research on oats suggests that higher sowing densities can impact grain development by increasing competition among plants. This can reduce grain formation within the panicles, potentially lowering the thousand-grain weight. Feng et al. (2024) found that higher sowing densities can influence the accumulation of dry matter and grain development, leading to a decrease in the individual grain weight. Oats with a low hull ratio are preferred in both the food industry and animal feeding. Additionally, the food industry favors oat grains that offer high efficiency and are easy to dehull (Doehlert et al., 2001). Previous studies have reported that the groat percentage of oat genotypes ranges from 56.30% to 81.10% (Buerstmayr et al., 2007; Mut et al., 2018b).

Ash content

The ash content was observed to be 1.78% in the first year and 2.49% in the second year. No significant differences were found among nitrogen levels regarding ash content, which varied between 2.11% and 2.15%. The highest ash content, at 2.24%, was observed at a sowing density of 600 seeds m², while the lowest, at 2.05%, was recorded at a sowing density of 150 seed m². A high mineral content in food is desirable due to its nutritional value. The ash content, which reflects the total accumulation of minerals in the grain, has been reported to be influenced by both genotype and environmental factors (Mut et al., 2018b). Ash content in seeds increases under drought conditions. In our study, since the total rainfall in the second year was lower (Table 1), it is thought that the ash content was also higher in the second year (Table 2). Furthermore, ash content is influenced not only by genetic and environmental factors but also by cultivation techniques. As the density of sowing increases, the plants enter into competition with one another, resulting in the formation of weaker grains within the panicle. Consequently, smaller grains tend to have a higher husk

percentage and a lower groat percentage, which may explain the increase in ash content with rising sowing density. Previous studies have reported ash content ranging from 1.73 to 2.90% (Erbaş Kose et al., 2021) and from 2.60 to 3.90% (Sandhu et al., 2017).

Protein content

The protein content was observed to be 14.32% in the first year and 15.49% in the second year. The highest protein content (15.55%) was recorded with an application of 120 kg nitrogen per hectare, while the lowest (14.45%) was observed in the untreated control group. Regarding sowing density treatments, the highest protein content (15.26%) was obtained at a density of 150 plants m², and the lowest (14.54%) at a density of 600 plants m² (Table 3). Protein content is a critical quality parameter for oat grains. Protein levels are influenced by both environmental and genotypic factors. The lower average protein content observed during the 2019-2020 growing season (Table 3) is likely due to the fact that cereals grown under high rainfall or irrigated conditions tend to have lower protein content. Rainfall in the growing regions, its monthly distribution, temperatures, and cultural practices all impact the protein content and quality of cereal grains (Buerstmayr et al., 2007). In our study, protein content in oat grains increased up to a nitrogen dose of 120 kg per hectare, after which a slight decrease was observed (Table 2). Nitrogen is a crucial element for protein synthesis, and at optimal levels, its applications can typically enhance protein content. However, the effects of excessive nitrogen are more complex. High nitrogen levels can disrupt plant metabolism, causing an imbalance in amino acid composition, which may reduce the efficiency of protein synthesis. Furthermore, excessive nitrogen can promote vegetative growth during critical phases such as flowering and grain filling, causing imbalances in growth and maturation processes. These disruptions are believed to negatively affect protein content. Consistent with our findings, Zhou et al. (1998) reported that the protein content in cereals increased with increasing sowing density.

Starch content

The starch content was observed to be 55.87% in the first year and 42.57% in the second year. Regarding nitrogen fertilization, the highest starch content (47.95%) was achieved with a nitrogen dose of 40 kg ha⁻¹, while the lowest (45.45%) was recorded with 160 kg ha⁻¹. All nitrogen treatments, except for the 160 kg ha⁻¹ dose, were statistically in the same group. The highest starch content (49.04%) was observed at a sowing density of 150 seeds m², and statistically, the starch contents at 150 and 300 seeds m² were in the same group. The lowest starch content (44.02%) was recorded at a sowing density of 600 seeds m² (Table 3). Starch, composed of amylose and amylopectin, is found in the endosperm of oat grains, which is surrounded by bran layers rich in β -glucan and protein. Since starch is the primary digestible carbohydrate in plants, it serves as a major energy source in both human and animal diets. Doeblert et al. (2001) reported that environmental factors have a much greater influence on starch content. In our study, the higher starch content observed during the 2019-2020 growing season (Table 3) is likely due to the increased total rainfall during that season (Figure 1). Mut et al. (2018b) noted that starch content is affected by both environmental and genetic factors, with values ranging from 42.7% to 49.6%. Previous study has reported starch content ranging from 45.7% to 46.3% (Brunava et al., 2014). In our study, starch content decreased with increasing nitrogen levels (Table 3). Similarly, Sterna et al. (2015) found that starch content in oat genotypes decreased with increasing nitrogen fertilizer doses. Furthermore, in our study, the decrease in starch content with increasing sowing density was likely due to the corresponding reduction in thousand-grain weight (Table 2).

β -glucan content

The starch content was observed to be 3.75% in the first year and 5.24% in the second year. The highest β -glucan content (4.73%) was obtained at a sowing density of 600 seeds m², while the lowest (4.32%) was recorded at a density of 150 seeds m². Regarding nitrogen fertilization, the highest β -glucan content (4.66%) was achieved with a nitrogen dose of 120 kg ha⁻¹, while the lowest (4.32%) was observed in the untreated control group (Table 3). Beta-glucan, which is present in significant amounts in oat grains, is a type of soluble dietary fiber important for human health. β -glucans are widely utilized in the cosmetics, food, and pharmaceutical industries. Consequently, oat varieties intended for human and animal nutrition are expected to have high beta-glucan content. Previous studies have reported that β -glucan content is influenced by genotype (Doeblert et al., 2001), environmental conditions (Mut et al., 2018b), and agricultural practices. In our study, conducted during the 2020-2021 growing season with low total rainfall (Figure 1), it is thought that the high β -glucan content is associated with a decrease in thousand-grain weight and an increase in husk percentage. Soil nitrogen levels and nitrogen fertilization are also considered as key factors affecting beta-glucan content. Fan et al. (2009) demonstrated that nitrogen application significantly enhanced the β -glucan content in oats. Mantai et al. (2016) highlighted that nitrogen fertilization improves the grain quality and increases the β -glucan content, which is an essential factor for the nutritional quality of oats. In our research, β -glucan content increased up to a nitrogen dose of 120 kg ha⁻¹, after which it slightly decreased (Table 2). Other studies have reported β -glucan content ranging from 1.02 to 6.33% (Rauf et al., 2019) and 1.27 to 3.48% (Erbaş Kose et al., 2021).

Fat content

The fat content was determined to be 4.91% in the first year and 4.99% in the second year. No statistically significant differences were observed in fat content across nitrogen fertilizer doses, which ranged from 4.83% to 5.02%. Some studies have shown that nitrogen application has little or no effect on the fat content of cereals. Devi et al. (2019) reported that while nitrogen doses increased protein content in oat, they generally had no significant impact on fat content. This may be because nitrogen is primarily utilized for protein synthesis, contributing less to fat synthesis. The highest fat content, 5.10%, was obtained at a sowing density of 600 seeds m², and the sowing densities of 150, 450 and 600 seeds m² were statistically grouped together in terms of fat content. The lowest fat content, 4.78%, was recorded at a sowing density of 300 seeds m² (Table 3). Fat content is largely determined by genetic factors, and cultivation practices such as nitrogen application or sowing density appear to have limited influence on this trait. Sowing density may indirectly affect fat content by influencing competition among plants for light, water, and nutrients (Zhang et al., 2023). The nutritional value of oats is primarily attributed to their high fat content. Carlson et al. (2019) reported that, in terms of fat content, oat grains are particularly rich in healthy unsaturated fatty acids compared to other cereals. However, breeders typically prefer low-fat genotypes for oats intended for human consumption (Mut et al., 2018b). The fat content of oats is influenced by both genetic and environmental factors. In a study examining the effects of different nitrogen doses on the chemical composition of oats, Pecio and Bichoński (2010) found that nitrogen doses had no statistically significant effect on fat content. Other studies have reported varying fat contents among oat genotypes: Martinez et al. (2010) recorded a range of 3.1 to 11.6%, Erbas Kose et al. (2020) reported values between 5.03 and 6.88%, and Erbas Kose et al. (2021) observed a range of 2.71 to 7.16%. Additionally, our study determined that fat content increased slightly with higher sowing densities. Similarly, Podolska et al. (2009) reported that increasing sowing densities enhanced fat content of oats.

Acid detergent fiber and Neutral detergent fiber

It was determined that both ADF and NDF values were higher in the first year than the second year. No statistically significant differences in ADF and NDF values were found among nitrogen doses and sowing densities with respect to ADF and NDF values. Based on sowing densities, ADF values ranged from 12.28% (600 seeds m²) to 14.19% (150 seeds m²), while NDF values ranged from 31.61% (600 seeds m²) to 32.74% (150 seeds m²). According to nitrogen doses, ADF and NDF values varied between 12.65% (N₁₂₀) and 13.46% (N₀), and 31.85% (N₁₂₀) and 32.89% (N₀), respectively (Table 3). In our study, neither sowing density nor nitrogen dose had a significant effect on ADF and NDF values in oats. This finding suggests that, within the range of nitrogen levels and sowing densities tested, these factors did not have a major impact on the fiber content of the oats. This could be attributed to the inherent genetic traits of oats, which may not be as sensitive to changes in these cultivation practices as other crops. Similar results were reported in previous studies, where changes in nitrogen application and plant density had minimal effects on fiber content in oats (Peltonen-Sainio et al., 2009). It is important to note that fiber content in oats may be more strongly influenced by genetic factors rather than agronomic practices such as nitrogen fertilization or sowing density. This aligns with findings from other research that suggests environmental and genetic factors play a more significant role in determining fiber composition (Mut et al., 2022; Ju et al., 2022). Our results also highlight that factors like genotype and environmental conditions may have a greater influence on fiber content than the specific agronomic practices applied in the study. Similarly, in another study involving different oat cultivars, the average ADF and NDF values were reported as 15.25% and 32.60%, respectively (Erbaş Kose et al., 2020).

4. CONCLUSION

Providing the necessary nutrients in appropriate amounts and at the correct time during the growing period, along with maintaining optimal plant density, is essential for achieving high yields. Fertilization and plant density are not only economically important for growers but are also critical for maximizing grain yield per unit area under specific ecological conditions. Based on the results of this two-year study conducted to determine the effects of different nitrogen doses and sowing densities on grain yield, yield components, and certain quality traits in oats, significant differences were found between the years, nitrogen doses, and sowing densities for many of the traits examined.

The highest grain yield was obtained at sowing densities of 300 and 450 seeds m², and since no statistically significant difference was observed between these densities, a sowing density of 300 seeds m² can be recommended. Additionally, a nitrogen dose of 80 kg ha⁻¹ was found to be sufficient for oat cultivation under these conditions. At the same time, the study demonstrated that oat grain quality, including thousand-grain weight, groat percentage, protein, starch, β -glucan, and ash content, is influenced by environmental factors such as rainfall and agronomic practices like nitrogen fertilization and sowing density. Higher rainfall in the first year resulted in better grain development and higher quality parameters. Nitrogen fertilization positively impacted protein and β -glucan content, while higher sowing densities generally reduced thousand-grain weight. These findings highlight the importance of considering local environmental conditions and management practices to optimize oat grain quality for various uses. Both fertilization

and sowing density are economically important factors. By optimizing these factors—sowing density and nitrogen application—farmers can achieve high yields without overinvesting in inputs, thereby ensuring both ecological and economic sustainability.

ACKNOWLEDGEMENT

This research was supported by the Bilecik Şeyh Edebali University Scientific Research Projects Coordination (project no. 2021-01.BŞEÜ.01-07). This manuscript is derived from Tuğçe Karakuzu master's thesis.

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A Flow Cytometry-Based Assessment of the Nuclear DNA Content and Ploidy Level of Wild Sunflower Species

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ARTICLE INFO

Research Article

Received: 4 March 2025

Accepted: 15 May 2025

Published: 23 June 2025

Keywords:

Flow cytometry

Helianthus

Nuclear DNA content

Ploidy

Wild sunflowers

ABSTRACT

Sunflower genus (*Helianthus* spp.), native to North America, includes 37 perennials and 14 annual species, many of which possess valuable genes related to biotic and abiotic stress tolerance that can enhance yield and yield-related traits in cultivated sunflower. However, it is crucial to characterize wild species to incorporate these beneficial traits into breeding programs. The study aim was to determine the nuclear DNA content of 133 sunflower accessions representing 52 species of the genus by flow cytometer and use the obtained information to determine their ploidy levels and verify their taxonomic identities. Based on flow cytometrical analysis, nuclear DNA content of the individual plants and the average values of accessions of the same species generally showed a high degree of similarity, except in some cases. However, the average 2C nuclear DNA content of *Helianthus* species was estimated to range from 5.61 pg (*H. porteri*) to 27.05 pg (*H. tuberosus*). The differences among the species were statistically significant. Based on the study results, it was determined that approximately 80% (41 species) of the wild species (15 annuals, 26 perennials) are diploid. The ploidy level of the remaining species exhibited variability, and 12 species were tetraploid (4x) while 2 species were hexaploid (6x). The study results indicated that some accessions of some species were either identified incorrectly or they are progenies of natural interspecific hybrids. The study's results confirm that flow cytometer can be used to characterize the sunflower genetic resources before including them in breeding programs.

Citation: Kaya, Y., Savas Tuna, G., Beser, N., & Tuna M. (2025). A Flow Cytometry-Based Assessment of the Nuclear DNA Content and Ploidy Level of Wild Sunflower Species. *Turkish Journal of Field Crops*, 30(1), 110-120. <https://doi.org/10.17557/tjfc.1650798>

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) ($2n=34$), a member of the genus *Helianthus* included in the Compositae family, is one of the most important crops grown mainly for its highly quality edible oil (Gül and Coban, 2020). The genus consists of 52 species (14 annual and 37 perennial) that contributed many desirable characteristics to cultivated sunflower (Kaya, 2014; 2016).

Due to their importance in sunflower breeding, wild sunflower species have been collected from the geographic areas where they are growing naturally through expedition trips for over 60 years and preserved in gene banks. Approximately 2500 accessions of wild sunflower species are currently stored in the USDA-ARS collection, the most complete collection in the world (Seiler and Gulya, 2004). However, it is well known that collection of genetic resources of the crop species are quite challenging due to difficulties in identification of the species in the nature when they are growing together with their relatives (Vižintin and Bohanec, 2008). Same is true for wild sunflower species since their identification is problematic especially in the field, due mainly to natural variation and interspecific hybridization. Ploidy is another important factor contributing to the complexity of the species classification as several ploidy series exists in the *Helianthus* genus (Seiler and Gulya, 2004; Natali et al., 2008; Makarenko et al., 2020). Therefore, taxonomic identity and ploidy of the sunflower gene bank accessions should be confirmed before they were included in the breeding programs. However, at the species level, reliable identification may require years of training and specialization on one plant taxon. On the other hand, the classical ploidy analysis method of counting chromosomes using a light microscope is time-consuming, requires skill and experience, and highly depends on rapidly dividing cells (Brummer et al., 1999; Tuna et al., 2001, 2004). Getting freshly growing root tips with rapidly dividing cells suitable for cytological preparations were also big problem in the current study. Additionally, the method is inadequate when many samples needed to be analyzed, as in the case of this study (Tuna et al., 2001).

Since chromosomes are primarily composed of nucleic acids and are in the cell nucleus, there is a strong positive correlation between ploidy level and nuclear DNA content. Due to this correlation, nuclear DNA content values can be used to estimate ploidy level. Nuclear DNA content in plants was previously determined by Feulgen microspectrophotometry of root tip or shoot tip mitotic cells (Bennett and Smith, 1976). In recent years, flow cytometry has become the preferred technique for estimating the nuclear DNA content because of its ease, quickness, and accuracy (Rayburn et al., 1989; Heslop-Harrison, 1995; Mabuza et al., 2023).

Carefully determined nuclear DNA content by flow cytometry remains generally constant among all individuals of a species and across all cells of an individual, while it can vary up to 10,000-fold among the species. Therefore, nuclear DNA content is specific to species information. This characteristic of the nuclear DNA content makes it an essential information to use in the characterization of species' genomes, investigating their relations and evolutions (Vogel et al., 1999; Tuna et al., 2001; Ozkan et al., 2003; Leitch and Bennett, 2004; Tuna et al., 2005; 2006; Ozkan et al., 2006). Hence, it is widely utilized for the taxonomic identification, classification, and determination of ploidy levels of species (Dolezel, 1997; Ohri, 1998; Lu et al., 1998. Baack et al., 2005; Tuna et al., 2004; Savas Tuna et al., 2016; 2017).

Few studies have previously employed flow cytometry to analyze *Helianthus* species. Michaelson et al., (1991) reported that the average DNA content in root and shoot tip nuclei of wild *Helianthus* species ranged from 6.01 to 7.95 pg in aneuploids ($2n = 17$ to 33) and diploids ($2n = 34$). Sujatha and Prabakaran (2006) utilized flow cytometry to evaluate interspecific hybrids derived from diploid cultivated sunflower and two hexaploid species (*H. tuberosus* and *H. resinosus*), aiming to identify plants with the desired diploid status. Kantar et al., (2014) investigated genomic diversity in sunflower accessions by assessing average nuclear DNA content using flow cytometry. Kallamadi and Sujatha (2016) measured the nuclear DNA content of 43 sunflower species out of 52 investigated in this study via flow cytometry using DAPI as fluorochrome and confirmed that the ploidy levels of the species were consistent with previous reports. However, preferential binding to AT-rich regions can cause DAPI to introduce up to a 15% deviation in nuclear DNA content analysis. This characteristic of DAPI make it unsuitable to use in determination of absolute nuclear DNA content. In addition, that study did not use an internal standard that eliminates errors due to random instrument drift and variation in the sample preparation and staining (Dolezel and Bartos, 2005). Due to these two drawbacks, the validity of the results of this recent study is low.

The objective of the current study is to determine the nuclear DNA content of 133 wild *Helianthus* accessions from 52 different species by flow cytometry analysis using propidium iodide as flourochrome, to confirm their taxonomic identity and ploidy level before incorporating them into sunflower research and breeding programs in Turkey.

2. MATERIALS AND METHODS

In the present study, 133 wild sunflower (*Helianthus spp.*) accessions, representing 52 species from the USDA gene bank, were planted in the Wild Sunflower Garden at Trakya University, Edirne, Turkey, and were investigated from 2020

to 2022. The nuclear DNA content analysis was performed in the Plant Genetics and Breeding Laboratory of the Department of Field Crops, Tekirdag Namik Kemal University, Tekirdag, Turkey by using a Partec CyFlow Space flow cytometer. Samples of intact nuclei were isolated from fresh leaf tissues of young (5-6 weeks old) and healthy plants using Partec commercial kit (CyStain® PI Absolute P). Nuclei isolations were done according to the manufacturer's protocol (Figure 1). Common vetch (*Vicia sativa*, cv. kara elci) has been used as internal standard in the study because it has the most suitable genome size for all sunflower species investigated. Briefly, the procedure is as follows:

1. Approximately 20 mg of fresh leaf tissues was collected from both young and healthy sunflower plants and standard and placed in a petri dish.
2. 500 µl of isolation buffer was added into the Petri dish.
3. both sample and standard leaf tissues were chopped simultaneously with a sharp razor blade into small pieces (30-60 seconds).
4. The cut leaf sample was further shaken in the petri dish for 15-20 seconds.
5. The leaf suspension was transferred to a glass tube, through a 30-33 µm mesh filter, to separate the nuclei from the residual plant tissue material.
6. 2 ml of flow cytometry staining solution was added to the isolated nuclei sample.
7. Samples were incubated in a dark environment at 37°C for approximately 1 hour.
8. After the incubation period is completed, the samples were loaded into the flow cytometry device and subjected to analysis. Minimum 3000 nuclei analyzed for each sample.

The absolute nuclear DNA content of a sample was calculated in picograms (1pg = 10⁻¹² g) using the values of the fluorescence intensities of the G1 peaks of the sample and the standard, using the formula given below.

Nuclear DNA content (pg) = (Fluorescence intensity of G1 peak of sample) / (Fluorescence intensity of G1 peak of the standard) × DNA content of the standard.

The leaves tissues of three single plants for each accession were analyzed separately and their average was used as mean of the accession. The confidence intervals which are a simple statistical procedure was used to analyze nuclear DNA content results. Nuclear DNA content of each accession was correlated with their ploidy by counting mitotic chromosomes of at least one plant from each group created based on their nuclear DNA content. It was assumed that the remaining plants within the same group, characterized by similar DNA content, possess the same chromosome number (ploidy level).

The average nuclear DNA content of all accessions was compared among themselves (1-3 samples for each accession) using confidence intervals, which is a simple statistical method. The confidence interval (0,95) for each mean was calculated using the equation below.

$$P(\bar{x} - t_{0.05} S_{\bar{x}} < \mu < \bar{x} + t_{0.05} S_{\bar{x}}) = 0.95$$

$$\text{In the formula, } t_{0.05} \text{ is the "t" statistic and } S_{\bar{x}} = \sqrt{\frac{S^2}{n}} = \frac{S}{\sqrt{n}}$$

n is the number of plants analyzed in each population and s is their standard deviation. It was assumed that the means with overlapping confidence intervals were not different from each other. In this respect, the analysis is the same as the t test performed to compare means (Steel and Torrie, 1960).

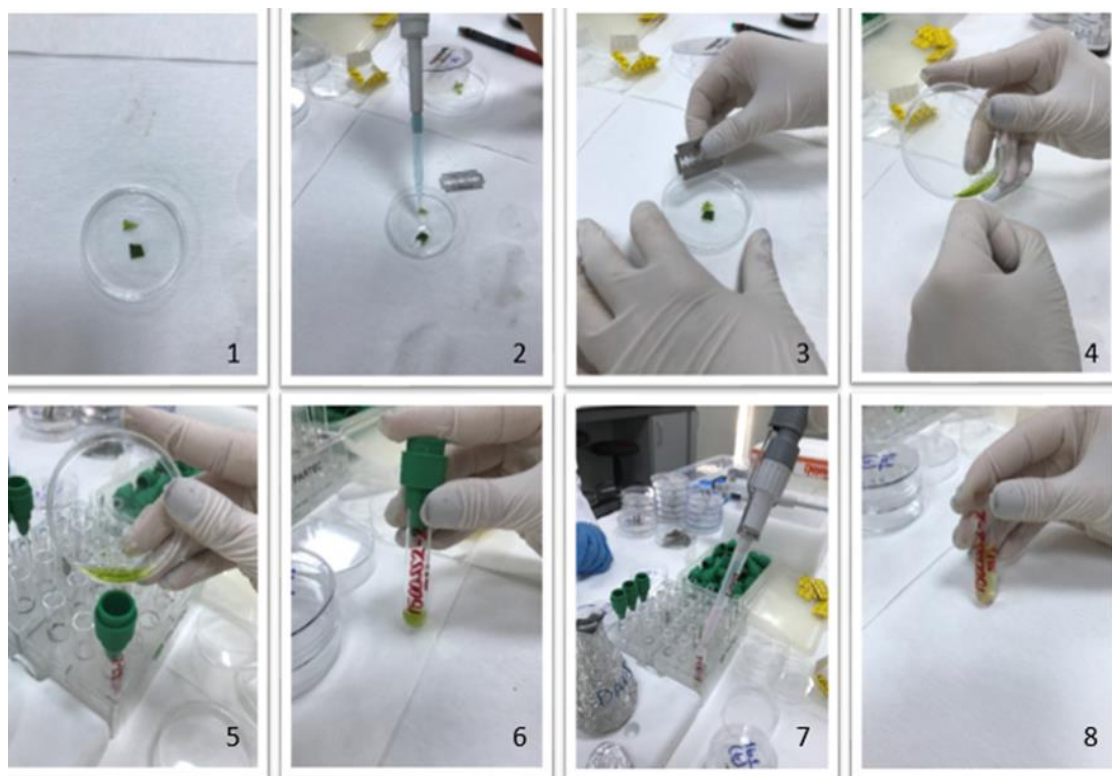


Figure 1. Isolation of nuclei from plant leaves for nuclear DNA content analysis

Cytological samples were prepared by using root tip meristem tissues. Root tips were harvested from adult plants growing in the pots, in early morning, treated with cold water for about 20 hours, fixed in farmer solution and stored at 4.0 °C until further use. Prior to squashing, the root tips were washed a few times, for 5 minutes, in 0.01 M citric acid – sodium citrate buffer (pH 4.8) and enzymatically digested at 37°C in an enzyme mixture comprising of 1% (w/v) cellulose (Calbiochem), 1% (w/v) cellulase, Onozuka R-10; (Serva) and 20% (v/v) pectinase (Sigma) for approximately 2 h.

The root tips were washed again in diluted citrate buffer (1X) to stop the enzyme reaction and remove the excess enzyme. Then, the root tips were carefully transferred to a container containing 45% acetic acid, and the meristem part of the root tips was taken out using special needles under a stereo microscope and transferred on a glass slide. A drop of 45% acetic acid was applied on the meristem, causing the tissue to disintegrate and further a coverslip was placed on it. The preparation was examined immediately under a phase-contrast microscope, and samples containing dividing cells were preserved for subsequent staining procedure.

Cytological preparations were stained using DAPI dye. For this purpose, the preparations containing dividing cells were kept at -80 °C for approximately 2 hours and the coverslip on the preparation was carefully removed with the help of a razor blade. The preparations were dried at room conditions for 24 hours, and then a small drop of DAPI dye was added to the preparation, to the area where the cells were located, and the coverslip was covered again. After this stage, the cytological preparation was stored in a cold and dark environment until chromosome counting was performed.

Chromosome counts were made using an epifluorescent microscope. Images of the cells with well distributed chromosomes were captured by an epifluorescence microscope (Olympus BX 51) with Rt Slider CCD digital camera attachment.

3. RESULTS AND DISCUSSION

Nuclear DNA content of 133 accessions from 52 different sunflower species investigated in the study was determined successfully. The nuclear DNA content of the following 25 wild sunflower species were determined for the first time in this study: *H. glaucophyllus*, *H. gracilentus*, *H. laciniatus*, *H. laetiflorus*, *H. longifolius*, *H. silphoides*, *H. nuttallii* s. *nuttallii*, *H. nuttallii*, *H. nuttallii* s. *rydbergi*, *H. occidentalis* s. *plantagenius*, *H. occidentalis* s. *occidentalis*, *H. pauciflorus* s. *subrhomboides*, *H. pauciflorus* s. *pauciflorus*, *H. pumilus*, *H. simulans*, *H. smithii*, *H. floridanus*, *H. californicus*, *H. ciliaris*, *H. cusickii*, *H. atrorubens*, *H. eggerti*, *H. strumosus*, *H. praecox* s. *hirtus* (Annual), *H. debilis* s. *silvestris* (Annual), *H. praecox* s. *runyonii*.

The mean 2C nuclear DNA content of the sunflower accessions ranged from 4,22 pg (PI 597908, *H. debilis* s. *cucumerifolius*) to 27,06 pg (PI 664699, *H. smithii*) as the mean 2C nuclear DNA content of the sunflower species ranged from 5.77 pg (*H. debilis* s. *cucumerifolius*) to 25.70 pg (*H. resinosus*) (Table 1 and 2). Unfortunately, seeds of some accessions had very low germination and therefore it was not possible to get 3 seedlings for each accession investigated in the study. The average value of the two or the only one seedling was used for those accessions based on the number of available seedling (Table 1). Due to the same reason, the average for some species was calculated based on three accessions, while for others it was based on two. Some species were represented by only one accession in the study; therefore, the value of that single accession was considered as the average for the species (Table 2). The difference between minimum and maximum mean nuclear DNA content of the species was approximately 5-fold. The differences observed among both accessions and species were found to be statistically significant. The flow histograms of two individuals from different species presented in Fig 2.

A careful examination of Table 1 and 2 reveals that the nuclear DNA content of individual plants within the same accession and the mean nuclear DNA content of accessions of the same species are notably similar. As a result of this similarity, SD values were generally low indicating the sensitivity of the nuclear DNA content analysis carried out in the study. However, it has been observed that some SD values were higher than the acceptable upper limit of 0.5 (Tuna et al., 2001). When accessions or species with such high SD values are reexamined, it has been observed that at least one sample of these accessions or species has a different nuclear DNA content value compared to the others. The reason for this difference may be mechanically mixed seeds during harvest, seed processing, packaging or misidentification of the species during the expedition trips. Some of those individuals with different nuclear DNA content value may even be interspecific hybrids (Kaya and Vasilevska-Ivanova, 2021). Those accessions or species have been accepted as mixed. In other words, they include interspecific hybrids or individuals from different species (Tuna et al., 2001).

The nuclear DNA content values of the species analyzed in the study were generally in agreement with the values reported in the literature (Sims and Price, 1985; Michaelson et al., 1991; Kantar et al., 2014; Kallamadi and Mulpuri, 2016; Sahin et al., 2022). The small differences between the results of the current and previous studies could be due to accessions, methods, fluorescent dye and internal standards used.

Table 1. The mean 2C nuclear DNA content (pg) of wild sunflower accessions

<i>Helianthus</i> species	Mean	SD	T*S _x	Confidence interval	
				Lower	upper
<i>H. smithii</i> -664699	27,06	0,023	0,019	27,044	27,082
<i>H. resinosus</i> -664678	25,70	1,081	0,884	24,816	26,584
<i>H. tuberosus</i> -664619	24,78	2,030	1,660	23,117	26,436
<i>H. laevigatus</i> -503226	24,52	0,023	0,019	24,504	24,542
<i>H. agrestis</i> -468415	23,83	0,404	0,330	23,503	24,164
<i>H. smithii</i> -664698	23,62	0,012	0,009	23,607	23,626
<i>H. agrestis</i> -673205	23,43	0,153	0,125	23,308	23,558
<i>H. decapetalus</i> -468697	23,20	0,755	0,617	22,579	23,814
<i>H. eggertii</i> -649978	22,73	0,032	0,026	22,707	22,760
<i>H. laevigatus</i> -503229	22,65	0,571	0,467	22,179	23,114
<i>H. atrorubens</i> -503206	21,41	0,193	0,158	21,252	21,568
<i>H. pauciflorus</i> s. <i>pauciflorus</i> -494613	21,37	0,035	0,028	21,342	21,398
<i>H. laetiflorus</i> -503236	20,98	0,012	0,009	20,974	20,993
<i>H. californicus</i> - 649941	20,48	0,189	0,155	20,329	20,638
<i>H. tuberosus</i> -650105	20,33	1,181	0,965	19,361	21,292
<i>H. hirsutus</i> -468735	18,71	0,609	0,498	18,212	19,208
<i>H. hirsutus</i> -435703	17,87	0,006	0,005	17,862	17,871
<i>H. decapetalus</i> -503245	17,38	0,040	0,033	17,350	17,416
<i>H. ciliaris</i> -653552	16,23	0,074	0,060	16,166	16,287
<i>H. strumosus</i> -547223	15,12	0,000	0,000	15,120	15,120
<i>H. floridanus</i> -664733	13,23	0,006	0,005	13,222	13,231
<i>H. floridanus</i> -468715	12,89	0,085	0,070	12,820	12,960
<i>H. simulans</i> -664806	12,40	0,035	0,029	12,368	12,425
<i>H. anomalus</i> -649861	11,60	0,361	0,295	11,305	11,895
<i>H. radula</i> -468876	11,36	0,295	0,241	11,119	11,601
<i>H. occidentalis</i> s. <i>plantagenius</i> -664789	11,29	0,318	0,260	11,027	11,546
<i>H. pumilus</i> -650048	11,01	0,069	0,057	10,953	11,067
<i>H. deserticola</i> -468702	10,90	0,458	0,375	10,525	11,275

Table 1. (continued)

<i>H. silphioides</i> -673159	10,79	0,020	0,016	10,774	10,806
<i>H. deserticola</i> -649883	10,67	0,231	0,189	10,478	10,856
<i>H. anomalus</i> -468638	10,40	0,173	0,142	10,258	10,542
<i>H. exilis</i> -649891	10,03	0,379	0,310	9,724	10,343
<i>H. longifolius</i> -650000	9,990	0,000	0,000	9,990	9,990
<i>H. paradoxus</i> -673239	9,927	0,046	0,038	9,889	9,964
<i>H. paradoxus</i> -673253	9,797	0,244	0,199	9,597	9,996
<i>H. longifolius</i> -664680	9,727	0,116	0,095	9,632	9,821
<i>H. exilis</i> -664633	9,533	0,252	0,206	9,328	9,739
<i>H. exilis</i> -649901	9,533	0,306	0,250	9,284	9,783
<i>H. bolanderi</i> -673280	9,500	0,361	0,295	9,205	9,795
<i>H. bolanderi</i> -673294	9,433	0,208	0,170	9,263	9,604
<i>H. mollis</i> -478309	9,287	0,100	0,082	9,205	9,369
<i>H. maximilliani</i> -613794	9,273	0,692	0,566	8,707	9,840
<i>H. bolanderi</i> -435641	9,200	0,346	0,283	8,917	9,483
<i>H. occidentalis s. occidentalis</i> -435788	9,037	0,055	0,045	8,992	9,082
<i>H. glaucophyllus</i> -468721	8,970	0,459	0,376	8,594	9,346
<i>H. giganteus</i> -468720	8,930	0,087	0,071	8,859	9,001
<i>H. argophyllus</i> -468651	8,833	0,404	0,330	8,503	9,164
<i>H. mollis</i> -441066	8,763	0,422	0,345	8,419	9,108
<i>H. niveus</i> -650020	8,700	0,361	0,295	8,405	8,995
<i>H. porteri</i> -649917	8,667	0,208	0,170	8,496	8,837
<i>H. cusickii</i> - 649962	8,590	0,036	0,029	8,561	8,619
<i>H. cusickii</i> - 649966	8,507	0,067	0,054	8,452	8,561
<i>H. anomalus</i> -664638	8,500	0,100	0,082	8,418	8,582
<i>H. argophyllus</i> -664803	8,500	0,608	0,497	8,003	8,997
<i>H. occidentalis s. plantagenius</i> -494592	8,493	0,248	0,203	8,290	8,696
<i>H. nuttallii</i> -592341	8,460	0,036	0,029	8,431	8,489
<i>H. grossesserratus</i> -586889	8,460	0,300	0,246	8,214	8,706
<i>H. niveus</i> -613758	8,433	0,379	0,310	8,124	8,743
<i>H. occidentalis</i> -664648	8,433	0,006	0,005	8,429	8,438
<i>H. gracilentus</i> -673286	8,427	0,012	0,009	8,417	8,436
<i>H. grossesserratus</i> -468727	8,427	0,214	0,175	8,252	8,602
<i>H. grossesserratus</i> -649994	8,407	0,031	0,025	8,382	8,432
<i>H. nuttallii s. rydbergii</i> -597918	8,390	0,104	0,085	8,305	8,475
<i>H. maximilliani</i> -613757	8,347	0,326	0,266	8,080	8,613
<i>H. salicifolius</i> -664759	8,340	0,216	0,177	8,163	8,517
<i>H. maximilliani</i> -468746	8,323	0,006	0,005	8,319	8,328
<i>H. nuttallii s. nuttallii</i> -586905	8,317	0,268	0,219	8,098	8,536
<i>H. pauciflorus s. subrhomboideus</i> -435869	8,310	0,269	0,220	8,090	8,530
<i>H. nuttallii</i> -420182	8,237	0,029	0,024	8,213	8,260
<i>H. argophyllus</i> -490291	8,233	0,208	0,170	8,063	8,404
<i>H. niveus</i> -435774	8,233	0,153	0,125	8,108	8,358
<i>H. giganteus</i> -649984	8,233	0,681	0,557	7,676	8,790
<i>H. argophyllus</i> -649865	8,200	0,100	0,082	8,118	8,282
<i>H. cusickii</i> - 649958	8,167	0,115	0,094	8,072	8,261
<i>H. nuttallii s. nuttallii</i> -531044	8,167	0,058	0,047	8,119	8,214
<i>H. divaricatus</i> -435675	8,163	0,144	0,117	8,046	8,281
<i>H. salicifolius</i> -664783	7,983	0,045	0,037	7,946	8,020
<i>H. porteri</i> -673214	7,900	0,400	0,327	7,573	8,227
<i>H. porteri</i> -649911	7,833	0,321	0,263	7,570	8,096
<i>H. grossesserratus</i> -468725	7,747	0,159	0,130	7,616	7,877
<i>H. argophyllus</i> -649863	7,733	0,153	0,125	7,608	7,858
<i>H. laciniatus</i> -653545	7,627	0,006	0,005	7,622	7,631
<i>H. annuus</i> - 597890	7,533	0,074	0,060	7,473	7,594
<i>H. debilis</i> -653611	7,433	0,321	0,263	7,170	7,696
<i>H. maximilliani</i> -586892	7,430	1,360	1,112	6,318	8,542
<i>H. praecox s. hirtus</i> – 435855	7,410	0,000	0,000	7,410	7,410
<i>H. winteri</i> -673290	7,333	0,058	0,047	7,286	7,381

Table 1. (continued)

<i>H. debilis</i> s. <i>Silvestris</i> -613754	7,307	0,025	0,021	7,286	7,327
<i>H. praecox</i> -435855	7,300	0,458	0,375	6,925	7,675
<i>H. debilis</i> -613753	7,300	0,000	0,000	7,300	7,300
<i>H. debilis</i> -597908	7,267	0,321	0,263	7,004	7,530
<i>H. debilis</i> -649870	7,233	0,058	0,047	7,186	7,281
<i>H. debilis</i> -613754	7,233	0,058	0,047	7,186	7,281
<i>H. praecox</i> -435853	7,100	0,436	0,356	6,744	7,456
<i>H. petiolaris</i> -586911	7,100	0,100	0,082	7,018	7,182
<i>H. bolanderi</i> -673141	7,067	0,252	0,206	6,861	7,272
<i>H. debilis</i> -435654	7,067	0,252	0,206	6,861	7,272
<i>H. debilis</i> -468672	7,067	0,208	0,170	6,896	7,237
<i>H. petiolaris</i> -586922	6,967	0,503	0,412	6,555	7,378
<i>H. petiolaris</i> -597922	6,967	0,208	0,170	6,796	7,137
<i>H. gracilentus</i> -664644	6,937	0,025	0,021	6,916	6,957
<i>H. praecox</i> -468846	6,900	0,100	0,082	6,818	6,982
<i>H. petiolaris</i> -597924	6,800	0,265	0,216	6,584	7,016
<i>H. petiolaris</i> -592355	6,800	0,100	0,082	6,718	6,882
<i>H. petiolaris</i> -586919	6,767	0,231	0,189	6,578	6,956
<i>H. petiolaris</i> -503232	6,767	0,115	0,094	6,672	6,861
<i>H. praecox</i> -435847	6,733	0,058	0,047	6,686	6,781
<i>H. petiolaris</i> -649910	6,700	0,100	0,082	6,618	6,782
<i>H. petiolaris</i> -468818	6,667	0,208	0,170	6,496	6,837
<i>H. petiolaris</i> -586928	6,633	0,416	0,340	6,293	6,974
<i>H. debilis</i> s. <i>cucumerifolius</i> -435654	6,627	0,029	0,024	6,603	6,650
<i>H. petiolaris</i> -597923	6,600	0,200	0,164	6,436	6,764
<i>H. petiolaris</i> -613769	6,600	0,300	0,245	6,355	6,845
<i>H. petiolaris</i> -592359	6,567	0,153	0,125	6,442	6,692
<i>H. petiolaris</i> -547210	6,533	0,029	0,024	6,510	6,557
<i>H. petiolaris</i> -613765	6,500	0,000	0,000	6,500	6,500
<i>H. petiolaris</i> -586931	6,500	0,173	0,142	6,358	6,642
<i>H. neglectus</i> -435769	6,467	0,289	0,236	6,231	6,703
<i>H. debilis</i> s. <i>cucumerifolius</i> -613753	6,460	0,329	0,269	6,191	6,729
<i>H. petiolaris fallax</i> -468817	6,400	0,100	0,082	6,318	6,482
<i>H. petiolaris</i> -468842	6,400	0,100	0,082	6,318	6,482
<i>H. petiolaris</i> s. <i>petiolaris</i> -586922	6,310	0,565	0,462	5,848	6,772
<i>H. neglectus</i> -468773	6,267	0,058	0,047	6,219	6,314
<i>H. praecox</i> -468846	6,250	0,221	0,181	6,069	6,431
<i>H. petiolaris fallax</i> -435817	6,233	0,115	0,094	6,139	6,328
<i>H. petiolaris</i> s. <i>petiolaris</i> -468842	6,197	0,237	0,194	6,003	6,390
<i>H. praecox</i> s. <i>praecox</i> - 435847	6,160	0,000	0,000	6,160	6,160
<i>H. petiolaris</i> s. <i>petiolaris</i> -503232	6,153	0,551	0,450	5,703	6,604
<i>H. praecox</i> s. <i>runyonii</i> -494606	6,123	0,080	0,066	6,058	6,189
<i>H. neglectus</i> -435763	6,000	0,200	0,164	5,836	6,164
<i>H. neglectus</i> -673249	5,933	0,153	0,125	5,808	6,058
<i>H. praecox</i> s. <i>runyonii</i> -435853	5,497	1,097	0,897	4,600	6,394
<i>H. debilis</i> s. <i>cucumerifolius</i> -597908	4,223	0,193	0,158	4,065	4,382

Table 2. The mean 2C nuclear DNA content (pg) of wild sunflower species

<i>Helianthus</i> species	Acces. No 1	Acces. No 2	Acces. No 3	Mean (pg/2C)	SD	T*S _x	Confidence intervals		Ploidy
							Lower	Upper	
<i>H. resinosus</i>	25,70			25,70					H
<i>H. smithii</i>	27,06	23,62		25,34	2,43	2,80	22,54	28,14	H
<i>H. tuberosus</i>	24,78	20,33		22,56	3,15	3,63	18,93	26,18	T
<i>H. agrestis</i>	23,83	23,43		23,63	0,28	0,39	23,24	24,02	T
<i>H. laevigatus</i>	24,52	22,65		23,59	1,32	1,52	22,06	25,11	T
<i>H. eggertii</i>	22,73			22,73					T
<i>H. atrorubens</i>	21,41			21,41					T
<i>H. pauciflorus</i> s. <i>pauciflorus</i>	21,37			21,37					T
<i>H. laetiflorus</i>	20,98			20,98					T
<i>H. californicus</i>	20,48			20,48					T
<i>H. decapetalus</i>	23,20	17,38		20,29	4,12	4,74	15,55	25,03	T
<i>H. hirsutus</i>	18,71	17,87		18,29	0,59	0,68	17,61	18,97	T
<i>H. ciliaris</i>	16,23			16,23					T
<i>H. strumosus</i>	15,12			15,12					T
<i>H. floridanus</i>	13,23	12,89		13,06	0,24	0,28	12,78	13,34	D
<i>H. simulans</i>	12,40			12,40					D
<i>H. radula</i>	11,36			11,36					D
<i>H. pumilus</i>	11,01			11,01					D
<i>H. silphioides</i>	10,79			10,79					D
<i>H. deserticola</i>	10,90	10,67		10,79	0,16	0,19	10,60	10,97	D
<i>H. anomalus</i>	11,60	10,40	8,50	10,17	1,56	1,80	8,37	11,97	D
<i>H. occidentalis</i> s. <i>plantagenius</i>	11,29	8,49		9,89	1,98	2,28	7,61	12,17	D
<i>H. paradoxus</i>	9,93	9,80		9,86	0,09	0,11	9,76	9,97	D
<i>H. longifolius</i>	9,99	9,73		9,86	0,19	0,21	9,64	10,07	D
<i>H. exilis</i>	10,03	9,53	9,53	9,70	0,29	0,33	9,37	10,03	D
<i>H. bolanderi</i>	9,50	9,43	9,20	9,38	0,16	0,18	9,20	9,56	D
<i>H. occidentalis</i> s. <i>occidentalis</i>	9,04			9,04					D
<i>H. mollis</i>	9,29	8,76		9,03	0,37	0,43	8,60	9,45	D
<i>H. glaucophyllus</i>	8,97			8,97					D
<i>H. maximilliani</i>	9,27	8,35	8,32	8,65	0,54	0,62	8,02	9,27	D
<i>H. giganteus</i>	8,93	8,23		8,58	0,49	0,57	8,01	9,15	D
<i>H. niveus</i>	8,70	8,43	8,23	8,46	0,23	0,27	8,19	8,73	D
<i>H. grossesserratus</i>	8,46	8,43	8,43	8,44	0,02	0,02	8,42	8,46	D
<i>H. occidentalis</i>	8,43			8,43					D
<i>H. cusickii</i>	8,59	8,51	8,17	8,42	0,22	0,26	8,16	8,68	D
<i>H. nuttallii</i> s. <i>rydbergii</i>	8,39			8,39					D
<i>H. nuttallii</i>	8,46	8,24		8,35	0,16	0,18	8,17	8,53	D
<i>H. pauciflorus</i> s. <i>subrhomboideus</i>	8,31			8,31					D
<i>H. argophyllus</i>	8,83	8,23	7,73	8,27	0,55	0,63	7,63	8,90	D
<i>H. nuttallii</i> s. <i>Nuttallii</i>	8,32	8,17		8,24	0,11	0,12	8,12	8,36	D
<i>H. salicifolius</i>	8,34	7,98		8,16	0,25	0,29	7,87	8,45	D
<i>H. divaricatus</i>	8,16			8,16					D
<i>H. porteri</i>	8,67	7,90	7,83	8,13	0,46	0,53	7,60	8,67	D
<i>H. gracilentus</i>	8,43	6,94		7,68	1,05	1,21	6,47	8,90	D
<i>H. laciniatus</i>	7,63			7,63					D
<i>H. annuus</i>	7,53			7,53					D
<i>H. winteri</i>	7,33			7,33					D
<i>H. praecox</i> s. <i>hirtus</i>	7,41			7,41					D
<i>H. debilis</i> s. <i>silvestris</i>	7,31			7,31					D
<i>H. debilis</i>	7,43	7,23	7,07	7,24	0,18	0,21	7,03	7,46	D
<i>H. praecox</i>	7,30	6,90	6,25	6,82	0,53	0,61	6,21	7,43	D
<i>H. petiolaris</i>	7,10	6,70	6,20	6,67	0,45	0,52	6,14	7,19	D
<i>H. praecox</i>	7,41	6,73	5,50	6,55	0,97	1,12	5,43	7,66	D
<i>H. petiolaris fallax</i>	6,40	6,23		6,32	0,12	0,14	6,18	6,45	D
<i>H. petiolaris</i> s. <i>petiolaris</i>	6,31	6,20	6,15	6,22	0,08	0,09	6,13	6,31	D
<i>H. neglectus</i>	6,47	6,27	5,93	6,22	0,27	0,31	5,91	6,53	D
<i>H. praecox</i> s. <i>praecox</i>	6,16			6,16					D
<i>H. praecox</i> s. <i>runyonii</i>	6,12	5,50		5,81	0,44	0,51	5,30	6,32	D
<i>H. debilis</i> s. <i>cucumerifolius</i>	6,63	6,46	4,22	5,77	1,34	1,55	4,22	7,32	D

H= hexaploid, T=Tetraploid, D=Diploid

At the onset of the study, obtaining root tips from plants, suitable to prepare cytological preparations (with plenty of dividing cells) was challenging due to high number of different sunflower species and their requirements. The problem was partly solved by transferring the potted plants to a climate chamber with lower temperatures. Eventually, chromosome counting was successfully conducted on a limited number of accessions from different species with varying nuclear DNA content except hexaploids (Fig 3). Based on those cytological investigations, all accessions with mean 2C DNA content ranging from 4.22 to 13.23 pg had $2n = 2x = 34$ chromosomes indicating that they were diploid (Figure 3). The chromosome number of *H. agrestis* accession (PI 468415) with a nuclear DNA content of 21 pg/2C was determined as $2n = 4x = 68$ indicating that it was tetraploid (Figure 3). Although it was not possible to count chromosomes for each species, based on the data obtained in this study it can be speculated that species with DNA content from 15.12 pg to 23.63 pg are tetraploid with $2n=68$ chromosome. It is possible to say this safely because accessions and species used in the study can be separated into 3 main groups based on their nuclear DNA content, and there is a clear gap (min. 2 pg) between groups as the data within each group is continuous type. In this case, the accessions and species with higher nuclear DNA content than 23.63 pg are hexaploids. However, this should still be confirmed by counting chromosomes of at least one individual plants for each species. Unfortunately, this was not possible in the current study since root tips of the plants for many species had very low dividing cells.

The results of chromosome count for the species used in the current study are generally consistent with previously reported results (Kallamadi and Mulpuri, 2016).

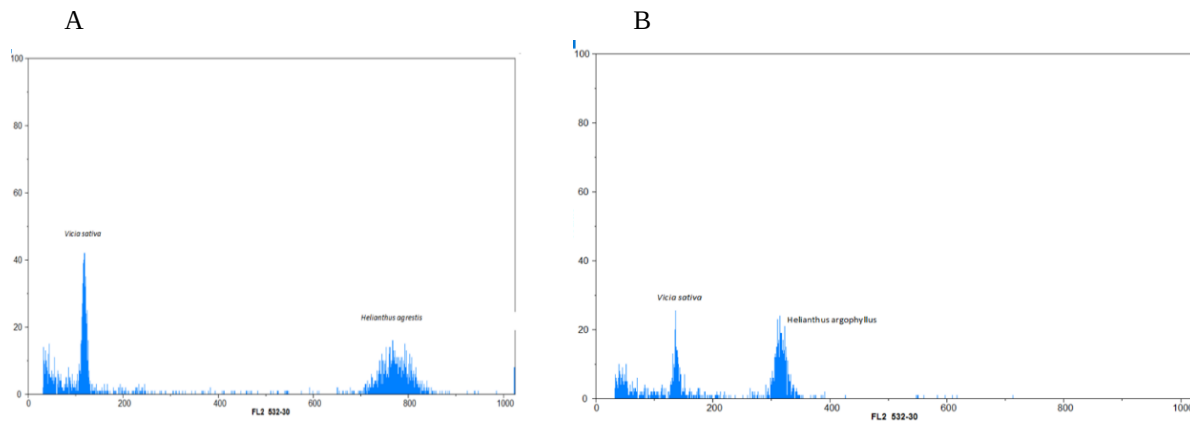


Figure 2. Histograms representing G1 peak position of samples and standards (A. PI 468415, *H. agrestis*; B. PI 468651, *H. argophyllus*)

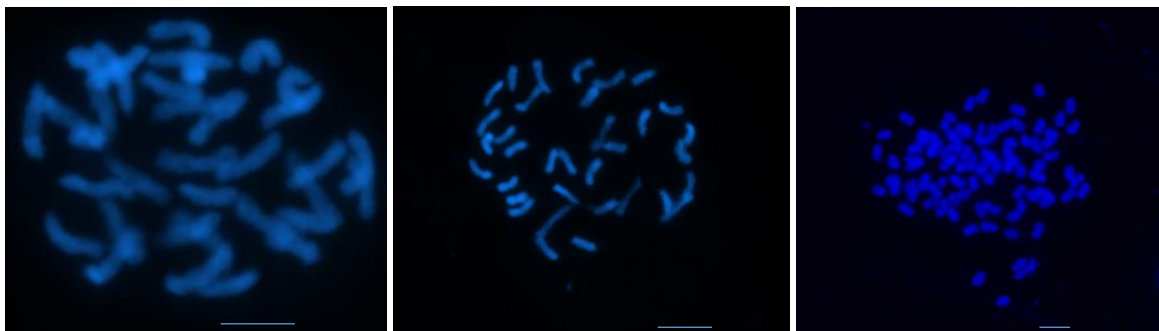


Figure 3. Mitotic chromosomes of PI 650001 (*H. longifolius*, $2n=2x=34$), PI 673147 (*H. mollis*, $2n=2x=34$) and PI 468415 (*H. agrestis*, $2n=4x=68$), Bar is 10 μ m.

4. CONCLUSION

In this study, nuclear DNA content and ploidy level of 52 wild sunflower species were determined using flow cytometry, the most accurate and reliable method. The performed analysis has shown that a significant variation (5-fold) in nuclear DNA content exists within the *Helianthus* genus. The study revealed that approximately 80% (41 species) of the 52 wild sunflower species (comprising 15 annuals and 26 perennials) were diploid ($2x$). The ploidy levels of the remaining species varied, with two species being hexaploid ($6x$) and twelve tetraploids ($4x$).

ACKNOWLEDGEMENT

The study results were funded by Trakya University with TUBAP 2020/62 project and by TUBITAK with 119O218 project. We would like to thank to Trakya University and TUBITAK for financial supports.

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Quality Determination in Confectionery Sunflower

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ARTICLE INFO

Research Article

Received: 12 March 2025

Accepted: 22 May 2025

Published: 23 June 2025

Keywords:

Confectionery sunflower

Hybrids

Quality characteristics

Seed

Taste

ABSTRACT

Confectionery sunflower is one of the most preferred and consumed snacks both in Turkey and worldwide. Turkey's confectionery sunflower planted area is approximately 80,000 to 100,000 hectares, with seed production around 160,000 to 170,000 tons, primarily located in Denizli, Kayseri, Aksaray, Bursa, and Konya provinces, where local populations such as Inegol are generally cultivated. However, hybrid varieties with high yield, quality, and homogeneous seeds should be integrated into Turkish production to replace these local varieties. Confectionery sunflower has higher export potential but has not developed sufficiently, resulting in economic losses due to low production and poor homogeneity during processing. Furthermore, Turkey imports sunflower seeds for around 100 million dollars annually from China; these imported seeds are preferred by consumers primarily due to their taste. No detailed scientific study has been conducted in Turkey or globally regarding the properties associated with flavor in confectionery sunflower. In this study, domestic candidate hybrids, white-striped local Inegol types, and Chinese varieties were used as materials. Based on the results, the seed yield of the varieties ranged between 1,280 and 4,010 kg ha⁻¹. Seed colors varied among black, white, and striped. Protein values ranged between 17.87% and 30.05%. For fatty acids, linolenic acid varied between 0.13% and 1.27%, and oleic acid between 31.25% and 48.25%. Consumer sensory analysis was performed, with varieties ranked from lowest to highest score, and the browning index was determined. As a result, the TG 400 variety stood out in terms of multiple quality parameters and can be used as a standard variety in future evaluations of taste and other yield elements.

Citation: Kaya, Y., Bagci, U., Beser, N., & Akpinar, E. (2025). Quality determination in confectionery sunflower. *Turkish Journal of Field Crops*, 30(1), 121-137. <https://doi.org/10.17557/tjfc.1655970>

1. INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most widely grown oil crops globally. However, sunflower is also cultivated for snack, birdseed, and ornamental purposes. Sunflower is one of the most preferred snacks in Turkey as well as in other countries such as Eastern Europe, USA, Canada, China, Pakistan, Iran, and Middle East countries. Except in Russia and some Slavic countries, the preferred sunflower seeds are generally white with gray stripes, and larger seeds. The main producer countries globally are China, USA, Spain, India, Ukraine, and Mexico, with China's market approximately 20 times larger than Turkey's. Worldwide, sunflower consumption preferences vary according to countries and regions. In Turkey, large sunflower types with white and gray stripes (Inegol Alaca) are preferred, while black colored sunflowers are preferred in the Balkans and Russia. In the USA, shorter grains (Dakota type) and mid longer gray and white stripe (Nevada type) varieties are consumed intensively (Gontcharov, 2011; Hladni, 2016; Hladni and Miladinović, 2019; Kaya, 2004; Velasco et al., 2014; Kaya and Beser 2018).

Confectionery sunflower yield is about 1000 kg ha⁻¹ under dry conditions, but in irrigated areas such as Konya and Kayseri, the yield ranges between 2500-3000 kg ha⁻¹. Turkey's seed requirement for sunflower is around 300 tons (0.5 kg of seed per capita), while the Chinese market, which is the largest globally, is about 20 times the size of Turkey's (Kaya and Beser 2018). When examining the consumption habits of Turkish society, nuts are frequently preferred as snacks. According to statistical data, 10.8 kg of nuts are consumed per person annually in Turkey. The most preferred snack is sunflower with an 84% household consumption rate and 50% volume in the sector. Sunflower, mostly produced as oilseed for vegetable oil production, also has development potential in Turkey due to its consumption as a snack.

Turkey's snack industry has highly developed modern facilities, but production is insufficient for the current capacity, especially since local populations with low yields are generally used in confectionery sunflower. Some companies even produce by renting lands in neighboring countries such as Ukraine, Russia, and Bulgaria. In addition to intensive domestic consumption, confectionery sunflower has high export potential due to Turkey's geographical location, as the Balkans and the Middle East are the regions with the highest confectionery consumption globally. While the confectionery consumption in Turkey is 4 kg per capita, it is 11 kg in Lebanon, and similar trends exist in Greece, Iran, and Iraq. Approximately 700 industrial companies are operating in the confectionery sector in Turkey, with products delivered to consumers through approximately 7-8,000 retail outlets. The sector's turnover exceeds 1 billion dollars. The leading producer countries of confectionery sunflower globally are China, USA, Spain, Russia, Ukraine, and Turkey, with different types consumed worldwide (Beser et al., 2020).

Sunflower is one of the most widely cultivated and consumed snacks globally and in Turkey. Imports of confectionery sunflower are increasing in recent years due to problems affecting yield and quality in domestic sunflower production, processing, and marketing. The most significant issue is the lack of domestic sunflower varieties that could compete with imported sunflower seeds preferred by consumers. For new variety development studies to be successful, there is an absolute need for sufficient genetic and agronomic knowledge for selection and breeding studies focused on desired traits, especially grain quality, which is the most important element after grain yield in sunflower (Kaya and Beser, 2018). However, no study on flavor and quality criteria in confectionery sunflower has been conducted globally, either directly determining the traits or examining the relationships between them comprehensively.

In this study, all quality characteristics in confectionery sunflower grain will be examined comprehensively, and the characteristics encompassing the concept of flavor, which is the most important criterion for consumer preferences, will be determined for the first time in Turkey and globally, making this an original and novel study. The results can directly apply to a product with both intensive domestic consumption and significant export potential.

Indeed, Beser et al. (2020) emphasized that it is possible to develop domestic sunflower varieties that can compete with imported sunflower in terms of yield. However, it is critically important for the Turkish economy and the prevention of imports that the quality criteria and prominent quality characteristics of these domestic varieties be thoroughly understood and that detailed quality studies be conducted for these domestic varieties to compete with imported varieties.

Seed quality is essential in confectionery sunflower because consumers mostly prefer larger sizes, good taste, lower oil content, and longer shelf life. In this context, in addition to seed yield, 1000 grain weight and larger seed size are the most important quality traits in confectionery sunflower production. Additionally, in the sunflower sector, the homogeneity of seeds in both size and color is significant, and both the industry and consumers consistently demand larger seeds (Jovanović et al., 1998; De Figueiredo, 2015). Since high quality and homogeneous seeds can only be obtained from hybrid seeds, hybrid sunflower varieties not only yield high quality seeds but also satisfy farmers by offering high yields (Velasco et al., 2014; De Figueiredo, 2015; Demurin, 2018; Li et al., 2024).

Although Turkey has high production capacity in sunflower, unfortunately, its domestic production of confectionery sunflower cannot fully meet domestic consumption. In 2019, 75,000 tons of confectionery sunflower were imported from China. Considering consumer habits, it is important to grow confectionery sunflower to contribute to the national economy and create employment for local producers. However, the productivity values and compliance with various

quality parameters of the sunflowers to be grown should be verified before local production becomes widespread. Plant breeding studies on confectionery sunflower production are ongoing, but various food-quality analyses are required to determine the chemical composition and sensory differences between imported and locally produced confectionery sunflower (Kaya and Beser, 2018). In this context, consumer preferences can be determined, and local production of confectionery sunflower can be supported. Consumer preferences for sunflower products are influenced by factors such as price, brand, and production method. For instance, consumers in Turkey prefer sunflower oil with attributes like domestic origin and organic production, which can be extrapolated to preferences for other sunflower products.

Sunflower is nutritious and healthy snack rich in fat, mostly 85% unsaturated fatty acids (14-43% oleic acid, 44-75% linoleic acid, and 0.7% linolenic acid) and 15% saturated fatty acids. It is also rich in antioxidants and various minerals, containing significant amounts of copper, zinc, iron, and fiber, and is especially rich in vitamin E (tocopherol) and selenium (Tesan et al., 2022; Rayman Ergun, 2024).

Sunflower quality is primarily related to its fatty acid composition, specifically the variability of its fatty acid content and susceptibility to oxidation. The possible oxidation process and the resulting products negatively affect the nutritional value, organoleptic properties, and shelf life of sunflower. The amount of peroxidase and hydrolytic activity in the seed cause the rancidity process. Therefore, the analysis of free fatty acids is one of the important quality criteria (Rayman Ergun, 2024). Additionally, antioxidant substances such as sterols, phenolics, and tocopherols in sunflower slow down the rancidity process by binding free radicals, making the determination of these substances' composition important for product quality. In summary, the oil composition and oxidative stability of sunflower oil are highly important quality factors (Muttagi and Joshi, 2021). Roasting is commonly used for nuts, and these process parameters may vary according to consumer expectations, affecting the color, odor, and aroma profile of the product (Zhu et al., 2015; Marzocchi et al., 2017).

Although hybrids developed by the Trakya Agricultural Research Institute (TARI), the only breeding program in Turkey for sunflower, are sold in the market, their market share is quite low because they lack the desired color and size. The market mostly demands Inegol type sunflower with white and black striped coloration. There are problems in the certified seed production of the white colored registered Inegol Alaca variety, and since it is not resistant to broomrape and some diseases, its yield is not very high. In the last few years, there has been significant demand for black types imported from China, and farmers cannot sell the white types they produce. There is an urgent need for high yielding, homogeneous, high quality hybrid varieties for producers, industrialists, and consumers. Additionally, the large, black striped sunflower variety imported from China has been highly appreciated and popular among consumers due to its large grains and flavor, leading to increased imports while demand for domestic white varieties has been declining (Kaya and Beser, 2018).

The goal of this study is to compare yield and quality traits of local and Chinese confectionery sunflower varieties widely sold and preferred in the market. Another aim is to analyze characteristics of Chinese hybrids to develop similar ones in local breeding programs, preventing imports from China by utilizing this study's data with various quality and food analyses to establish flavor criteria.

2. MATERIALS AND METHODS

A total of 25 varieties were used in the study, including 18 local candidate hybrids developed by Tragen R&D Co located in Trakya Technopark and Trakya Agricultural Research Institute (TARI), three Chinese hybrids, and four confectionery controls. TG400 (Chinese type) developed by Tragen R&D Co, Palanci and Ahmetbey sunflower hybrids developed by TARI and Denizli Beyazi cultivars, which is an open pollinated local population and still cultivated in the market were used as controls.

The experiment was conducted in Edirne in 2022 as 4 rows, 4 reps with 7.5 m plot length, 70 cm between and 50 cm above rows, according to RCBD design. The plot area was 6 m x 2.8 m = 16.80 m² and the harvested area was 5.4 m x 1.4 m = 7.56 m² by hand harvesting the middle two rows. The trial was sown on March 11, 2022, and harvested on September 22, 2022. 250 kg ha⁻¹ 20-20-0 Compose fertilizer was applied to the soil at 50 kg ha⁻¹ N and 50 kg ha⁻¹ P2O5 dose before soil preparation in the trial.

Field observations taken in the research are as follows.

Grain yield (kg ha⁻¹): The threshed grains were cleaned and weighed. Moisture content was determined with a moisture meter and seed yields were calculated according to 10% moisture. Table Diameter (cm): 5 plants in each plot were measured at physiological maturity (R-6). Plant Height (cm): At physiological maturity (R-6), the distance from the root collar on the soil surface of five plants in each plot. Days to Flowering: It is the time when the plants in each plot reach 50% flowering (R.5.5) after emergence. Physiological Maturity: The time when the plants in each plot reached the physiological maturity stage (R.9) after emergence. Grain width and length (cm); the seeds were measured with calipers

after harvesting and threshing. Grain color: the grain color was determined according to the RHS color scale. Hectoliter weight (g); the seeds were determined with a hectoliter measuring device after threshing. Oil Ratio (%); The oil ratios were determined by NMR. Thousand Grain Weight (TSW) (g); seeds were determined using grain counting machine.

Sunflower seeds were roasted in a commercial nut roaster at 145 °C for 30 min (Guo et al., 2019). Comparative analyses were performed on raw and roasted samples. The dry matter was determined based on the method of Kashaninejad et al. (2006) as 4-5 g seeds at petri dishes dried at 105 °C.

Total Phenolic Content

The phenolic content was determined by spectrophotometer (UV1601, Shimadzu) according to the Folin-Ciocalteu colorimetric method. Phenolic substances were extracted by taking 1 gram of ground unshelled sunflower seed samples, adding 10 mL hexane and 12 mL methanol-water (60:40 V/V) and waiting for 30 minutes. The sample was then passed through a coarse filter, and the filtrate was centrifuged at 3500 rpm for 10 minutes to separate phases. The methanol phase was taken, and Folin-Ciocalteu solution and saturated sodium carbonate solution were added to measure total phenolic content spectrophotometrically. The total phenolic content was determined according to the gallic acid standard curve (Karamac et al., 2012; Ali et al., 2023).

Dry Matter and Water Activity

The dry matter content of dehulled sunflower seeds was determined based on Kashaninejad et al. (2006). The homogenized samples (4-5 g) were placed in petri dishes and dried at 105°C until constant weight to determine moisture content. Water activity values were determined using an Aqualab 4TE device.

Protein Content

Protein content of dehulled sunflower seeds was determined using the Kjeldahl method described by Petraru (2021).

pH

After grinding the unshelled kernel samples, 9 mL of pure water was added to 1 gram of sample and homogenized. The pH values were determined using a Mettler-Toledo pH meter (Akgun et al, 2017).

Elemental Analysis

The elemental content of sunflower seeds (Ag, Ca, Cu, Fe, Mg, Mn, Na, Zn, P, Se, Cr, and Pb) was determined using Agilent 7700 ICP-MS. Unshelled sunflower seeds (1 g) were first incinerated in a microwave incinerator (CEM, MARS6) using nitric acid. Elemental matter content in the incinerated samples was determined by ICP-MS and concentrations were calculated according to standard curves (Karlsson et al., 2015).

Determination of fatty acid composition

Fatty acid profiles were determined by GC-FID (Agilent 6850). Extracted fatty acids (0.1 g) were mixed with 10 mL of hexane and 100 µL of KOH (2N) solution in methanol, mixed for 30 s, and centrifuged at 2500 g for 5 min. GC-FID analysis was performed according to Ayyildiz et al. (2015). The area under each fatty acid peak relative to the total area of all peaks was used to quantify the percentage of each fatty acid.

Textural Analysis

Hardness properties of sunflower samples were determined using a Stable Microsystems TA. HD Plus texture analyzer with P/36R probe according to Mosayebi et al. (2018).

Color Analysis

The color analysis of sunflower kernels was measured according to the CIE Lab* scale using a Minolta CM-5 colorimeter. After peeling, the seeds were placed in a petri dish for color measurement (Mosayebi et al., 2018).

Sensory Analysis

The sensory analysis of the samples (appearance, firmness, taste, aroma, and overall acceptability) was determined using a 5-point hedonic scale with 20 participants. Samples (20 g) were presented to the panelists in white plates (Mosayebi et al., 2018).

Statistical Analysis

The data were calculated with mean values and standard deviations determined from triplicate trials. All statistical analyses were performed with SPSS 17.0 (SPSS, ;2008).



Figure 1. Quality analysis in confectionery sunflower varieties in the study

3. RESULTS AND DISCUSSION

The seed yield of varieties in the experiment was statistically significant, ranging between 1280-4010 kg ha⁻¹, with the TG-400 hybrid ranking first, followed by CRZ-116, CRZ-318, CRZ-101, Affan, CRZ-213, G1, CRZ-104, and CRZ-113 hybrids respectively (Table 1). The lowest yields were obtained from Ahmetbey and Denizli Beyazi controls. The seed colors varied among black, white, and black stripe, with black stripe being most common. The earliest variety to flower was Ahmetbey (69 days), and TG-400 was earliest to reach physiological maturity (PM). The latest varieties were Palanci and IMI-CRZ-16 for flowering, and CRZ-112, CRZ-113, and CRZ-318 based on PM. TG 400 was both early maturing and high yielding as desired, CRZ-116, Affan, Stripi, and CRZ-213 varieties also had similar characteristics.

Taste evaluation of raw grains was performed with notes from three participants (5: Best, 1: Worst). The highest grade was obtained by TG 400, followed by IMI-CRZ-3, IMI-CRZ-16, IMI-CRZ-6, CRZ-19, CRZ-24, CRZ-103, and CRZ-105 (Table 1). High oleic acid ratio in confectionery sunflower is desired as it prevents oil burning during frying, prolongs shelf life, and enhances flavor. The oleic acid content measured by Spinlock NMR ranged between 54.8% and 74.4%, with the highest in Ahmetbey and lowest in CRZ-111. Higher oil content above 30% is undesirable in confectionery sunflower; oil contents ranged from 22.8-35.6%, with the highest in Palanci and lowest in CRZ-104.

Nutritional quality, including protein content and oleic acid levels, is another critical factor. High sugar content and oleic acid levels positively correlate with consumer acceptance, as they enhance taste and health benefits (Latif et al., 2024). The sensory characteristics of foods, including confectionery sunflower, are essential for consumer acceptance. Factors such as taste, texture, and appearance significantly influence consumer choices and product success in the market. While the primary focus is on seed size, nutritional content, and sensory attributes, it is important to consider the broader context of consumer acceptance.

Seed size, length, and thickness are the most significant features in confectionery sunflower, with consumers generally preferring long and large-grained varieties. Seed width ranged between 0.6-1.0 cm, with CRZ-116 and CRZ-30 having the widest seeds. Seed length varied between 1.6-2.5 cm, with CRZ-116, CRZ-30, CRZ-19, Ahmetbey, IMI-CRZ-6, G1, TG400, Affan, and CRZ-110 ranking highest. Seed thickness ranged from 0.27-0.50 cm, with CRZ-110 and CRZ-318 having the highest values. In confectionery sunflower, TSW is a critical quality determinant affecting price. Seeds weighing 150 g and above are acceptable in the market, with that 200 g and above considered large and most preferred. TSW ranged between 130.0-248.5 g, with CRZ-116 having the highest weight. CRZ-30, CRZ-19, CRZ-318, CRZ-105, and CRZ-109 candidates all exceeded 200 g (Table 1).

Based on chemical analyses, the pH values of raw sunflower seed samples ranged between 7.04-6.59 (Table 2). After roasting, this range shifted to 6.17-5.32. In sunflower seeds, pH value varies depending on roasting temperature and duration. Sen and Gokmen (2022) reported that initial pH value decreased from 7.1 to 6.25 after thermal treatment at 180°C for 45 minutes. The water activity values were below 0.6 in all samples, ranging between 0.470-0.442 (Table 2), indicating microbiological safety from spoilage. Moisture content ranged between 3.3-2.41%. Soleimanieh et al. (2015) determined that moisture content in sunflower seeds decreased from approximately 4.5% to as low as 1%, depending on

roasting conditions. The same roasting parameters were used for all samples, and a decrease in moisture content of approximately 2-3% was observed after roasting.

Table 1 Seed quality characteristics of sunflower varieties, measured in the current study.

#	Variety Name	Taste	Seed Color	Seed Yield Kg ha ⁻¹	Flower Period	Phy M Period	Oleic Acid (%)	Oil Cont (%)	Seed Width (cm)	Seed Length (cm)	Seed Thick (cm)	TSW (g)
1	CRZ-113	4-3-2	Black Stripe	3167 DEFG	72	110	64.52	29.79	0.8	2.0	0.4	161.5
2	CRZ-116	4-4-4	Black Stripe	3612 AB	70	105	64.28	27.88	0.9	2.2	0.4	226.0
3	CRZ-213	3-2- 2	White	3330 CDE	70	104	55.61	31.75	0.6	2.0	0.4	161.7
4	CRZ-30	4-4	Black Stripe	2052 KL	70	107	61.72	31.36	0.9	2.3	0.4	223.7
5	CRZ-19	4-4-5	Black Stripe	2566 J	70	109	62.01	30.15	0.8	2.2	0.4	205.5
6	CRZ-24	5-5	White	2870 GHI	69	105	60.34	26.77	0.7	2.2	0.4	194.3
7	CRZ-318	4-3	Black Stripe	3434 ABC	72	110	62.89	28.62	0.9	2.3	0.5	214.2
8	CRZ-103	5-4-5	Black Stripe	2998 FGH	72	107	63.23	29.93	0.8	2.0	0.4	192.8
9	CRZ-101	4-4	Black Stripe	3332 BCD	71	107	60.37	26.82	0.8	2.1	0.4	185.2
10	CRZ-110	3	Black Stripe	2895 GH	71	105	64.30	26.20	0.9	2.4	0.5	193.3
11	CRZ-112	3	White	3050 EFG	71	107	61.32	33.99	0.7	2.1	0.3	161.5
12	CRZ-104	2	Black Stripe	3140 DEFG	73	110	60.41	22.82	0.8	1.9	0.3	151.5
13	CRZ-105	4-5	Black Stripe	2161 KL	72	104	63.83	29.30	0.9	2.1	0.4	207.3
14	CRZ-111	4-4	White	2285 K	71	105	54.83	34.54	0.6	2.0	0.3	155.7
15	CRZ-109	5	Black Stripe	2607 IJ	72	105	63.29	29.94	0.9	2.2	0.3	203.7
16	STRIPI	4-4	Black Stripe	3000 FGH	70	105	66.97	32.59	0.7	2.0	0.3	138.7
17	DENIZLI B(C)	4-4	White	1715 M	71	105	60.14	31.57	0.7	1.6	0.3	140.5
18	AHMETBEY(C)	4-4	Stripe	199.0 LM	69	105	74.39	27.56	0.9	2.1	0.4	172.3
19	IMI-CRZ-6	5-4-5	Stripe	2720 HIJ	71	105	62.71	34.79	0.8	1.9	0.4	142.2
20	G1	5-4	Black Stripe	3218 CDEF	71	106	64.16	30.25	0.9	2.0	0.4	153.2
21	TG400 (C)	5-5-5	Stripe	3690 A	70	104	63.39	30.94	0.8	2.1	0.3	151.2
22	AFFAN	4-4	Black Stripe	3330 CDE	70	104	62.51	29.40	0.9	2.1	0.4	155.2
23	IMI-CRZ-3	4-5-5	Stripe	2613 IJ	73	108	64.59	34.22	0.8	2.0	0.4	143.5
24	IMI-CRZ-16	4-5-5	Stripe	2754 HIJ	74	108	65.55	30.90	0.9	1.8	0.4	170.0
25	PALANCI 1 (C)	3-3	Black Stripe	2220 KL	74	109	64.74	35.59	0.7	1.8	0.4	134.7
Average				283,0	71.2	106.4	62.9	30.3	0.8	2.1	0.4	173.6
Std Deviation				532	1.4	2.0	3.7	3.0	0.1	0.2	0.0	28.5
Maximum				3690	74	110	74.4	35.6	0.9	2.4	0.5	226.0
Minimum				1715	69	104	54.8	22.8	0.6	1.6	0.3	134.7

The peroxide value indicates the degree of oxidation in oil-containing products and generally increases with heat treatment. The peroxide values of the samples ranged between 4.5-124.5, with high values indicating significant oxidation in some samples. In confectionery seeds, high protein content is desirable from a nutritional perspective, and high-protein snacks are preferred. The protein values ranged between 17.87-30.05%, with the highest value in the Ahmetbey variety and the lowest in the Denizli Beyazi control variety (Table 2). The most preferred control variety, TG400, and some candidate lines had protein values above the average.

Under normal conditions, high phenolic content in confectionery sunflower seeds is desirable due to beneficial compounds such as antioxidants and nutrients. However, some phenolic compounds may cause bitterness or undesirable tastes and sensory perceptions for consumers. Detailed phenolic compound analyses were not conducted in this study. The total phenolic content ranged between 943-2505 mg GAE 100 g⁻¹ DM (Table 2). Among the varieties, CRZ-103 had the highest phenolic content, while IMI-CRZ-16 had the lowest. Generally, the control varieties and foreign candidate lines, except for Affan, had phenolic contents below the trial average.

Nadeem et al. (2011) determined that total phenolic content ranged between 2185-2965 mg 100 g⁻¹ in hulled seed samples and 3162-3614 mg GAE 100 g⁻¹ in dehulled samples. Fiska et al. (2006) found the total phenolic content of hulled sunflower seed samples to be 2700 mg GAE 100 g⁻¹ of dry matter. Ali et al. (2023) reported values between 1930-2310 mg GAE/100g. The total phenolic content in this study was generally consistent with literature. The macro and micro element contents in the sunflower seed samples are presented in Table 3. The heavy metal contents, such as arsenic, lead, and cadmium, were below 2 ppm in all samples. In a study conducted in Iran, the lead and cadmium contents in sunflower seed samples ranged between 10-381 ppb and 73-731 ppb, respectively (Torki et al., 2018). In all samples examined in this study, much lower amounts of cadmium and lead were detected compared to these values. No high values of heavy metals were identified in the microelement analyses of the varieties (Table 3).

The magnesium content ranged between 1181-2694 mg kg⁻¹, potassium between 3576-6855 mg kg⁻¹, and calcium between 315-803 mg kg⁻¹. In a study conducted in Argentina, the magnesium, potassium, and calcium contents in

sunflower seeds were approximately 5000-6000 mg kg⁻¹, 1300-1000 mg kg⁻¹, and 1200-1600 mg kg⁻¹, respectively (Tesan et al., 2022). For confectionery sunflower seeds, high levels of certain macro elements such as Mg, K, and Ca are desirable from a nutritional perspective. However, it is difficult to interpret which of these elements influence consumers' taste preferences or play a definitive role. In terms of fatty acids, linolenic acid (Omega 3), which is highly preferred in nutrition, ranged between 0.13-1.27%, with the highest ratio in CRZ-113. Oleic acid, which significantly affects quality and extends shelf life, ranged between 31.25-48.25%, with the highest value in IMI-CRZ-3 (Table 4).

Table 2. Moisture and water activity values of hulled sunflower and total phenolic substance amounts in unhulled seed samples

Variety Name	Humidity %	Protein %	Water Activity	pH (Raw)	pH (roasted)	Peroxide # (meq O ₂ kg ⁻¹ sample)	Tot Phenolic Subst (mg GAE 100 g ⁻¹ DM)
CRZ-113	3.15	25.48	0.465	7.00	6.17	14.7	2262
CRZ-116	3.20	25.11	0.447	6.77	5.96	57.5	1403
CRZ-213	3.30	18.42	0.421	6.78	5.85	21.5	2207
CRZ-30	2.98	20.72	0.424	6.63	5.42	62.5	1782
CRZ-19	2.91	24.10	0.459	6.81	5.77	16.5	1536
CRZ-24	2.81	27.14	0.470	6.83	5.72	83.5	1855
CRZ-318	3.02	22.68	0.448	6.68	5.56	4.50	1701
CRZ-103	2.68	20.74	0.462	6.64	5.64	12.5	2505
CRZ-101	3.06	23.78	0.447	6.72	5.32	16.5	1230
CRZ-110	2.69	21.05	0.443	6.79	5.82	19.5	1352
CRZ-112	2.41	23.23	0.454	6.84	5.49	13.5	1768
CRZ-104	3.13	26.89	0.443	6.41	5.60	9.50	1570
CRZ-105	2.47	23.71	0.445	6.95	5.60	7.50	2212
CRZ-111	3.00	21.82	0.447	6.68	5.38	32.5	2215
CRZ-109	2.62	24.41	0.450	6.59	6.01	27.5	2112
STRIPI	3.09	22.12	0.453	6.90	5.85	26.5	1543
DENIZLI(C)	2.63	17.87	0.442	6.80	5.67	124.5	1386
A.BEY (C)	2.92	30.05	0.450	6.92	5.86	83.5	1394
IMI-CRZ-6	2.92	20.50	0.446	6.94	5.87	7.5	1317
G1	2.43	19.67	0.454	6.99	6.14	7.5	1731
TG400 (C)	2.27	24.82	0.465	6.77	6.04	8.5	1505
AFFAN	2.82	21.15	0.454	7.01	5.94	3.5	2143
IMI-CRZ-3	2.67	21.63	0.455	7.04	6.04	9.5	1219
IMI-CRZ-16	2.80	22.84	0.454	6.97	5.80	7.5	943
PALANCI(C)	2.58	19.63	0.452	7.01	5.75	8.5	1396
Average	2.82	22.78	0.45	6.82	5.77	27.5	1707.9
Std Deviation	0.27	2.86	0.01	0.16	0.23	30.8	398.0
Maximum	3.30	30.05	0.47	7.04	6.17	124.5	2505.0
Minimum	2.27	17.87	0.42	6.41	5.32	4.5	943.0

The hardness values of the sunflower seed samples were determined both with and without hulls. In all samples, the hardness values of the hulled samples were higher than those of the dehulled samples. While the hardness value in hulled samples ranged between 30-81 kg, in dehulled samples, this value ranged between 12-43 kg (Table 5). Seed hardness is an undesirable characteristic in confectionery sunflower seeds. When seeds are too hard, they are less preferred due to both excessive energy consumption during roasting and reduced ease of cracking during consumption. The seed hardness values ranged between 30.17-81.26 kg for hulled seeds and 12.86-43.07 kg for dehulled seeds. The hardest seeds, both hulled and dehulled, were found in CRZ-116, while the softest were in CRZ-112.

There appears to be a nuanced relationship when comparing instrumental hardness measurements with sensory evaluation scores, particularly for crispness. For instance, CRZ-19, which received the highest overall sensory score (4.3) and maximum crispness score (4.0), displays a relatively high hardness value (69.28 kg with shell). However, CRZ-116, which has the highest instrumental hardness value (81.26 kg), received only a moderate crispness score (3.7) and overall acceptability score (3.2).

The samples with highest consumer acceptance scores demonstrate an interesting pattern. Samples CRZ-19 and CRZ-105, which received the highest overall acceptance scores (4.3 and 4.2 respectively), show different hardness values. CRZ-19 has a relatively high hardness (69.28 kg with shell, 27.82 kg without shell), while CRZ-105 has a moderate hardness (55.71 kg with shell, 21.84 kg without shell). This suggests that consumers may prefer sunflower seeds within a specific hardness range, rather than simply favoring extremely hard or soft seeds. The data indicates that sunflower seeds with shell hardness between approximately 55-70 kg may align with higher consumer acceptance. There is generally a parallel between hulled and dehulled values in terms of seed hardness, and the control varieties mostly exhibited values close to the average in both measurements.

Table 3. Macro and micro element contents in sunflower seed samples (mg kg⁻¹).

Variety	Mo	Ag	Cd	Sn	Ba	Pb	Ni	Cu	Zn	As	Se	Li	Cr	Mn	Fe	Co	Na	Mg	Al	K
CRZ-113	8.5	1.41	1.27	2.18	1.115	0.163	7.1	64.8	269.8	0.602	0.174	0.147	0.321	62.3	179.6	0.314	9559.8	1515354	5844.6	3755460
CRZ-116	5.2	0.83	0.90	1.74	0.537	0.085	6.8	78.2	218.6	0.493	0.272	0.735	0.620	66.7	165.9	0.091	8485.7	1297360	589.1	4396577
CRZ-213	3.4	0.59	0.62	1.32	0.725	0.065	6.0	60.3	208.3	0.399	0.23	0.399	0.175	57.5	158.1	0.073	9432.1	1181626	439.1	3118582
CRZ-30	3.2	0.35	1.03	1.10	0.7	0.093	6.1	81.2	237.1	0.262	0.292	0.210	0.260	64.1	166.8	0.142	9087.9	1449102	487.9	4691641
CRZ-19	2.8	0.25	1.11	1.04	0.748	0.067	8.3	78.9	211.1	0.282	0.334	0.830	0.469	66.1	177.7	0.351	9290.1	1373562	659.3	4579551
CRZ-24	2.9	0.19	0.79	0.85	0.78	0.087	7.8	84.1	243.4	0.231	0.167	0.084	0.166	79.1	223.5	0.093	9460.1	1632988	954.5	4150464
CRZ-318	3.0	0.16	1.16	0.81	0.567	0.068	8.2	93.6	235.4	0.225	0.23	0.105	0.197	73.7	255.2	0.094	9012.0	1647868	1388.9	5685108
CRZ-103	2.1	0.16	0.99	0.58	0.438	0.122	7.9	75.2	226.7	0.191	0.271	0.231	0.048	65.5	173.3	0.131	3155.5	1427551	526.0	4214615
CRZ-101	2.3	0.11	0.98	0.55	0.583	0.104	6.6	87.7	261.9	0.172	0.272	0.457	0.040	88.3	200.8	0.080	2966.6	1731217	612.5	4807492
CRZ-110	2.1	0.07	0.97	0.55	0.314	0.110	6.5	81.6	210.5	0.193	0.209	0.200	0.080	74.3	181.2	0.084	2651.8	1288442	358.2	3909625
CRZ-112	2.0	0.07	0.99	0.52	0.931	0.146	5.7	91.7	265.6	0.159	0.272	0.851	0.154	62.7	185.2	0.139	3569.0	1418324	491.5	3576585
CRZ-104	2.6	0.05	0.87	0.39	0.497	0.109	9.2	74.7	251.8	0.137	0.195	0.672	0.036	80.9	173.8	0.119	1703.5	1600003	351.1	6855340
CRZ-105	2.5	0.04	1.63	0.37	0.569	0.150	6.7	95.9	255.1	0.115	0.091	0.021	0.041	79.5	172.5	0.111	2040.7	1489421	441.5	4915618
CRZ-111	1.7	0.04	0.85	0.44	0.653	0.131	9.9	71.1	219.3	0.142	0.202	0.336	0.110	51.5	153.7	0.106	3523.1	1279586	423.6	3710743
CRZ-109	2.3	0.03	1.30	0.34	0.77	0.126	5.9	94.7	268.0	0.2	0.056	0.168	0.047	76.3	192.7	0.112	2623.2	1381208	2363.9	4421664
STRIPI	1.3	0.02	0.92	0.28	0.615	0.113	15.2	67.1	210.8	0.162	1.086	0.200	0.125	95.9	146.4	0.196	3159.5	2694150	372.5	6176063
DENIZ B(C)	0.9	0.03	0.69	0.26	0.737	0.120	22.1	84.7	210.9	0.132	1.128	0.084	0.034	117.9	180.9	0.821	2400.4	2569147	360.6	5827747
A.BEY (C)	1.0	0.02	0.62	0.25	0.653	0.114	21.9	82.5	204.8	0.12	1.316	0.231	0.092	124.2	181.5	0.475	2233.5	2476237	276.1	5728024
IMI-CRZ-6	1.0	0.02	0.66	0.23	1.209	0.113	15.5	74.5	217.2	0.159	1.504	0.630	0.062	96.2	180.9	0.330	2856.9	2375885	229.7	5510162
G1	1.1	0.01	0.79	0.19	1.271	0.129	13.8	71.6	230.8	0.105	1.337	0.389	0.034	76.8	175.0	0.283	1933.7	2562309	197.5	6652478
TG400 (C)	1.4	0.01	0.78	0.19	1.229	0.125	13.3	86.8	247.8	0.145	1.086	0.000	0.062	97.2	190.9	0.195	1390.2	2712822	1802.0	6524797
AFFAN	1.3	0.02	0.72	0.16	1.233	0.114	12.8	72.2	218.5	0.105	1.149	0.389	0.047	70.0	157.5	0.268	2151.0	2680067	387.9	6750759
IMI-CRZ-3	0.9	0.01	0.75	0.15	1.415	0.119	14.7	76.1	241.9	0.108	1.316	0.273	0.036	96.1	178.1	0.287	3911.2	2637617	483.2	5908922
IMI-CRZ-16	0.9	0.01	0.61	0.15	2.123	0.112	14.7	72.0	215.1	0.085	0.815	0.567	0.049	95.2	175.7	0.195	3085.7	2304126	253.5	5928708
PALANCI(C)	1.1	0.00	0.85	0.15	1.396	0.175	13.3	81.0	271.0	0.164	1.107	0.021	0.046	68.2	185.7	0.305	2310.7	2750421	2577.0	6661903
Average	2.3	0.18	0.91	0.59	0.9	0.1	10.6	79.3	234.1	0.2	0.6	0.329	0.134	79.4	180.5	0.216	4479.8	1899055.8	914.9	5138345.1
Std Dev	1.6	0.32	0.24	0.53	0.4	0.0	4.8	9.4	22.1	0.1	0.5	0.256	0.147	18.1	22.1	0.166	3058.5	584102.5	1210.5	1139849.3
Maximum	8.5	1.41	1.63	2.18	2.1	0.2	22.1	95.9	271.0	0.6	1.5	0.851	0.620	124.2	255.2	0.821	9559.8	2750421.2	5844.6	6855340.2
Minimum	0.9	0.00	0.61	0.15	0.3	0.1	5.7	60.3	204.8	0.1	0.1	0.000	0.034	51.5	146.4	0.073	1390.2	1181626.1	197.5	3118581.5

When examining the overall liking scores, CRZ-19 and CRZ-105 received the highest scores from panelists. The sample with the lowest score was CRZ-30. CRZ-19 received the highest score in all sensory analysis parameters (Table 5). Among the control and candidate varieties, TG 400 received the highest score, while Palanci and Ahmetbey control varieties also scored high in all parameters except general appearance. Among the candidate lines, CRZ-105 and CRZ-111 stood out with high scores, while Stripi was notable for its strong aroma and taste, Affan for its taste, and Denizli Beyazi for its crispness.

Table 4. Fatty acid profiles of sunflower seeds from the varieties (%).

Variety	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Arachidic	Eicosenoic	Behenic
CRZ-113	4.99	3.33	38.79	47.99	1.27	0.62	0.27	1.41
CRZ-116	6.27	2.66	43.24	46.42	0.27	0.08	0.19	0.63
CRZ-213	6.02	3.38	36.60	52.76	0.22	0.04	0.10	0.70
CRZ-30	6.61	2.62	39.92	49.39	0.20	0.05	0.13	0.70
CRZ-19	5.94	2.13	42.22	48.47	0.14	0.11	0.14	0.58
CRZ-24	5.98	3.80	38.43	50.26	0.25	0.12	0.12	0.81
CRZ-318	6.23	2.32	38.87	51.38	0.15	0.05	0.14	0.54
CRZ-103	5.98	2.36	43.58	44.76	0.13	0.78	0.13	0.53
CRZ-101	6.23	3.01	37.51	51.82	0.22	0.06	0.13	0.56
CRZ-110	6.21	3.21	42.77	46.36	0.22	0.11	0.14	0.58
CRZ-112	5.60	3.80	45.73	43.27	0.23	0.06	0.11	0.91
CRZ-104	7.19	2.45	31.25	57.59	0.15	0.08	0.13	0.35
CRZ-105	6.21	2.72	41.68	46.86	0.18	0.04	0.12	0.58
CRZ-111	5.47	2.93	40.09	50.18	0.17	0.01	0.15	0.77
CRZ-109	6.00	2.73	45.38	44.58	0.20	0.01	0.12	0.63
STRIP	5.62	2.89	46.34	43.49	0.19	0.06	0.18	0.65
DENİZ B(C)	6.17	2.73	36.36	53.25	0.19	0.09	0.16	0.64
AHMETBY(C)	5.42	4.12	47.60	40.99	0.28	0.05	0.14	0.78
IMI-CRZ-6	6.01	2.60	41.25	48.25	0.21	0.06	0.16	0.89
G1	6.17	2.91	40.06	49.30	0.23	0.06	0.14	0.67
TG400 (C)	6.06	2.89	43.24	46.25	0.21	0.01	0.13	0.69
AFFAN	6.23	2.92	39.68	49.64	0.21	0.06	0.13	0.73
IMI-CRZ--3	4.96	3.10	48.25	42.00	0.22	0.04	0.15	0.86
IMI-CRZ-16	5.54	2.98	40.98	48.91	0.20	0.06	0.13	0.75
PALANCI(C)	5.48	3.12	41.47	48.57	0.21	0.05	0.14	0.61
Average (%)	5.94	2.95	41.25	48.11	0.25	0.12	0.14	0.70
Std Dev (%)	0.48	0.47	3.85	3.79	0.22	0.19	0.03	0.19
Maximum (%)	7.19	4.12	48.25	57.59	1.27	0.78	0.27	1.41
Minimum (%)	4.96	2.13	31.25	40.99	0.13	0.04	0.10	0.35

Based on correlation analysis, significant relationships were observed among measured traits in the study, as shown in blue in Figure 2. Significant positive and negative relationships were determined especially between seed width and thousand seed weight and some micro elements such as Mo, Ag, Sn, Se, and Ni, and macro elements Na, Mg, K, Al, as well as palmitic and oleic acid contents with some quality traits. Mostly negative relationships were observed between Mg, Se and Ni elements and palmitic acid with other traits. In contrast, generally positive relationships were determined between oleic acid, Ag and TSW with other traits (Figure 2).

The grain yield values of the varieties included in the study ranged between 1280 and 4010 kg ha⁻¹, with the TG-400 hybrid ranking first followed by CRZ-116, CRZ-318, CRZ-101 hybrids, etc. Since earliness is an important and desirable trait, the earliest variety in the study was Ahmetbey, while TG-400 was the earliest to reach physiological maturity. The TG-400 hybrid stood out for being both early-maturing and high-yielding, as desired. Similarly, the varieties CRZ-116, Affan, Stripi, and CRZ-213 also exhibited similar characteristics.

In the taste evaluation of raw kernels of the varieties included in the study, the TG-400 variety received the highest score, followed by IMI-CRZ-3, IMI-CRZ-16, IMI-CRZ-6 hybrids, etc. The highest oleic acid content was measured in Ahmetbey and Palanci control varieties. Kernel size, consisting of kernel width (cm), kernel length (cm), and kernel thickness (cm), is the most important desired trait in confectionery sunflowers.

The kernel size of sunflower seeds significantly influences consumer preference in the confectionery market. Larger seeds are generally preferred for direct consumption as snacks, a key market for confectionery sunflower seeds. This preference is driven by both aesthetic appeal and the ease of consumption associated with larger seeds (Reinert et al., 2020).

Consumers primarily prefer large kernels, with kernel width ranging between 0.6 and 1 cm, and the widest kernels observed in the CRZ-116 and CRZ-30 lines. Kernel length ranged between 1.6 and 2.5 cm, with the largest kernels found in CRZ-116, CRZ-30, CRZ-19, Ahmetbey (C), IMI-CRZ-6, G1, TG400 (C), Affan, CRZ-110, and CRZ-110 hybrids. Kernel thickness ranged between 0.27 and 0.50 cm, with the thickest kernels identified in CRZ-110 and CRZ-318. Another measure of kernel size is the 1000-seed weight, which ranged between 130.0 and 248.5 g, with the highest value observed in the CRZ-116 line, followed by CRZ-30, CRZ-19, CRZ-318, CRZ-105, and CRZ-109 lines.

Table 5. Sensory and Color analysis results of sunflower seed samples.

#	Variety	Color	Aroma	Taste	Crispness	General	Seed Hardness		Color Analysis			
							Hulled	Un-hulled	L*	a*	b*	Browning index
1	CRZ-113	3.0	3.9	4.0	3.0	3.3	41.2	18.1	63.35	1.36	14.65	3,83
2	CRZ-116	1.8	3.0	4.2	3.7	3.2	81.3	43.1	60.22	2.95	17.94	6,48
3	CRZ-213	1.3	4.1	4.0	3.0	3.2	30.2	16.5	61.18	2.21	14.00	4,86
4	CRZ-30	2.8	2.2	4.0	4.0	2.0	68.6	27.2	57.4	2.85	16.25	6,37
5	CRZ-19	3.0	4.4	4.2	4.0	4.3	69.3	27.8	62.08	1.54	15.62	4,28
6	CRZ-24	3.0	1.0	1.4	3.1	2.9	47.9	20.7	64.49	1.54	16.97	4,33
7	CRZ-318	1.2	2.3	1.2	3.2	1.2	68.7	24.9	60.73	2.03	15.60	4,95
8	CRZ-103	1.0	3.9	3.8	3.9	3.0	65.0	22.0	61.74	2.39	16.88	5,49
9	CRZ-101	1.3	3.3	4.1	3.1	2.9	73.0	28.8	60.97	2.19	14.89	4,99
10	CRZ-110	2.5	3.4	3.8	3.9	2.8	69.8	26.0	62.35	1.53	15.49	4,22
11	CRZ-112	3.0	3.4	3.1	3.6	2.3	30.9	12.9	60.86	3.12	13.53	5,87
12	CRZ-104	3.0	3.8	1.7	3.9	3.5	58.7	21.6	59.45	2.4	14.17	5,25
13	CRZ-105	3.0	4.1	4.2	3.9	4.2	55.7	21.8	59.00	2.84	14.68	5,92
14	CRZ-111	2.9	4.0	4.0	3.9	3.7	31.8	14.6	58.35	2.95	13.82	5,97
15	CRZ-109	3.0	3.2	4.0	3.2	2.5	51.9	21.5	59.63	1.87	12.82	4,38
16	STRIPI	2.8	4.6	4.2	1.0	3.2	59.9	20.6	61.48	3.2	16.86	6,45
17	DENİZ B(C)	2.0	1.8	1.8	4.0	2.6	55.2	26.0	63.02	2.21	16.01	5,04
18	AHMETBEY (C)	2.6	3.7	3.8	3.8	2.7	59.7	26.3	62.85	1.82	17.14	4,79
19	IMI-CRZ--6	2.5	1.5	1.8	3.0	2.2	53.2	19.5	61.02	2.19	14.73	4,97
20	G1	2.3	2.7	3.3	3.2	2.1	51.0	18.7	62.73	2.76	16.36	5,74
21	TG400 (C)	2.9	4.3	3.8	3.8	3.9	51.9	22.8	67.02	2.03	15.43	4,45
22	AFFAN	2.8	4.3	4.2	3.1	2.9	53.0	23.0	64.22	2.85	16.16	5,67
23	IMI-CRZ--3	2.9	2.5	3.8	3.2	3.8	40.8	17.9	62.10	1.35	17.85	4,41
24	IMI-CRZ--16	3.0	3.7	2.8	3.8	2.7	47.4	21.5	62.63	2.09	15.39	4,83
25	PALANCI 1 (C)	3.0	4.0	4.0	3.9	3.1	41.7	19.4	64.44	2.23	14.77	4,76
Average		3,0	3,9	4,0	3,0	3,3	54,31	22,52	61,73	15,52	2,26	5,13
Std Deviation		1,8	3,0	4,2	3,7	3,2	41,2	18,1	2,14	1,34	0,56	0,76
Maximum		1,3	4,1	4,0	3,0	3,2	81,3	43,1	67,02	17,94	3,20	6,48
Minimum		2,8	2,2	4,0	4,0	2,0	30,2	16,5	57,40	12,82	1,35	3,83

The peroxide value is an analysis indicator that reflects the degree of oxidation in oil-containing products, generally increasing with the application of heat treatment. In our study, the peroxide values of the samples ranged considerably between 4.5 and 124.5 meq O₂ kg⁻¹ sample, with high peroxide values indicating that some samples were exposed to significant oxidation levels. This wide range of peroxide values provided an opportunity to evaluate the relationship between oxidative status and consumer acceptance.

The results of the sensory evaluation showed an inverse relationship between peroxide value and overall acceptability scores. Samples with lower peroxide values generally received higher scores in taste, aroma, and overall acceptability parameters. For instance, Affan, TG400, CRZ-318, and CRZ-105 varieties, which had comparatively lower peroxide values (3.5, 8.5, 4.5, and 7.5 meq O₂ kg⁻¹, respectively), received higher sensory scores. In contrast, varieties such as CRZ-24 and Denizli Beyazi, with significantly higher peroxide values (83.5 and 124.5 meq O₂ kg⁻¹, respectively), were rated lower in sensory evaluations, particularly in taste attributes.

The relationship between peroxide value and off-flavor development is well-established in the literature. Peroxide value measures the concentration of peroxides formed during the initial stages of lipid oxidation, which are unstable and can decompose into various volatile compounds that contribute to off-flavors, particularly rancidity (Muresan et al., 2010; Manzocco et al., 2020). In sunflower seeds, the lipid fraction is rich in polyunsaturated fatty acids, which are highly susceptible to oxidation due to their multiple double bonds, making sunflower oil and its derivatives prone to oxidative deterioration.

Figure 2. Correlation analysis of measured traits

Several volatile compounds resulting from lipid oxidation contribute to off-flavors in sunflower seeds, including hexanal (associated with rancid flavor), pentane (linked to rancidity), and deca-2,4-dienals (contributing to a deep-fried or painty flavor). Our findings align with other research showing that higher peroxide values correlate with increased consumer detection of off-flavors and reduced acceptability (Villiere et al., 2007).

Multiple factors, including fatty acid composition influence the oxidative stability of sunflower seeds. Interestingly, varieties with higher oleic acid content in our study, such as AHMETBEY (74.39%) and IMI-CRZ-3 (64.59%), demonstrated better sensory scores despite having moderate peroxide values. This observation is consistent with research suggesting that high-oleic sunflower varieties, with their higher proportion of monounsaturated fatty acids, are more resistant to oxidation and subsequent off-flavor development compared to varieties with higher polyunsaturated fatty acid content (Sabolová et al., 2017; Marmesat et al., 2009).

The total phenolic content of the varieties may also play a role in oxidative stability and consumer acceptance. Phenolic compounds act as natural antioxidants, potentially reducing peroxide formation and delaying the development of off-flavors. In our study, CRZ-103, which had the highest phenolic content (2505 mg GAE 100 g⁻¹), exhibited a relatively low peroxide value (12.5 meq O₂ kg⁻¹) and received favorable sensory scores. This observation supports previous findings that natural antioxidants can mitigate lipid oxidation and improve consumer acceptance (Athanasiadis et al., 2023). Storage conditions, though not specifically measured in this study, also significantly impact the rate of lipid oxidation and the formation of off-flavors. Higher temperatures and light exposure accelerate the oxidation process, leading to faster increases in peroxide value and the formation of volatile compounds (Sadeghi et al., 2020). This factor should be considered when interpreting our results, as variations in storage conditions prior to analysis could have influenced the peroxide values observed.

From a practical perspective, understanding the relationship between peroxide value and consumer acceptance is essential for the confectionery sunflower industry. Our findings suggest that maintaining low peroxide values through appropriate selection of varieties with favorable fatty acid compositions (higher oleic acid content), higher phenolic content, and implementing proper storage conditions could significantly improve consumer acceptance of confectionery sunflower products.

The TG 400 variety, which stood out in our study for its overall performance, demonstrated a favorable balance between peroxide value (8.5 meq O₂ kg⁻¹), oleic acid content (63.39%), and phenolic content (1505 mg GAE/100g DM), resulting in high consumer acceptance scores. This variety could serve as a benchmark for developing new confectionery sunflower varieties with improved oxidative stability and consumer acceptability.

Soil pH, organic matter content, and the presence of other elements can significantly influence the accumulation of heavy metals and microelements in sunflower seeds. For example, lower soil pH has been associated with higher accumulation of Cu and Zn in sunflower seeds (Sabudak et al., 2007). In this study, heavy metal contents such as Ar, Pb, and Cd in the samples were found below 0.002 mg kg⁻¹. Cadmium is one of the most studied heavy metals in sunflower seeds. Research indicates that sunflowers have a high capacity to accumulate Cd, particularly in their seeds. Studies have shown that Cd concentrations in sunflower seeds can range from 0.052 to 0.234 mg kg⁻¹, with some samples exceeding the maximum permissible levels (Sorour et al., 2024). The bioaccumulation of Pb in sunflower seeds is relatively low compared to other heavy metals, suggesting that sunflowers may not be as efficient at accumulating Pb as they are for Cd or Zn (Chaves et al., 2011).

Sunflowers have been shown to accumulate Zn efficiently, with higher concentrations in seeds compared to other plant parts. This makes sunflowers a good candidate for Zn biofortification. In this study, the highest microelement found in samples was also Zn, with concentrations ranging between 201-271 mg kg⁻¹. Copper is an essential microelement for plant growth, but excessive levels can be toxic. The second highest microelement found in samples was Cu, with concentrations ranging between 67-95 mg kg⁻¹. Sunflower seeds have been found to accumulate Cu, with concentrations ranging from 1.21 to 42.9 mg kg⁻¹ (Gul and Kul, 2021).

The micronutrients in sunflower seeds also affect the fatty acid profile of the seed. The concentrations of oleic acid were primarily influenced positively by manganese, phosphorus, and nitrogen. This indicates that these nutrients play a vital role in enhancing the quality of oil in terms of its fatty acid composition. The findings emphasize the importance of balanced nutrient management in sunflower cultivation to optimize oil quality and fatty acid composition. Understanding these relationships can help farmers and agronomists make informed decisions regarding fertilization practices to enhance oil yield and quality (Bozkurt and Karaca, 2001).

The pH values of raw sunflower kernel samples ranged between 7.04 and 6.59, water activity values between 0.470 and 0.442, and moisture content between 3.3% and 2.41%, with no significant impact on kernel quality. Another critical factor is the moisture content of the seeds. Maintaining optimal moisture levels is essential, as excessive moisture can lead to spoilage, while insufficient moisture can result in undesirable texture. Moisture content significantly affects the texture of roasted sunflower seeds. Roasting at specific temperatures and times reduces moisture, enhancing crispiness. For instance, roasting at 125°C for 45 minutes yields seeds with optimal texture and minimal off-flavors (Guo et al.,

2019). High protein content is a desirable trait in confectionery sunflowers, and the protein values of the varieties ranged between 17.87% and 30.05%, with the highest value observed in the Ahmetbey variety. Protein content is a key factor in the texture of sunflower seeds. Varieties with higher protein content (18.82-29.81%) tend to have a firmer texture, which is desirable for snacking and culinary uses (Ozcan et al., 2024).

The total phenolic content of hulled sunflower kernel samples ranged between 943 and 2505. Among the varieties, the CRZ-103 candidate line had the highest phenolic content, while the lowest value was observed in the IMI-CRZ-16 candidate line. Control and foreign candidates, except for the Affan variety, generally had phenolic content below the trial average. Numerous studies have investigated the phenolic compounds in sunflower seeds, highlighting their strong antioxidant properties. The primary phenolic components in sunflower seeds include chlorogenic acid, caffeic acid (in smaller amounts), as well as cinnamic, coumaric, syringic, and hydroxy-benzoic acids. These phenolic compounds exhibit high antioxidant potential and technofunctional properties that may offer biofunctional benefits (Rayman Ergun, 2024).

The differences in the total phenolic content of sunflower kernels are likely influenced by factors such as variety, location, growing and climatic conditions, and harvest time. Nadeem et al. (2011), in their study with sunflower seeds, found that the total phenolic matter content of the samples varied between 2185-2965 mg/100g in shelled seed samples and 3162-3614 mg GAE100 g⁻¹ in unshelled seed samples. Fiska et al. (2006) determined the total phenolic matter content of unshelled sunflower samples as 2700 mg GAE100 g⁻¹ dry matter. Ali et al. (2023) found the total phenolic matter content of unshelled sunflower seed samples between 1930-2310 mg GAE100 g⁻¹. The total phenolic matter content was found to be generally compatible with the literature. In another study, Ozcan et al. (2024) found that total phenolic content of the seeds varied between 99.64-130.75 GAE 100 g⁻¹ sample.

No direct correlation was found between the total phenolic content in the samples and the results obtained from sensory analysis regarding taste, aroma, and color. Phenolic compounds in olive oil and other plant-based foods contribute to their aroma and flavor profiles, which are critical for consumer acceptance. For example, lower molecular weight phenolic acids, such as ferulic and vanillic acids, tend to have a sourer and bitter taste, whereas larger polymeric phenols are associated with astringency (Duizer & Langfried, 2016). Phenolic compounds can contribute to the color of nuts and seeds. For example, chlorogenic acid, a phenolic compound found in sunflower seeds, can react with proteins to form green-colored complexes, which may affect the appearance of sunflower-based food products (Wildermuth et al., 2016). Some phenolic compounds, such as tannins, can impart a bitter or astringent taste to nuts and seeds, which may be undesirable to some consumers (Ozcan et al., 2024). Detailed research should be carried out on which phenolic components affect consumers' appreciation of sunflower seeds.

The fatty acid composition of confectionery sunflower seeds plays a crucial role in determining their consumer acceptance, not only affecting nutritional value but also significantly influencing flavor, texture, oxidative stability, and shelf life (Yin et al., 2022; Li et al., 2024). In our study, the fatty acid profiles of the different varieties showed considerable variation (Table 4), with palmitic acid ranging from 4.96-7.19%, stearic acid from 2.13-4.12%, oleic acid from 31.25-48.25%, linoleic acid from 40.99-57.59%, and linolenic acid from 0.13-1.27%. These variations have important implications for consumer acceptance of confectionery sunflower varieties.

The relationship between fatty acid composition and sensory characteristics is particularly notable in confectionery sunflower seeds. High-oleic varieties, with their lower polyunsaturated fatty acid content, are more resistant to oxidation and less prone to developing rancidity and off-flavors (Sabolová et al., 2017). Our study showed that varieties with higher oleic acid content generally received higher sensory scores and had lower peroxide values, indicating better oxidative stability. Varieties with higher oleic acid content, such as IMI-CRZ-3 (48.25%), Ahmetbey (47.60%), and Stripi (46.34%), generally received favorable scores in taste and overall acceptability. This correlation can be attributed to the enhanced oxidative stability and improved flavor profile associated with high-oleic varieties. The moderate oleic acid content of TG 400 (43.24%), combined with its balanced fatty acid profile, contributed to its high consumer acceptance ratings, making it a promising variety for confectionery applications.

Notably, the ratio of saturated to unsaturated fatty acids also had a significant impact on consumer acceptance in the present study. Varieties exhibiting moderate concentrations of saturated fatty acids—specifically palmitic and stearic acids—alongside elevated levels of oleic acid, appeared to offer an optimal balance, aligning well with consumer preferences. For example, CRZ-19, which received high sensory scores (4.3 overall acceptability), had a balanced fatty acid profile with 5.94% palmitic acid, 2.13% stearic acid, and 42.22% oleic acid. This balance provides improved oxidative stability while maintaining desirable sensory properties. The linolenic acid content varied significantly among the varieties in our study, with CRZ-113 showing the highest content (1.27%). While linolenic acid is nutritionally beneficial, its high degree of unsaturation makes it susceptible to oxidation, potentially affecting flavor stability and shelf life. This may explain why CRZ-113 received moderate rather than exceptional sensory scores despite having the highest linolenic acid content.

The aroma of sunflower seeds is significantly influenced by their fatty acid composition, with variations in oleic acid content being particularly impactful on the sensory experience and consumer acceptance. Our findings demonstrate that the aroma profile of confectionery sunflower seeds is closely tied to both the underlying fatty acid composition and the

oxidative processes that occur during storage and processing. Higher oleic acid content in sunflower seeds is consistently associated with a more desirable aroma profile, particularly following thermal treatment such as roasting (Yin et al., 2022). As evidenced in our analysis, varieties with higher oleic acid content, such as IMI-CRZ-3 (48.25%) and Ahmetbey (47.60%), received higher sensory scores for aroma (2.5 and 3.7, respectively).

Our data align with previous research indicating that the volatile compounds present in sunflower oil are significantly influenced by its fatty acid composition. High oleic sunflower varieties tend to have fewer volatile aldehydes associated with rancidity and off-flavors, such as E-2-heptenal, compared to traditional varieties with higher linoleic acid content (Xu et al., 2017). The CRZ-104 variety in our study, with the lowest oleic acid content (31.25%), received a relatively lower aroma score (3.8) compared to varieties with higher oleic acid content.

This extended shelf-life is vital for maintaining product quality and consumer satisfaction, as it allows for longer storage without degradation (Pajin et al., 2011). Among fatty acids, linolenic acid (Omega 3), which is highly preferred in nutrition, showed the highest rate in CRZ-113, as well as oleic acid, which extends shelf-life quality and taste, with the highest value measured in IMI-CRZ-3. Regarding kernel hardness, the CRZ-116 line showed the highest hardness in both measurements, while the CRZ-112 line had the lowest. Fatty acid composition is a critical quality parameter. High oleic sunflower seeds have higher oleic acid (C18:1) and lower linoleic acid (C18:2) content, which improves oxidative stability and shelf life. Processing methods like roasting and microwave pretreatment can influence fatty acid profiles, with high-oleic varieties being more resistant to oxidation (Soleimanieh et al., 2015; Yin et al., 2022). The taste of sunflower seeds is largely determined by volatile compounds formed during processing. Roasting generates terpenes, pyrazines, and aldehydes, which contribute to nutty and roasted flavors. Key compounds include 2,5-dimethylpyrazine and 2,3-dimethylpyrazine, which are enhanced by higher roasting temperatures and times (Guo et al., 2019; Yin et al., 2022). During storage, sunflower seeds can develop off-flavors due to lipid oxidation. The formation of compounds like hexanal and octanal contributes to rancidity. Proper processing and storage conditions are essential to minimize these changes (Yin et al., 2022).

Our findings revealed a positive correlation between oleic acid content and consumer acceptability scores. Varieties with higher oleic acid content, such as IMI-CRZ-3 (48.25%), Ahmetbey (47.60%), and Stripi (46.34%), generally received favorable scores in taste and overall acceptability. This correlation can be attributed to the enhanced oxidative stability and improved flavor profile associated with high-oleic varieties. The moderate oleic acid content of TG 400 (43.24%), combined with its balanced fatty acid profile, contributed to its high consumer acceptance ratings, making it a promising variety for confectionery applications.

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The fatty acid composition of sunflower seeds significantly influences their acceptance as a confectionery food, both in terms of overall and sensory acceptance. Sunflower seeds with modified fatty acid profiles, such as high oleic and high stearic varieties, offer distinct physical properties that enhance their functionality in confectionery applications. These modifications affect the texture, melting behavior, and sensory characteristics of the final product, which are crucial for consumer acceptance. Interestingly, the ratio of saturated to unsaturated fatty acids also influenced consumer acceptance in our study. Varieties with moderate levels of saturated fatty acids (palmitic and stearic acids) combined with high levels of oleic acid seemed to achieve the best balance for consumer preference. For example, CRZ-19, which received high sensory scores (4.3 overall acceptability), had a balanced fatty acid profile with 5.94% palmitic acid, 2.13% stearic acid, and 42.22% oleic acid. This balance provides improved oxidative stability while maintaining desirable sensory properties.

The application of sunflower seeds in confectionery products is further enhanced by specific fatty acid compositions. High stearic sunflower oils have been developed to improve the physical properties of sunflower oil, making it suitable for confectionery applications. These oils have a higher content of solid fats, which are essential for creating the desired texture and stability in confectionery products. Several varieties in our study, particularly Ahmetbey with 4.12% stearic acid, show potential for such applications due to their relatively higher stearic acid content. In our sensory evaluations, this characteristic may have contributed to the higher acceptability of varieties with balanced fatty acid profiles, such as TG 400 and CRZ-19.

The linolenic acid (Omega-3) content varied significantly among the varieties in our study, with CRZ-113 showing the highest content (1.27%). While linolenic acid is nutritionally beneficial, its high degree of unsaturation makes it susceptible to oxidation, potentially affecting flavor stability and shelf life. This may explain why CRZ-113 received moderate rather than exceptional sensory scores despite having the highest linolenic acid content. However, from a nutritional perspective, moderate levels of linolenic acid are desirable and could be promoted as a health benefit in marketing confectionery sunflower seeds. While the modification of fatty acid composition in sunflower seeds can enhance their application in confectionery products, it is important to consider the nutritional implications. High levels of

saturated fats, although beneficial for texture and stability, may pose health risks if consumed in excess. Therefore, balancing the functional benefits with health considerations is crucial for the overall acceptance of sunflower seed-based confectionery products.

According to sensory analyses of the varieties, the CRZ-19 line received the highest score, while among control and foreign candidate varieties, the TG-400 variety received the highest score. Among control varieties, Palanci and Ahmetbey, and among candidate varieties, CRZ-105 and CRZ-111 lines also received high scores. The CRZ-113 line had the lowest browning index among the varieties, while the TG-400 variety had the lowest among control varieties. In general, samples with lower peroxide values had higher sensory acceptability.

4. CONCLUSION

In summary, from the producer's perspective, grain yield and earliness are important. In terms of yield, the TG-400 hybrid and, respectively, CRZ-116, CRZ-318, CRZ-101, Affan, and CRZ-213 varieties stood out. For earliness and high yield, TG-400, CRZ-116, AFFAN, and CRZ-213 varieties were prominent. From the consumer's perspective, based on raw taste evaluation, TG-400 and IMI-CRZ-3, IMI-CRZ-16, IMI-CRZ-6, CRZ-19, and CRZ-24 lines were notable. For kernel size, CRZ-116 and CRZ-30 lines, as well as TG400, CRZ-110, CRZ-318, and CRZ-19 lines, were prominent. According to sensory analyses, the CRZ-19 line stood out, and the TG-400 variety, along with CRZ-105 and CRZ-111 lines, were among the most preferred. In conclusion, when evaluated from the perspectives of consumers, industry, and producers together, it is preferred that newly developed varieties be high-yielding and early maturing like the TG-400 hybrid, with high oleic and linolenic acid and protein content, but low browning index and hardness.

ACKNOWLEDGEMENT

The study results were funded by Trakya University with TUBAP 2022/19 project. We would like to thank to Trakya University or financial supports.

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Effects of Different Seeding Rates on Growth Performance, Yield, and Quality of *Calendula officinalis* L. in Mediterranean Conditions

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ARTICLE INFO

Research Article

Received: 4 November 2024

Accepted: 6 January 2025

Published: 23 June 2025

Keywords:

Calendula officinalis L.

Climate change impact

Flower yield

Pot marigold

Seed yield

Citation: Boztas, G., & Bayram, E. (2025). Effects of different seeding rates on growth performance, yield, and quality of *Calendula officinalis* L. in Mediterranean conditions. *Turkish Journal of Field Crops*, 30(1), 138-150. <https://doi.org/10.17557/tjfc.1578727>

ABSTRACT

"*Calendula officinalis* L., commonly known as pot marigold and a member of the *Asteraceae* family, is widely used in cosmetics, medicine, and pharmacy due to its rich bioactive compounds. This study investigated the effects of varied seeding rates on the growth, yield, and quality of *Calendula officinalis* L. in Mediterranean climates, focusing on morphological, quality, and agronomic characteristics, including seed and biological yield. The research was conducted at the experimental fields of the Ege University, Faculty of Agriculture, Department of Field Crops, over two growing seasons (2019–2020 and 2020–2021). The experiment followed a randomized complete block design with a factorial arrangement, incorporating three replicates. The factors included (a) year (2020 and 2021) and (b) five seeding rates (5, 10, 15, 25, and 35 kg ha⁻¹). Results indicated that seeding rate significantly impacted agronomic yield. While plant density per m² increased with higher seeding rates, there was a decline in plant branching, plant height, seed yield, biological yield, fresh flower yield, and dried flower yield at higher seeding rates. The optimum seeding rate for achieving high flower and seed yields was determined to be 10 kg ha⁻¹. Rainfall during the flowering period was found to be critical for maximizing drug flower and seed yield, highlighting the importance of careful consideration of seeding percentage and precipitation to achieve the optimum yield of *C. officinalis*.

1. INTRODUCTION

Calendula officinalis L., also known as pot marigold, is an annual or rarely perennial plant of the Asteraceae family. Native to the Mediterranean region, it is a medicinal and ornamental herb with yellow and orange flowers. It is a herbaceous (slightly woody at the base), 15-50 cm tall, multi-branched (Webb et al., 1988), usually secretory and aromatic plant (European Pharmacopoeia, 1975). Primarily, the flowers are used, but the stems, younger leaves, seeds and roots of *C. officinalis* L. also have medicinal properties. This versatile plant is used extensively the manufacture of pharmaceuticals (pain relief, calming the nervous system, promoting digestion, and treating skin diseases), cosmetics, culinary in medicinal, cosmetic, culinary and ornamental purposes (Warner and Erwin, 2005; Khalid and Teixeira da Silva, 2012; Ercetin et al., 2012). The essential oil of *Calendula officinalis* has been found to have various beneficial effects, including pain relief, calming nerves, promoting digestion, and treating skin diseases (Daniil and Kashchenko, 2013). Therefore, the essential oil and total flavonoid content of *C. officinalis* are significant factors that contribute to its pharmaceutical and medicinal importance.

The quantity and quality of crop products are significantly affected by cultural treatments like sowing date and seeding percentage, mineral fertilization, biotic stresses (diseases, pests and weeds), abiotic stress (harvest date, drying and storage conditions). These conditions significantly impact the production and concentration of secondary metabolites percentage. (Hussain et al., 2011; Berimavandi et al., 2011). Plants cannot fully utilize light, water and soil at extremely low densities. Similarly, intraspecific competition reduces yields at high densities (Seghatoleslami and Mousavi, 2009). Optimum plant density has been shown to provide more favorable conditions for crop development for higher flower yields (Karuppaiah and Krishna, 2005). Therefore, seeding rate is a critical element that impact on crop management components and yields. Low plant populations can diminish product by reducing seed yield and essential oil production (Panhwar et al., 2017).

The optimal seeding rate for crop production is influenced by several factors including soil moisture content and fertility, as well as the genotype or variety of the crop (Bukharov et al., 2023; Namdeo et al., 2020). The uniformity of seeding rate across the field is also important, as it affects the distribution of nutrition and moisture contents in the plants (Gupta, 2022). Additionally, economic factors such as the cost of seed and the price received from marginal yield increases can impact the optimal seeding rate (Lidsey et al., 2018). Other factors influencing the optimal yield are seeding percentage, planting date, soil type, drainage, soil fertility, pest pressure, and growing season conditions (Sabeti et al., 2019). Precision agriculture techniques, such as variable seeding rate can help optimize seeding rate by taking into account field conditions and variability. Overall, achieving the optimal seeding rate requires considering a combination of biological, environmental, and economic factors.

Climate change poses additional challenges to seed production, including impacts on germination and plant development due to fluctuating temperatures and rainfall patterns (Maity et al., 2023; Bailly and Gomez Roldan, 2023; Moharana et al., 2023). Additionally, its can lead to changes in crop physiology and yield potential, with rising temperatures and weather extremes affecting (Roy and Rakshit, 2022). As climate change induces an upward shift in mean temperatures, the likelihood of supra-optimal conditions intensifies, potentially necessitating revisions in seeding rates to secure sufficient stand establishment (Cochrane, 2020).

The objective of study was to evaluate how different seeding rate treatments impact the productivity of plant flowers and seeds in response to varying climatic conditions in the Mediterranean region. This is an important area of research, as understanding the relationship between seeding rates and plant productivity can provide valuable insights for agricultural practices in regions with diverse Mediterranean climate.

2. MATERIAL AND METHODS

Climatic and soil characteristics

Izmir Province, located in the Mediterranean climate zone, is situated in the Aegean Region in the west of Türkiye, between the latitudes of 37° 45' and 39° 15' northern parallels and the longitudes of 26° 15' and 28° 20' eastern meridians. The study area was characterized by clay-loam soil (pH 7.88, and predominantly calcareous), with an organic matter content of 1.99%. The soil is rich in medium lime, with a lime content of 14.18%. Soil analysis indicated insufficient levels of organic matter, total nitrogen, and available phosphorus, but excessive available potassium in the field. According to long-term climatic data for Izmir province, the average maximum temperature is 27.0°C, the average minimum temperature is 3.9°C, the annual average temperature is 14.4°C, and the average yearly precipitation is 733 mm (Turkish State Meteorological Service, 2023). The climatic diagram of the experimental field for the years 2019, 2020, and 2021 is presented in Figure 1.

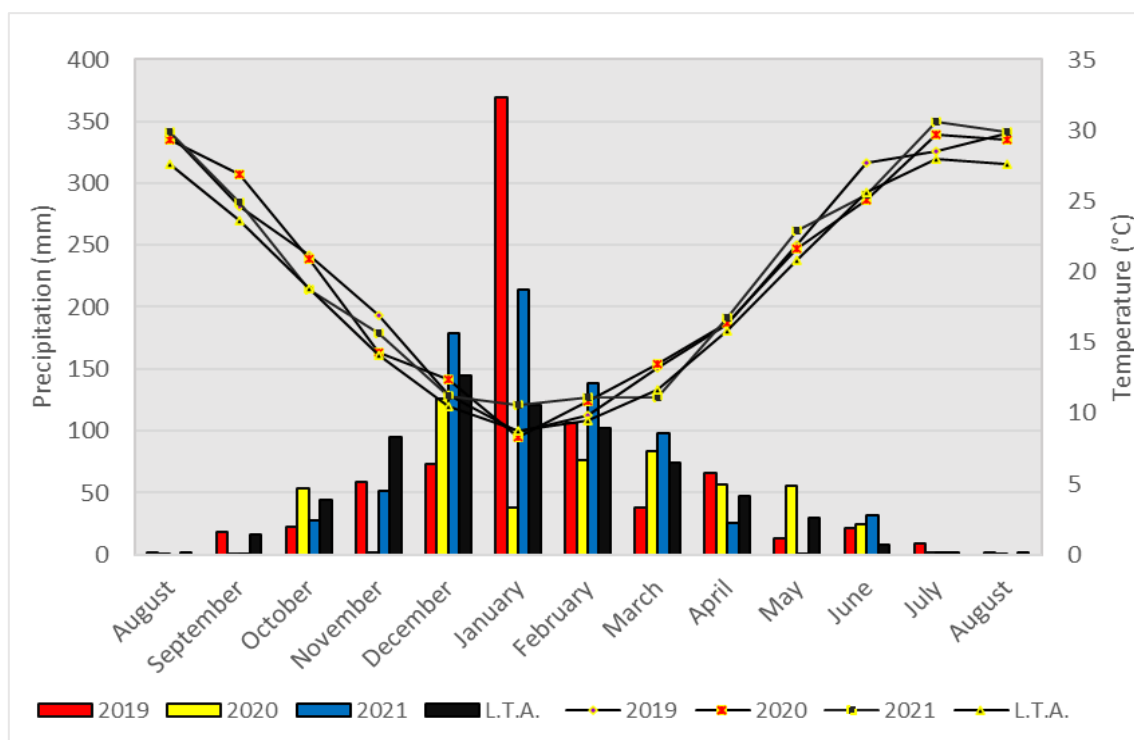


Figure 1. Precipitation and temperature of the experimental area.

In general, it is noteworthy that the temperature values observed in 2019, 2020, and 2021 were above the long-term averages (L.T.A.). For example, the temperatures in August were 29.8°C in 2019 and 29.9°C in 2021, compared to the long-term average of 27.6°C. This indicates a significant temperature increase in the summer months. Temperatures in January and February were also higher than the L.T.A. In particular, the temperature reached 10.6°C in January 2021, which is a significant increase compared to the long-term average of 8.8°C. There were considerable fluctuations in rainfall amounts. In particular, while 58.2 mm of precipitation was measured in November 2019, only 2.2 mm was recorded in the same month in 2020. This demonstrates an increase in the imbalance of the precipitation regime.

Additionally, significant increases in precipitation were observed in December. In 2021, December rainfall was 178.3 mm, well above the long-term average of 144.1 mm. Notably, 369.3 mm of rainfall was recorded in January 2019, nearly three times the long-term average of 121.0 mm (Fig. 1).

Plant material and field studies

The *C. officinalis* L. population seeds were used supplied by Pharmasaat GmbH (Germany) as research material. The field experiments were conducted on the trial area of the Field Crops Department, Faculty of Agriculture in Ege University in 2019-2020 and 2020-2021 growing seasons in Izmir-Turkey. The experiment followed a randomized complete block design with a factorial arrangement, incorporating three replicates. The factors included (a) year (2020 and 2021) and (b) five seeding rates (5 [SR1], 10 [SR2], 15 [SR3], 25 [SR4], 35 [SR5], kg ha⁻¹). Each plot consisted 4 rows, 40 cm apart and 3 m long. Plot sizes was 4.8 m², the total trial area was 96 m² in both the 2019-2020 and 2020-2021 growing seasons. The sowing dates were 8 November 2019 and 24 November 2020, in the same sequence. Seeding depth were 1-2 cm, and the seeds was covered using harrow. Sixty kilograms per hectare each of nitrogen and phosphate were applied to the seedbed. As basal fertilizer, phosphorus fertilizer TSP (triple super phosphate) was applied before sowing. Nitrogen (Urea) was given two times: as 30 kg ha⁻¹ at the sowing stage, 30 kg ha⁻¹ at the beginning of flowering period. During the experimental years, the plants were watered by a drip irrigation system. The weeds were controlled by hand and hoeing. No pesticides were used in used in the experiments.

Morphological and yield parameters

Ten plants were randomly sampled from each plot, and their morphological properties [(plant height (PH), total number of branches (TNB)]. Flower harvest, (the onset of flowering 12 April in 2020, 4 May in 2021) at the full bloom periods were measured at the time of harvest. Flower yield was determined by hand harvesting 1,6 m² area of the centre rows of each plots. The harvested plants whose fresh flowers yields (FFY) were calculated in each plot were left to dry in a drying cabinet at 30 °C for 2 days. The dry weights of the dried flowers were weighed on a precision balance and drug flowers yield (DFY) was determined in kg ha⁻¹.

Number of plant (NP), biological yield (BY), seed yield (SY) and harvest index (HI) were determined by collecting aboveground biomass. Plant samples were counted in the designated area using a 0.25 m² scale, and the number of plants per square meter was calculated. Seed yield was determined by hand threshing. The harvest index was determined by dividing the hand-threshed dry seed biomass by the total aboveground dry biomass.

Quality parameters

The essential oil rate (EOR) was measured by extraction from 10 g of air-dried (35 °C) drug material by hydro-distillation (100 mL water) with a Clevenger-type apparatus for a period of three hours (European Pharmacopoeia, 1975).

The total flavonoid content (TFC) was quantified using the aluminium chloride colorimetric assay. A solution of 5% NaNO₂ (0.3 mL) was added to the dry plant extract (0.3 mL), followed by the addition of 0.3 mL of 10% AlCl₃ solution after 5 minutes. Subsequently, 2 mL of 1 M NaOH was added, and the total volume was made up to 10 mL with distilled water. The solution was thoroughly mixed and incubated in the dark for 30 minutes. Absorbance was then measured spectrophotometrically at 510 nm. The total flavonoid content of plant samples was expressed as mg rutin per gram of dry matter. All spectrophotometric measurements were conducted using a Cary 50® spectrophotometer (Zhishen et al., 1999).

Statistical analysis

The statistical analyses were conducted using the JMP 14® statistical program (SAS Institute Inc., 2018). Statistically significant differences in seeding rate treatments were analyzed using a two-way analysis of variance (ANOVA) with the separation of means by a least significant difference (LSD) test ($p < 0.05$). Grouped boxplots were created in R Studio via the 'tidyverse' (Wickham et al. 2019), 'ggplot2' (Wickham et al. 2016) and 'gapminder' (Bryan, 2022) packages. Additionally, correlations between all pairs of measured characters were calculated in R Studio.

3. RESULTS AND DISCUSSION

Morphological and yield parameters

Plant height (cm)

The year factor was statistically and significantly different ($p < 0.05$). The maximum average plant height was 51.8 cm in 2021 and the minimum average was 43.9 cm in 2020 (Fig 2). The maximum plant height among the seeding rates was found using 10 kg ha⁻¹ (48.8-54.8 cm) in both years, although the effect of seeding rate was not significantly different.

Rahmani et al. (2008) reported that drought stress in *C. officinalis* L. reduced plant height and caused smaller leaf area during the early vegetative growth period. Similarly, the decrease in plant height with drought stress was associated with an increase in the partitioning of assimilates between shoots and roots in agreement with Moosavi et al. (2014). Martin and Deo (1999) studied different seeding rates on flower production of *C. officinalis* L. in New Zealand. The study found the minimum plant height (54.4 cm) at 3 kg ha⁻¹ seeding rate and the maximum (73.4 cm) at 48 kg ha⁻¹. Mollafilabi and Hosseini (2012) reported that the maximum plant height was found in 10 kg ha⁻¹ seeding rates treatment and this value was found to be 26.4 cm. Mirzaei et al. (2016) determined the maximum plant height as 51.01 cm (60 plants m⁻²). The researchers mentioned that the increase in plant height with increasing plant density can be explained by the increased activity of stem growth hormone due to lack of light. Kwiatkowski et al. (2020) came to the conclusion that an increase in plant height is associated with an increase in plant density, with the maximum height increase occurring at densities of up to 70-90 plants per m².

Plant height of *Calendula officinalis* showed a significant response to climate variability during the 2019-2020 and 2020-2021 growing seasons, particularly in terms of precipitation and temperature. Precipitation in the 2019-2020 season (November-May), in which the experiment was carried out, totaled 384.4 mm, while 603.1 mm was recorded in the same months of the 2020-2021 growing year. The results of the second year, the amount of precipitation effective in the period from sowing to flowering, increased approximately two times more than in the first year (Fig 1). The effects of the climatic conditions, which varied according to the year, and of increasing the seed rate up to a certain level on the plant height were significant ($p < 0.05$). Increased rainfall in 2021 resulted in increased plant height, presumably due to increased water availability, facilitating cell elongation and overall growth. The increased height of the plants is beneficial for harvesting, but also makes them more susceptible to lodging in strong winds, especially if the height is excessive. Conversely, the reduced rainfall in 2020 resulted in less than optimal growth, highlighting the critical role of consistent water availability in maintaining healthy plant height.

Total number of branches (pcs plant⁻¹)

The effect of seeding rates on total number of branches was found to be significant ($p < 0.05$). The maximum value in total number of branches per plant was obtained from 10 kg ha⁻¹ (11.7 pcs) and the minimum value was obtained from 35

kg ha⁻¹ (5.67 pcs) seeding rate treatment. At low seeding rate, branching increased with the expansion of the unit area per plant. This may be attributed to the decrease in intraspecific competition with the decrease in the amount of seed per unit area. It is well established that the decrease in intraspecific competition results from lower seed density (Fig 2.).

The findings of study revealed a significant effect of seeding rates on the total number of branches (TNB) per plant ($p<0.05$), with the maximum TNB (11.7 branches) observed at a seeding rate of 10 kg ha⁻¹ and the minimum (5.67 branches) at 35 kg ha⁻¹. These results suggest that lower seeding rates reduce intraspecific competition by expanding the unit area available per plant, thereby promoting branching. Our findings are consistent with the literature, where Martin and Deo (1999) established that increased plant density correlates with a decrease in branching and the harvest index. Similarly, Berimavandi et al. (2011) reported a TNB maximum of 9.44 branches per plant under conditions comparable to those in this study. Additionally, studies conducted in Türkiye, Bicen (2020) and Barut et al. (2022) reported TNB values ranging from 5.02 to 6.7 and 7.04 to 7.43 branches per plant, respectively. While these findings align with the trends observed in this research, variations may stem from differences in research methodologies and the influence of specific environmental conditions. Climatic variability during the study period also played a critical role in determining TNB. It is hypothesized that the reduced rainfall in 2020 may have restricted branching due to water stress affecting meristem activity. However, the higher and more variable rainfall in 2021 may have alleviated some of this stress, allowing plants to utilize available resources more effectively at optimal seeding rates.

Number of plants (plants m⁻²)

The interaction between year and seeding rate had a significant effect on the number of plants ($p<0.05$). Figure 2 illustrates that the maximum number of plants per square meter was observed in both 2020 and 2021 at a seeding rate of 35 kg ha⁻¹. Specifically, in 2020, this seeding rate resulted in a value of 98.7 plants m⁻², while in 2021, it yielded 82 plants m⁻². Conversely, the minimum number of plants was consistently recorded at a seeding rate of 5 kg ha⁻¹ in both years. Upon considering the seeding rate concerning the average number of plants per year, it is evident that in 2021, the number of plants decreased compared to 2020. Nonetheless, the overall average number of plants across both years was calculated to be 41.4 plants m⁻².

The significant interaction between year and seeding rate on number of plant ($p<0.05$) observed in our study is consistent with findings in the literature, where plant density influences various growth parameters. The results showed that the maximum number of plants was recorded at a seeding rate of 35 kg ha⁻¹, with a decrease in 2021 compared to 2020. These findings align with studies like Cromack and Smith (1998), who noted that 40 plants m⁻² or higher densities have minimal effects on crop growth and yield. Their findings suggest that increasing plant density does not significantly enhance the yield beyond a certain threshold, which may explain why a higher seeding rate (35 kg ha⁻¹) did not lead to a proportional increase in plant numbers in 2021. Similarly, Martin and Deo (1999) observed a decline in the number of flowers as plant density increased. This suggests that while the number of plants may rise with higher seeding rates, factors like competition for resources (light, water, nutrients) may negatively impact flower production, a key yield component. This may explain the observed differences in plant number and flower production in our study, as the seeding rates of 35 kg ha⁻¹ did not result in the highest flower yields. Mollafilabi and Hosseini (2012) found that the maximum number of plants occurred at a seeding rate of 15 kg ha⁻¹, but higher densities did not significantly affect yield parameters. This observation further supports the concept that plant density may not always translate into higher yield, as other factors such as soil fertility, water availability, and plant spacing may play more critical roles in determining the final yield. Furthermore, the findings by Mirzaei et al. (2016) on dry flower yield in relation to plant density provide valuable insight into how plant density can influence not only the number of plants but also their biomass. They found a positive correlation between plant density and dry weight, with higher densities resulting in increased dry weight. This suggests that higher seeding rates might enhance certain aspects of growth (such as dry weight) even if the number of plants per square meter decreases, which could be a key consideration when optimizing seeding rates for flower yield. In conclusion, while higher seeding rates like 35 kg ha⁻¹ lead to more plants per square meter in some years, the overall yield and quality depend on a complex interaction of factors, including year, density, and environmental conditions. The literature supports the idea that optimal plant density does not always equate to higher yields, and in some cases, there are diminishing returns as plant competition for resources increases.

Fresh flower yield (kg ha⁻¹)

The statistical analysis results revealed a significant interaction between year and seeding rate on fresh flower yield ($p<0.05$). Notably, in 2021, the maximum fresh flower yield was observed at a seeding rate of 10 kg ha⁻¹, reaching 6.277 kg ha⁻¹, followed by a seeding rate of 5 kg ha⁻¹, yielding 6.088 kg ha⁻¹. Conversely, the minimum fresh flower yield occurred in 2020 at a seeding rate of 5 kg ha⁻¹, with a yield of 3.509 kg ha⁻¹. The average fresh flower yield across treatments was 4.186 kg ha⁻¹ in 2020 and increased to 5.393 kg ha⁻¹ in 2021. Over the study period, there was a notable 28.9% increase in the average fresh flower yield in 2021 compared to 2020. The average fresh flower yield across all seeding rate treatments was 4.789 kg ha⁻¹, with the maximum yield recorded at 10 kg ha⁻¹ (5440 kg ha⁻¹). Remarkably, both years exhibited a decreasing trend in fresh flower yield beyond the 10 kg ha⁻¹ seeding rate, with yields of 4.600 kg ha⁻¹ and 6.277 kg ha⁻¹ in 2020 and 2021, respectively (Fig 2.).

In 2019–2020, although rainfall was low but regular, it supported the water needs of *Calendula officinalis* L. during its growth stages, positively affecting fresh flower yield. However, insufficient water limits flower biomass and yield, especially during flowering. Higher seeding rates (e.g., 20 kg ha⁻¹) led to increased competition, which likely contributed to the reduction in yield. In 2020–2021, while rainfall was higher but irregular, the plants' growth was influenced by fluctuating water availability during flowering (Fig 1.). Despite this, the fresh flower yield was higher than in 2019–2020, particularly at lower seeding rates (e.g., 10 kg ha⁻¹), where plants showed improved flower biomass and yield (Fig 2.).

In the previous studies, Martin and Deo (1999) noted that lower plant densities increased flower weight per plant but decreased overall yield per hectare. Similarly, in our study, the highest yield was obtained at 10 kg ha⁻¹, while higher seeding rates beyond this point led to a decline in yield. This suggests that while plant weight might increase at higher densities, resource competition negatively impacts overall yield. Ganjali et al. (2010) and Mollafilabi and Hosseini (2012) also reported that increased plant density led to reduced flower yield. This supports the idea that higher seeding rates cause plant competition for resources like water, nutrients, and light, which ultimately lowers yield. In conclusion, the results of our study indicate that the relationship between seeding rate and flower yield is complex. The 10 kg ha⁻¹ seeding rate optimizes fresh flower yield, while higher seeding rates lead to yield loss due to increased competition.

Drug flower yield (kg ha⁻¹)

The statistical analysis unveiled a significant interaction between year and seeding rate concerning drug flower yield ($p < 0.05$). The findings suggest that increasing the seeding rate positively influences drug flower yield each year. Specifically, in 2021, the maximum flower yield of 1.571 kg was attained with a seeding rate of 10 kg ha⁻¹. Likewise, in the same year, a seeding rate of 5 kg ha⁻¹ resulted in a yield of 1.479 kg ha⁻¹, which statistically matched the yield obtained with the 10 kg ha⁻¹ application. Conversely, the lowest drug flower yield was observed in 2020 with a seeding rate of 5 kg ha⁻¹ (Fig 2.).

The findings are consistent with the broader literature but also reflect variations based on the experimental conditions. Martin and Deo (1999) identified optimal seeding rates ranging from 3 to 24 kg ha⁻¹, thereby establishing a foundational relationship between plant density and yield. However, Ganjali et al. (2010) and Mollafilabi and Hosseini (2012) reported no significant differences in drug flower yield under varying plant density treatments, suggesting that the effect of density may be context-dependent. Berimavandi et al. (2011) noted a maximum yield of 1.320 kg ha⁻¹ at a density of 60 plants m⁻², while Mirzaei et al. (2016) observed a positive correlation between plant density and dry flower weight, achieving an exceptional yield of 211.19 kg ha⁻¹ at similar densities. The differences observed in this study and previous research underscore the complex interplay of factors such as seeding rate, environmental conditions, and management practices. The high yields achieved in 2021, particularly at lower seeding rates, may be attributed to favorable climatic conditions, which mitigated resource competition and enhanced plant productivity. Conversely, the lower yields in 2020 highlight the adverse impact of water stress and variable precipitation on flower development. The findings emphasise the need for tailored agronomic practices, including precise seeding rate adjustments, to optimise drug flower yield under varying environmental conditions. Selecting an appropriate seeding rate, such as 10 kg ha⁻¹, coupled with effective water management, can ensure sustainable production while mitigating risks associated with climatic variability.

Seed yield (kg ha⁻¹)

The effect of seeding rates has been found to be significant on seed yield ($p < 0.05$). In trial years, the average seed yield values for seeding rate applications ranged from 1.020 to 1.724 kg ha⁻¹, with the peak yield occurring in 2020. The maximum yield was recorded in the 10 kg ha⁻¹ seeding rate application (1.671 kg ha⁻¹), followed by 35 kg ha⁻¹ (1.535 kg ha⁻¹) and 25 kg ha⁻¹ (1.500 kg ha⁻¹) applications, respectively. Conversely, the minimum seed yield of 919 kg ha⁻¹ was observed in the 5 kg ha⁻¹ seeding rate application. However, the maximum seed yield per hectare was achieved in 2020, with 207 kg ha⁻¹ from the 10 kg ha⁻¹ seeding rate application, while the minimum yield was noted in 2021, with both 5 kg ha⁻¹ and 15 kg ha⁻¹ seeding rates (Fig 2.). Despite this variation, the interaction effect of year x seeding rates on seed yield did not reach statistical significance.

Seed yield varied between years, with a decrease in the second year's values compared to the first year. Climatic factors during the growing years were observed to limit seed yield, but the 10 kg ha⁻¹ seeding rate consistently maximized seed yield, regardless of the year. A comparison of total precipitation from seed sowing to the last harvest date in the trial years (2019–2020 and 2020–2021) showed that the first trial year received 464.9 mm of precipitation, lower than the second year's 635.1 mm. Despite this, the first trial year exhibited more consistent rainfall during the plant's emergence and flowering period, while the second year had more fluctuation (Fig 1). The reduced rainfall, especially during the flowering period, is thought to limit plant productivity. Even with the decrease in average seed yield due to climatic factors, the 10 kg ha⁻¹ seeding rate maximized seed yield in both years (Fig 2.).

The findings of Rahmani (2008), Jevdovic et al. (2013), and Telci et al. (2023) support the current study, highlighting the importance of irrigation and precipitation for seed yield in *C. officinalis* L.. Rahmani (2008) showed that delayed irrigation significantly reduces seed yield, which aligns with the reduced yields observed in the second year of this study due to fluctuating rainfall. Jevdovic et al. (2013) and Telci et al. (2023) emphasized the role of precipitation during the

vegetative phase, which was less stable in the second year and may have hindered plant growth. Additionally, studies by Seghatoleslami and Mousavi (2009) and Mollafilabi and Hosseini (2012) suggest that high seeding rates lead to increased plant density, which can reduce seed yield by limiting factors like branch number and canopy cover. This finding is consistent with the current study, where the 10 kg ha⁻¹ seeding rate produced the highest seed yield, emphasizing the importance of balanced plant density for optimal production. In the instance, with changing climatic conditions, it is important to carefully manage the of seeding rate and irrigation needed. The variability in yields, exacerbated by extreme meteorological phenomena, necessitates an increase in optimal seed production volumes, thereby affecting the financial requirements for seed producers (Serhatli et al., 2019). As the probability of extreme events escalates, anticipated profits diminish while the ideal planting volumes expand, thereby complicating supply chain logistics and market distribution (Serhatli et al., 2020).

Biological yield (kg ha⁻¹)

The statistical analysis showed a significant interaction between seeding rate and year on biological yield ($p < 0.05$). The maximum biological yield value was obtained from the 10 kg ha⁻¹ seed application in 2020 (11.180 kg ha⁻¹). The minimum biological yield value was determined in the 15 kg ha⁻¹ and 25 kg ha⁻¹ seed treatments in 2020. Based on the results obtained for biological yield, variability was observed in the biological yield values for the different seed treatments during the trial years. In the first year, the biological yield ranged from 5.508 to 11.180 kg ha⁻¹, while in the second year it varied from 6.350 to 8.660 kg ha⁻¹. In particular, the maximum biological yield in 2020 was achieved with a seeding rate of 10 kg ha⁻¹, indicating that this specific seeding rate resulted in optimal yield performance in that year. Similarly, in 2021, the maximum biological yield was achieved at seeding rate of 35 kg ha⁻¹ and 10 kg ha⁻¹, suggesting that this seeding rate was most conducive to maximizing yield in that year (Fig 2.).

The findings align with Martin and Deo (1999), who emphasized that seeding rates significantly influence biomass production, with higher yields observed at higher seeding rates. However, the observed variation in yield across different seeding rates in this study parallels the work of Seghatoleslami and Mousavi (2009), who found that optimal biological yields are not always achieved at the highest densities but depend on specific plant density thresholds. Mirzaei et al. (2016) reported increased plant dry weight with higher plant density, which appears partially consistent with the 2021 findings of this study, where a seeding rate of 35 kg ha⁻¹ produced the maximum biological yield. Nevertheless, the results from 2020, where the 10 kg ha⁻¹ seeding rate outperformed others, underscore the influence of environmental factors, such as precipitation patterns and temperature variations, on yield. The variability in biological yield between the two trial years highlights the role of climatic conditions (Fig 1.). In 2020, regular rainfall patterns during critical growth stages likely contributed to the superior yield at the 10 kg ha⁻¹ seeding rate. Conversely, in 2021, despite higher total rainfall, its irregular distribution may have influenced the results, favoring a broader range of seeding rates, including the 35 kg ha⁻¹ rate. This supports Jevdovic et al.'s (2013) findings that rainfall significantly affects biomass production during plant growth and flowering. Rahmani (2008) similarly emphasized the importance of water availability, showing that irrigation scheduling significantly impacts biomass production.

Harvest index (%)

The effects of the year x seeding rates interaction on the harvest index parameter were found statistically significant ($p < 0.05$). The maximum harvest index value was determined at 25 kg ha⁻¹ in the first year, followed by 35 kg ha⁻¹ and 15 kg ha⁻¹ seeding rate treatments. The minimum harvest index value was determined at 15 kg ha⁻¹ seeding rate in the second year (Fig 2.). The harvest index reflects the ratio of seed yield to biological yield. In 2020, the higher HI values likely resulted from favorable climatic conditions that allowed for increased seed yield relative to biological yield. Conversely, the lower HI values in 2021 can be attributed to higher biological yields combined with a decline in seed yield. This result underscores the influence of environmental factors, particularly the irregular rainfall patterns observed in 2021, which likely limited seed production while promoting vegetative growth. In addition, it was determined that the harvest index values of the seed treatments in 2020 were twice as high as those in 2021, except for the 10 kg ha⁻¹ seeding rate. It is noteworthy in this regard that the 10 kg ha⁻¹ seed application yielded similar results in both years.

The findings of this study partially align with Martin and Deo (1999), who reported no statistically significant effect of seeding rates on HI but observed an optimal value at a seeding rate of 24 kg ha⁻¹. However, the negative correlation between increased plant density and HI observed in studies by Seghatoleslami and Mousavi (2009) and Mollafilabi and Hosseini (2012) was not fully supported here. While the HI decreased at higher seeding rates (e.g., 35 kg ha⁻¹) in 2021, in 2020, higher seeding rates such as 25 kg ha⁻¹ and 35 kg ha⁻¹ produced higher HI values. The consistent performance of the 10 kg ha⁻¹ seeding rate across both trial years is noteworthy, as it suggests a robust adaptability to varying environmental conditions. This rate may provide a more stable harvest index compared to higher seeding rates, which were more sensitive to climatic fluctuations. In contrast, higher seeding rates exhibited greater variability in HI, potentially due to competition among plants for limited resources, particularly under suboptimal conditions.

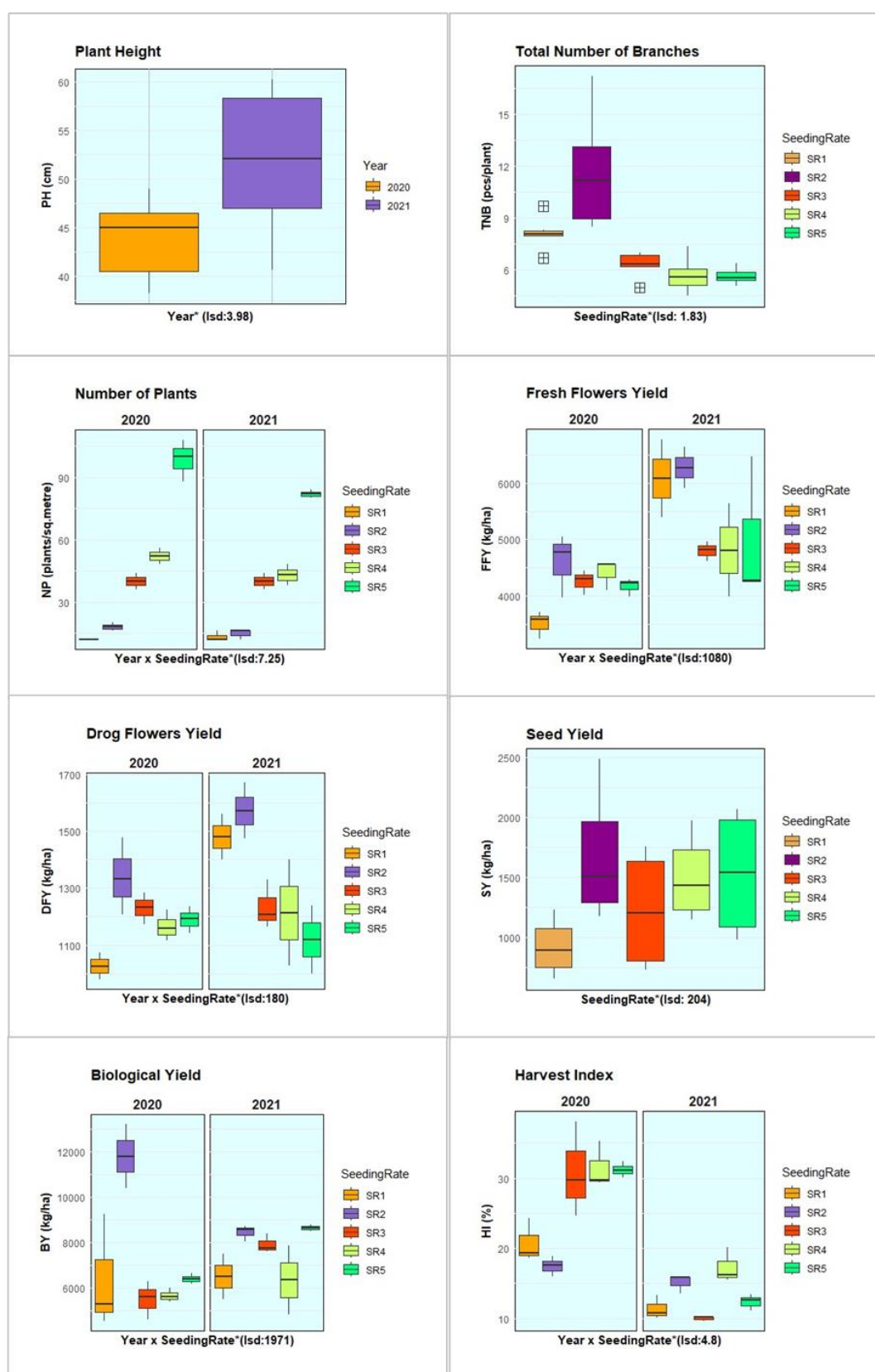


Figure 2. Effects of different seeding rates treatments on morphological and yield parameters of *Calendula officinalis* L.

Quality parameters

Essential oil rate (kg ha⁻¹)

The statistical analysis revealed a significant interaction between the year and seeding rates on the essential oil rate ($p < 0.05$). Fig. 3 shows that the essential oil content ranged from 0.06% to 0.11%, with the maximum value of 0.11% observed in 2021 at a seeding rate of 25 kg ha⁻¹. In the trial years, no statistically significant difference was observed among the other seeding rates applied. The minimum essential oil rate was identified at 35 kg ha⁻¹ seeding rate in the first year. The first year showed more favorable climatic conditions, including temperature and rainfall, for *Calendula officinalis* L., a cool climate plant, compared to the second year. Observations throughout the growing season showed a tendency for the flower to shrink in the second year due to weather conditions (Fig 1). This change in flower size is thought to potentially affect the essential oil rate (Lastra Valdés and Piquet García, 1999).

The essential oil yield of *Calendula officinalis* L. is known to vary depending on cultivation conditions and cultivar characteristics. Research by Raal et al (2016), reported oil yields ranging from 0.10% to 0.43%, with the 'Double Ball' cultivar producing the highest yield. Similarly, studies by Lastra Valdés and Piquet García (1999) and Okoh et al. (2008) demonstrated varied oil yields depending on the plant's part, with fresh flowers yielding 0.09% oil. Additionally, Berimavandi et al. (2011) and Mollafilabi et al. (2014) found that increasing plant density reduced essential oil yield, a trend also observed in our study, where the seeding rate of 35 kg ha⁻¹ in the first year resulted in the lowest oil content. Research's findings align with earlier studies, suggesting that essential oil yield is sensitive to environmental factors, plant density, and cultivar differences. The observed decrease in essential oil content at higher plant densities further emphasizes the role of plant arrangement in optimizing oil production, with lower densities potentially leading to better yield outcomes (Shakib et al., 2010). The variation in results across different studies reflects the complexity of essential oil production, which is influenced by a combination of genetic, environmental, and agronomic factors.

Total flavonoid content (mg rutin g⁻¹)

Flavonoid levels were analyzed concerning seeding rates over the years. The impact of years on seeding rates was deemed insignificant, whereas the influence of seeding rate treatments proved statistically notable ($p < 0.05$). Figure 3 illustrated that the maximum total flavonoid content was achieved at a seeding rate of 35 kg ha⁻¹ (72.1 mg rutin g⁻¹), followed closely by 25 kg ha⁻¹ (72.0 mg rutin g⁻¹), 15 kg ha⁻¹ (71.4 mg rutin g⁻¹), and 10 kg ha⁻¹ (66.9 mg rutin g⁻¹) treatments. Specifically, the minimum total flavonoid content was recorded at 5 kg ha⁻¹ (ranging from 35.8 to 54.8 mg rutin g⁻¹), with the maximum concentration observed at 10 kg ha⁻¹, totalling 77.8 mg rutin g⁻¹ in 2021. On average, the total flavonoid content across treatments amounted to 62.2 mg rutin g⁻¹ in 2020 and increased to 68.9 mg rutin g⁻¹ in 2021. Notably, a trend emerged where higher seeding rates correlated positively with increased flavonoid content.

Calendula officinalis L. exhibits varying total flavonoid contents across different plant parts. The ligulate ray-florets and tubular disc-florets of *C. officinalis* collectively contain 0.88% and 0.25% of flavonoids, respectively (Masterova, 1991). The peripheral florets of *C. officinalis* contain the maximum flavonoid content at 36.66 mg g⁻¹, followed by tubular florets at 9.95 mg g⁻¹, and leaves at 9.65 mg g⁻¹ (Daniil and Kashchenko, 2013). Harvest time significantly influences flavonoid content, with the maximum levels found three days after anthesis, recommending a harvest every three days for optimal yield (Isabela et al., 2016). The total flavonoid content in aqueous-methanolic extracts of *C. officinalis* ranges between 44.91 and 76.44 mg QE g⁻¹ dry weight, with rutin, quercetin-3-O-glucoside, and other compounds identified (Rigane et al., 2013). The differences observed in the total flavonoid content across literature studies can be attributed to variations in the applied analysis methods and influencing factors.

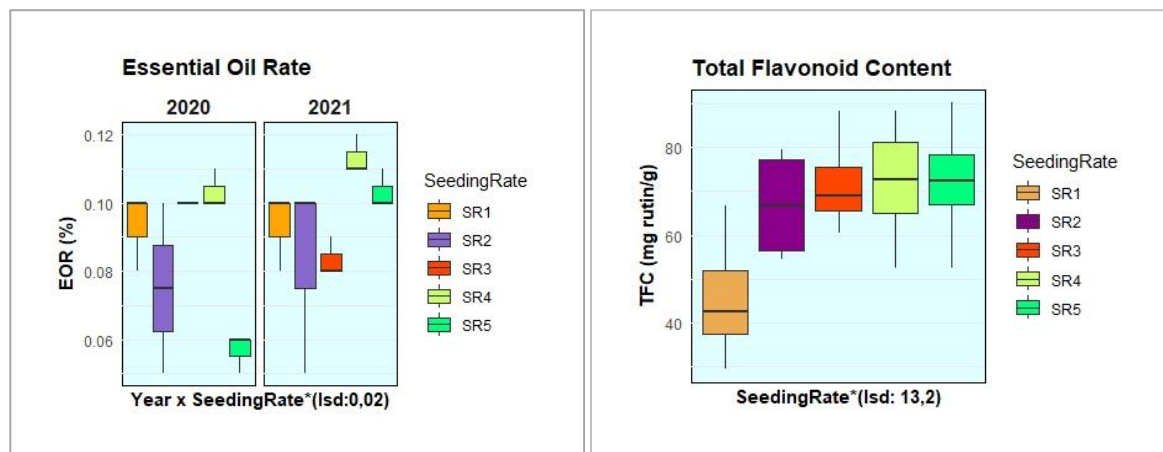


Figure 3. Effects of different seeding rates treatments on quality parameters of *Calendula officinalis* L.

a critical climate variable, also influences seed development. This necessitates adjustments in seeding strategies to maintain optimal plant density and yield (Bailly et al., 2023). Studies show that varying seeding rates can mitigate some adverse effects of climate change. For instance, higher seeding rates can increase the number of reproductive structures, while lower rates may enhance grain yield per structure (Kuhling et al., 2017). Understanding the interaction between climate change and seeding rates is essential to ensure agricultural productivity and food security in an evolving environmental landscape. However, the success of these strategies depends on local climate conditions and soil management practices (Garnier et al., 2021).

4. CONCLUSION

Significant relationships were identified between seeding rate treatments and the examined traits of *C. officinalis* L. An increase in the seeding rate led to a higher number of plants. This was positively correlated with the harvest index and total flavonoid content. However, traits such as plant height, fresh flower yield, and biological yield decreased with increasing plant density. A similar trend was observed in drug flower yield, which declined as plant density per unit area increased. Considering flower yield, one of the most critical traits, the 10 kg ha⁻¹ seeding rate produced the best results. This rate (16.3 plants m⁻²) allowed for higher branching, likely due to a wider distribution of plants compared to other treatments. The increase in branching contributed to greater plant biomass per unit area. Climate conditions, which varied by year, also had a significant impact. Changes in thermal conditions and vegetative density influenced the optimal seeding rate for *C. officinalis* L., a cool-climate species grown in the Mediterranean region. These findings emphasize the importance of understanding the interplay between climate and agronomic practices to optimize productivity. In conclusion, the 10 kg ha⁻¹ seeding rate was determined to be the most suitable for maximizing the agronomic productivity of *Calendula officinalis* L. under Mediterranean climate conditions in Türkiye.

ACKNOWLEDGEMENTS

This article is a part of Gülsüm BOZTAŞ's PhD thesis carried out at Ege University in Izmir titled "Effects of Different Agro-technical Practices on Yield, Quality and Physiological Characteristics of *Calendula officinalis* L".

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Characterization and Multivariate Assessment of Faba Bean (*Vicia faba* L.) Varieties Based on Mineral, Agro-Morphological and Technological Characteristics

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ARTICLE INFO

Research Article

Received: 12 January 2025

Accepted: 31 May 2025

Published: 23 June 2025

Keywords:

Biochemical content

Biplot

Cluster Analysis

Correlation Analysis

Faba bean

ABSTRACT

Faba bean (*Vicia faba* L.) is the oldest cultivated plant in the world, rich in protein, vitamins, minerals and antioxidant compounds. This study aimed to characterize some economically important faba bean cultivars based on agro-morphological, technological, and mineral properties using multivariate analysis methods. The research was carried out for two years in the trial land of Erciyes University Agricultural Research and Application Center located in the central campus of Erciyes University in 2021 and 2022. The trial was conducted using a randomized complete block design with three replications. The spacing was set at 50 cm between rows and 20 cm within rows, with 1 m spacing between plots and blocks, and a plot length of 4 m. The study determined significant positive correlations with plant height trait, wet volume, wet weight, dry weight, water absorption capacity, dry volume, 100-grain weight, and Na content of the grain. Based on the angle and correlation values of the axes in the same region with the Zn axis, Cu, protein ratio, Ca, P, cooking time, and S showed positive correlations, while the number of pods, Mn, and hydration coefficient showed negative correlations. Based on the angle and correlation values from the axes in the same region as the swelling index, water absorption index, Mn, hydration coefficient, the number of pods, Mg, and unite weight showed a positive correlation, while it showed a negative correlation with cooking time. The cluster analysis performed to define the genetic relationship between the varieties determined that 11 varieties were divided into 3 different clusters based on their morphological, technological, and mineral characteristics.

Citation: Ozaktan, H., & Erol, O. (2025). Characterization and multivariate assessment of faba bean (*Vicia faba* L.) varieties based on mineral, agro-morphological and technological characteristics. *Turkish Journal of Field Crops*, 30(1), 151-163.

<https://doi.org/10.17557/tjfc.1618510>

1. INTRODUCTION

Legume crops are widely cultivated throughout the world. Edible legumes are important plant groups in human diets due to their high protein content (Dhull et al., 2021). Faba bean (*Vicia faba* L.), which belongs to the *Fabaceae* family, is an annual plant that can grow to a height of 1-2 m, grows upright and unbranched. The stem is hollow and ridged. The flowers are found in short panicles in the leaf axils, typically containing 1 to 8 flowers in each panicle. It is divided into two subspecies, *V. faba* ssp. *paucijuga* and *V. faba* ssp. *eufaba*. The second subspecies consists of three botanical species in terms of seed size. These are; small-seeded species (*V. faba* minor), with a 1000-seed weight of less than about 500 g, found in the Ethiopian region and preferred by Northern European agriculture. Medium-seeded species (*V. faba* equina) in the Middle East and North Africa, and large-seeded species (*V. faba* major), with a 1000-seed weight of more than 1000 g, concentrated in Egypt (Duc, 1997). Faba bean is one of the oldest widely cultivated crops worldwide (Mínguez and Rubiales, 2021). Mediterranean countries, China, India, Afghanistan, Northern Europe, Ethiopia, Egypt, and North Africa are the primary producers of faba beans in the world (Rahate et al., 2020; Dhull et al., 2021). Faba bean is a versatile crop that contributes to various ecosystems and can be used for human nutrition, animal feed, and soil improvement. Faba bean is crucial in crop rotations due to its ability to fix atmospheric nitrogen symbiotically to increase the amount of available phosphorus and nitrogen-rich soil for the following crop (Köpke and Nemecek, 2010; Neugschwandtner et al., 2015). Faba bean grain plays an essential role in human nutrition as it contains 26.1% protein, 58.3% carbohydrate, and 25.0% dietary fiber. In addition, faba beans contain a wide variety of bioactive compounds (total phenolics, flavonoids, and antioxidants). Faba bean grain contains antinutrients such as lectins, trypsin inhibitors, saponins, phytic acids, tannins. Oligosaccharides are also present in faba bean grain. They may produce gas, leading to abdominal discomfort. Lectins can also be destroyed during normal cooking due to high heat or by germination, making faba beans suitable for human consumption (Singh et al., 2013; Labba et al., 2021; Dhull et al., 2021). In addition, broad beans are a promising source of plant-based protein with health benefits such as improved gut function and reduced risk of chronic diseases (Martineau-Côté et al., 2022). Faba beans are utilized in different ways around the world. They are consumed fresh or cooked and canned. They are also consumed as a breakfast food in the Middle East and Mediterranean Region, China, and Ethiopia. The most popular foods made from faba beans are Medamis (boiled faba beans), Falafel (deep-fried), Bissara (cotyledon puree), Hummus (faba bean puree), and Nabet soup prepared from boiled and germinated faba beans (Dhull et al., 2021; Singh et al., 2013).

Faba bean, an essential legume, had 6,144,394 tons of dry bean production from 2,684,296 hectares and 1,642,153 tons of fresh bean production from 257,815 hectares in 2022. China is the leading country with the highest cultivation area in the world with 810,356 hectares of cultivation area. China is followed by Ethiopia and Australia (FAO, 2024). In Türkiye, 4,268 tons of faba beans were produced from 1.7554 hectare of land in 2023. Çanakkale, which has the highest production area in Türkiye, is at the top of the list with 444.9 hectare of land. Çanakkale is followed by Balıkesir and Kütahya provinces (TurkStat, 2024).

Multivariate analyses are used to characterize, evaluate, and classify genetic resources when assessing the heritability of various traits with morphological, agronomic, and physiological significance (Astaraki et al., 2020). Multivariate analysis methods such as cluster analysis and principal component analysis (PCA) are frequently used to reduce the dimensionality and visualization of variable datasets. PCA provides a detailed insight into the relationships of traits and is an effective method for identifying key characteristics of high importance for phenotypic characterization. Cluster analysis is used to determine the genetic diversity of genotypes based on trait similarities (Alam et al., 2024). These analyses are the most appropriate tools for selecting suitable parents for future breeding studies. In addition to these analyses, correlation analysis provides information about the relationship between the examined characters. It contributes to determining characters that have direct or indirect effects on the examined characters (Benlioglu and Ozkan, 2021). This study aimed to characterize some economically important faba bean varieties based on agro-morphological, technological, and mineral characteristics using multivariate analysis methods.

2. MATERIALS AND METHODS

Material

In the study, 11 registered faba bean (*Vicia faba* L.) varieties were used. The varieties were Emiralem, Eresen-87, Filiz-99, Gölyaka, Hilal, Kıtık-2003, Lara, Luz De Otono, Sakız, Salkım, and Sevil.

Experiment location

The experiment was conducted at Erciyes University Agricultural Research and Application Center (ERUTAM) Yıldırım Beyazıt Farm in the central campus of Erciyes University during the 2021 and 2022 growing season. The test site is located at an altitude of 1094 m above sea level, between 34° 56' and 36° 59' east longitude and 37° 45' and 38° 18' north latitude.

Climate Characteristics of the Experimental Area

Table 1 summarizes the climatic data on average, minimum and maximum temperatures, rainfall and average relative humidity values for the months covering the growing period in the 2021 and 2022 growing seasons. When table 1 is examined, it is observed that the temperatures in 2021, the first production year, were within the climatological normals, and the amount of rainfall in 2021 was lower than the average of many years. The rainfall in August was above the long-term average. The average relative humidity value was below the long-term average. In 2022, the second growing season, the temperature was around the long-term average. Considering the rainfall patterns in the second year, precipitation in April was below the long-term average, while rainfall in May and June exceeded the climatological normals. Relative humidity remained close to the long-term average.

Table 1 Climate data for Kayseri province for 2021, 2022, and long-term average

Climate data	April	May	June	July	August	September
			2021			
T _{avg} (°C)	10.7	15.9	18.8	23.8	21.9	16.9
T _{max} (°C)	16.8	23.6	25.8	31.8	30.7	24.2
T _{min} (°C)	4.5	7.4	12.1	14.6	13.5	10.4
RH _{avg} (%)	63.5	56.4	53.1	44.7	41.5	49.4
Rainfall (mm)	22.7	21.3	37.2	0.0	17.1	16.7
			2022			
T _{ort} (°C)	13.4	13.8	19.9	21.6	25.1	
T _{max} (°C)	29.7	32.3	34.9	36.3	37.2	
T _{min} (°C)	-4.8	1.0	9.0	7.6	13.6	
RH _{ort} (%)	46.0	60.7	54.5	46.9	40.9	
Rainfall (mm)	23.1	72.1	82.9	0.6	***	
			Long Term Average			
T _{avg} (°C)	10.6	15.0	19.0	22.2	22.0	17.3
T _{max} (°C)	17.7	22.5	26.8	30.6	30.7	26.5
T _{min} (°C)	3.1	6.8	9.7	11.9	11.4	7.3
RH _{avg} (%)	62.3	61.2	55.8	49.5	49.2	54.1
Rainfall (mm)	52.1	51.8	39.5	10.5	8.8	15.0

T_{avg}: Average Temperature T_{max}: Maximum temperature T_{min}: Minimum temperature RH_{avg}: Average relative humidity

Soil Characteristics of the Experimental Area

Table 2 Soil analysis results of the experimental area

Years	Clay (%)	Silt (%)	Sand (%)	Texture Class	pH	EC (Mmhos cm ⁻¹)	Organic Matter (%)	P ₂ O ₅ (kg ha ⁻¹)	Lime (%)
2021	16.64	14.56	68.80	Sandy Loam	7.41	0.272	0.83	67.6	1.28
2022	14.56	9.78	75.66	Sandy Loam	7.97	0.184	0.77	61.7	1.60

Soil samples taken from the experimental field were analyzed in the laboratory of Erciyes University, Department of Soil Science and Plant Nutrition. Soil analysis results are given in Table 2. Based on the first-year soil analysis in Table 2, it was determined that the soil contained 16.64% clay, 14.58% silt, 68.80% sand, and the soil class was sandy loam. The soil was slightly alkaline, salt-free, low in organic matter content, medium in useful phosphorus, and calcareous. According to the soil analysis results in the second year of the experiment, the soil contained 14.56% clay, 9.78% silt, and 75.66% sand, and was classified as sandy loam. The soil was classified as medium alkaline, salt-free, low in organic matter, medium in useful phosphorus, and calcareous.

Method

The experiment was conducted in 2021 and 2022 with 3 replications according to the randomized complete block design. In the experiment, a 1 m distance was left between the plots and blocks. Plot lengths were 4 m, 6 rows in each plot, 50 cm between the rows, and 20 cm between plants in the same row. Before sowing, fertilization was made with DAP at a rate of 20 kg ha⁻¹ N and 50 kg ha⁻¹ P₂O₅ and fertilizers were mixed with the soil. Stones and materials preventing emergence were removed from the area using a rake. The experiments were performed by sowing the faba bean seeds on April 29, 2021, in the first year, and May 13, 2022, in the second year. After sowing, sprinkler irrigation was used to ensure uniform plant emergence. After the plants reached a height of about 30 cm, mechanical control was carried out with a hand hoe for weed control and hilling. After the hilling process, a drip irrigation system was installed by placing one drip irrigation pipe in each row. In the experiment, the varieties that reached harvest maturity were removed 1 row from the edges of the parcel, 0.5 m from the beginning and end of the parcel. Ten sample plants were taken from the remaining part to represent the parcel, measurements were made, and data were recorded.

Characteristics Analyzed

Morphological Characteristics

Plant height, first pod height, number of main branches, number of pods, number of grains per pod, and number of grains per plant were determined on 10 plants selected from each plot. When the moisture content of the seeds became constant, seed yield was calculated by converting the yield obtained from the plot into hectare. Grain protein content was determined according to Kjeldahl method.

Technological Characteristics

Fresh weight, dry weight, fresh volume, swelling index, swelling capacity, water absorption index, water absorption capacity, cooking time, hydration coefficient, and bulk density characteristics were determined according to Ozaktan and Doymaz (2022).

Mineral Content

Macronutrients (Ca, Mg, K, P, S, Na) and micronutrients (Zn, Mn, Fe, Cu) were determined using the Agilent 5800 VDV model ICP-OES. 0.5 g of ground grain underwent acid digestion in a microwave digestion unit by adding 10 ml of Nitric Perchloric acid. Mineral compositions of the samples were determined in ICP-OES spectrometer.

Statistical Analysis

Multivariate analysis methods were used to determine the relationship between the studied characteristics. The effects of the varieties on agronomic traits, technological properties and grain mineral compositions were analyzed using the JMP Pro 17 statistical package program. Significant means were compared using Tukey's test ($p < 0.05$) PCA, and correlation analyses were performed using two-year averages of the analyzed characters. R² (Coefficient of Determination) is the model's accuracy rate decision coefficient. A high value of this coefficient indicates that the prediction relationship is good. RMSE (Root Mean Square Error) is a measure of error, so low results are inversely proportional to performance, and a measure that shows high performance (Wang and Xu, 2004). When the R² value approaches 1 and the RMSE value approaches 0, it indicates that the data received is successful. Evaluating these two criteria together provides comprehensive information about both the accuracy of the model and the error size (Gultepe, 2019).

3. RESULTS AND DISCUSSION

The analysis of variance table for morphological parameters, technological parameters and mineral matter contents are given in Table 3. The effect of each examined parameter on the source of varietal variation was found statistically significant. In addition, the effects of plant height, grain protein content, grain dry weight, grain wet weight and volume, water uptake capacity, hydration coefficient, swelling capacity and water uptake index values on year*variety variation source were found statistically insignificant (Table 3).

Table 3. Analysis of variance table for morphological parameters, technological parameters and mineral contents

Source of Variation	Morphological parameters F values											
	Degree of Freedom	Plant Height (cm)	Number of Main Branches (branch plant ⁻¹)	First Pod Height (cm)	Number of Pods (pod plant ⁻¹)	Number of Seeds Per Plant (seeds plant ⁻¹)	Number of Seeds Per Pod (seeds pod ⁻¹)	Seed Yield (kg ha ⁻¹)	100 Seed Weight (g)	Seed Protein Rate (%)		
Year	1	1.235	1264.800**	220.253**	35.805*	53.083*	27.169*	183.417*	11.785*	26.401*		
Rep. (Block)	4	0.939	5.358*	4.877*	0.819	1.506	3.307*	2.545	2.036	0.991		
Cultivars	10	10.463**	14.748**	2.616*	14.697**	6.335**	6.515**	73.211**	24.560*	6.860**		
Year*Cultivars	10	0.471	13.459**	2.690*	2.370*	2.299*	7.357**	10.731**	3.615*	0.862		
Technological parameters F values												
		Dry Weight (g)	Dry Volume (ml)	Fresh Weight (g)	Fresh Volume (ml)	Cooking Time (min.)	Water Absorption Capacity (g seed ⁻¹)	Swelling Index (%)	Hydration Coefficient (g seed ⁻¹)	Swelling Capacity (ml seed ⁻¹)	Water Absorption index (%)	Unit Weight (g ml ⁻¹)
Year	1	25.450*	118.478*	83.433*	52.494*	2.784	16.040*	12.855*	0.545	1.486	2.141	245.226*
Rep. (Block)	4	1.400	0.790	0.285	0.722	1.480	0.545	1.533	2.819*	1.094	2.085	0.240
Cultivars	10	15.088**	16.525**	16.334**	24.548**	21.483**	15.843**	18.007**	11.837**	27.003**	18.607**	3.884**
Year*Cultivars	10	1.396	2.262*	1.608	1.692	2.938*	1.619	2.091*	1.107	1.330	1.215	3.090*
Mineral Contents F values												
		B (ppm)	Ca (ppm)	Cu (ppm)	Fe (ppm)	K (ppm)	Mg (ppm)	Mn (ppm)	Na (ppm)	P (ppm)	S (ppm)	Zn (ppm)
Year	1	974.301**	1133.640**	11.828*	166.271*	29912.950*	768.921**	106.447*	2670.667**	475.110**	4814.714**	41.452*
Rep. (Block)	4	2.636*	1.485	7.335*	0.286	0.174	1.814	0.615	3.422*	1.592	1.334	1.786
Cultivars	10	8.860**	20.573**	74.155**	58.498**	2.505*	8.872**	6.865**	29.905**	6.873**	17.326**	79.858**
Year*Cultivars	10	4.053*	37.319**	65.380**	25.839**	5.617**	5.823**	6.160**	42.523**	16.533**	25.391**	23.249**

While the number of pods per plant varied between 6.95 and 20 pod plant⁻¹, grain yield varied between 1790 and 4690 kg ha⁻¹. Filiz-99 variety had the highest values in terms of both the number of pods per plant (18.18 pod plant⁻¹) and grain yield (4472.5 kg ha⁻¹), and R² values were recorded as 0.812 and 0.950, respectively. Kebede et al. (2022) reported the number of pods per plant to be between 8.9 and 22.7, and Oncan Sumer and Erten (2022) observed it to be between 14.6 and 16.2. When the total number of grains per plant was analyzed, the values varied between 18.75 and 56.45 seeds plant⁻¹ and the highest average number of grains per plant was the Hilal variety with 43.65 seeds plant⁻¹, and the R² value was 0.672. In addition, the Hilal variety was in the same statistical group as the Filiz-99 variety in terms of grain yield. The number of grains per plant was reported by Ton (2021) as 15.9-20.1 seeds plant⁻¹, Soysal et al. (2020) as 18.8-25.6 seeds plant⁻¹, and by Karakoy et al. (2017) as 1.2-40.4 seeds plant⁻¹. The minimum number of grains in pods was recorded as 1.7 seeds pod⁻¹ and the maximum as 3.4 seeds pod⁻¹. In terms of mean values, the Sevil variety had the highest value at 2.98 seeds pod⁻¹. RMSE and R² values were 0.26 and 0.672, respectively. In the literature, Abdel-aziz and Ismail (2023) found the number of grains per pod to range from 3.8 to 4.1 seeds pod⁻¹ in the control group, while Dewangan et al. (2022) reported a range of 2.22 to 4.89 seeds pod⁻¹. The 100-grain weight varied between 80.07 and 132.48 g, and the R² value was 0.897. The Eresen-87 variety had the highest average 100-grain weight. 100-grain weight was recorded as 108.8 - 110.9 g by Oncan Sumer and Erten (2022), 120.3 - 142.6 g by Ton (2021), and 47.93 - 193.37 g by F. Sheikh et al. (2015). Grain protein ratio values varied between 20.98% and 29.88%. The Lara variety had the lowest average grain yield with 1964.2 kg ha⁻¹, while the average grain protein value was 28.19%. RMSE and R² values were 1.21 and 0.727, respectively. In the literature, the grain protein ratio was reported as 23.5 - 28.4% by Segers et al. (2022), and 25.71- 31.15% by Zulkadir et al. (2022). When comparing the values obtained from the examined morphological parameters with those in the literature, it is seen that the study's results are compatible with the literature.

Figure 2, which presents the technological characteristics, shows that the minimum and maximum values for dry weight were 98.06 - 157.34 g, for dry volume 97 - 158 ml, and for fresh weight 172.98 - 314.5 g. The Eresen-87 variety had the highest average value in these three characteristics. The Luz De Otono variety had the lowest average values for dry weight (101.4 g) and dry volume (102.3 ml), while the Hilal variety recorded the lowest average for wet weight (186.9 g). The fresh volume values varied between 144 and 294 ml, and the Filiz-99 variety had the highest average at 282.7 ml. Various factors such as seed differences, location, variety characteristics, environmental factors, soil properties, and storage conditions also affect cooking quality of chickpeas (Wang et al., 2017). Cooking times of desi chickpea varies between 55 – 200 minutes (Williams et al., 1983) and cooking times of kabuli chickpeas vary between 33–72 minutes (Ozer et al., 2010). Consumers demand legumes with shorter cooking times (Ozaktan, 2021). When evaluating the cooking times, the average cooking time varied between 32 and 42 minutes, with the Gölyaka variety exhibiting the shortest average cooking time, recorded at 33 minutes. Conversely, the Hilal variety showed the longest average cooking time, recorded at 40.8 minutes. RMSE and R² values were recorded as 1.10 and 0.911, respectively. Water absorption capacity values varied between 0.612 and 1.573 g seed⁻¹, with an RMSE value of 0.11 and an R² value of 0.835. The Eresen-87 genotype recorded the highest mean value of 1.419 g seed⁻¹. While the swelling index values varied between 1.262% and 2.283% and unit weight values ranged from 0.958 to 1.149 g ml⁻¹. RMSE and R² values for swelling index and unit weight parameters were 0.11-0.03 and 0.867-0.704, respectively. When the mean values of hydration capacity and water absorption index were analyzed, the Gölyaka genotype had the highest values, with 2.151g seed⁻¹ and 1.159%, respectively. R² values were recorded as 0.742 and 0.704, respectively. The swelling capacity values varied between 0.31 and 1.5 ml seed⁻¹, and the highest value was obtained from the Salkım (1.45 ml seed⁻¹) genotype. RMSE and R² values were 0.11 and 0.912, respectively. In the literature, Cilesiz et al. (2023) determined the swelling capacity to range from 0.45 to 1.72 ml seed⁻¹. They reported that the hydration capacity values varied between 0.59 and 2.07 g seed⁻¹ and that the swelling index varied between 1.30% and 5.35%.



Figure 1. Data on morphological parameters of faba bean varieties (Min value, Max value, Range, CV, RMSE, R²)

In Figure 3, which analyzes the mineral matter contents of faba bean grains, the boron contents of the genotypes varied between 12.37 ppm (Luz de Otono) and 14.93 ppm (Hilal). The R^2 value was recorded as 0.744. Ca content values varied between 1267 ppm (Lara) and 1617 ppm (Gölyaka), with an R^2 value of 0.896 and an RMSE value of 37.89. The highest mean value of Cu content was obtained from the Lara genotype with 17.5 ppm, while the lowest mean value was obtained from the Eresen-87 genotype with 12.6 ppm. RMSE and R^2 values were 0.29 and 0.966, respectively. Fe content values varied between 34.0 and 49.8 ppm, with the highest average value observed in the Kıtık 2003 genotype and the lowest in the Gölyaka genotype. The R^2 value was recorded as 0.971. When the average content amounts of potassium (10193-11742 ppm) and mg (1399-1761 ppm) were analyzed, the highest amount was obtained from the Sakız genotype. R^2 values were 0.551 and 0.774, respectively. Mn contents varied between 12.7 and 14.9 ppm, and the R^2 value was recorded as 0.770. Na contents varied between 297.2 and 387.3 ppm, and the R^2 value was recorded as 0.923. While the highest average Mn content was obtained from the Eresen-87 genotype, the highest average Na content was obtained from the Salkım genotype. Phosphorus content values varied between 6320.1 and 7639.6 ppm, and the R^2 value was recorded as 0.773. Sulfur content values varied between 1303.5 and 1584.6 ppm, and the R^2 value was recorded as 0.892. Zn content values varied between 47.4 and 78.7 ppm, and the R^2 value was recorded as 0.969. In addition, while the highest average values for P and Zn were obtained from the Lara variety, the lowest average content for S and Zn was obtained from the Eresen-87 genotype. When the literature was examined, Karakoy et al. (2018) reported that phosphorus in faba bean grain ranged from 1000 to 9900 ppm, potassium from 9400 to 56000 ppm, copper from 8,13 to 34,23 ppm, and zinc from 28,42 to 64,33 ppm. Khazaei and Vandenberg (2020) reported that boron content in the chemical composition of faba bean skin ranged from 10.35 to 12.32 ppm, sodium content from 21.85 to 163.74 ppm, magnesium content from 1232 to 1478 ppm, iron content from 48.57 to 52.54 ppm, and manganese content from 13.38 to 15.87 ppm. Baloch et al. (2014) reported that the amount of potassium in faba bean grain varied between 4500 and 19300 ppm, the amount of iron between 29.7 and 96.3 ppm, the amount of copper between 10.3 and 33 ppm, and the amount of zinc between 10.4 and 49.3 ppm. As a result of the literature evaluation, it was found that the results obtained from the study were compatible with the literatures.



Figure 2. Data on technological parameters of faba bean varieties (Min value, Max value, Range, CV, RMSE, R^2)

When the scatterplot matrix for the overview of correlations and fit lines is examined, blue circles indicate the positive correlation between the examined parameters, red circles denote the negative correlation, and the circle's diameter represents the strength of the relationship. In addition, Figure 5 shows the scatter plot of varieties and fit lines in terms of the examined parameters. Furthermore, in the biplot (Figure 4), formed from the mean values of all examined parameters, the colored circles formed by the groups formed in the dendrogram (Figure 6) are also given on the biplot. Both the axis lengths and angles from the biplot analysis and the scatter of varieties from the dendrogram are presented together in Figure 4. In this context, a long axis length and a narrow angle between the other axis define a highly positive relationship between the axes, while the opposite indicates a negative relationship (Okumus et al., 2023).

Based on the angle values and correlation values of the axes in the same region with the plant height axis, wet volume ($r=0.795$), wet weight ($r=0.758$), dry weight ($r=0.742$), water absorption capacity ($r=0.637$), dry volume ($r=0.636$), swelling capacity ($r=0.669$), 100 grain weight ($r=0.624$) and Na ($r=0.521$), while it showed a positive relationship between the number of grains in the pod ($r=0.649$) and Fe ($r=0.629$).

Based on the angle and correlation values of the axes in the same region as the Zn axis, Cu ($r=0.902$) showed a positive correlation with protein ratio ($r=0.595$), Ca ($r=0.592$), P ($r=0.572$), cooking time ($r=0.443$), and S ($r=0.391$), while it showed a negative correlation with the number of pods ($r=-0.833$), Mn ($r=-0.775$), and hydration coefficient ($r=-0.743$).

Based on the angle and correlation values of the axes in the same region as the swelling index, water absorption index ($r=0.987$), Mn ($r=0.463$), hydration coefficient ($r=0.744$), number of pods ($r=0.471$), Mg ($r=0.457$), and unite weight ($r=0.455$) showed a positive correlation, while cooking time ($r=-0.762$) showed a negative correlation.

According to the literature, Erten and Oncan Sumer (2023) reported that there is a negative correlation between seed yield and protein content in the seed, Baloch et al. (2014) reported a significant positive correlation between K content and Mn, Fe, Cu, and Zn, as well as a strong positive correlation between the Zn content of grain and P, K, Fe, Mn, and Cu. Ozaktan and Doymaz (2022) reported significant positive correlations between Fe and first pod height, seed yield, number of pods per plant, number of seeds per plant, and plant height, as well as notable positive correlations between the amount of zinc in grain and the number of main branches and grain protein content.

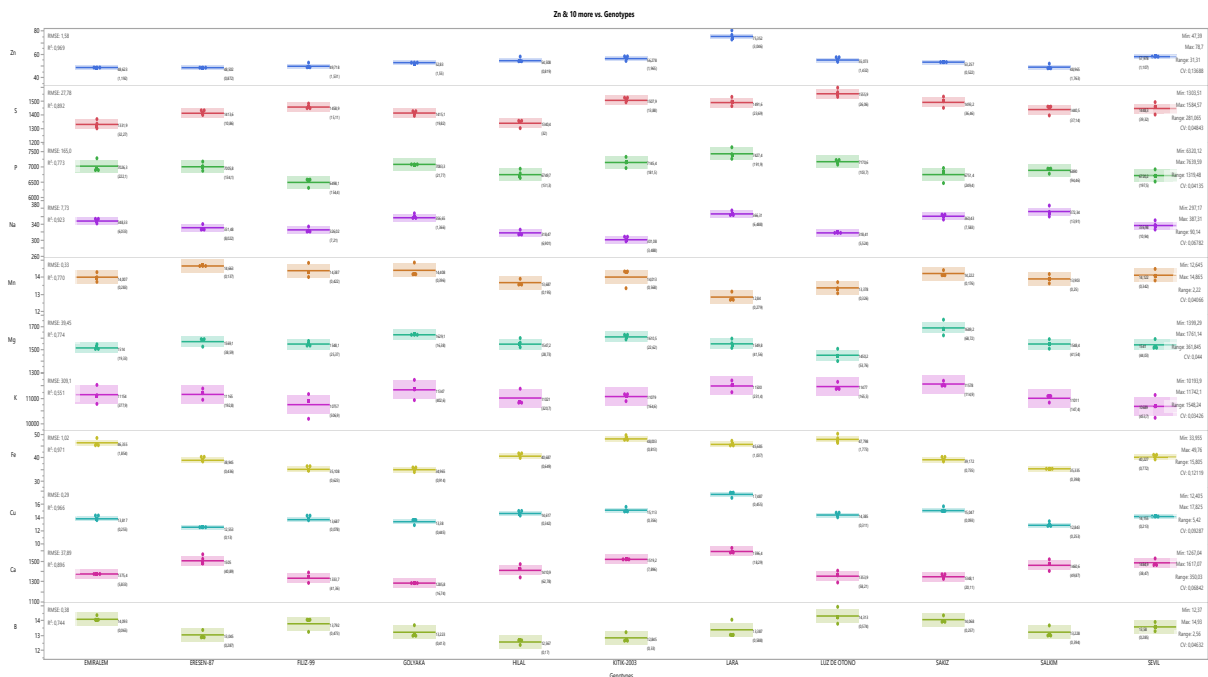


Figure 3. Data on mineral contents of faba bean varieties (Min value, Max value, Range, CV, RMSE, R^2)

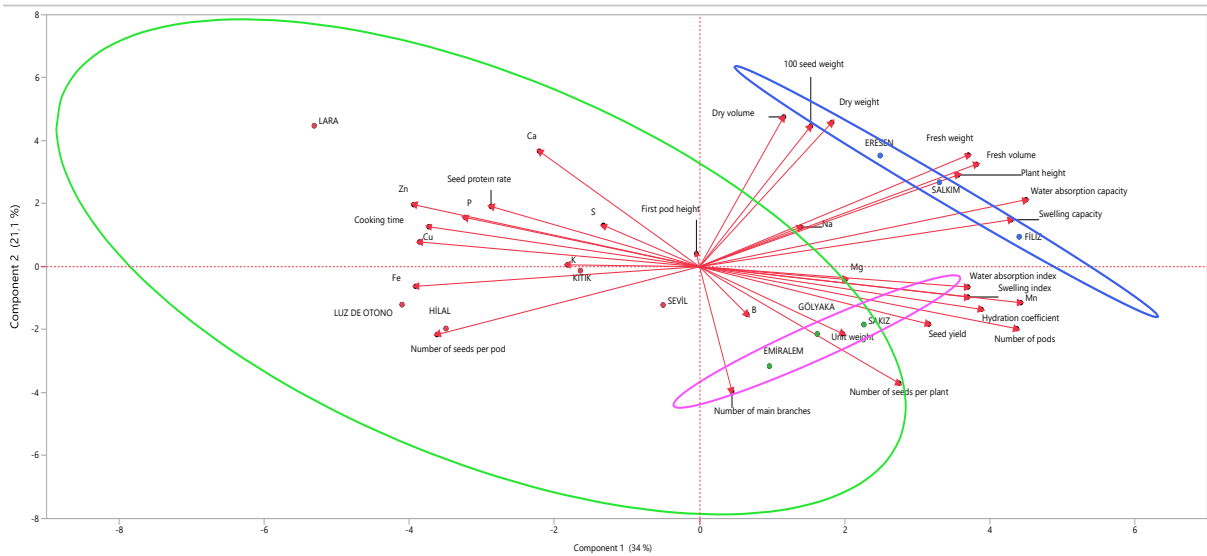


Figure 4. Biplot analysis on the examined parameters and clustering of varieties

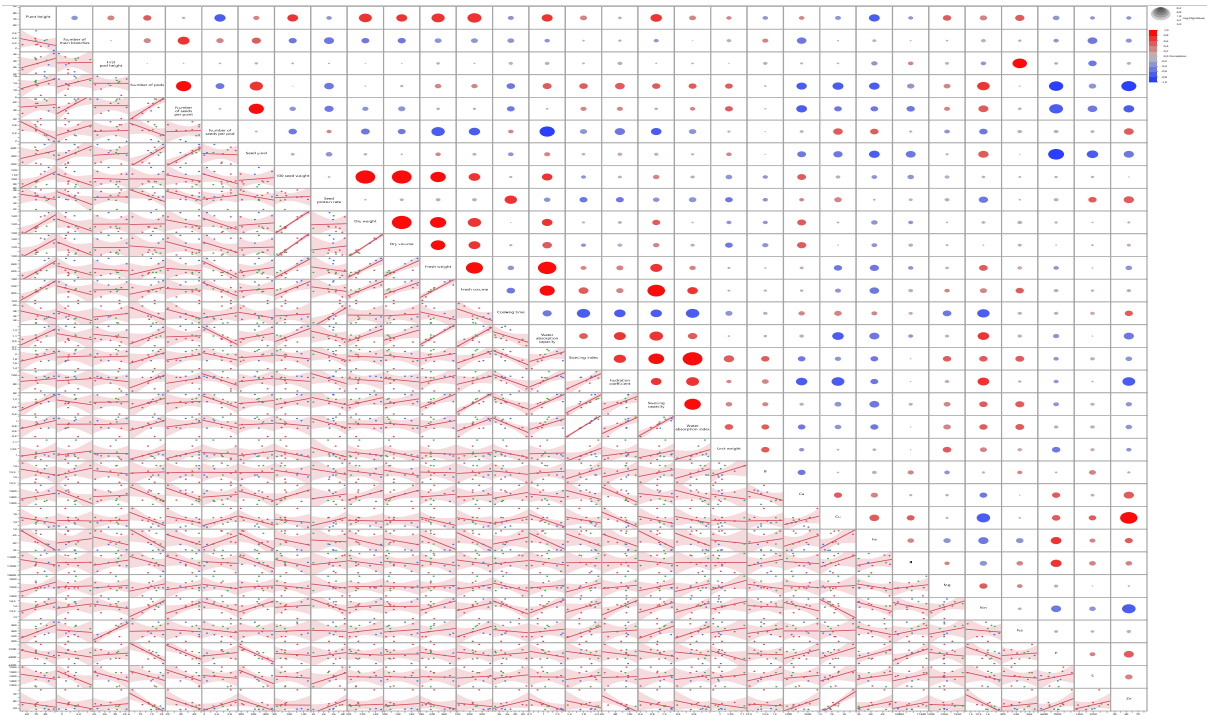


Figure 5. Scatterplot matrix for an overview of correlations and fit lines

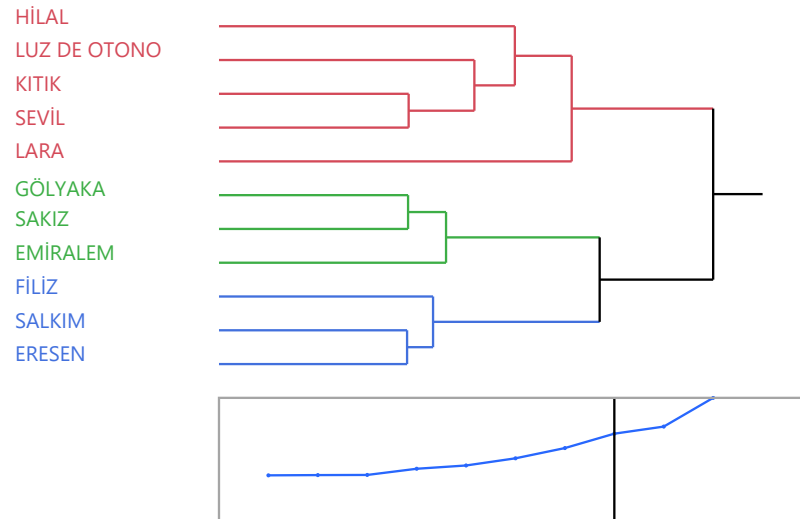


Figure 6. Dendrogram for 11 varieties of faba bean based on the examined parameters

4. CONCLUSION

The study which characterized morphological, technological, and mineral contents of faba bean varieties using multivariate statistical analysis methods, identified a wide variation among the faba bean varieties. The correlation analysis performed to determine the relationship between the traits revealed significant positive correlations with plant height trait, wet volume, wet weight, dry weight, water absorption capacity, dry volume, 100-grain weight, and Na content of grain. Significant positive correlations were determined between Zn content and Cu, grain protein content and Ca, and P and S contents, while negative correlations were determined between pod number and hydration coefficient. A significant positive correlation was determined between the swelling index trait and water uptake index, Mn content, hydration coefficient, number of pods per plant, and grain Mg content, while a negative correlation was determined with cooking time. The cluster analysis performed to identify the genetic relationship between the varieties determined that 11 varieties were divided into 3 different clusters based on their morphological, technological, and mineral characteristics.

For similar ecological conditions, Filiz 99 variety with the highest grain yield, Gölyaka variety with the shortest cooking time and Lara variety with the highest protein content and zinc content in the grain can be recommended.

ACKNOWLEDGEMENT

This study was supported by Erciyes University Scientific Research Projects Unit, Türkiye (Project Number: FYL- 2022- 11793). We also would like to thank the Proofreading & Editing Office of the Dean for Research at Erciyes University for the copyediting and proofreading service for this manuscript.

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Grain Yield Stability of Durum Wheat Genotypes: A Graphical Approach

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ARTICLE INFO

Research Article

Received: 7 April 2025

Accepted: 22 May 2025

Published: 23 June 2025

Keywords:

AMMI

Biplot

Durum Wheat

Elite Line

Stability

ABSTRACT

The study was conducted in the Thrace Region in 2019 and 2020. Fifteen elite lines and 10 varieties of durum wheat were used as material. The aim of this study was to determine the stability of elite lines and varieties by using the graphical AMMI and GGE biplot analyses. Elite lines 3, 4, 5, 7, 8, 9, 10, 12, 15 and varieties Cabato and NKU Ziraat had values above the general mean. The grain yield results of the elite lines indicate that the selected breeding materials possess high yield potential, demonstrating the effectiveness of the selection process. Stability analysis of the genotypes indicated that three out of eight environments contributed positively to grain yield, whereas the remaining five had a negative impact. These data reveal the importance of identifying suitable environments for high grain yield in durum wheat. Among the durum wheat genotypes examined, elite lines 7 and 4 had better stability and grain yield for all environments. When genotype performance was assessed across individual environments, elite lines 3, 9 and 10 showed the most superior characteristics for environments 1 and 4, while elite lines 12 and 15 were most suitable for environments 7 and 8.

Citation: Balaban Gocmen, D. (2025). Grain yield stability of durum wheat genotypes: a graphical approach. *Turkish Journal of Field Crops*, 30(1), 164-174. <https://doi.org/10.17557/tjfc.1671073>

1. INTRODUCTION

Durum wheat (*Triticum durum* Desf.), which is grown on a limited basis in certain countries around the world, is a grain product with high prices and plays an important role in world trade. Durum wheat is well adapted to the Mediterranean climate since it is heat and drought tolerant and performs well in poor soils (Mastrangelo et al., 2005). Thus, durum wheat is cultivated as the main crop in four countries in southern Europe (Spain, France, Italy, and Greece), three countries in northern Africa (Morocco, Algeria, and Tunisia) and two countries in southwest Asia (Türkiye and Syria) (Martínez-Moreno et al., 2022).

Türkiye was the largest producer of durum wheat (*Triticum durum* Desf.) after Russia, with a cultivation area of 1.6-2 million hectares in the 19th and early 20th centuries. Durum wheat accounted for 80% of the wheat area in Southeast Anatolia, 60% in Thrace, and 40% in the coastal areas (Ozberk et al., 2005). As in many other countries, the introduction of high-yield bread wheats into the region, as a reflection of the "Green Revolution" caused an increase in bread wheat production and a rapid decrease in durum wheat production.

As a result of the high adaptation to new bread wheat varieties in the Thrace Region within the scope of the National Wheat Project, the proportion of durum wheat varieties in wheat production was approximately 60% in the 1960s but decreased to 2.5% after the 1980s (Sehirali & Genctan, 1985; Ada, 1993). Today, durum wheat production is at the level of 1%. The main reason for this situation is the lack of new genotypes with high yield and quality. Finding and developing cultivars with improved grain yields has been the main agenda of many durum wheat breeding programs worldwide (Khayatnezhad & Gholamin, 2020), which are expected to play a crucial role in climate change adaptation (De Vita & Taranto, 2019). Therefore, it is important to select genotypes that have the desired agronomic characteristics and are well adapted to the environment (Bilgin et al., 2008).

The different responses of genotypes to various environmental conditions, that is, genotype $\times$ environment interactions, make it difficult to select stable and well-adapted genotypes. Genotype selection depends on understanding genotype, environment, and genotype $\times$ environment interactions (Jat et al., 2017). Yan et al. (2000) developed a "genotype main effect and genotype by environment interaction (GGE) biplot" methodology for graphical analysis of multi-environment trials (MET) data. The GGE biplot is constructed by plotting the first two principal components (PC1 and PC2) derived from singular value decomposition of the environment-centered data. Increasingly, plant breeders/agronomists have found GGE useful in mega-environment analysis (Yan & Tinker, 2005; Kang et al., 2006; Fan et al., 2007), test-environment evaluation (Dimitrios et al., 2008), trait association and trait profile analyses (Yan & Rajcan, 2002).

The Additive Main Effects and Multiplicative Interaction (AMMI) model, a widely adopted approach in stability analysis, incorporates both additive main effects and genotype-by-environment (G $\times$ E) interaction effects in its assessment. In contrast, the GGE biplot method graphically represents multi-environment trial data, facilitating the identification of superior genotypes across test environments. Through GGE biplot analysis, genotypes can be evaluated for mean performance, stability, and general or specific adaptations in individual environments or between environments. A stable genotype is a genotype that has high yield with minimum fluctuation in performance when grown in diverse environments (Zerihun et al., 2016). Additive main effects and multiplicative interactions analysis indicated that the yield performances of bread wheat cultivars were highly affected by the major environmental factors. The first two principal component axes (PCA 1 and PCA 2) were significant and they explained 60.9% of the total genotype $\times$ environment interaction (Ilker et al., 2012).

These graphical tools play a special role in the simultaneous evaluation of yield and stability, as well as the selection of multi-environments (ME) and specific adaptability (Ajay et al., 2020). Among those models, the AMMI model and the GGE biplot are statistical methods most extensively used for modeling genotype $\times$ environment interactions (GEI) and increasing the efficiency of selection in yield trials and group environments in multi-environment trials of cereal crops, including wheat. GGE-Biplot analysis showed that although the durum wheat yield trials were conducted in many environments, outcomes alike can be obtained from one or two representatives of each ME (Kaya, 2022). According to GGE-Biplot analysis, it was determined that two mega-environments instead of four environments would be sufficient for the stability of Lucilla and Glosa genotypes (Gungor et al., 2022).

This study, which was carried out for 2 years in 4 different locations with 15 elite durum wheat lines and 10 durum wheat varieties, aimed to determine appropriate elite lines to be bred for different environments and varieties to be produced in the region by using AMMI and GGE biplot graphics.

2. MATERIALS AND METHODS

Field trials and materials

The trials were conducted for two years (2019 and 2020) under natural rainfall conditions in the Thrace region in Suleymanpasa (Tekirdag), Hayrabolu (Tekirdag), Luleburgaz (Kırklareli) and Edirne locations to represent different ecologies. Geographical descriptions of the locations are given in Table 1.

Table 1. Geographical characteristics of test locations

Locations	Longitude Latitude	Altitude, m	Soil Texture	Temperature (°C)		Rainfall, mm	Temperature (°C)		Rainfall, mm
				Min.	Max.		Min.	Max.	
				2019			2020		
Suleymanpasa	40° 59′ 37" N 27° 34′ 53" E	20	Silt Loam	3.1	28.2	404.4	2.8	25.1	394.1
Hayrabolu	41° 12′ 49" N 27° 6′ 22" E	81	Clay Loam	0.8	31.7	381.8	- 0.7	30.2	411.2
Luleburgaz	41° 24′ 10" N 27° 21′ 56" E	45	Clay Loam	- 0.2	32.0	350.4	- 2.4	28.5	394.4
Edirne	41° 40′ 38" N 26° 33′ 21" E	252	Clay Loam	1.1	32.0	478.3	- 0.9	30.1	393.2

Soil classification system (Ulgen and Yurtsever, 1995).

Fifteen elite lines of durum wheat developed by combination breeding in the Department of Field Crops of the Faculty of Agriculture and 10 commonly cultivated durum wheat varieties were used as material in the study (Table 2). The experiments were conducted according to a randomized complete block design with 4 replications. Sowing was made with a parcel sowing machine on 7.5 m long and 1.02 m wide plots with 500 plants per square meter. In the trials, 250 kg of 20.20.0 compound fertilizer was given per hectare with sowing, 180 kg of urea (46%) was given in the tillering period, and 200 kg of ammonium nitrate fertilizer (26%) was given during stem elongation. Herbicide was applied to the weeds during the tillering period.

Table 2. Pedigrees of the durum wheat elite lines and varieties used in the experiment

eno. n	Elite lines	Pedigree	Geno. no	Elite lines	Pedigree
1	NZFM 8	IDSN261/Svevo	14	NZFM 45	Tunca79/Svevo
2	NZFM 22	Ged75//Ged75/Yav79	15	NZFM 49	Epidur/Zenit
3	NZFM 32	IDSN165/Kızıltan91	Varieties		
4	NZFM 33	Fuatbey2000/Zenit	16	Caboto	Saragolla/Line D97.15
5	NZFM 35	Zenit/YP43	17	NKU Ziraat	Gediz75/Yavaros79
6	NZFM 37	Zenit/Tunca79	18	Mirzabey 2000	GD-2/D-1184528
7	NZFM 38	Svevo/Hacimestan	19	Zenit	Valriccardo/Vic.
8	NZFM 39	Zenit/Hacimestan	20	Saragolla	Iride/Linea-PSB-0114
9	NZFM 40	Zenit/7113M5	21	Pitegora	V702/Levante
10	NZFM 41	Ç 1252/Svevo	22	Kızıltan 91	UVY 162/61-130//BY2E/TC
11	NZFM 42	Fuatbey2000/Svevo	23	Svevo	Cimmyt-Selection/Zenit
12	NZFM 43	Ionia/Zenit	24	Ç 1252	61-130//Kunduru414-44/377-2
13	NZFM 44	Ged75/Yav79//Zenit	25	Maestrale	Iride/Svevo

Statistical analysis

The grain yield values obtained in the research conducted with 25 durum wheat genotypes were analyzed via variance analysis using the JMP 16.0 statistical program. Significant differences between genotypes were determined using the least significant difference TUKEY_{HSD} test. The obtained data were analyzed using the BAFR statistical package program (Kahrıman, 2020) according to the AMMI and GGE biplot methods, which allow two-way evaluation by combining the effects of genotype and genotype × environment (G × E) interaction on the same graph. The performance of durum wheat genotypes was assessed using stability models (1) AMMI (Gauch & Zobel, 1997) and (2) GGE biplot (Yan & Kang, 2002). The AMMI analysis is based on two previously discovered simple models. AMMI first analyzes the main effects of genotypes and environments (additive) using analysis of

variance (ANOVA) and then analyses the residual from this model (namely the interaction) using principal components analysis (PCA). The model for AMMI analysis is given.

$$Y_{ij} = \mu + \delta_i + \beta_j + \sum_k \lambda_k \delta_{ik} \beta_{jk} + \varepsilon_{ij} \quad (1)$$

where Y_{ij} is the average yield of the i^{th} variety in the j^{th} environment, μ is the general mean, δ_i is the genotypic effect of the i^{th} cultivar, β_j is the j^{th} environment effect, λ_k is the eigenvalue of the principal component axis k , δ_{ik} is the genotype eigenvector value for PC axis n , β_{jk} is the environment eigen vector value for PC axis k , and ε_{ij} is the residual error. The following linear-bilinear GE biplot model was used to explore G plus GE variability in durum wheat's grain yield.

$$Y_{ij} = m + b_j + \sum_{n=1}^k \lambda_n \xi_{in} \eta_{jn} + \varepsilon_{ij} \quad (2)$$

where Y_{ij} is the mean of genotype i in environment j ; μ is the grand mean; β_j is the main effect of environment j ; n is the singular value; λ_n and ζ_{in} are the singular vectors for genotypes and environments for $\lambda = 1, 2, \dots, k$, respectively; and ε_{ij} is the residual effect. The dataset was then subjected to graphical analysis using the GGE biplot in the BAFR statistical package program (Kahrıman, 2020). The GGE biplots were drawn using the first two symmetrically scaled principal components for generating average tester coordinate and polygon view graphs (Yan & Kang, 2002). The vector view biplot was obtained to visualize relationships between locations.

3. RESULTS AND DISCUSSION

In this study, the mean grain yields of 15 elite durum wheat lines and 10 durum wheat varieties at 4 different locations over 2 years ranged from 705.44 to 551.94 kg ha⁻¹ (Table 3). While 9 durum wheat elite lines and 2 durum wheat varieties had grain yields above the general mean, 6 elite lines and 8 durum wheat varieties had grain yields below the general mean. The mean yield of the elite lines was 654.74 kg ha⁻¹ and the mean yield of the varieties was 626.88 kg ha⁻¹. The environmental impact on grain yield was positive in the Suleymanpasa, Hayrabolu and Luleburgaz locations in 2019, while the environmental impacts were negative in the Edirne location in 2019 and in the Suleymanpasa, Luleburgaz, Hayrabolu and Edirne locations in 2020. The genotypic effects of elite lines 3, 4, 5, 7, 8, 9, 10, 12, 15 with Caboto and NKU Ziraat durum wheat varieties on grain yield were positive, while the genotypic effects of the other elite lines and durum wheat varieties were negative.

Table 3. Mean grain yield, genotypic and environmental effect values

Genotype no	Genotypes	2019				2020				Mean		Genotypic effect
		Tekirdag	Hayrabolu	Luleburgaz	Edirne	Tekirdag	Hayrabolu	Luleburgaz	Edirne			
1	NZFM 8	845,75	754,00	808,00	615,75	533,50	576,00	560,50	451,75	643,16	c-h	-0,44
2	NZFM 22	869,00	679,50	830,00	546,50	516,00	520,50	546,25	443,25	618,88	ghı	-24,72
3	NZFM 32	871,00	825,25	923,50	663,25	500,25	650,25	624,75	535,50	699,22	a	55,63
4	NZFM 33	935,00	766,75	897,00	524,00	593,00	610,50	557,00	454,25	667,19	a-e	23,59
5	NZFM 35	828,00	701,75	908,75	503,75	583,50	525,00	607,25	551,50	651,19	b-g	7,59
6	NZFM 37	702,25	638,75	711,50	381,50	545,25	535,75	562,50	497,00	571,81	k	-71,78
7	NZFM 38	882,50	774,00	1013,50	576,00	654,25	623,75	534,00	466,50	690,56	ab	46,97
8	NZFM 39	795,25	817,75	866,00	510,50	612,00	668,50	622,25	508,25	675,06	a-d	31,47
9	NZFM 40	891,25	721,75	966,50	657,25	584,25	559,25	639,00	487,00	688,28	ab	44,69
10	NZFM 41	921,75	834,25	972,25	622,75	600,75	541,25	539,50	507,00	692,44	ab	48,84
11	NZFM 42	838,75	660,25	811,00	496,00	609,25	562,75	521,00	450,75	618,72	ghı	-24,88
12	NZFM 43	819,75	721,75	929,25	579,00	676,75	599,50	617,25	525,50	683,59	abc	40,00
13	NZFM 44	777,00	622,00	775,75	426,75	618,75	703,50	633,25	531,25	636,03	d-h	-7,56
14	NZFM 45	917,00	683,00	837,75	571,00	546,75	486,75	489,00	431,00	620,28	f-ı	-23,31
15	NZFM 49	819,75	711,25	893,75	569,50	666,25	589,50	547,75	519,25	664,63	a-f	21,03
16	CABOTO	849,25	799,50	898,00	594,00	740,50	586,50	629,25	546,50	705,44	a	61,84
17	NKU ZIRAAT	861,75	753,25	973,25	562,00	734,50	614,00	601,00	504,50	700,53	a	56,94
18	MIRZABEY	840,75	612,50	720,25	529,00	480,50	427,75	568,25	461,75	580,09	ijk	-63,50
19	ZENIT	840,25	615,75	802,00	532,50	457,00	579,75	526,75	448,00	600,25	hıj	-43,34
20	SARAGOLLA	841,25	717,00	764,00	565,00	497,25	512,50	568,25	517,00	622,78	e-ı	-20,81
21	PITEGORA	786,25	710,00	809,00	634,75	501,25	479,25	589,00	488,25	624,72	e-ı	-18,88
22	KIZILTAN 91	799,75	604,75	673,25	510,75	494,00	346,25	569,00	417,75	551,94	jk	-91,66
23	SVEVO	778,50	763,50	863,25	539,50	511,00	560,00	511,00	471,50	624,78	e-ı	-18,81
24	Ç 1252	848,75	720,50	772,25	537,75	620,25	464,25	591,00	434,75	623,69	e-ı	-19,91
25	MAESTRALE	828,25	744,00	819,00	542,50	564,00	541,00	544,75	493,25	634,59	d-h	-9,00
Mean of lines		847,60	727,47	876,30	549,57	589,37	583,52	573,42	490,65	654,74		
Mean of varieties		827,48	704,08	809,43	554,78	560,03	511,13	569,83	478,33	626,88		
Environments mean		839,55	718,11	849,55	551,65	577,63	554,56	571,98	485,72	643,59		
Environmental effect		195,96	74,52	205,96	-91,94	-65,96	-89,03	-71,61	-157,87			

E1, Tekirdag-Suleymanpasa location in 2019; E2, Tekirdag-Hayrabolu location in 2019; E3, Kırklareli-Luleburgaz location in 2019; E4, Edirne location in 2019; E5, Tekirdag-Suleymanpasa location in 2020; E6, Tekirdag-Hayrabolu location in 2020; E7, Kırklareli-Luleburgaz location in 2020 ve E8, Edirne location in 2020.

The AMMI variance analysis and principal component analyses performed on the grain yield data obtained from 8 environments are given in Table 4. When the main effects and variance AMMI regarding grain yield values obtained in eight environments were examined, environment, genotype, and genotype $\times$ environment interactions were found to be statistically significant at the 0.01 level (Table 4).

Table 4. AMMI variance analysis and principal component analysis for grain yield values

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Impact Rate
Environments	7	137000	196000	398.00 **	80.07
Replication (E)	24	1180	492	1.73*	0.69
Genotypes	24	13900	5770	20.3**	8.12
Genotype $\times$ Environment Int	168	19000	1130	3.99**	11.11
PC1	30	6620	2210	7.77**	34.8
PC2	28	4870	1740	6.12**	25.6
PC3	26	3190	1230	4.32**	16.8
PC4	24	1850	773	2.72**	9.70
PC5	22	1300	589	2.07**	6.80
PC6	20	736	368	1.30	3.90
PC7	18	461	256	0.90	2.40
Residuals	576	16400	284		
Total	967	207000	2140		

The result of the analysis of variance of the AMMI model revealed that grain yield was significantly ($p < 0.01$) affected by environment, genotype, and genotype-environment interaction, which explained 80.07%, 8.12% and 11.11% of the variation, respectively. Moreover, three PCs (PC1, PC2 and PC3) explained 77.2%, a significant part of the genotype-environment interaction, as shown in Table 4. AMMI analysis showed that much of the variation in grain yield was due to environmental differences, indicating that the environments varied significantly. This finding is consistent with findings of studies by Mohammadi et al. (2017), Ngailo et al. (2019) and Bhardwaj et al. (2020). The highest proportion of variation from the environment, followed by GEI is in agreement with results for barley (Pour-Aboughadareh et al., 2022) and durum wheat grain yield (Mohammadi et al., 2015).

According to the results obtained by the AMMI graphic method, PC1 explained 34.8% of the variation in grain yield, PC2 explained 25.6% and PC3 explained 16.8%. The fact that PC1, PC2 and PC3 explain 77.2% of the grain yield shows that these three main components are sufficient to visually represent the grain yield of durum wheat varieties. The grain yield explanation rate was determined as 9.7% for PC4, 6.8% for PC5, 3.9% for PC6 and 2.4% for PC7. Temesgen Bacha et al. (2015) stated that two-way principal component analysis explained 66.56% of the genotype and environment interaction.

In the AMMI1 analysis, the abscissa and ordinate of the biplot show the 1st principal component (PC1) term and the significant effect of the trait, respectively. The AMMI model describes the position of genotypes relative to each other and to the studied environments (Elakhdar et al., 2017). In the AMMI1 biplot, genotypes or environments that appear on a perpendicular line on a graph had similar mean yields, and those that fall almost on a horizontal line had similar interactions (Crossa et al., 1990). Genotypes or environments on the right side of the midpoint of the perpendicular line have higher mean values than those on the left side. AMMI2 is a principal component (PC1 and PC2) scores-based graphical representation of summarized information, which has advantages over joint regression-based analysis. AMMI2 allows inferences to be made about complicated GEI that involves significant multi-environments and detection of genotypes with either broad or narrow spectrum adaptability. The AMMI results for the mean grain yield of the 25 durum wheat genotypes are shown in Figure 1 and Figure 2.

When 25 durum wheat genotypes are examined according to the AMMI1 biplot analysis results, 8 environments were grouped into 5 mega-environments. According to the AMMI1 biplot environments, environments 1 and 2 were the 1st mega-environment, environment 3 was the 2nd mega-environment, environments 6 and 5 were the 3rd mega-environment, environments 8 and 7 were the 4th mega-environment and environment 4 was the 5th mega-environment. These results reveal that the study can be carried out in 5 mega-environments instead of 8 environments. Mohamed et al. (2022) reported in their studies that AMMI and GGE biplot results constituted four mega-environments and durum wheat genotypes were also in two mega-environments in terms of grain yield.

In the mega-environment formed by environments 1 and 2, there are genotypes with mean grain yields above the general mean and regression coefficients below one. Elite lines 3, 9 and 10 in this mega-environment are genotypes that do not respond strongly enough to improvements in environmental conditions. The mega-environment created by environment number 3 was located on elite line 4, above the general mean. Although this elite line has high stability, it did not have a high response to improving environmental conditions. Elite line 7, Caboto and NKU Ziraat durum wheat varieties were genotypes with yield above the general mean and a regression coefficient above 1. These genotypes were the most suitable genotypes in terms of stability and had strong responses to changing environmental conditions. The mega-environment containing environments 6 and 5 contained genotypes that show good results in specific environments.

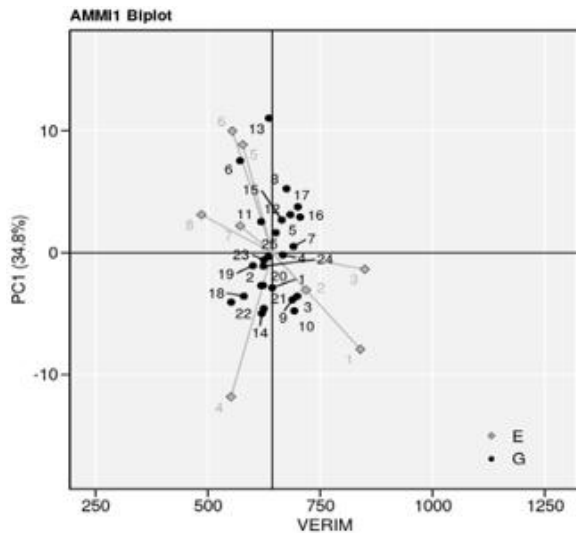


Figure 1. AMMI1 biplot graph for grain yield

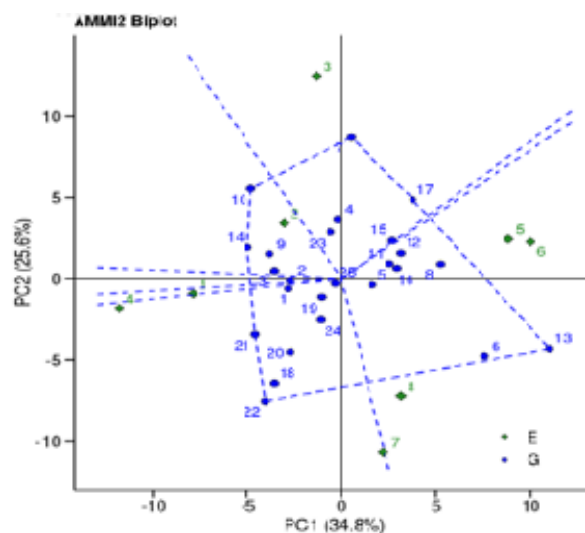


Figure 2. AMMI2 biplot graph for grain yield

Elite lines 6 and 13, which had values below the general average, showed specific adaptation to the mega environment created by environments 6 and 5, and reached high grain yields, while elite line 11, which had a yield below the general average, was the genotype with higher stability in this mega environment. Elite line 14, Mirzabey, Zenit, Pitegora and Kızıltan 91 varieties, which had values below the general mean and adapted to adverse environmental conditions, were located in the 5th mega-environment where the 4th environment is located. Lines that were superior in terms of grain yield, as determined by GGE biplot analysis, could be included in regional yield trials to be evaluated at the registration stage (Tekdal et al., 2017).

The AMMI2 biplot polygon relates genotypes to each other and to environments in terms of grain yield values obtained under multiple environmental conditions and shows which genotype is most compatible with which environment. It also provides more detailed information by dividing the environment into different sectors. If the genotype and environment are located in the same sector, the interaction of these two factors is positive; if they are located in different sectors, the interaction of the two factors is negative; and if they are both located in the same sector, the interaction of the two factors is a mixed interaction (Islam et al., 2015). If the genotypes appear very close to each other, all environments are similar to each other, and if the genotypes are located at opposite points, then the genotypes have different results from each other (Akter et al., 2014).

In the analysis graph (Figure 2), 8 environments were divided into 7 sectors. In the first sector (environments 1, 5 and 6), there were elite lines 5, 6, 8, 11, 12 and 13; in the third sector (environment 3), there were elite lines 4 and 7, and durum wheat varieties NKU Ziraat and Svevo; in sector number 4 (environment 2), there were elite lines 2, 3, 9, 10 and 14; in sector number 6 (environment 8), there was elite line 2; and in sector number 7 (environment 4), there were elite line 1 and durum wheat varieties Zenit, Saragolla, Pitegora, Kızıltan 91 and Ç 1252. The fact that the genotypes used in the study are located in different sectors reveals a genetic difference in grain yield. Genotypes that are close to each other in the same sector are genetically closer to each other. There were no environments in the second and fifth sectors. The fact that the environments in the study from the same year were located in different sectors shows the differences between the environments. In this study, the fact that elite lines 5, 6, 8, 11, 12 and 13 are in the same sector indicates that these genotypes are well adapted to environments 1, 5 and 6. Genotypes 2, 5, 19 and 25, which are closer to the center of the polygon, are better adapted to all environments than the other genotypes, while elite line 5 is above the general mean. Khan et al. (2020), Mekonnen et al. (2020) and Verma and Singh (2021) examined the stability of wheat genotypes and

reported that the AMMI method, which has high fitting power is more suitable than other methods for determining the adaptability of genotypes at different locations.

The GGE biplot showing the mean and stability values (Figure 3), and the which-won-where (Figure 4) in terms of grain yield for the 25 genotypes included in the experiment at 8 different environments are given in graphs.

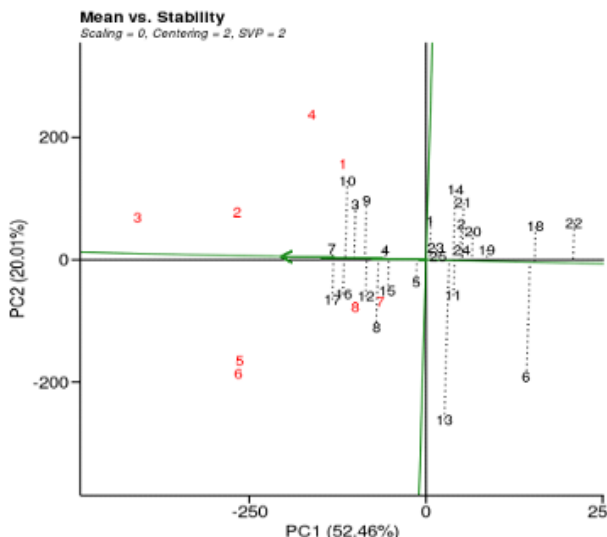


Figure 3. Mean and stability graph for grain yield

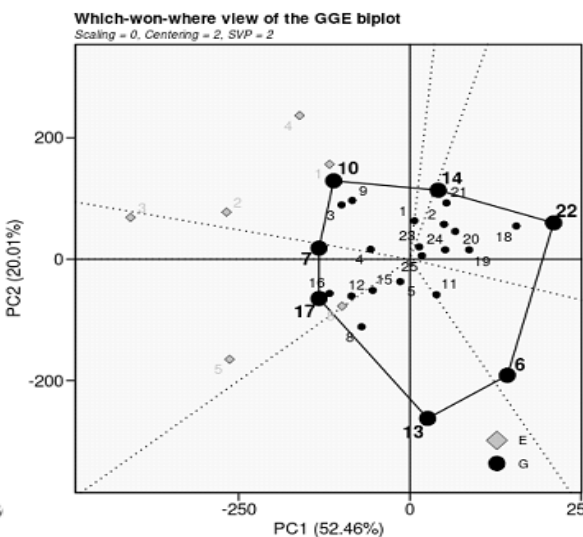


Figure 4. Which-won-where graph for grain yield

The length of the abscissa gives the grain yields of genotypes with above-mean and below-mean grain yields to the right and left of the origin, respectively. The length of the ordinate approaches the GEI associated with the genotype, where more length corresponds to higher variability and lower stability. GGE is the distribution created by a model when the genotype is suitable for the environment, which is one of the most important approaches for biplot analysis. The genotypes at the corners of the polygon drawn in the GGE biplot chart are the most preferred genotypes for the environments in that sector (Yan & Thinker, 2006). According to the GGE biplot analysis, the shorter the projection of a genotype with a vertical line passing through the starting point, the greater the stability of the genotype. The genotypes and environments the closest to the vector ends on the abscissa are those with closest to ideal performance (White & Broadley, 2009).

In this study, environment (E) contributed to 80% of the total variation in the data, while the contribution rates of genotype (G) and genotype $\times$ environment interaction (GEI) were lower, at 8.12% and 11.11% of the total variation in the data, respectively.

The GGE biplot has been used in some studies to analyze the MET data for wheat in Turkey (Tekdal et al., 2017; Kendal, 2019). Putto et al. (2008) revealed that 50-80% of the total variation was attributed to environment, while the main effect of genotype contributed to 15-46% of the total variation.

A GGE biplot is constructed by plotting the first principal component (PC1) scores of the genotypes and the environments against their respective scores for the second principal component (PC2) that results from SVD of environment-centered or environment-standardized GED (Yan et al. 2007). According to the GGE biplot analysis result, the total variation for grain yield was 72.47%, of which 52.46% was represented by component 1 (PC1) and 20.01% by component 2 (PC2) (Figures 3 and 4). The total variability explained by the two principal components, PC1 and PC2, in the available GE data is greater than 60%, and the variability explained by GE is greater than 10% in determining the stability and effectiveness of genotypes (Yang & Holland, 2010). Jedzura et al. (2022) reported that the main effects of the environment explained 89.0% of the sum of squared deviations for yield means. The high value of this parameter indicates that the means for yield differ greatly according to the environments. The study's results are largely similar to those of other studies.

The GGE biplot combines traits with yield and can help visually identify the best cultivars (Kendal, 2019). In this study, while elite lines 4 and 7, which had above-general mean values and the shortest projection lines, were the most stable, elite lines 5, 12 and 15, varieties Caboto and NKU Ziraat, which had above-general mean values, follow these elite lines (Figure 3). In contrast, the Zenit, Ç 1252 and Maestrals varieties showed high stability, but their grain yields were not high. Megerssa et al. (2024) indicated that genotypes with the highest stability did not have the highest yield in their study. The genotypes with the lowest stability are those with the longest projection

line and values below the general mean. According to the obtained data, the longest projection lines were found for elite lines 6 and 13, followed by elite lines 8, 10, 14, which had low stability and yielded above the general mean. Elite lines 6, 13 and 14 had yields below the general mean.

The which-won-where biplot is established by combining the furthest (i.e., vertex) genotypes that form a polygon (Yan, 2001). Genotypes at the polygon's vertices are either the best or the lowest in one or more environments. The genotype at the vertex of the polygon performs best in the environment entering the sectors. The which-won-where biplot for grain yield is presented in Figure 4. The biplot demonstrates the presence of the cross-over GEI and mega-environments (MEs) for grain yield.

The equality line in the polygon was divided into 6 sectors in the which-won-where graph for grain yield. While there was no environment in sectors 2, 3 and 4, the environments were distributed in sectors 1, 5 and 6. Environment 7 was located in the 1st sector, environments 1, 2 and 4 were in the 5th sector, and environments 3, 5, 6 and 8 were in the 6th sector. When the genotypes at the corner of the polygon were evaluated according to the environments, although elite lines 13 and 6 had values below the general mean, elite line 13 showed more stability than elite line 6 for environment 7. Elite line 10, which had a value above the general mean, showed more stability in environments 1, 2 and 4 than elite line 14, which had a value below the general mean. The stability of the genotypes for environments 1, 2 and 4 was $10 > 14 > 22$, while for environments 3, 5, 6 and 8, the stability of the genotypes was $17 > 7 > 10$. According to the obtained data, elite lines 3, 9 and 10 had grain yields above the general mean in environments 1, 2 and 4, while elite lines 7 and 12, varieties NKU Ziraat and Caboto had grain yields above the general mean in environments 5, 6 and 8. Elite line 8 had grain yield above the general mean, while elite line 13 had grain yield below the general mean in environment 7.

High-yielding newly bred durum wheat varieties may have higher yield potential due to better adaptation and stability in different environments compared to previous ones (Ayed et al., 2021; Frankin et al., 2021). These findings confirm the results of this study.

The study reveals that all test environments, except environment 3 are closely related, and most (especially 1, 4, 7 and 8) have values close to the mean. Environments 1, 4, 7 and 8 were the most representative and suitable test environments to select widely adapted genotypes. Environment 3, which was the most distant from the origin, was more discriminative than environments 1, 4, 7 and 8. Discriminative environment 3 helped identify genotypes adapted to specific environments. Plavsin et al. (2021) also stated that graphical representation can easily identify genotypes or genotypes specifically adapted to a particular environment.

4. CONCLUSION

The results obtained from a study conducted with 25 durum wheat genotypes in 8 environments show that most of the elite lines had higher grain yield and stability than the varieties. Nine elite lines had performance above the mean and high stability. Although 3 environments had positive effects on grain yield, the negative effects of 5 environments indicate that it is important to determine the appropriate environment for high grain yield in durum wheat. The fact that the majority of elite lines had a positive effect on grain yield shows that the selection of elite lines with good yield potential among breeding lines was successful. Regarding stability, elite lines 7 and 4 were determined to have the highest performance in all environments. Elite lines 3, 9 and 10 were most suitable for environments 1 and 4, elite line 8 and variety NKU Ziraat were most suitable for environments 5 and 6, and elite lines 12 and 15 was most suitable for environments 7 and 8. Environments 1, 4, 7 and 8 were the most representative and suitable test environments to select widely adapted genotypes. Environment 3, which was the most distant from the origin, was more discriminative than environments 1, 4, 7 and 8. Discriminative environment 3 helped identify genotypes adapted to specific environments. The AMMI and GGE biplot analysis results provided very effective and accurate information for the selection of superior elite lines for plant breeding studies selecting regionally adapted varieties.

ACKNOWLEDGMENTS

The author thanks Prof. Dr. İsmet BASER, Prof. Dr. Oguz BİLGİN and Prof. Dr. Alpay BALKAN from Department of Field Crops of Agricultural Faculty of Tekirdag Namık Kemal University for their contribution in the field experiments, helpful suggestions and critically reviewing the manuscript.

DISCLOSURE STATEMENT

The author declares no conflict of interest.

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Physiological and Agronomic Responses of Peanut (*Arachis hypogaea* L.) to Melatonin Seed Pretreatment and Gamma-Aminobutyric Acid Foliar Application under Accelerated Aging

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ARTICLE INFO

Research Article

Received: 13 July 2024

Accepted: 1 May 2025

Published: 23 June 2025

Keywords:

Amino acid

Antioxidant

Osmopriming

Seed deterioration

ABSTRACT

This research aims to investigate the effects of melatonin and gamma-aminobutyric acid (GABA) on improving plant quality from aged peanut seeds, a factorial experiment was conducted in 2021–2022 using a randomized complete block design (RCBD) with three replications. Treatments included two seed quality levels (normal and aged), three melatonin pretreatment concentrations (0, 50, or 100 μ M), and two GABA foliar application levels (0, or 1 mM). Seed aging was induced at 40°C and 96–100% humidity for 96 hours, followed by 8-hour melatonin soaking. Aging reduced pegs and pods per plant, 100-seed weight, and relative water content while increasing MDA, anthocyanin, and antioxidant enzyme activities (SOD, CAT). Pretreatment with 100 μ M melatonin improved yield traits and enzyme activity in aged seeds. Both 50 and 100 μ M melatonin increased anthocyanin and reduced MDA. GABA foliar application (1 mM) enhanced pegs per plant, 100-seed weight, RWC, shell yield, and antioxidant enzyme activity, while reducing MDA. Seed aging reduced yield by 45.81%, but melatonin pretreatments (50 and 100 μ M) improved it by 9.91% and 11.33%, respectively. In normal seeds, these treatments increased yield by 7.53% and 14.66%. GABA application improved yield by 6.53%. Path analysis showed that pod number and 100-seed weight had the strongest positive effects on yield, while SOD and CAT had indirect adverse effects. Overall, pretreatment with 100 μ M melatonin and 1 mM GABA foliar application is recommended to mitigate seed aging effects and improve peanut yield.

Citation: Khoshkarvandani, N. H., Firouzabadi, M. B., Esfahani, M., Safaei, S., & Makarian, H. (2025). Physiological and agronomic responses of peanut (*Arachis hypogaea* L.) to melatonin seed pretreatment and gamma-aminobutyric acid foliar application under accelerated aging. *Turkish Journal of Field Crops*, 30(1), 175-191. <https://doi.org/10.17557/tjfc.1515618>

1. INTRODUCTION

Seed deterioration is one of the most important factors that reduce the physiological quality of seeds, and its damage increases under adverse environmental conditions (Zhang et al., 2021). Seed deterioration begins with a chain of biochemical events, including damage to the membrane and disruption of biochemical reactions, as a result of which many vital properties of the seed, the rate of germination and seedling establishment reduce, and abnormal seedlings increase, leading to the death of the seed (Arab et al., 2022). Membrane damage is considered as a basic and primary factor in seed deterioration. Loss of integrity of the cell membrane and increase in membrane permeability causes an increase in the leakage of seed compounds and loss of seed vitality. As a result, seed cells cannot maintain their natural physical conditions and function properly (Ebone et al., 2019). The composition inside a seed affects its aging rate. The more oxidizing substances such as oil are in the seeds, the more they are subject to deterioration and require special storage methods and treatment. Therefore, oilseeds are more sensitive (Wang et al., 2021). For example, peanut is an annual plant belonging to the legume family, which has an essential place among industrial products and is one of the most important oil plants in the world. Peanut seed contains 44-56% oil and 22-30% protein and is a valuable source of vitamins E, K, and B (Li et al., 2022a). Peanut seed is an oil seed that contains saturated fatty acids such as palmitic acid and stearic acid and unsaturated fatty acids such as oleic acid and linoleic acid. The rapid oxidation of unsaturated fatty acids of peanut causes rapid seed deterioration and reduction in seed vigor during the period of drying and storage, and one of the problems of its cultivation is the reduction in the percentage of germination and the emergence of seedlings of peanut in the field (Lin et al., 2022). Therefore, it is necessary to find solutions to reduce or improve the damage caused by peanut seed deterioration.

Today, there are many solutions to reduce seed deterioration. Seed pretreatment is one of the important methods of seed empowerment (Kaya et al., 2022) So that the seeds are first watered using different methods and then dried again for ease of transportation and planting. This practice increases the rate and uniformity of germination and greening, increases germination in a wider range of environmental factors, and improves the growth and establishment of seedlings (Elsanatawy et al., 2021). Melatonin can be one of these substances. Melatonin (N-acetyl 5-methoxytryptamine) is an indole compound derived from tryptophan that is synthesized in the chloroplast and mitochondria of plant cells (Aydin et al., 2021) One of the important functions of melatonin in plants is its significant effect on auxin, gibberellin, cytokinin, and abscisic acid hormones (Jiang et al., 2017). Melatonin also activates the antioxidant response of cells and antioxidant enzymes including SOD, CAT and ascorbate peroxidase (APX) and scavenges reactive oxygen species (ROS) and thereby reducing the effects of stress on plants (Li et al., 2022b). Pretreatment using 200 μ M of melatonin improves the germination percentage, seed vigor index, germination index, seedling length, seedling vigor index, and seedling weight in the deteriorated oat seeds (Yan et al., 2020).

Sugars and amino acids are the most frequent organic metabolites that play an important role in signal transmission, osmotic regulation, energy supply, and free radical inhibition in plants (Sharma et al., 2019). Gamma-aminobutyric acid (GABA) is a four-carbon non-protein amino acid that is produced in plants to activate defense responses and regulate biochemical functions, especially under stress (Malekzadeh et al., 2014). Treatment with bioactive compounds such as GABA can be considered as a practical method to increase the seedling establishment potential in old seeds. The GABA is a specific plant growth regulator that acts as an internal signal in response to environmental stresses (Hu et al., 2015; Rousta et al., 2023). Under salinity stress, increasing the accumulation of GABA in pumpkin (*Cucurbita pepo*) leaves significantly reduced lipid peroxidation and improved the activity of antioxidant enzymes (Nejad-Alimoradi et al., 2019). In a study, researchers investigated the use of GABA on the deteriorated seeds of pumpkin. The results showed that by increasing the deterioration the percentage of saturated fatty acids increases and the amount of unsaturated fatty acids reduces. The use of GABA reduces the harmful effects of the deterioration on seedling growth and fatty acid compounds (Tavakkol Afshari and Seyyedi, 2020).

Studies have shown that the occurrence of any undesired conditions such as environmental stress (Delijani et al., 2022; Matinizadeh et al., 2024; Moshki et al., 2024) in the way of seed production or the use of inappropriate methods of production and after that seed storage can cause early deterioration and reduce seed vigor, which will cause irreparable damage (Demir et al., 2023). Therefore, it is necessary to find an efficient solution to reduce the negative impacts caused by seed deterioration for any reason. In this study, the use of melatonin and GABA as compounds that have shown positive effects on inhibiting the aging process of plants have been considered. The effect of these substances was investigated in the form of seed pretreatment and foliar application in peanut seeds and plants.

2. MATERIALS AND METHODS

Experimental overview

This study was conducted in peanut field in Astaneh Ashrafieh and the genetics and breeding laboratory of Tea Research Institute on peanut during two years (2021 and 2022). The cultivar used for planting was North Carolina 2 (NC₂). A factorial experiment was performed based on a RCBD with three replications. Planting operation was done in the first year on May 14, 2021 and in the second year on May 15, 2022 at a depth of 4 cm. Each plot consisted of 4 lines with a length of 6 meters. The distance between the rows was 40 cm and the distance on the row was 40 cm

The experiment was conducted using a factorial arrangement that included: (1) two seed quality levels (normal and aged seeds); (2) three melatonin seed pretreatment concentrations (0, 50, and 100 μ M); and (3) two GABA foliar application levels (0 and 1 mM). These treatments were applied in combination to evaluate their individual and interactive effects. To apply the seed deterioration treatment, peanut seeds were placed in an incubator at 40 °C and 96–100% relative humidity for 96 hours. This method was used to simulate aging conditions, following the guidelines of ISTA (1999). Prior to the deterioration treatment, the seeds were pretreated by soaking in different concentrations of melatonin. For this purpose, seeds were placed in containers and soaked in melatonin solutions at the desired concentrations. After soaking, they were aerated for 8 hours to allow for oxygen exchange and then dried in the shade under ambient conditions.

Foliar application of treatments was performed early in the morning at the beginning of the flowering stage, which occurred 38 days after planting, under optimal environmental conditions. Care was taken to ensure that the leaves were completely wetted during application. Trait measurements were conducted two weeks after foliar application. Tables 1 and 2 present the physicochemical properties of the field soil and the meteorological data for the two study years, respectively.

Table 1. Physico-chemical properties of the experimental soil

Characteristics	Units	Values
Organic carbon	%	1.1
Phosphorus (P)	mg kg ⁻¹	10.5
Potassium (K)	mg kg ⁻¹	229
Electronic content (EC)	dSm ⁻¹	0.506
pH		7.3
Sand	%	55
Clay	%	12
Silt	%	33
Soil texture		Silty-loam

Table 2. Meteorological parameters for the field sites during the experiment (Astaneh Ashrafieh Meteorological Office)

	Mean temperature (°C)		Relative humidity (%)		Precipitation (mm)	
Month	2021	2022	2021	2022	2021	2022
March	14.95	13.7	75.82	75.20	0.67	1.1
April	18.51	17.5	83.5	82.30	2.65	1.4
May	23.82	22.4	80.62	76.10	0.52	0.1
June	26.79	25.6	78.72	74.00	1.35	0.2
July	27.35	26.7	75.33	77.8	2.82	1.3
August	24.98	24.9	79.04	76.4	3.22	2.7
Long-term average value	Mean temperature (°C)		Relative humidity (%)		Precipitation (mm)	
	20		72		1500	

Measured traits

Leaf RWC

Leaf RWC was measured two weeks after foliar application and before irrigation. The leaves were weighted by a scale with accuracy of 0.001 (fresh weight), then cut into equal pieces and stored in disposable plastic cups for 24 hours in 150 mL of double distilled water and in a refrigerator at 4°C. The lids of the glasses were covered entirely using aluminum foil (Kramer, 1983). Next, the leaves were removed from the distilled water and after the water was dried with filter paper, they were weighed again (saturated weight). Then, they were placed in an oven at 70 °C for 72 hours and weighed (dry weight). Leaf RWC was calculated using Equation (1).

$$RWC = \left(\frac{Fw - Dw}{Sw - Dw} \right) \times 100 \quad (1)$$

Where Fw: leaf fresh weight, Dw: leaf dry weight, Sw: leaf turgid weight.

Lipid peroxidation

Lipid peroxidation was assessed by measuring the concentration of MDA, following the method of Du and Bramley (1992). 0.25 g of plant sample was extracted with 5 ml of 0.1% trichloroacetic acid (w/v). The homogenate was centrifuged at 20 °C and 10,000 g for 20 minutes. 2 mL of 0.25% thiobarbituric acid was added to 250 µl of the extract. The resulting mixture was placed in a 100 °C water bain-marie for 30 minutes, and then the tubes were removed and cooled in ice for 15 minutes. It was immediately centrifuged at 20 °C at 10,000 g for 10 minutes. The absorbance was read at wavelengths of 440, 532 and 600 nm. To calculate the concentration of MDA, the extinction coefficient equal to 157 M cm⁻¹ was used.

Leaf anthocyanin

The leaf anthocyanin was measured using the method of Mita et al. (1997). 0.2 g of fresh plant tissue was homogenized in liquid nitrogen, and then 4 mL of 1% hydrochloric acid solution in methanol was added and placed in the refrigerator for 24 hours. The resulting mixture was centrifuged at 13,000 g for 10 minutes. The absorbance in the supernatant was read using spectrophotometry at wavelengths of 657, and 530 nm.

Antioxidant enzymes

SOD activity

The reaction mixture for measuring SOD activity included 1.5 mL of potassium phosphate buffer (pH = 7.8), 0.1 mL of 2.25 mM nitro blue tetrazolium chloride (NBT), 0.1 mL of 3 mM ethylenediaminetetraacetic acid (EDTA), 0.2 mL of 200 mM methionine, 0.1 mL of 1.5 mM sodium carbonate, 1 mL of distilled water, and 0.05 mL of enzyme extract. A blank was used to zero the device and a control was used for the standard curve. The reaction in the extracts prepared at 25 °C was started by turning on the fluorescent lamp (15 W) and adding 0.1 mL of 60 µM riboflavin, and after 15 minutes, the reaction was stopped by turning off the lamp. The blank tube contained the above substances, with the difference that the enzyme extract was not added and it was not placed in the light. In the control tube, there was also the above mixture and the enzyme extract was not added, but it was placed in the light. The absorbance was recorded at the wavelength of 560 nm. The difference in absorbance of the samples and the control at the wavelength of 560 nm indicates the inhibition of NBT photo-reduction in the presence of SOD. By calculating the absorbance difference, the enzyme unit of the samples was calculated and the enzyme activity was expressed in enzyme unit/total protein (mg) in 50 microliters of the extract (Sairam et al., 2002).

CAT activity

CAT activity was measured at 25°C by the method of Cakmak and Horst (1991). A spectrophotometer at the wavelength of 240 nm was used to measure CAT enzyme activity. The substances used included 3000 microliters of 50 mM potassium phosphate buffer (pH=7), 5 microliters of 3.41 M hydrogen peroxide, and 100 microliters of enzyme extract. CAT converts H₂O₂ into O₂ and H₂O without the need for a reducing agent. The maximum absorbance of hydrogen peroxide occurs at the wavelength of 240 nm. Therefore, with the start of the reaction by CAT, the amount of hydrogen peroxide in the reaction mixture gradually reduces, and as a result, the amount of absorption at the wavelength of 240 nm also reduces. The specific activity of CAT was calculated based on micromoles of hydrogen peroxide per minute per milligram of soluble protein.

APX activity

The reaction medium for measuring APX activity contained 600 microliters of 0.1 mM EDTA, 1500 microliters of 50 mM phosphate buffer (pH=7), 400 microliters of 0.5 mM ascorbic acid, 400 microliters of 30% H₂O₂, and 100 microliters of enzyme extract. Enzyme activity measurement was recorded for 1 minute at the wavelength of 290 nm with an extinction coefficient of 2.8 mM cm⁻¹ (Nakano and Asada, 1981).

Grain yield

The number of pegs per plant, the number of ripe pods per plant, the shell yield and 100 seed weight were also measured by taking 10 plants in 1 m² and finally reported in g m⁻².

Statistical analysis

For the data of two years, Bartlett's test was performed first. Bartlett's test was used to ensure the homogeneity of variances, which is a key assumption for conducting ANOVA. When an experiment is conducted across two locations

and/or years, it is essential to test the homogeneity of variances prior to performing combined analysis of variance (ANOVA). For this purpose, Bartlett's test was used to assess the equality of error variances across environments (Steel et al., 1997). This test is based on the error mean squares obtained from each environment separately. If Bartlett's test is non-significant ($P > 0.05$), the variances are considered homogeneous, and a combined ANOVA can be validly performed. However, if the test is significant ($P \leq 0.05$), indicating heterogeneity of variances, a combined analysis is not statistically valid, and the data are analyzed separately for each environment. For the traits studied in this article, this test was not significant and the homogeneity of the experimental error was confirmed, as a result, composite analysis was performed. Data was analyzed using SAS 9.1 and mean comparison of data was done by LSD method at the probability level of 5%.

3. RESULTS AND DISCUSSION

The effect of year and foliar application at the probability level of 5% and the effect of deterioration, the interaction of pretreatment $\times$ foliar application and year $\times$ deterioration at the probability level of 1% had an effect on leaf RWC. The effect of year, deterioration, pretreatment, and foliar application, and the interaction of deterioration $\times$ pretreatment, deterioration $\times$ foliar application, and year $\times$ deterioration was significant at the probability level of 1% on MDA. At the same time, year $\times$ pretreatment was significant at the probability level of 5% on MDA. Seed deterioration and melatonin pretreatment at the probability level of 1% and GABA foliar application at the probability level of 5% were effective on the amount of leaf anthocyanin. Seed deterioration, melatonin pretreatment and the interaction of these two factors affected CAT activity at the probability level of 1%. The number of pegs per plant was affected by the year, seed deterioration, melatonin pretreatment, GABA foliar application and interactions of deterioration $\times$ pretreatment, year $\times$ pretreatment, year $\times$ deterioration $\times$ pretreatment and year $\times$ deterioration. The year at the probability level of 5% and the treatments of seed deterioration, pretreatment and the interaction of year $\times$ deterioration at the probability level of 1% significantly affected the number of pods in the peanut plant. According to the analysis of variance, all main effects and interaction of deterioration $\times$ melatonin, deterioration $\times$ GABA, year $\times$ deterioration, year $\times$ melatonin, and deterioration $\times$ melatonin $\times$ GABA on 100 seed weight were significant. The results showed that the seed deterioration ($P < 0.01$), melatonin and GABA ($P < 0.05$), and the interaction of melatonin $\times$ GABA ($P < 0.01$) had an effect on shell yield. Seed deterioration, seed pretreatment with melatonin and GABA foliar application influenced grain yield at the probability level of 1%. The interaction of seed deterioration $\times$ melatonin also influenced grain yield at the probability level of 5% on grain yield (Table 3).

Leaf RWC

All treatment compounds resulting from the interaction of seed pretreatment by melatonin $\times$ foliar application by GABA caused an increase in leaf RWC compared to the control (Figure 1). In the first and second years, seed deterioration reduced leaf RWC by 6.86 and 11.60% (Figure 2). Seed aging caused a reduction in leaf RWC in the plants grown from these seeds. Melatonin pretreatment and GABA foliar application caused a significant increase in leaf RWC. Melatonin and GABA can improve leaf RWC by reducing lipid peroxidation, various ROS and maintaining membrane stability (Farouk and Al-Amri, 2019).

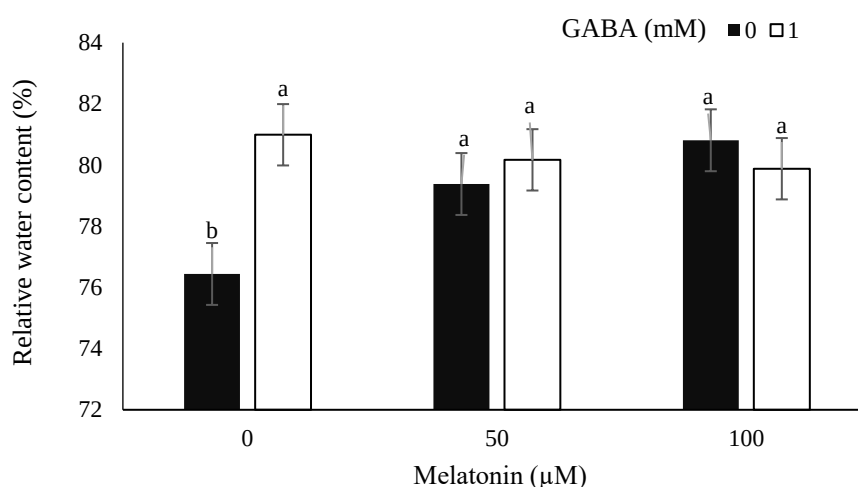


Figure 1. Effect of pretreatment with melatonin and foliar application with gamma-aminobutyric acid (GABA) on relative water content in peanut. Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p < 0.05$.

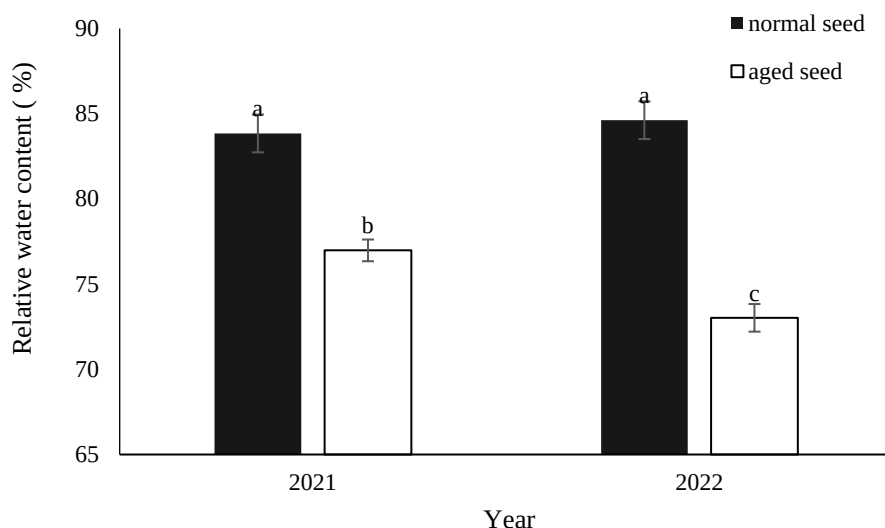


Figure 2. Effect of year and seed deterioration on relative water content in peanut

Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p < 0.05$

MDA

The amount of MDA in the leaves of plants grown from aged seeds was 92.57% higher than that of normal seeds. The use of 50 and 100 μM of melatonin as seed pretreatment under deterioration caused a significant reduction by 10.66 and 23.78% respectively, in the amount of MDA in the leaves of plants obtained from these seeds, but melatonin had no effect in normal seeds (Figure 3).

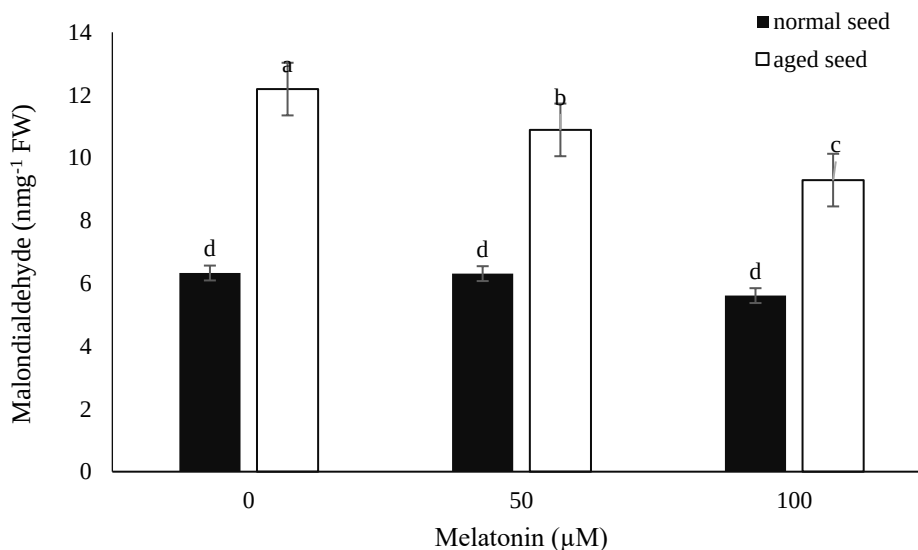


Figure 3. Effect of seed deterioration and pretreatment with melatonin on malondialdehyde in peanut

Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p < 0.05$

Mean comparison of MDA under the influence of deterioration and GABA foliar application showed that foliar application of 1 mM of GABA reduced the amount of MDA by 12.01% in the leaves of plants grown from aged seeds compared to the conditions of not using this substance (Figure 4). Seed aging increased the amount of MDA in the leaves of tested plants, indicating that deterioration caused membrane damage and increased lipid peroxidation. The increase in the amount of MDA under deterioration can be considered as the result of the increase in ROS production, which may be caused by the weakness of the antioxidant system in plants (Liu et al., 2023).

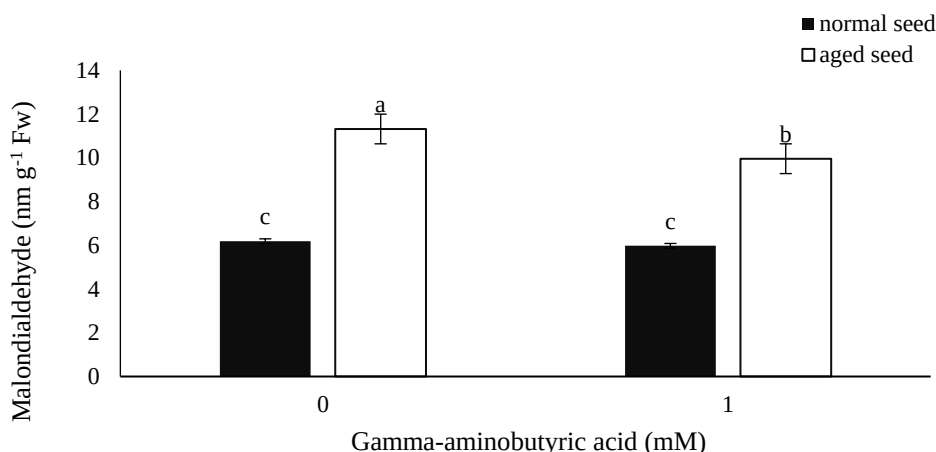


Figure 4. Effect of seed deterioration and foliar application with gamma-aminobutyric acid (GABA) on malondialdehyde in peanut. Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p < 0.05$.

Melatonin and GABA have probably prevented the peroxidation of cell membrane lipids and the increase of MDA by cleaning ROS. The reduction in cell membrane damage in response to receiving these two substances can indicate the induction of the antioxidant defense system by these substances. By removing free radicals, these two substances reduce the damage caused, and as a result, membrane lipid peroxidation reduces. The physiological role of GABA in stress tolerance is related to osmotic regulation, membrane stability, and removal of ROS (Shelp et al., 2012).

Anthocyanin

The results showed that deterioration increased the amount of anthocyanin in the leaves by 84.61%. Seed pretreatment by 50 and 100 μ M of melatonin increased anthocyanin by 6.89 and 14.94%, respectively, which was statistically significant. The GABA spraying also increased the amount of anthocyanin in the leaves by 5.26% (Table 4).

The effect of seed deterioration, melatonin pre-treatment, GABA foliar application, interaction of deterioration $\times$ melatonin and deterioration $\times$ GABA was significant at the probability level of 1% on SOD activity (Table 3). Seed aging increased SOD activity by 11.04, 27.15, and 32.91 percentage in the 0, 50, and 100 μ M levels of melatonin, respectively. Soaking normal seeds in 100 μ M of melatonin increased anthocyanin by 9.66%, and in aged seeds, the use of 50 and 100 μ M of melatonin increased the activity of this enzyme by 20.60 and 31.26% compared to the non-use of this substance in plants grown from these seeds (Figure 5). Anthocyanins are a group of water-soluble flavonoids, and their increase indicates an increase in the main pathway of flavonoid production. In the present study, seed deterioration increased the amount of anthocyanin and the use of melatonin and GABA increased anthocyanin. The increase in the amount of anthocyanin under seed deterioration in this study can also be due to the activation of the internal mechanisms of the plant to reduce the destructive effects of deterioration stress. Researchers found that GABA stimulates the activity of phenylalanine ammonia lyase (PAL) and starts the phenylpropanoid pathway, as a result of which the synthesis of phenolic compounds and the antioxidant activity increases (Soleimani Aghdam et al., 2015). The reason for the increase in anthocyanin by using melatonin is related to the increased expression of genes involved in the synthesis of flavonoids by using this substance (Liang et al., 2018).

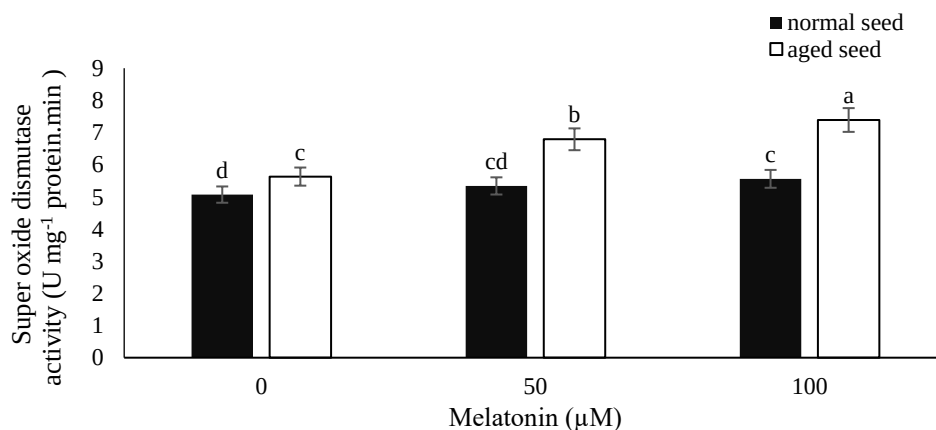


Figure 5. Effect of seed deterioration and pretreatment with melatonin on super oxide dismutase in peanut. Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p < 0.05$.

Table 3. Variance analysis of measured traits in peanut affected by seed deterioration, seed pretreatment with melatonin and gamma-aminobutyric acid (GABA) foliar application

S.O.V	d.f.	RWC	MDA	Anthocyanin	SOD	CAT	APX	Number of pegs per plant	Number of pods per plant	100 seed weight	Shell yield	Grain yield
Year	1	45.51*	12.56**	0.0001	0.205	0.001	0.060	125.34**	113.37*	110.95**	2.02	5635.68
Error	4	67.17	6.52	0.0001	0.428	0.017	0.003	5.11	27.76	11.75	1849.95	7.91
Seed deterioration (A)	1	1533.79**	399.23**	0.055**	29.49**	2.81**	0.060	292.82**	2116.29**	10594.34**	12695.75**	1644.68**
Melatonin (B)	2	16.47	20.17**	0.001**	7.77**	0.16**	0.056	221.99**	583.64**	412.03**	1549.69*	652538.89**
GABA (C)	1	36.68*	15.67**	0.0005*	1.35**	0.01*	0.047	20.69*	66.99	141.68**	2402.59*	460000.35**
A*B	2	2.02	7.19**	0.00007	2.57**	0.06**	0.013	42.60**	30.25	100.33**	259.90	116180.17*
A*C	1	1.27	9.40**	0.00002	1.38**	0.00008	0.018	5.12	34.37	47.04*	5.60	8646.12
B*C	2	47.08**	0.74	0.00010	0.07	0.001	0.020	0.54	17.76	11.97	2081.71**	19500.72
A*B*C	2	8.38	0.37	0.00011	0.05	0.003	0.005	1.20	4.07	0.38	530.50	30472.17
Y*A	1	101.44**	23.15**	0.00015	0.10	0.003	0.0008	13.17*	214.76**	200.60**	0.0001	4884.01
Y*B	2	8.10	4.89*	0.00006	0.09	0.002	0.009	16.34**	13.10	62.29**	273.06	2206.89
Y*C	1	13.91	1.06	0.000008	0.21	0.008	0.022	0.09	15.17	0.02	139.11	52542.01
Y*A*B	2	5.29	1.00	0.00005	0.012	0.009	0.001	20.50**	5.60	84.42**	105.99	49268.72
Y*A*C	1	22.39	0.15	0.00003	0.08	0.003	0.001	0.72	0.19	0.22	17.91	95120.68
Y*B*C	2	21.90	1.90	0.000015	0.005	0.0004	0.003	0.97	13.09	1.65	254.12	10518.72
Y*A*B*C	2	12.76	2.68	0.000006	0.04	0.0005	0.013	0.50	7.43	2.04	304.36	9108.72
Error	44	7.36	1.09	0.00008	0.13	0.003	0.02	3.09	19.19	8.39	352.39	26302.29
C.V (%)	-	3.40	12.39	10.15	6.22	9.77	10.40	3.72	16.44	4.21	5.51	6.24

Table 4. Mean comparison of measured traits in peanut affected by year, seed deterioration, seed pretreatment with melatonin and gamma-aminobutyric acid (GABA) foliar application

Treatment	RWC	MDA	Anthocyanin	SOD	CAT	APX	Number pegs plant	of per pod	Number of plant	100 seed weight	Shell yield	Grain yield
	(%)	(nm g ⁻¹ leaf Fw)	(µm g ⁻¹ leaf Fw)	(U mg ⁻¹ protein.min)						(g)	(kg ha ⁻¹)	(kg ha ⁻¹)
Year												
2021	80.41 a	8.02 b	0.094 a	6.02 a	0.55 a	1.41 a	46.00 b		25.38 b	67.46 b	340.78 a	2605.44 a
2022	78.82 b	8.85 a	0.091 a	5.91 a	0.56 a	1.37 a	48.63 a		27.89 a	69.94 a	340.44 a	2587.25 a
LSD 5%	1.289	0.497	N.S.	N.S.	N.S.	N.S.	0.836		2.08	1.376	N.S.	N.S.
Deterioration												
normal seeds)	84.23 a	6.087 b	0.065 b	5.32 b	0.36 b	1.40 a	49.33 a		32.06 a	80.83 a	353.89 a	3371.72 a
aged seeds	75.00 b	10.797 a	0.120 a	6.60 a	0.76 a	1.37 a	45.30 b		21.21 b	56.57 b	327.33 b	1821.47 b
LSD 5%	1.289	0.497	0.004	0.176	0.026	N.S.	0.836		2.081	1.376	8.917	77.04
Melatonin (µM)												
0	78.71 b	9.26 a	0.087 c	5.35 c	0.47 c	1.38 a	44.13 c		21.23 c	64.81 c	331.71 b	2420.21 c
50	79.78ab	8.60 b	0.093 b	6.06 b	0.56 b	1.36 a	47.63 b		27.78 b	68.22 b	342.79 a	2622.71 b
100	80.35 a	7.45 c	0.100 a	6.47 a	0.64 a	1.37 a	50.19 a		30.90 a	73.06 a	347.33 a	2746.88 a
LSD 5%	1.579	0.609	0.005	0.216	0.032	0.085	1.024		2.549	1.686	10.921	94.35
GABA (mM)												
0	78.88 b	8.90 a	0.090 b	5.83 b	0.55 b	1.38 a	46.78 b		25.67 a	67.29 b	334.83 b	2516.67 b
1	80.35 a	7.97 b	0.095 a	6.10 a	0.57 a	1.43 a	47.85 a		27.60 a	70.10 a	346.38 a	2676.53 a
LSD 5%	1.289	0.497	0.004	0.176	0.026	N.S.	0.836		N.S.	1.376	8.917	77.04

Superoxide Dismutase (SOD) Activity

Mean comparison of SOD activity affected by seed aging and GABA foliar application showed that only under seed deterioration conditions, the use of GABA caused a significant increase in SOD activity. In contrast, there was no significant difference under normal conditions compared to the control (Figure 6). GABA spraying also had a considerable effect on SOD activity at the 5% probability level (Table 3). Overall, the activity of SOD was higher in the leaves of plants grown from aged seeds. Under seed aging, an imbalance occurs between reactive oxygen species (ROS) production and antioxidant scavenging capacity, leading to oxidative stress. In such conditions, enhancing SOD activity is crucial to dismutase superoxide radicals into hydrogen peroxide and mitigate oxidative damage (Govindaraj et al., 2017).

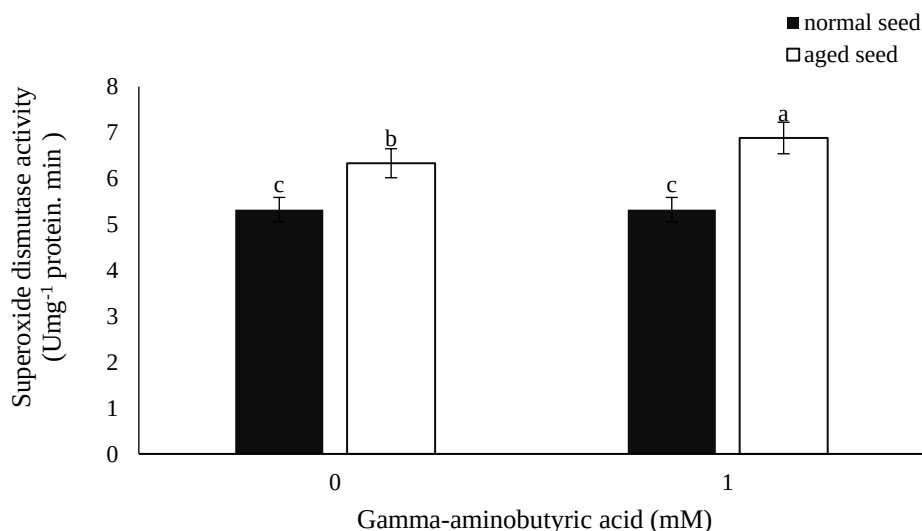


Figure 6. Effect of seed deterioration and foliar application with gamma-aminobutyric acid (GABA) on super oxide dismutase in peanut
Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p < 0.05$

Catalase (CAT) Activity

Seed aging caused a 79.41% increase in CAT activity at zero melatonin level, and this effect was also evident at two other melatonin concentrations (Figure 7). Pretreatment of normal seeds with a low concentration of melatonin had no significant effect on CAT activity; however, the application of 100 μ M melatonin significantly increased CAT activity by 44.11%. The highest catalase activities were observed in leaves of plants grown from aged seeds pretreated with melatonin, where concentrations of 50 and 100 μ M increased CAT activity by 27.86% and 32.78%, respectively (Figure 7). Foliar application of GABA led to a 3.63% increase in CAT activity compared to the control (Table 3). Catalase is essential for decomposing hydrogen peroxide into water and oxygen, thereby preventing oxidative damage caused by ROS accumulation (Govindaraj et al., 2017). One possible reason for the increase in CAT under seed aging may be the elevated levels of hydrogen peroxide under such stress.

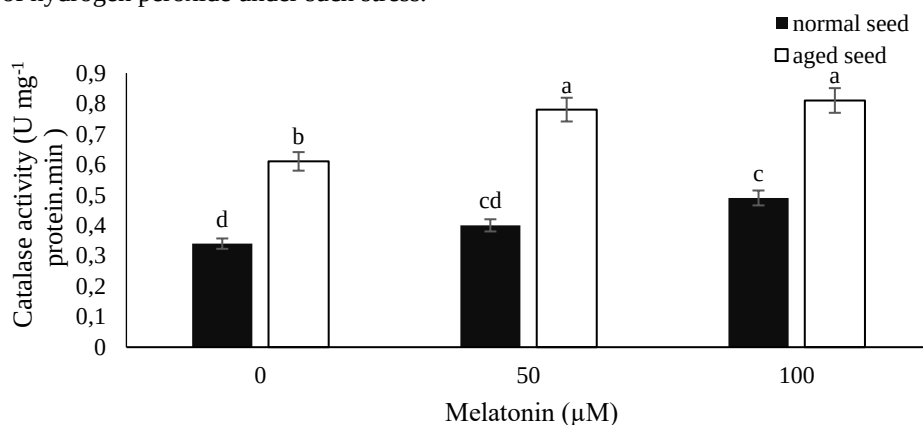


Figure 7. Effect of seed deterioration and pretreatment with melatonin on catalase activity in peanut.
Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p < 0.05$

Ascorbate Peroxidase (APX) Activity

In contrast to SOD and CAT, APX activity was not significantly affected by any of the experimental treatments (Tables 2 and 3), indicating that this enzyme may play a less prominent role under the specific stress and treatment conditions tested.

It is known that melatonin can enhance antioxidant capacity via several mechanisms, including direct scavenging of free radicals, upregulation of antioxidant enzymes such as SOD and CAT, protection of enzymes from oxidative damage, and boosting mitochondrial efficiency to reduce ROS production (Gong et al., 2017). Additionally, GABA has been shown to increase antioxidant enzyme activity under stress conditions. This effect is likely due to GABA's role in enhancing polyamine biosynthesis and preventing their degradation, which in turn supports CAT activity by reducing lipid peroxidation and enhancing ROS scavenging (Wang et al., 2014). GABA-treated corn plants have exhibited elevated SOD activity (Shi et al., 2010), and this activation of the antioxidant defense system is considered vital for improving plant tolerance to oxidative stress (Ramalho et al., 2018; Shi et al., 2010).

Number of pegs per plant

During both years, the number of pegs in plants grown from aged seeds was significantly lower than plants grown from normal seeds. The highest number of pegs was recorded in plants whose seeds were pretreated by 100 μ M of melatonin. Interestingly, in the second year, the number of pegs in the plants obtained from normal and aged seeds that were pretreated by 100 μ M of melatonin was at the first statistical level. Investigation of the interaction of year $\times$ deterioration $\times$ pretreatment showed that in aged seeds, both levels of melatonin increased the number of pegs in the plant to a significant level in both years, while in normal seeds, only the application of 100 μ M of melatonin increased Number of pegs per plant (Figure 8). Melatonin likely enhanced peg formation by mitigating oxidative damage during early seedling development and supporting reproductive tissue differentiation. Its known role in modulating auxin signaling and improving root/shoot architecture may also indirectly support peg initiation in peanuts (Arnao & Hernández-Ruiz, 2015; Zhang et al., 2022).

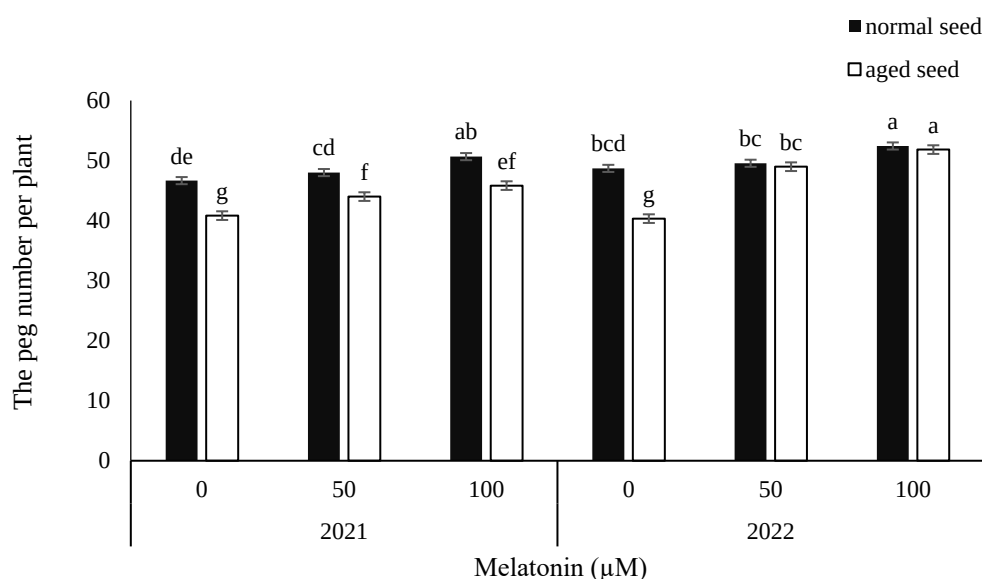


Figure 8. Effect of seed deterioration and pretreatment with melatonin on the peg number per plant in peanut. Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p < 0.05$.

Number of pods per plant

The results of mean comparison of the number of pods per plant under the influence of the interaction of year $\times$ deterioration showed that seed aging in the first and second year caused a significant reduction by 43.95 and 23.36% in Number of pods per plant, respectively (Figure 9). The decline in pod number may result from impaired floral and peg development due to reduced seed vigor. Stress-induced disruption in hormonal balance and increased lipid peroxidation in aged seeds likely impair resource allocation to reproductive growth (Bewley et al., 2013). This highlights the critical role of seed quality in determining yield components.

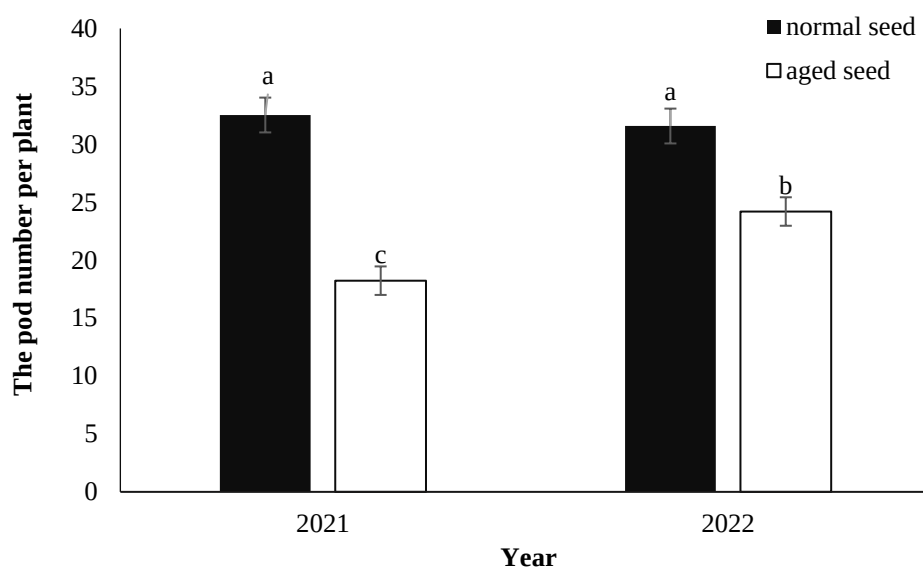


Figure 9. Effect of seed deterioration and year on the pod number per plant in peanut
Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p<0.05$.

100 seed weight (HSW)

The highest 100 seed weight in the plants was obtained from normal seeds with pretreatment by 100 μM of melatonin in the second year (94.22 g) and compared to the control (normal seeds without pretreatment in the second year) showed an increase by 24.53%. In both years, the application of deterioration caused a significant reduction in 100 seed weight. Soaking normal seeds in 50 and 100 μM of melatonin in both years increased 100 seed weight, but in aged seeds, only the use of 100 μM of melatonin in the first year could improve 100 seed weight and in other cases showed no difference with the control (Figure 10). Melatonin's antioxidant properties may have protected cellular structures and enhanced seed filling by preserving photosynthetic capacity and improving assimilate transport, especially in high-concentration treatments (Li et al., 2016). In aged seeds, only higher melatonin doses may overcome the threshold of damage.

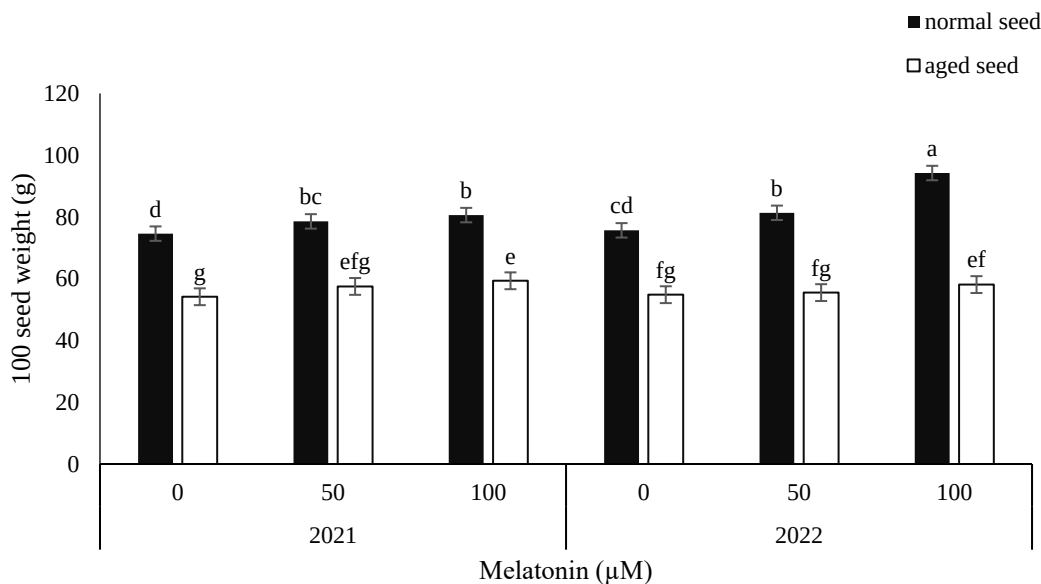


Figure 10. Effect of seed deterioration, pretreatment with melatonin and foliar application with gamma-aminobutyric acid (GABA) on 100 seed weight in peanut
Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p<0.05$

Shell yield

Seed aging caused a 50.7% reduction in shell yield (Table 4). The interaction of melatonin×GABA showed that all the treatment combinations increased shell yield compared to the control. Shell yield

increase varied between 8.25 and 10.44 percent but was statistically similar (Figure 11). Shell yield reduction likely mirrors overall plant biomass reduction under stress. The observed improvement due to combined treatment suggests potential synergism between melatonin and GABA in enhancing stress tolerance and biomass allocation (Bouché & Fromm, 2004; Hasan et al., 2021).

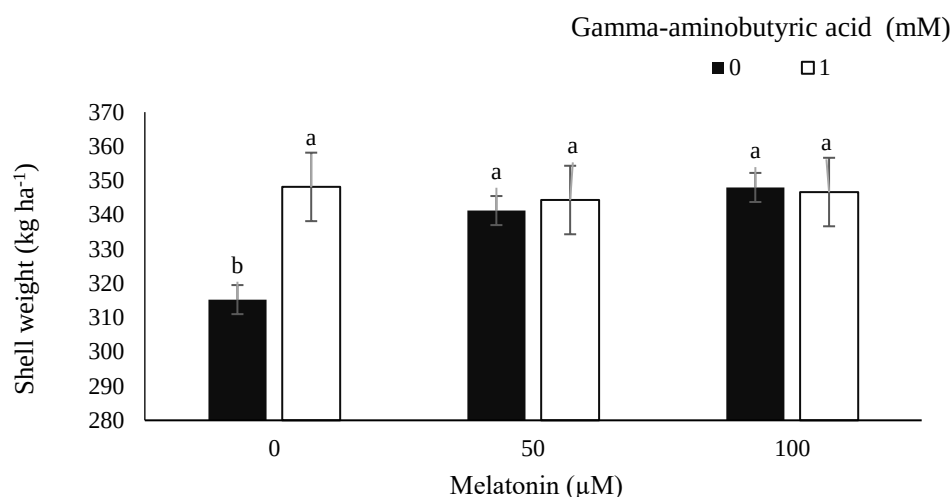


Figure 11. Effect of seed pretreatment with melatonin and foliar application of gamma-aminobutyric acid (GABA) on shell weight in peanut

Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p < 0.05$

Grain yield

Mean comparison of grain yield showed that seed aging caused a 45.81% reduction in grain yield in control without melatonin. Pretreatment of normal seeds with 50 and 100 μM of melatonin increased 7.53 and 14.66% grain yield. Whereas, pretreatment of aged seeds by 50 and 100 μM of melatonin could improve grain yield by 9.91% and 11.33% under such conditions, respectively (Figure 12). GABA foliar application increased grain yield by 6.53% compared to the control (Table 4). Melatonin and GABA may contribute to yield improvement by reducing oxidative stress, preserving chlorophyll content, and enhancing enzymatic antioxidant defense. These effects collectively sustain photosynthesis and seed filling, especially under the oxidative stress associated with seed aging (Zhang et al., 2019; Hasan et al., 2021).

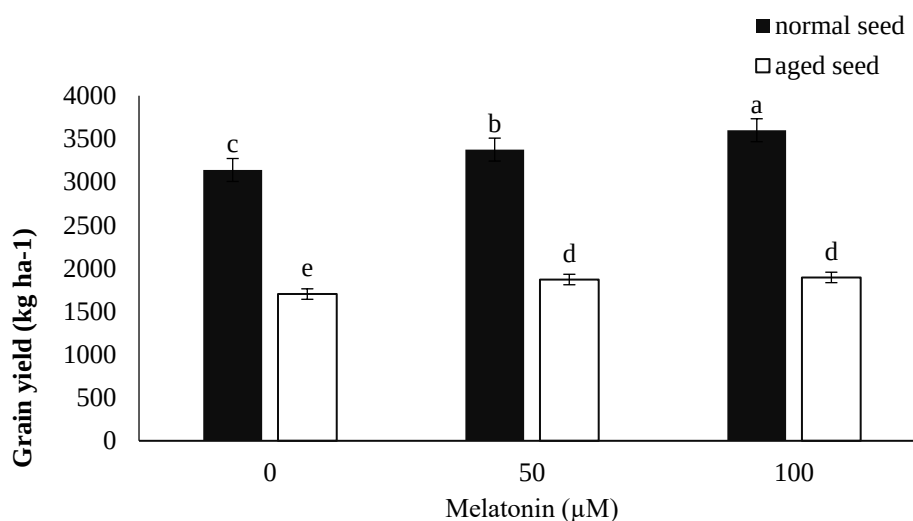


Figure 12. Effect of seed deterioration and pretreatment with melatonin on grain yield in peanut

Error bars represent the standard error (n=3). The mean values in a group with the same letter are not significantly different at $p < 0.05$

Path analysis

Path analysis was used to determine the contribution of direct and indirect effects of variables. As shown in Table 5, when the trait of seed yield was considered as a dependent variable in the plants obtained from normal seeds, the number of pods per plant (0.71), 100 seed weight (0.67) and SOD (-0.52) were entered the model as the main variables. According to the coefficient of determination (R^2), these three traits explained 61.57% of grain yield changes. The results of causal analysis of seed yield under normal conditions (Table 5) showed that the number of pods per plant and 100 seed weight had the most direct and positive effect. SOD indirectly caused a reduction in the yield through the reduction in the number of pods per plant and 100 seed weight.

Table 5. Path analysis of yield- related traits in plants derived from normal seeds

Plant traits	Number of pods per plant	100 seed weight	Super oxide dismutase	Total effect
Number of pods per plant	0.4829	0.2138	0.0151	0.7120
100 seed weight	0.2677	0.3858	0.0150	0.6686
Super oxide dismutase	-0.2756	-0.2184	-0.0265	-0.5206
R-Square	0.6157			

Causal analysis of plants obtained from aged seeds showed that traits of 100 seed weight (0.45), SOD (0.34) and CAT (-0.32) were entered the model as the main variables. R^2 showed that 23.06% of the variable changes in grain yield in plants obtained from aged seeds were explained by these three traits. 100 seed weight had the most direct and positive effect on grain yield. SOD had the most negative impact on Path. CAT also caused a reduction in grain yield through an indirect effect on 100 seed weight (Table 6).

Table 6. Path analysis of yield- related traits in plants derived from aged seeds

Plant traits	100 seed weight	Super oxide dismutase	Catalase	Total effect
100 seed weight	0.4316	0.1403	-0.1122	0.4597
Super oxide dismutase	-0.2413	-0.2510	0.1425	-0.3498
Catalase	-0.2867	-0.2117	0.1689	-0.3295
R-Square	0.2306			

The reduction in HSW in this study due to the deterioration of the seeds may be due to the reduction in the growth of the photosynthetic organs due to the low quality of the seeds. Therefore, the decrease in the leaf area under the deterioration through the reduction in the net photosynthesis rate has finally reduced HSW significantly. Researchers stated that HSW depends on the amount of photosynthetic matter available and the capacity of the seeds for storage, and the reduction in each reduces HSW (Li et al., 2022). Researchers reported that HSW and the number of pods in *Vigna radiata* L. grown from seeds pretreated with 50 μ M of melatonin were higher than the control (MariaJanas and Maria Posmyk, 2013). Other studies have also shown the positive effect of melatonin pretreatment on increasing the number of pods per plant in soybean plants (Wei et al., 2015).

Peanut grain yield in this study showed a reduction under deterioration, which can be due to the decrease in the number of pods per plant, the number of pegs per plant, leaf RWC and HSW, and the increase in the activity of antioxidant enzymes, anthocyanin and MDA. Melatonin and GABA also increased grain yield in this plant by increasing leaf RWC, the number of pods per plant and HSW, and reducing MDA. The researchers announced that seed priming by melatonin improves seed quality, seedling development, plant growth, reduces the accumulation of ROS, protecting photosynthetic pigments, improves the performance of photosynthetic components, higher leaf RWC, and finally increases the yield of the product (Szafranska et al., 2016). Researchers found that indole compounds such as melatonin affect the growth and performance of plants by affecting different physiological mechanisms such as photosynthesis, respiration, ion absorption, membrane permeability, enzyme activity and hormones (Zhang et al., 2015). GABA increases growth and yield in various crops. Through increasing the level of endogenous plant hormones, this substance plays a positive role in improving factors related to vegetative and reproductive growth, as well as physiological traits and plant performance (Ramos-Ruiz et al., 2018).

4. CONCLUSIONS

According to the study results, accelerated aging of seeds caused changes in physiological processes of peanut, such as increasing the activity of antioxidant enzymes, anthocyanins, and reducing 100 seed weight, the number of pods per

plant, and seed yield. The use of melatonin as seed pre-treatment and GABA as foliar application had a positive effect on normal and aged seeds and reduced the effects of seed deterioration. Finally, within the scope of the research, pretreatment of aged seeds with 100 μmol of melatonin and foliar application with 1 mM of GABA is suggested to improve the yield of peanut seeds under normal and seed aging conditions.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest or personal relationships.

STATEMENTS AND DECLARATIONS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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The Study on *Calendula officinalis* L. In Mediterranean Climatic Conditions: Impact of Different Nitrogen and Phosphorus Fertilization Levels on Morphological, Yield and Physiological Characteristics

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ARTICLE INFO

Research Article

Received: 30 October 2024

Accepted: 1 June 2025

Published: 23 June 2025

Keywords:

Calendula officinalis

Fertilization

Nitrogen use efficiency (NUE)

Phosphorus use efficiency (PUE)

Pot marigold

Yield

ABSTRACT

Pot marigold, *Calendula officinalis* L., a plant widely utilized in cosmetics, medicine, and pharmacy due to its rich bioactive compounds, was the subject of this study. The research aimed to investigate the effects of varying nitrogen (0, 50, 100, and 150 kg ha⁻¹ N) and phosphorus (0, 50, and 100 kg ha⁻¹ P₂O₅) fertilizer doses on the morphological, physiological, and yield-related characteristics of *C. officinalis* cultivated under Mediterranean climatic conditions. Field experiments were conducted during the 2019-2020 and 2020-2021 growing seasons in İzmir Province, western Türkiye, using a randomized complete block design with factorial arrangements and three replications. Morphological traits, seed and flower yields, and nutrient use efficiencies were assessed.

The results revealed that nitrogen and phosphorus doses, year, and their interactions significantly influenced plant growth, yield performance, and nutrient use efficiency. The highest agronomic nitrogen use efficiency based on seed yield (35.7 kg/kg N ha⁻¹) and flower yield (49.3 kg/kg N ha⁻¹) was recorded at 0-50 kg ha⁻¹ N in 2020. Similarly, phosphorus use efficiency peaked at 34.5 kg/kg P ha⁻¹ under the same conditions but declined sharply in the following year due to reduced and irregular rainfall.

These findings underscore the critical influence of soil moisture on nutrient uptake and utilization. Moderate fertilizer inputs (50 kg ha⁻¹ N and 50 kg ha⁻¹ P₂O₅) provided a favourable balance between yield and nutrient efficiency. Fertilization strategies for *Calendula officinalis* should be based on integrated evaluations of both yield performance and nutrient use efficiency to ensure sustainable cultivation under Mediterranean agroecological conditions.

Citation: Boztas, G., & Bayram, E. (2025). The study on *Calendula officinalis* L. in Mediterranean climatic conditions: Impact of different nitrogen and phosphorus fertilization levels on morphological, yield and physiological characteristics. *Turkish Journal of Field Crops*, 30(1), 192-205.

<https://doi.org/10.17557/tjfc.1575836>

1. INTRODUCTION

Pot marigold (*Calendula officinalis* L.) is an annual, or occasionally short-lived perennial, herbaceous plant belonging to the Asteraceae family. Characterized by its aromatic and highly branched stem reaching heights of 15-50 cm, *C. officinalis* has been traditionally used for treating a wide range of diseases, with particular emphasis on its antitumor and cytotoxic activities (Webb et al., 1988; Preethi et al., 2010). The pharmacological significance of this species is recognized by international authorities such as the German Commission E, British Herbal Pharmacopoeia, EMA, ESCOP, and WHO, which document both in vivo and in vitro studies and recent clinical research (Egeli, 2022).

Environmental conditions and agronomic practices substantially influence the production and concentration of secondary metabolites in medicinal plants (Hussain et al., 2011; Berimavandi et al., 2011). Under nutrient stress, plant growth tends to be more constrained by mineral nutrient availability than by photosynthetic capacity (Chapin, 1980). Nitrogen (N) and phosphorus (P) are essential macronutrients that play critical roles in promoting vegetative growth, flowering, and secondary metabolite synthesis (Pandey and Mishra, 2005; Ahirwar et al., 2012). Adequate nitrogen improves vigor and flower quality, whereas phosphorus plays a vital role in various plant metabolic processes, including energy transfer (ATP), nucleic acid synthesis (DNA and RNA), membrane structure (phospholipids), and overall growth regulation (Verma et al., 2017; Defforey and Paytan, 2018; Odoom & Ofosu, 2024). Deficiencies in these nutrients often result in poor plant growth and reduced flowering (Kumar et al., 2015). While appropriate nitrogen fertilization enhances crop yield by increasing leaf area index, photosynthetic duration, and flower formation potential (Hao et al., 2024), excessive nitrogen inputs can alter the biochemical composition of medicinal plants, compromising their therapeutic properties (Rahemi, 2004; Ganjali et al., 2010; Zhu et al., 2023). Thus, determining the optimal nutrient management strategy is critical for maintaining productivity and quality in pot marigold.

Efficient nutrient use is essential for improving crop yields, ensuring fertilizer cost-effectiveness, and reducing environmental impact (Rawal et al., 2022). Nitrogen use efficiency (NUE) refers to the crop yield produced per unit of nitrogen available in the soil (Moll et al., 1982), while phosphorus use efficiency (PUE) represents biomass or yield obtained per unit of phosphorus absorbed. Variations in nitrogen application rates are known to significantly affect both nitrogen uptake and crop yield (Baligar and Barber, 1979). Understanding the agronomic and physiological responses of high-yielding crops to fertilization regimes is critical for advancing breeding efforts and optimizing crop management practices (Yuan et al., 2011; Xue et al., 2013). Improving NUE is particularly important at the production level for balancing economic profitability and environmental sustainability (Cassman et al., 1996; Novoa and Loomis, 1981). Economic optimum nitrogen rates can be determined by considering fertilizer costs, yield responses, and market prices (Hong et al., 2007; Kyveryga et al., 2011; Morris et al., 2018). Recent studies indicate that *C. officinalis* exhibits notable plasticity in nitrogen uptake and biomass accumulation depending on nitrogen levels and harvest timing (Johnson et al., 2018; Marek et al., 2018). While seed yield tends to increase with nitrogen application, NUE declines beyond a certain threshold, suggesting diminishing returns (Serra et al., 2013). Additionally, the combined effects of nitrogen and phosphorus fertilization significantly influence floral characteristics and overall yield (Samoon et al., 2018).

It is imperative to comprehend these aspects to optimize nitrogen and phosphorus management tactics for enhancing the growth and productivity of pot marigold while mitigating environmental risks. This investigation delves into the influence of changing environmental conditions on agronomic nitrogen and phosphorus utilization efficiency to establish suitable fertilization approaches and enhance plant performance for maximizing product output from a unit area of *Calendula officinalis* L.

2. MATERIALS AND METHODS

Experiment site and plant material

This study was conducted over two consecutive years (2019–2020 and 2020–2021) under the coastal Mediterranean climate zone of İzmir, in the experimental fields of the Department of Field Crops, Faculty of Agriculture, Ege University. The experimental material, consisting of seeds of the *Calendula officinalis* L. species, was sourced from a population provided by the PHARMASAAT seed company in Germany. İzmir province, situated in the Mediterranean climate region, lies within the Aegean Region in the western part of Turkey, spanning between 37° 45' and 39° 15' northern latitudes, and 26° 15' and 28° 20' eastern longitudes.

Climatic and soil characteristics

The experimental site displays a soil composition of clay, loam, and alluvial structure. The pH level of the experimental site is 7.88, indicating a slightly alkaline environment. With a water-soluble salt content of 0.091%, there are no hindrances to plant growth. The lime concentration stands at 14.18%, suggesting a moderate presence of lime in the soil. However, the experimental site shows a deficiency in organic matter and total nitrogen, insufficiency in available phosphorus, and an abundance of available potassium. During the years of the study, air temperatures began to increase in March, peaking in August of 2019, July of 2020, and 2021 at 29.8, 29.7, and 30.6 °C, respectively. Conversely, the lowest average temperatures for the three years were observed at 8.7, 8.3, and 10.6 °C in January. The climatic diagram of the experimental field during years in 2019, 2020 and 2021 was given in Figure 1.

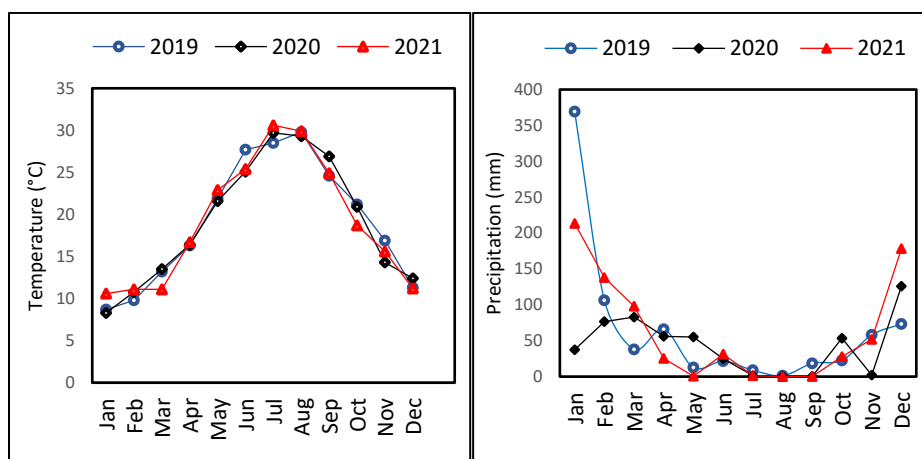


Figure 1. Experimental field climatic diagram

Experimental design and treatments

The study was conducted using a factorial experimental design arranged in randomized complete block design with two main factors. These factors included nitrogen doses (0 [N0], 50 [N5], 100 [N10], and 150 [N15] kg ha⁻¹ N) and phosphorus doses (0 [P0], 50 [P5], and 100 [P10] kg ha⁻¹ P₂O₅). Fertilizers used in the experiment were Urea with 46% N content and Triple Super Phosphate with 44% P₂O₅. Half of the nitrogen doses were split into two applications: at planting and at the start of flowering. All phosphorus fertilizer was applied to the rows prior to planting. The research spanned two years, from 2019 to 2021.

The study consisted of 36 plots arranged in a factorial design with 4 nitrogen levels, 3 phosphorus levels, and 3 replications. Each plot measured 3.0 meters in length and 1.6 meters in width, with 1.5-meter spacing between blocks and 1.0 meter between nitrogen plots, totaling an experimental area of 266.4 m². Seed sowing was conducted on November 13, 2019, in the first year and on November 12, 2020, in the second year. Seedlings were transplanted into rows spaced 40 cm apart, with 30 cm between plants within rows. Each plot included four rows, totaling 1,440 seedlings across the experiment, with a planting density of 40 plants m⁻². Transplanting was carried out on March 19, 2020, and April 8, 2021, respectively. Seedling establishment was supported by irrigation during the first three days after transplanting. Weed control was maintained manually throughout both seasons until seed harvest. Post-planting, irrigation was applied at regular intervals according to plant needs and prevailing weather conditions.

Morphological and yield measurements

Before harvest, plant height (cm), total branch number, and number of flowering branches (per plant) were recorded from ten randomly selected plants in each plot. The seed yields were determined using the first and fourth rows of the trial, while the second and third rows were allocated for drug flower yields (kg ha⁻¹). Following the initiation of flowering in the trial period (April 12, 2020, and May 4, 2021), the flowers in the remaining section were collected and weighed weekly from the onset to the conclusion of flowering after removing edge effects from the 2nd and 3rd rows of each plot. Subsequently, the fresh flower yield (kg ha⁻¹) was computed. The plants with quantified fresh flower yields underwent a drying process in a drying cabinet at 30 °C for 3-4 days. The seeds from the plant samples were then isolated and weighed, and the seed yield (kg ha⁻¹) was ascertained.

Physiological measurements

Nitrogen Use Efficiency (NUE) was determined by analysing seed and flower yield data collected from each plot using the agronomic efficiency measure [(yield in kilograms per kilogram of nitrogen input)] using the following formula:

$$\square \text{ NUE}_{\text{seed}} (\text{kg/kg N ha}^{-1}): (\text{TN}-\text{T0})/\Delta\text{N}$$

TN: Seed yield obtained from nitrogen application, kg

T0: Seed yield obtained from control (0 kg N ha⁻¹) application, kg

ΔN: Difference between nitrogen applications, kg

$$\square \text{ NUE}_{\text{flower}} (\text{kg/kg N ha}^{-1}): (\text{TN}-\text{T0})/\Delta\text{N}$$

TN: Drug flower yield obtained from nitrogen application, kg

T0: Drug flower yield obtained from control (0 kg N ha⁻¹) application, kg

ΔN: Difference between nitrogen applications, kg

Phosphour Use Efficiency (PUE) was determined by analyzing seed and flower yield data collected from each plot using the agronomic efficiency measure [(yield in kilograms per kilogram of phosphorus input)] using the following formula:

$$\square \text{ PUE}_{\text{seed}} (\text{kg/kg P ha}^{-1}): (\text{TP}-\text{T0})/\Delta\text{P}$$

TP: Seed yield obtained from phosphorus application, kg

T0: Seed yield obtained from control (0 kg P₂O₅ ha⁻¹) application, kg

ΔP: Difference between phosphorus applications, kg

$$\square \text{ PUE}_{\text{flower}} (\text{kg/kg P ha}^{-1}): (\text{TP}-\text{T0})/\Delta\text{P}$$

TP: Drug flower yield obtained from phosphorus application, kg

T0: Drug flower yield obtained from control (0 kg P₂O₅ ha⁻¹) application, kg

ΔP: Difference between phosphorus applications, kg

Statistical analysis

The statistical analysis was performed using the software JMP 14® by SAS Institute, Inc. (2018). Significantly different fertiliser treatments were examined through a two-way analysis of variance (ANOVA) with mean separation conducted using a least significant difference (LSD) test (p<0.05). Boxplots grouping was carried out in R Studio utilizing the 'tidyverse' (Wickham et al., 2019), 'ggplot2' (Wickham et al., 2016), and 'gapminder' (Bryan, 2022) packages.

3. RESULTS AND DISCUSSION

Morphological characteristics

Plant height

Plant height was significantly influenced by the interaction between year and nitrogen fertilization (p<0.01) (Fig. 2). The maximum plant height was recorded in the first year at a nitrogen level of 50 kg ha⁻¹ (58.2 cm), whereas in the second year, the tallest plants (38.6 cm) were observed at 150 kg ha⁻¹ N. Nitrogen application enhanced plant height in both years; however, the optimal nitrogen rate varied between seasons, indicating a strong dependency on annual environmental conditions such as temperature, soil moisture, and precipitation patterns. Seasonal variability also affected phosphorus responsiveness. While the highest plant height in 2020 was achieved at 50 kg ha⁻¹ P₂O₅ (56.8 cm), phosphorus fertilization had no statistically significant effect on plant height in 2021 (p>0.05), likely due to limited root activity and impaired nutrient translocation under drought conditions.

Drought restricts nitrogen use efficiency through several physiological mechanisms. One of the most critical is stomatal closure, which limits CO₂ uptake and reduces photosynthetic capacity, thereby decreasing the nitrogen requirement for metabolic activities (Ullah et al., 2019; Zhang, 2024). Given the central role of nitrogen in chlorophyll synthesis and photosynthesis, disruptions to these processes under water-deficit conditions constrain biomass accumulation and overall plant growth (Ye et al., 2022). In addition, drought stress negatively impacts

root development and function, both of which are essential for efficient nitrogen uptake. A reduction in root surface area and volume impairs nitrogen acquisition from the soil (Wang et al., 2024). Drought conditions also modify the composition of root exudates, which play a crucial role in nutrient mobilization within the rhizosphere, thereby further inhibiting nitrogen uptake (Wang et al., 2024).

Although supplemental irrigation was implemented during both growing seasons, extremely low rainfall during critical developmental stages-particularly in May 2021 (0.6 mm compared to 55.2 mm in May 2020)-likely intensified water stress, reducing nitrogen mobility in the soil and limiting its biological availability (Fig. 1). This highlights the limitation of irrigation in fully compensating for severe drought stress, especially in Mediterranean climates where rainfall distribution and timing are key factors for supporting vegetative growth. In 2020, favorable precipitation enabled efficient nitrogen utilization at moderate application rates, resulting in increased plant height. In contrast, in 2021, despite higher nitrogen inputs, reduced growth was observed, suggesting not an increased nitrogen requirement but a diminished capacity for nitrogen absorption and assimilation under drought conditions.

Overall, the benefits of nitrogen fertilization on plant height appear to be significantly constrained under drought, primarily due to physiological limitations in uptake and utilization rather than nitrogen deficiency per se. These findings are consistent with earlier reports. Moosavi et al. (2014) demonstrated that drought stress reduces biomass and plant height by impairing photosynthetic activity and altering assimilate distribution. Rahmani et al. (2008) also reported that drought during early vegetative stages significantly suppresses plant height in *Calendula officinalis*. Similarly, Kwiatkowski et al. (2020) noted that while nitrogen levels between 60 and 80 kg ha⁻¹ enhanced plant height, morphological responses were strongly influenced by seasonal weather conditions and water availability.

Total number of branches

The interaction between year, nitrogen dose, and phosphorus dose had a statistically significant effect on the total number of branches in *Calendula officinalis* L. ($p < 0.01$) (Fig. 2). The highest number of branches was recorded in the first year under the 50 kg ha⁻¹ N $\times$ 100 kg ha⁻¹ P₂O₅ and 100 kg ha⁻¹ N $\times$ 100 kg ha⁻¹ P₂O₅ fertilizer combinations. In contrast, the lowest branching (5.07 branches per plant) was observed in the second year at 0 kg ha⁻¹ N $\times$ 50 kg ha⁻¹ P₂O₅. These results suggest that both nitrogen and phosphorus application promote vegetative branching in *C. officinalis*, although the magnitude of response varied between growing seasons.

Comparative findings in the literature support these observations. Kumar and Singh (2011), in a study conducted under Indian conditions, reported the highest total branch number (20.7 branches per plant) at a nitrogen dose of 150 kg ha⁻¹. Other studies have documented a broad range in branching capacity: Kareem et al. (2014) reported values between 3.67 and 15.3, while Pazoki et al. (2016) found a range of 5.7 to 13.0 branches per plant. Additionally, Doğramacı and Arabacı (2010) observed that both organic and inorganic fertilizers positively influenced branching in *Pimpinella anisum*.

Although the branch numbers recorded in the present study were lower than those reported by Kumar and Singh (2011), they align closely with the ranges observed by Kareem et al. (2014) and Pazoki et al. (2016). These variations in branching responses may be attributed to differences in environmental conditions, soil fertility, genotype, and fertilizer timing, emphasizing the importance of site-specific management in optimizing vegetative growth traits in *Calendula officinalis*.

Number of flowering branches

A statistically significant three-way interaction was observed among year, nitrogen dose, and phosphorus dose on the number of flowering branches in *Calendula officinalis* L. ($p < 0.01$) (Fig. 2). The number of flowering branches varied across phosphorus levels depending on nitrogen doses and growing seasons. The highest number of flowering branches per plant (89.0) was recorded in 2021 under the 150 kg ha⁻¹ N $\times$ 50 kg ha⁻¹ P₂O₅ treatment, while the maximum value in 2020 (83.2) was observed with the 50 kg ha⁻¹ N $\times$ 50 kg ha⁻¹ P₂O₅ combination. The lowest number of flowering branches (52.9) was noted in the control (unfertilized) treatment in 2020. When yearly averages were considered, the second year yielded a higher average number of flowering branches (79.5 per plant) compared to the first year (65.0 per plant). This suggests that the response to nitrogen fertilization was more pronounced in 2021, potentially due to more favourable environmental conditions. However, although the number of flowering branches increased in the second year, a reduction in flower diameter was also observed, indicating a potential trade-off between branch quantity and flower size (Fig. 2).

Previous studies have reported a wide range of flowering branch numbers in *C. officinalis* depending on fertilizer regimes and environmental factors. For instance, Nagmote et al. (2020) reported a maximum of 55.16 flowering branches per plant under 100 kg ha⁻¹ N $\times$ 50 kg ha⁻¹ P application, while Kumar et al. (2015) observed fewer branches (35.7) with 150 kg ha⁻¹ N. Other researchers reported even lower figures: Crnobarac et al. (2009) found approximately 30 branches, Ganjali et al. (2010) noted 38.29-45.83, and Król (2011) reported 41.6-69 flowering branches per plant.

The discrepancies between the literature findings and our results regarding this particular characteristic can be attributed to factors such as environmental conditions, variations in growing seasons, the genetic diversity of the plant materials (population or variety), and discrepancies in the experimental variables examined.

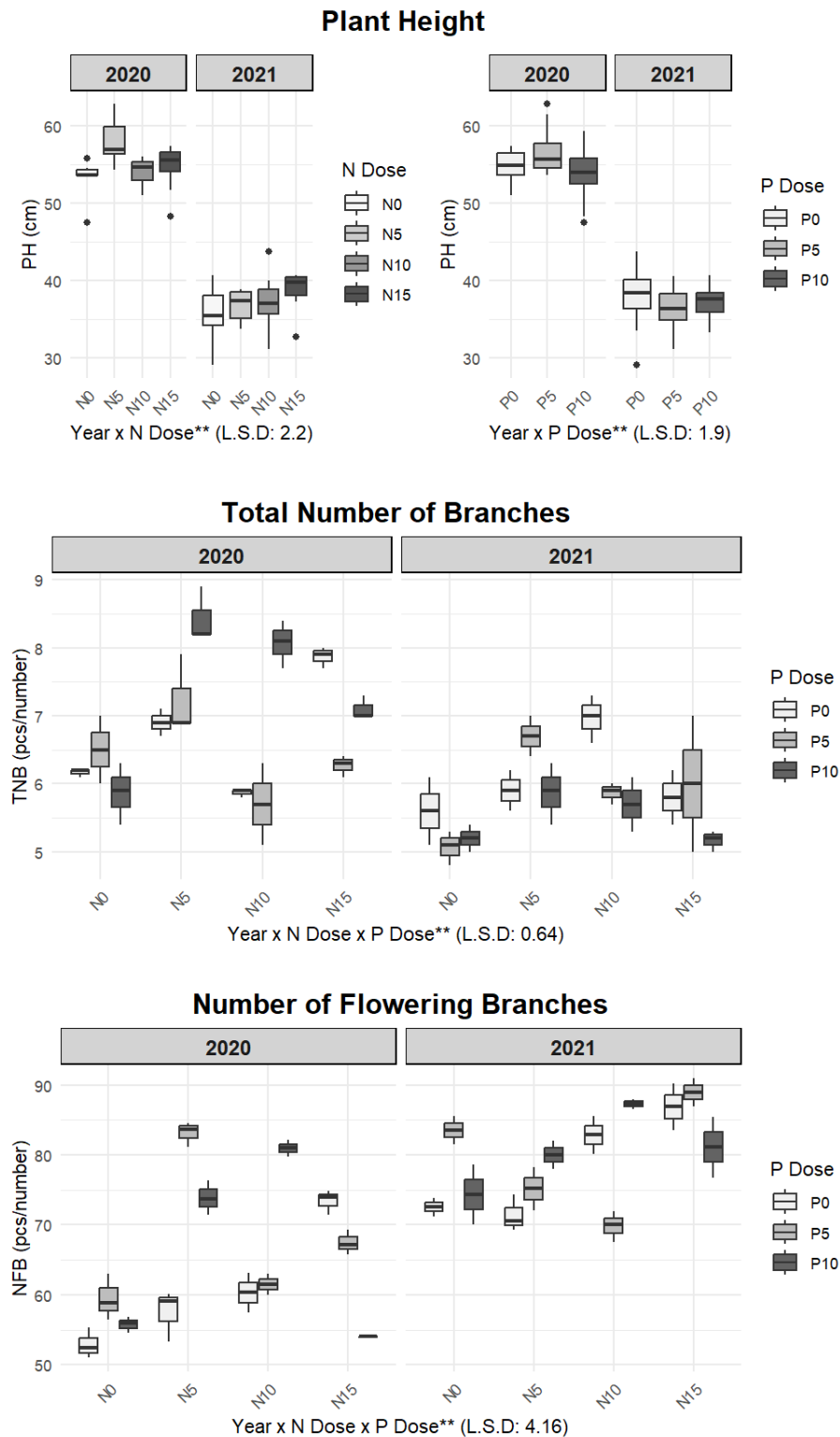


Figure 2. The effect of nitrogen and phosphorus fertilization on morphological characteristics of *Calendula officinalis* L. during 2020 and 2021 years. (* significant at $p < 0.05$ level, ** significant $p < 0.01$ level)

Yield characteristics

Fresh flowers yield

Figure 3 illustrates that the three-way interaction between year, nitrogen, and phosphorus levels significantly influenced fresh flower yield ($p < 0.01$). The highest yield was recorded in 2020, with 4.812 kg ha^{-1} under the combined application of $50 \text{ kg ha}^{-1} \text{ N}$ and $50 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$, while the lowest yield (2.177 kg ha^{-1}) was observed in the unfertilized control treatment. The overall average fresh flower yield across treatments was 3.163 kg ha^{-1} . In the first year, fresh flower yields ranged from 2.551 to 4.812 kg ha^{-1} , whereas in the second year they ranged between 2.177 and 3.741 kg ha^{-1} , indicating a general decline in yield during the second year. This decline was attributed to less favorable climatic conditions. Notably, the fertilizer combination of $50 \text{ kg ha}^{-1} \text{ N} \times 50 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$ consistently produced the highest fresh flower yields across both growing seasons, while control plots resulted in the lowest values.

These findings are supported by previous studies that have documented yield increases with increasing nitrogen inputs. Mollafilabi et al. (2014) reported the highest fresh flower yield at a nitrogen dose of 90 kg ha^{-1} . Similarly, Moosavi et al. (2014) observed a 36.8% increase in yield as nitrogen application increased from 0 to 180 kg ha^{-1} (ranging from 4.581 to 6.269 kg ha^{-1}). Under Türkiye agroecological conditions, Akkoç (2021) reported fresh flower yields between 2.486 and 5.019 kg ha^{-1} , with the highest yield achieved at $150 \text{ kg ha}^{-1} \text{ N}$.

Changes in environmental factors, such as temperature, precipitation, and soil moisture, have the potential to impact both the growth and yield of plants. Variations in yield might be attributed to fluctuations in temperature and rainfall patterns observed between consecutive years. Moreover, the composition of the soil and its fertility levels could also play a role in the variability of yields. Several research studies cited underscore the significance of the amount of fertilizer applied in relation to the yield of fresh flowers. The findings suggest that an optimal amount of fertilizer, for instance $90 \text{ kg ha}^{-1} \text{ N}$ or 150 kg ha^{-1} , can exert a considerable influence on the yield outcomes. Furthermore, the timing of fertilizer application during different growth stages is another factor that could affect the yield results.

Drug flowers yield

According to the results obtained from the drug flower yield, the effect of the trial years on the fertilizer combinations was found to be significant ($p < 0.05$) (Fig. 3). When the values of the year $\times$ nitrogen $\times$ phosphorus interaction are examined, the highest value in drug flower yield was found in $50 \text{ kg ha}^{-1} \text{ N} \times 50 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$ application in 2020 (1.320 kg ha^{-1}), whereas the lowest yield was obtained in control application in 2021 (587 kg ha^{-1}). In the second year, the average drug flower yield decreased by approximately 30% compared to the first year. Depending on climatic factors, this situation is thought to be caused by rainfall during the flowering period, which is critical for plant development. Although the average drug flower yield varied in the trial years, the highest values were obtained from $50 \text{ kg ha}^{-1} \text{ N}$ and $50 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$ applications in both years (Fig. 3).

In fertilization studies conducted on pot marigold, several researchers have reported that drug flower yield increases with rising nitrogen doses (Al-Badawy et al., 1995; Krol, 2011; Kumar et al., 2015; Samoon et al., 2018). However, other studies have indicated that this response plateaus beyond a certain nitrogen threshold, with no further yield enhancement observed (Nagmote et al., 2020; Akkoç, 2021). When the results of the present study are compared with those reported under different cultivation techniques, the flower yields obtained here are notably higher (Ayran, 2017; Yetis, 2019). This discrepancy may be attributed to various factors, including regional climatic conditions, soil properties, differences in cultivation practices, and genetic variation among plant materials.

Seed yield

As shown in Fig. 3, the two-way interactions between year $\times$ nitrogen dose and year $\times$ phosphorus dose significantly affected seed yield ($p < 0.01$). The average seed yield across the year $\times$ nitrogen interaction was 688 kg ha^{-1} . Within this interaction, the highest seed yield was obtained from the $100 \text{ kg ha}^{-1} \text{ N}$ treatment in the first year (884 kg ha^{-1}), closely followed by the $50 \text{ kg ha}^{-1} \text{ N}$ treatment (876 kg ha^{-1}). Conversely, the lowest yield was observed in the second year under the control ($0 \text{ kg ha}^{-1} \text{ N}$) condition, with 476 kg ha^{-1} .

Regarding the year $\times$ phosphorus interaction, seed yields reached 902 kg ha^{-1} and 893 kg ha^{-1} in the 100 and $50 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$ treatments, respectively, in 2020. The lowest seed yield (524 kg ha^{-1}) was recorded in the control treatment of the second year. On average, seed yield was 824 kg ha^{-1} in the first year and declined to 552 kg ha^{-1} in the second year, corresponding to a 33% reduction. These results emphasize the impact of both fertilizer input and seasonal conditions on seed productivity in *Calendula officinalis* (Fig. 3).

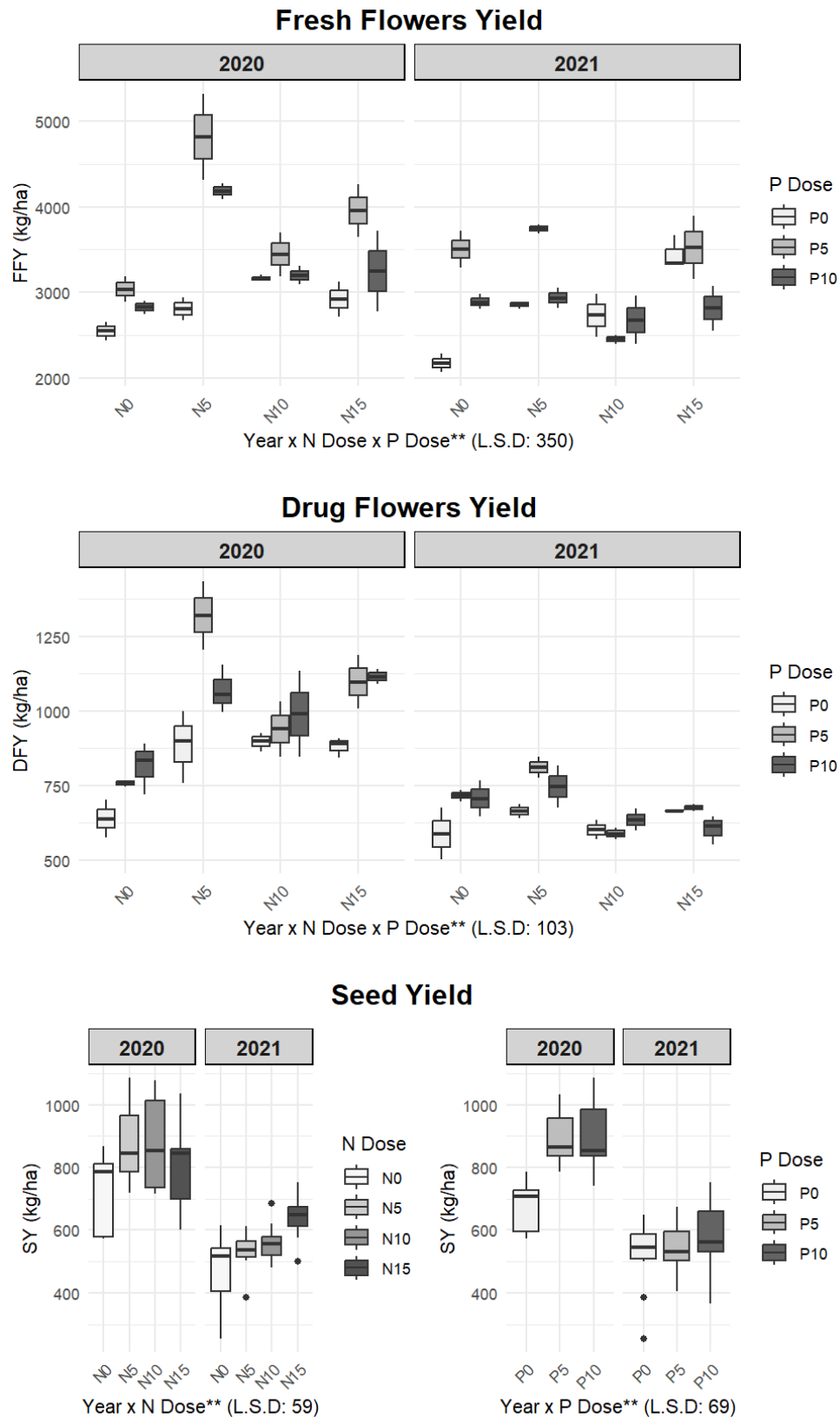


Figure 3. The effect of nitrogen and phosphorus fertilization on yield characteristics of *Calendula officinalis* L. during 2020 and 2021 years. (** significant $p < 0.01$ level)

Seed yield is influenced by numerous environmental and agronomic factors, particularly nitrogen and phosphorus fertilization. Król (2017) reported that the highest seed yields were obtained in seasons with moderate

and well-distributed rainfall, particularly with nitrogen applications ranging from 60–90 kg ha⁻¹. Similarly, Rahmani et al. (2011) observed that the application of 90 kg ha⁻¹ N led to the highest yield under drought conditions. Moreover, combined N × P applications were found to be effective in maximizing seed yield, with Samoon et al. (2018) and Kumar et al. (2015) recording the highest seed yields at 150 kg ha⁻¹ N × 80 kg ha⁻¹ P₂O₅. In another study, Jevdovic et al. (2013) reported a yield of 672.84 kg ha⁻¹ with 400 kg ha⁻¹ NPK, while significantly lower values were obtained under unfertilized conditions.

In the present study, both fertilizer dose and growing season significantly influenced seed yield, with nitrogen application playing a particularly prominent role. Our results support earlier findings indicating that nitrogen fertilization mitigates drought stress by enhancing root development, chlorophyll synthesis, and overall plant physiological performance. While water stress adversely affected yield components, adequately irrigated conditions promoted significantly higher seed yields, reinforcing the importance of water availability alongside optimal fertilization for maximizing *C. officinalis* productivity under Mediterranean conditions.

Physiological characteristics

Nitrogen Use Efficiency_{Seed} (NUE_{seed})

The effects of nitrogen doses and year interaction on nitrogen use efficiency (NUE_{seed}) were found to be statistically significant. The highest NUE_{seed} was determined to be 35.7 kg/kg N ha⁻¹ between 0 and 50 kg ha⁻¹ nitrogen doses in the first year. In the first year, it was seen that nitrogen use efficiency decreased in response to increasing nitrogen doses at 0-50 kg N ha⁻¹ compared to 50-100 and 100-150 kg N ha⁻¹ doses. NUE_{seed} values for 2021 decreased significantly compared to 2020 (Fig. 4).

When the NUE_{seed} values of the trial years were examined, it was seen that 2021 decreased significantly compared to 2020 (Fig. 4). As a result of this situation, increasing nitrogen doses in the pot marigold in 2020 reduced the efficiency of nitrogen use and did not make a significant difference in 2021. Nitrogen, due to its structure, dissolves better in soils that are saturated with water and is used more effectively by the plant. Dry conditions not only reduce plant growth and yield but also reduce water infiltration below the rooting zone, nutrient leaching, and losses by surface runoff, possibly increasing nutrient use efficiency (Ullah et al., 2019). In contrast, nutrient uptake and yield in wetter conditions are higher, but the potential for nutrient leaching also increases, reducing nutrient use efficiency (Rupp et al., 2021). When the rainfall amount in April, May, and June of the trial years was examined, it was seen that 2020 was higher than 2021. In this context, it was concluded that the amount of precipitation depending on the year factor was effective in the change in NUE values (Fig. 1).

Nitrogen Use Efficiency_{flower} (NUE_{flower})

The effects of nitrogen dose and year factors on NUE_{flower} were found to be significant. During the research years, the highest value was obtained in 2020 with 30.5 kg/kg N ha⁻¹. It was determined that the average nitrogen use efficiency in 2021 decreased by approximately 76% compared to 2020. The average NUE_{flower} value was 18.9 kg/kg N ha⁻¹, and the highest value was detected at 0-50 kg ha⁻¹ nitrogen fertilizer dose (32.3 kg/kg N ha⁻¹). As with NUE_{seed} value, increasing nitrogen doses resulted in a decrease in nitrogen use efficiency. Although the interaction of year × nitrogen dose was not found to be statistically significant, it was observed that the highest NUE_{flower} value among nitrogen doses in the first trial year was obtained at a nitrogen dose of 0-50 kg ha⁻¹ (49.3 kg/kg N ha⁻¹) (Fig. 4).

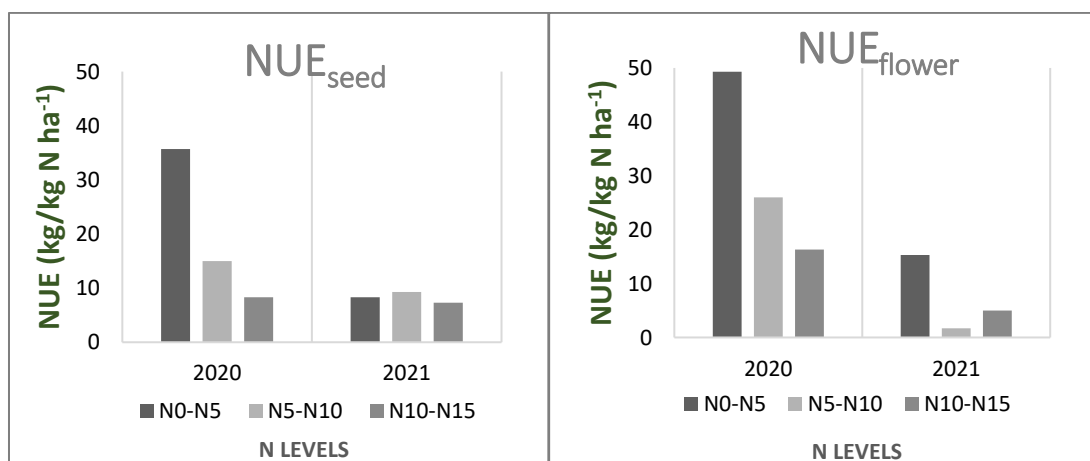


Figure 4. The effects of different nitrogen fertilizers on NUE_{seed} and NUE_{flower} in *Calendula officinalis* L. during 2020 and 2021.

Available water content, which is one of the factors limiting nitrogen use efficiency, is important for soil saturation (Gupta et al., 2012). Rainfall, which is especially effective in the spring period when nitrogenous fertilizers are applied and cover the first development stages of plants, increases the field water capacity and ensures that nitrogen reaches the plant effectively (Ahmad et al., 2014). Considering the trial years of NUE_{flower} , it was seen that the values for 2020 were higher than 2021. When evaluated in terms of the effect of the year factor on NUE_{flower} , it is thought that the resulting difference depends on climatic conditions. When the rainfall values of April, May, and June, which are critical for the growth and development of pot marigold in both trial years, were compared, the total rainfalls were 136.2 and 57.4 mm in 2020 and 2021, respectively. Precipitation values, which showed a more regular course in the first trial year, showed an irregular distribution in the second year (Fig. 1). It can be said that this precipitation change seen in the trial years in the spring had an impact on NUE_{flower} values and limited nitrogen use efficiency in the second trial year, which was relatively dry (Statham, 2012; Nematpour et al., 2019; Raza et al., 2019a). It has been observed that drought stress during the reproductive phase causes a shortening of the time between seed formation and pollen shedding and therefore a decrease in grain filling time (Shil & Dewanjee, 2022).

Phosphorus Use Efficiency_{Seed} (PUE_{seed})

In terms of PUE calculated based on seed yield, the 2020 value (34.5 kg/kg P ha⁻¹) was the year in which the highest results were achieved. The average PUE_{seed} value for 2021 was recorded at 3.0 kg/kg P ha⁻¹ (Fig. 5). Limiting water needs in plants during their development periods is important for the effective use of phosphorus. In the second trial year of the study, it is thought that the insufficient amount of precipitation in the months covering this period caused a decrease in phosphorus use efficiency (Meisinger and Delgado, 2002).

The effect of the year factor on PUE_{seed} was found to be statistically significant, and the values obtained in the two trial years differed greatly. It is thought that the amount of precipitation due to climatic factors during the trial years was effective in this situation. Plants absorb most of the phosphorus they need at the beginning of their development period, and their uptake can be limited due to factors such as soil pH, structure, moisture content, and climatic factors effective throughout the season (Ullah et al., 2019). When the total precipitation values in January and June of the research years are examined (Fig. 1), the amount of precipitation, which had a regular distribution until June in 2020, was largely effective in January and February in 2021 and then tended to decrease. Although the amount of precipitation in 2021 is higher than the precipitation in 2020 and the long-term average, it has decreased significantly, especially compared to the precipitation that is effective in the spring period. Considering that phosphorus fertilizers were applied with planting in March (2020) and April (2021) in the trial, it is thought that the water content in the soil was effective on phosphorus uptake and the soil water content caused changes in PUE_{seed} values (Kacar, 2020; Scaini et al., 2023).

Phosphorus is found to be an immobile element in the soil. In order for plants to take it up, phosphorus in the soil must be transported to the root area. However, this transport process depends on the presence of water. Water is located in the spaces between soil particles, and these spaces are used by the roots of plants. As the amount of water decreases, the space volume between soil particles decreases. In this case, it becomes difficult for water to reach the roots, and therefore, it becomes difficult for the roots to absorb phosphorus. This means that water is held so strongly in the soil that it cannot be taken up by the roots of plants. In this case, the roots of the plants can hardly take in water, and phosphorus uptake becomes difficult. Decreasing the amount of water negatively affects phosphorus transport in the soil and reduces the phosphorus use efficiency of plants (Kacar, 2020).

Phosphorus Use Efficiency_{Flower} (PUE_{flower})

Based on flower production, phosphorus use efficiency was investigated, and it was found that there was no significant relationship between PUE and the year, phosphorus dose, and year $\times$ phosphorus interaction. However, compared to 50-100 kg ha⁻¹ P₂O₅ fertilizer dose administration in both years, PUE_{flower} values between 0-50 kg ha⁻¹ P₂O₅ doses increased the consumption efficiency (Fig. 5).

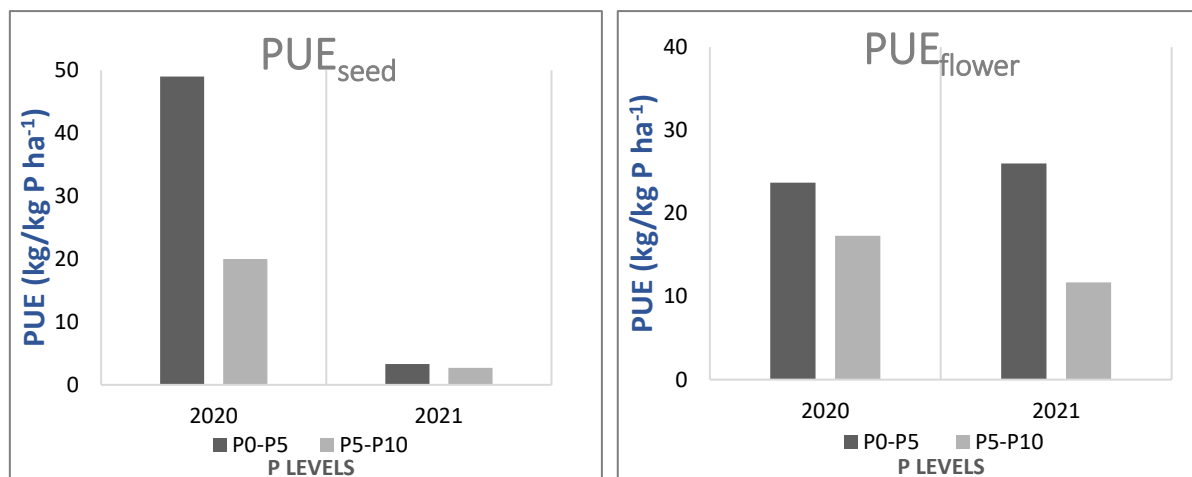


Figure 5. The effects of different phosphorus fertilizers on PUE_{seed} and PUE_{flower} in *Calendula officinalis* L. during 2020 and 2021.

The investigation conducted by Wang et al. (2011) delves into the effects of different levels of phosphorus application on crop yield, water consumption, and nitrogen use efficiency in dryland regions of the loess plateau across varying precipitation patterns. In these areas, the inadequacy of soil phosphorus poses a barrier to crop productivity and the effective utilization of water and nitrogen resources. It is imperative to apply phosphorus fertilizers appropriately to boost crop yield and enhance water and nitrogen use efficiency in dryland farming. The findings indicate an overall increase in most agricultural parameters at lower phosphorus rates, with a subsequent decrease at higher rates, except for water consumption and harvesting index (HI). Elevated phosphorus levels have been shown to have adverse effects on crop yield, water usage, and nitrogen use efficiency in both precipitation scenarios, underscoring the recommendation for a moderate application to achieve optimal productivity and resource utilization. Furthermore, even at moderate levels, phosphorus application leads to a reduction in HI and water consumption compared to non-phosphorus treatments during dry years, emphasizing the significance of implementing balanced fertilization practices.

In order to effectively use phosphorus fertilizers, Kacar (2020) lists a number of elements that must be taken into consideration. These include careful assessment of the qualities of the soil, balanced nutrient use, optimal plant selection, water management, and increased microbial activities. Assessing the aforementioned variables regarding calendula plant phosphorus uptake will aid in obtaining precise data regarding the degree and conditions of PUE_{flower} alterations.

4. CONCLUSION

This study demonstrated that *Calendula officinalis* L., a medicinal and aromatic plant of high therapeutic and economic value, responds significantly to nitrogen and phosphorus fertilization under Mediterranean conditions. Agronomic parameters such as flowering branch number, drug flower yield, seed yield, and nutrient use efficiency (NUE and PUE) were strongly influenced by fertilizer dose and seasonal variation, particularly rainfall. Key findings highlighted that the highest agronomic nitrogen use efficiency (NUE) based on seed and flower yield was achieved at 0-50 kg ha⁻¹ N in 2020, with values of 35.7 kg/kg N ha⁻¹ and 49.3 kg/kg N ha⁻¹, respectively. Similarly, PUE peaked at 34.5 kg/kg P ha⁻¹ in the same year and dose level. However, NUE and PUE decreased considerably in 2021 due to lower and irregular rainfall, indicating the strong influence of environmental conditions -especially rainfall during flowering- on nutrient uptake efficiency. Although the highest nutrient use efficiencies were recorded at the lowest nitrogen and phosphorus levels, this aligns with the well-established agronomic principle that NUE and PUE tend to decrease as nutrient input increases. In conclusion, *C. officinalis* does not require high nutrient inputs to reach its agronomic potential. The application of 50 kg ha⁻¹ nitrogen and 50 kg ha⁻¹ phosphorus can be recommended as an efficient and environmentally sound fertilization strategy for sustainable production of *C. officinalis* in the Aegean region and other areas with similar agroecological conditions.

ACKNOWLEDGEMENT

This article is a part of Gülsüm BOZTAŞ's PhD thesis carried out at Ege University in Izmir titled "Effects of Different Agro-technical Practices on Yield, Quality and Physiological Characteristics of *Calendula officinalis* L".

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Trait-Based Characterization of Barley Genotypes under Simulated Early Drought Stress Conditions

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ARTICLE INFO

Research Article

Received: 24 March 2025

Accepted: 4 June 2025

Published: 23 June 2025

Keywords:

Barley

Early drought resistance

Germination

Osmotic potentials

ABSTRACT

This study was carried out to determine the barley varieties that can be used as parents in drought resistance breeding and can be grown in regions where drought is experienced during the early growth and development periods. Seedling survival after drought, coleoptile length, seedling vigor, cell membrane damage and germination parameters at low water potential were measured. The seedling survival after drought rates of the varieties ranged from 8.0% to 76.0%, the coleoptile lengths ranged from 45.47 mm to 94.60 mm, the specific leaf area ranged from 100.1 cm² g⁻¹ to 255.8 cm² g⁻¹, the first leaf width ranged from 3.11 mm to 8.93 mm, and the cell membrane damage rates ranged from 2.34% to 37.79%. In our study, the germination rate, root length, shoot length and seed vigor index decreased as the osmotic potential increased. The 74 barley varieties used in the study were divided into four groups, resistant, medium resistant, medium sensitive and sensitive, according to the rank total values calculated over 6 selection criteria. Accordingly, the Konevi, İnce-04 and Fahrettinbey varieties were determined to be resistant to early drought. Twenty-six varieties of medium hardness with a rank total ranging from 29.1 to 40.3 were identified. Thirty-two varieties with a rank total between 40.4 and 51.6 were determined to be moderately sensitive, and 13 varieties with a rank total between 51.7 and 62.8 were determined to be sensitive.

Citation: Kodaz, S., Haliloglu, K. & Ozturk, A. (2025). Trait-Based Characterization of Barley Genotypes Under Simulated Early Drought Stress Conditions. *Turkish Journal of Field Crops*, 30(1), 206-222.

<https://doi.org/10.17557/tjfc.1664378>

1. INTRODUCTION

Stress in plant production can be defined as abiotic and biotic factors that negatively affect plant growth and development and consequently decrease yield (Kusvuran, 2010). Some environmental factors can become very stressful in a short period of time, while others can become very stressful days later. Some parts of the plant may be resistant to a stressor, while others may be sensitive (Boyer, 1982). Drought is a major abiotic stress that limits agricultural production and significantly threatens the world's food supply. Drought ranks first among abiotic stresses, with a share of 26% (Blum & Ebercon, 1981). It is a global problem that restricts agricultural production in arid and semiarid areas, which make up approximately 35% of the world's land. Drought is defined as the occurrence of a significant water deficit in the soil or atmosphere that depletes soil moisture and stresses plants. In plants, growth occurs through cell division, cell growth and differentiation and depends on genetic, physiological, biochemical, ecological and morphological events and their complex interactions. The quality and quantity of plant growth depend on these events, which are affected by water deficit (Ahmed et al., 2016). Barley production is generally carried out in dry agricultural areas in Türkiye, and irregular or insufficient rainfall throughout vegetation causes serious problems in these areas. Drought first reduces the water potential of the soil and then the plant. In subsequent periods, low turgor pressure, stomatal closure, a decrease in leaf growth and a decrease in the photosynthesis rate occur. Drought is a complex abiotic stress that varies according to the degree of severity and can be effective in any period of plant growth. Van Oosterom et al. (1993) reported that drought is the most important abiotic stress factor that reduces the grain yield of barley, but this decrease depends not only on the duration and severity of the drought but also on the period of development during which the plant is exposed to drought. Drought can be effective in three periods in cereals: the vegetative period (germination, emergence, seedling and tillering), anthesis period (stem elongation, booting and heading) and after anthesis period (anthesis and grain filling) (Shavrukov et al., 2017). In plants where drought stress is observed, there are conditions such as limited growth, decreased dry matter production, increased susceptibility to diseases and pests, and decreased product quantity and quality (Monti, 1987). Drought in autumn and winter causes the plant to enter the winter without a good seedling plant or tillering. Drought that occurs for a long time in the winter months and spring causes a decrease in the number of tillers and a decrease in the number of spikelets and flowers due to the reduction in plant size, spikelets and flowers, as it will include the tillering, jointing and heading periods, even if timely and adequate emergence occurs. In this case, since drought lasts in winter and spring, earliness is no longer a mechanism for escaping drought and is the reason for being more affected by drought. Barley is more affected by this situation due to its early age (Kutlu, 2010).

Drought tolerance occurs as a function of morphological (coleoptile length, first leaf emergence, root characteristics, tillering, flowering, awn formation, stomatal density, and cell membrane stability), physiological (low transpiration rate, relative water content, stomatal density, high water use efficiency, osmotic pressure, and leaf turgor) and biochemical (increased nitrate reductase activity and proline deposition) characteristics (Mitra, 2001). Tolerance to drought stress is a quantitative trait controlled by many different genes (Ahmed et al., 2016). The drought resistance of plants can vary depending on the genetic background of the cultivar, the duration and severity of drought, and the stage of development (Beltrano & Ronco 2008; Hu et al., 2007). In plants exposed to drought in early development periods, earlier flowering, plant height, leaf area, and the number of fertile tillers are reduced (Robertson & Giunta 1994). Drought-tolerant varieties are defined as those that can produce relatively high yields under drought conditions (Hall, 1993). Drought tolerance can be divided into three categories: drought escape, drought avoidance and drought tolerance (Blum, 2011).

This study was carried out to investigate the effectiveness of early drought resistance selection criteria based on seedling survival, coleoptile length, seedling vigor, cell membrane damage and germination in barley (*Hordeum vulgare* L.) at different osmotic potentials after drought and to determine suitable varieties that can be used as parents in drought-related breeding programs and can be grown in drought-prone regions in the early stages of plant development.

2. MATERIALS AND METHODS

In this study, 74 barley varieties included in the 2015 National Variety List of Türkiye were used as plant material (Table 1). Among the 74 barley varieties included in the experiment, 34 were alternative, 26 were winter, and 14 were spring varieties.

Table 1. Season character and spike type of the barley genotypes used in the study

Cultivar	Institution	Release Date	Spike Type	Season Character
Akar	FCCRI	2012	Two-Rowed	Alternative
Akdane	AEBMI	2011	Two-Rowed	Alternative
Akhisar 98	AARI	1998	Six-Rowed	Spring
Altıkat	GAPIAREC	2011	Six-Rowed	Spring
Arcanda	PSI	2014	Two-Rowed	Alternative
Atılır	AEBMI	2005	Two-Rowed	Alternative
Avcı-2002	FCCRI	2002	Six-Rowed	Winter
Aydanhanım	FCCRI	2002	Two-Rowed	Winter
Balkan 96 (Igri)	TARI	1996	Two-Rowed	Winter
Barış	GAPIAREC	2015	Two-Rowed	Spring
Başgül	AEBMI	2003	Two-Rowed	Alternative
Bayrak	AARI	2014	Six-Rowed	Spring
Bilgi-91	TZARI	1991	Two-Rowed	Spring
Bolayır	TARI	2007	Two-Rowed	Winter
Burakbey	FCCRI	2013	Two-Rowed	Alternative
Bülbül 89	FCCRI	1989	Two-Rowed	Alternative
Cervoise	ASI	2011	Six-Rowed	Alternative
Clarica	ASI	2013	Two-Rowed	Winter
Cumhuriyet 50	TZARI	1973	Two-Rowed	Alternative
Çatalhöyük 2001	AEBMI	2001	Two-Rowed	Spring
Çetin 2000	FCCRI	2000	Six-Rowed	Winter
Çıldır 02	TZARI	2002	Two-Rowed	Alternative
Çumra 2001	AEBMI	2001	Two-Rowed	Alternative
Durusu	AEBMI	2007	Two-Rowed	Winter
Efes 98	AEBMI	1998	Two-Rowed	Alternative
Emon	TFFI	2014	Two-Rowed	Winter
Erciyes	AEBMI	2006	Two-Rowed	Alternative
Erginel 90	TZARI	1990	Six-Rowed	Alternative
Escadre	ASI	2013	Six-Rowed	Winter
Fahrettinbey	KARI	2004	Two-Rowed	Spring
Fırat	AEBMI	2005	Two-Rowed	Spring
Gazda	TAPI	2013	Two-Rowed	Winter
Harman	TARI	2011	Two-Rowed	Winter
Hasat	TARI	2014	Two-Rowed	Winter
Hilal	AARI	2010	Two-Rowed	Spring
İnce-04	TZARI	2004	Two-Rowed	Alternative
Kalaycı-97	TZARI	1997	Two-Rowed	Alternative
Karatay 94	BDIARI	1996	Two-Rowed	Alternative
Kendal	GAPIAREC	2013	Six-Rowed	Spring
Keser	TZARI	2007	Six-Rowed	Alternative
Kıral-97	BDIARI	1997	Six-Rowed	Alternative
Konevi	BDIARI	1998	Two-Rowed	Alternative
Larende	BDIARI	2006	Two-Rowed	Alternative
Lord	TAPI	2011	Six-Rowed	Winter
Manava	ASAFI	2014	Two-Rowed	Winter
Martı	TARI	2009	Six-Rowed	Alternative
Meriç	AEBMI	2005	Six-Rowed	Winter
Olgun	EAARI	2011	Six-Rowed	Winter
Oliver	TAPI	2013	Six-Rowed	Winter
Orza 96	FCCRI	1996	Two-Rowed	Alternative
Özdemir-05	TZARI	2005	Two-Rowed	Alternative
Özen	FCCRI	2012	Two-Rowed	Spring
Premium	ASI	2013	Two-Rowed	Winter
Ramata	ASAFI	2015	Six-Rowed	Winter
Samyeli	GAPIAREC	2011	Two-Rowed	Spring
Sancak	AARI	2014	Two-Rowed	Spring
Scarpia	MSDI	2015	Six-Rowed	Winter
Seymen	SSI	2015	Two-Rowed	Winter

Table 1. (continued)

Sladoran	TARI	1998	Two-Rowed	Winter
Sultan	TSFI	2015	Six-Rowed	Winter
Sur-93	GAPIAREC	2002	Two-Rowed	Alternative
Şahin-91	GAPIAREC	1991	Two-Rowed	Alternative
Tarm-92	FCCRI	1992	Two-Rowed	Alternative
Tokak 157/37	FCCRI	1963	Six-Rowed	Winter
Toprak	AEBMI	2011	Two-Rowed	Alternative
Ünver	TZARI	2013	Two-Rowed	Alternative
Vamikhoca 98	AARI	1998	Six-Rowed	Spring
Yalın	FCCRI	2014	Two-Rowed	Alternative
Yerçil-147	TZARI	1976	Two-Rowed	Alternative
Yesevi 93	FCCRI	1993	Two-Rowed	Alternative
Yıldız	AEBMI	2007	Two-Rowed	Winter
Zeus	PSI	2014	Six-Rowed	Winter
Zeynel Ağa	FCCRI	2003	Two-Rowed	Alternative

AARI: Aegean Agricultural Research Institute; BDIARI: Bahri Dağdaş International Agricultural Research Institute; Eaari: East Anatolian Agricultural Research Institute; FCCRI: Field Crops Central Research Institute; TARI: Trakya Agricultural Research Institute; TZARI: Transition Zone Agricultural Research Institute; GAPIAREC: GAP International Agricultural Research and Education Center; KARI: Karadeniz Agricultural Research Institute, EAARI: Eastern Anatolia Agricultural Research Institute, AEBMI: Anadolu Efes Beer and Malt Industry, ASI: Ata Seed Industry, TFFI: Tarar Flour and Food Industry, PSI: Progen Seed Industry, TAPI: Tareks Agricultural Production Industry, ASAFI: Alfa Seed, Agricultural and Food Industry, MSDI: Marmara Seed Development Industry, SSI: Sari Seed Industry, TSFI: Tekcan Seed and Food Industry

Seedling survival after drought

The experiment was conducted according to a completely randomized block design with 4 repetitions. Fifty seeds of each genotype were planted in wooden crates with dimensions of 80x100x12 cm, width 5 cm, 2 cm row and intrarow spacing at depths of 3 cm, respectively. After planting, the plants were sufficiently irrigated until they reached 3 leaves. After this period, the plants were not watered until most of the plants died from drought. When most of the seedlings died from drought, the moisture content of the soil was determined with the help of a soil moisture meter (HH2 Soil Moisture Meter) on the basis of volume at a depth of 10 cm; the plants were given enough water again, and their regrowth was ensured. Seedlings that remained viable approximately 10 days after redevelopment were counted in the crates, and the percentage of seedlings remaining viable after drought was calculated as a percentage for each genotype (Winter et al., 1988).

Coleoptile length

The experiment was conducted according to a completely randomized design with 4 replications. Fifty seeds of each genotype were planted in wooden crates with dimensions of 80x100x12 cm at depths of 3 cm and 5 cm and with 2 cm row and intrarow spacing. The crates, which were given sufficient water after planting, were placed in a growth cabinet set to 15°C and kept for 10 days in darkness. Then, 10 plants of each genotype were carefully removed from the soil, and the distance between the seed and the tip of the coleoptile, from which the first leaf emerged, was measured with a millimeter ruler (Rebetzke et al., 1999).

Seedling vigor

The experiment was carried out in research fields of the Atatürk University Plant Production and Application Center Directorate according to the randomized complete block design with 4 replications. When the number of leaves per plant averaged 4-5, 10 plants were randomly selected. The maximum width of the first leaf of each plant was measured using an electronic caliper. Then, the first 3 leaves of the plants were separated, and their total area was measured with a leaf area meter (LI-COR LI-3000C). The leaves were kept in a drying oven set to 70°C for 72 hours and then weighed on a 0.001 g sensitive balance, and their total dry weight was determined. Using the obtained values, the specific leaf area (cm² g⁻¹) was calculated with the following formula (Rebetzke et al., 2004):

$$\text{Specific leaf area (cm}^2 \text{ g}^{-1}\text{)} = (\text{Total leaf area}) / (\text{Total leaf dry weight})$$

Cell membrane damage

The experiment was conducted in triplicate according to a randomized completely block design. After the seeds were sterilized, 50 seeds of each genotype were placed in Petri dishes. Distilled water (5 ml) was added to the Petri dish and subsequently incubated at 22°C for 15 days. At the end of this period, samples approximately 2 cm in length were taken from the middle parts of the first leaves of 20 plants and were subsequently washed with distilled water. Leaf samples were kept at 10°C for 24 hours in 30 ml of 30% polyethylene glycol (PEG, average molecular

weight 6000) solution in test tubes for drying application (T) and in 30 ml of distilled water for control application (C). At the end of this period, both the drying and control samples were washed three times with distilled water, 30 ml of distilled water was added again, and the samples were kept in a water bath with a thermostat set to 25°C for 1 hour. Afterwards, the samples were mixed thoroughly, and the electrical conductivity of the solution was measured at 25°C with an EC meter. At the end of this measurement, T1 values were obtained from the dried samples, and C1 values were obtained from the control samples. After the first measurement, all sample tubes were autoclaved at 121°C for 15 minutes under 1 kg cm⁻² pressure to measure the total electrolyte concentration. Then, the sample tubes were kept in a water bath with a thermostat set to 25°C for 1 hour and mixed thoroughly, and their electrical conductivity was measured again at 25°C with an EC meter. At the end of this measurement, after autoclaving, T2 values were obtained from the dried samples, and C2 values were obtained from the control samples.

$$\text{Damage \%} = 1 - [(T1:T2)) / (1 - (C1:C2))] \times 100$$

Germination in Different Osmotic Potentials

The experiment was conducted in 3 replications according to a complete randomized block design. After the seeds were sterilized, 50 seeds were placed in petri dishes with osmotic potential conditions of 0 (control), -4 and -6 bar prepared using PEG 6000 solution. Five milliliters of distilled water were added to each Petri dish for control application, and 5 ml of the corresponding PEG solution was added to each Petri dish to osmotic stress application. All Petri dishes were placed in a plant growth cabinet at a temperature of 22°C and kept at that temperature for 10 days. At the end of this period, the root, shoot and coleoptile lengths of 10 plants of each genotype were measured under a stereomicroscope. Seeds with a rootlet length of at least 2 mm were considered germinated and counted, and the germination percentage was calculated. The seed vigor index was calculated by multiplying the embryonic root and shoot lengths sum by the germination percentage (Dhanda et al., 2004).

$$\text{Seed Vigor Index} = (\text{Root length} + \text{Shoot length}) \times \text{germination\%}$$

Statistical analysis

In the early drought resistance study, the data obtained under field, laboratory and greenhouse conditions were analyzed using the SAS GLM (SAS Ins., Cary, NC) computer program, and the LSD multiple comparison test was used to compare the means of the varieties. The parameters of seedling survival, coleoptile length, seedling vigor and cell membrane damage after drought were compared among the varieties. For the germination parameters at low water potentials, the varieties and applications were compared, and the applications were evaluated separately in cases where variety $\times$ application interactions were important. The relationships between the selection criteria used in the study were examined by correlation analysis. Selection criteria and varieties were grouped principal component analysis. In addition, according to the Rank method, the varieties were grouped in terms of early drought resistance.

3. RESULTS AND DISCUSSION

Seedling survival after drought

In the present study, there were significant differences between barley varieties in terms of seedling survival after drought ($P < 0.05$) (Table 2). After drought treatment, the seedling survival rates of the varieties varied between 8.0% and 76.0%, and the average seedling survival rate was 51.4%. While the Scarpia variety had the highest seedling survival rate after drought, the Tarm-92 variety had the lowest seedling survival rate (Table 2). Ozturk et al. (2014) reported that the seedling survival rate after drought was between 18.5% and 51.1% in their study of 64 bread wheat genotypes. Voltaire (2003) reported that the seedling survival rate was 100% in orchardgrass variety, as a result of his study investigating the responses of a drought-tolerant orchardgrass and a drought-tolerant and sensitive barley genotype to drought during the seedling period. He also reported that the seedling retention rates of the Tadmor and Plaisant barley varieties were 66% and 69%, respectively, when there was no irrigation for 44 days, and the seedling retention rate of both barley varieties was 4% when there was no irrigation for 53 days; moreover, there was no difference between the barley varieties in terms of the seedling survival rate. On the other hand, Tomar & Kumar (2004) reported that post drought seedling survival, which is a dominant character controlled by a single gene, can be used as a selection criterion to improve drought tolerance in early development. Accordingly, it can be demonstrated that the Scarpia, Hilal, Meriç, Kalaycı-97, Hasat and Erciyes varieties, which are in the first place in terms of seedling survival after drought, can better adapt to places where drought occurs in early development stages.

Coleoptile Length

There were significant differences between barley varieties in terms of coleoptile length ($P<0.05$) (Table 2). The coleoptile length of the varieties ranged from 45.47 mm to 94.60 mm, and the average coleoptile length was 63.76 mm. The Hilal variety had the longest coleoptile, while the Premium variety had the shortest coleoptile length (Table 2). In this experiment, one variety had a coleoptile length greater than 90.1 mm, five varieties had a coleoptile length between 75.01 and 90.00 mm, 39 varieties had a coleoptile length between 60.01 and 75.00 mm, and 29 varieties had a coleoptile length between 45.00 and 60.00 mm (Table 2). In the research conducted by Paynter & Clarke (2010) on 44 summer barley varieties, significant differences were detected between the varieties in terms of coleoptile length. Radford (1987) reported that the results for the coleoptile are in agreement with the results we obtained from our study. Under dry farming conditions, varieties with long coleoptiles can increase yield by showing better emergence. Therefore, it can be demonstrated that the Hilal, Balkan 96 (Igri), Çatalhöyük 2001, Akdane and Başgöl varieties, which have long coleoptiles, can better adapt to dry farming areas, with lengths ranging from 60-80 mm in most varieties, while Paynter & Clarke (2010) reported lengths ranging from 39 to 93 mm. Ozturk et al. (2014), on the other hand, reported that the length of the coleoptile varied between 39 and 74 mm in their study of 64 bread wheat genotypes.

Seedling vigor

Differences between barley varieties in terms of specific leaf area and first leaf width, which are elements of seedling vigor, were significant ($P<0.05$) (Table 2). The specific leaf area of the cultivars ranged from 100.1 $\text{cm}^2 \text{g}^{-1}$ to 255.8 $\text{cm}^2 \text{g}^{-1}$, with an average of 151.7 $\text{cm}^2 \text{g}^{-1}$. The Olgun variety had the greatest specific leaf area, while the Çetin 2000 variety had the lowest specific leaf area (Table 2). Ozturk et al. (2014) reported that the specific leaf area ranged from 164.4-204.2 $\text{cm}^2 \text{g}^{-1}$. Amanullah (2015) reported that the particular leaf areas measured 30, 60 and 90 days after emergence in barley were 710.0 $\text{cm}^2 \text{g}^{-1}$, 558.3 $\text{cm}^2 \text{g}^{-1}$ and 699.6 $\text{cm}^2 \text{g}^{-1}$, respectively. In our study, lower values were found. This difference may have occurred because the leaf shapes of the varieties used in our study differed. The first leaf width varied between 3.11 and 8.93 mm, with an average of 4.97 mm. The Bolayır variety had the greatest first leaf width, while the Kendal variety had the shortest first leaf width (Table 1). Ozturk et al. (2014) reported that the 1st leaf width ranged between 3.27 and 4.71 mm in their study of 64 bread wheat genotypes. Seedling vigor is defined as the rapid development of leaf area in the early development period (Richards & Lukacs, 2002). Seedling vigor increases the plant growth rate and grain yield by reducing evaporation (López-Castañeda & Richards 1994), increasing the plant's ability to compete with weeds (Coleman et al., 2001) and increasing the efficiency of water and light use under dry farming conditions (Rebetzke et al., 2004). Accordingly, in terms of the elements of seedling vigor; Olgun, Gazda, Atılır, Yercil-147, Ramata and Tokak 157/37 varieties were in first place for specific leaf area, thus Bolayır, Burakbey, Keser, Toprak and Çıldır 02 varieties, were in the first place in terms of first leaf width, and it can better adapt to places where drought occurs in early development periods.

Table 2. Seedling survival after drought, coleoptile length, specific leaf area, first leaf width, and cell membrane damage of barley varieties and variance analysis results of these characteristics

Cultivars	Seedling survival after drought (%)	Coleoptile length (mm)	Specific leaf area ($\text{cm}^2 \text{g}^{-1}$)	First leaf width (mm)	Cell membrane damage (%)
Akar	60.0	57.62	197.0	4.19	9.18
Akdane	56.0	77.87	150.4	5.21	2.34
Akhisar 98	34.0	66.73	129.9	5.17	3.11
Altukat	40.0	64.60	119.4	5.85	7.60
Arcanda	52.0	58.27	131.1	3.85	4.14
Atılır	62.0	57.47	223.0	6.06	5.45
Avcı-2002	24.0	61.13	118.7	5.27	5.44
Aydanhanım	54.0	68.07	207.2	4.85	3.26
Balkan 96 (Igri)	64.0	81.00	110.0	5.21	6.23
Bariş	62.0	47.13	155.4	5.13	5.92
Başgöl	30.0	77.60	201.3	3.81	7.10
Bayrak	64.0	56.27	154.8	4.49	8.72
Beyşehir	64.0	74.00	102.7	5.16	5.14
Bilgi-91	46.0	57.33	123.6	4.67	4.44
Bolayır	70.0	55.47	163.2	8.93	6.32
Burakbey	64.0	68.20	127.0	7.79	9.40
Bülbül 89	58.0	61.00	129.4	5.21	12.70
Cerveise	50.0	57.00	113.4	6.28	7.59
Clarica	40.0	64.20	103.1	4.30	8.82
Cumhuriyet 50	52.0	71.67	104.1	5.83	4.95
Çatalhöyük 2001	60.0	79.00	105.2	6.09	5.13

Table 2. (continued)

Çetin 2000	40.0	64.67	100.1	4.12	3.69
Çıldır 02	54.0	76.87	132.3	6.48	8.62
Çumra 2001	54.0	68.47	101.6	4.90	3.39
Durusu	60.0	69.13	127.8	4.59	4.28
Efes 98	66.0	73.00	118.6	6.10	18.82
Emon	64.0	62.40	114.2	5.66	9.79
Erciyes	70.0	73.47	168.4	4.10	7.62
Erginel 90	64.0	70.00	152.6	4.13	10.62
Escadre	50.0	69.00	136.8	5.30	10.33
Fahrettinbey	54.0	68.87	206.0	4.83	18.98
Fırat	64.0	63.33	166.4	5.06	10.25
Gazda	48.0	57.27	241.5	5.32	20.62
Harman	44.0	62.80	107.0	5.21	8.31
Hasat	70.0	69.80	107.1	4.53	12.57
Hilal	74.0	94.60	123.9	3.95	17.36
İnce-04	52.0	69.07	198.5	4.83	14.86
Kalaycı-97	70.0	72.53	116.8	4.28	10.52
Karatay 94	42.0	72.40	170.6	3.22	9.12
Kendal	34.0	69.00	174.0	3.11	10.84
Keser	52.0	68.07	153.4	7.09	12.15
Kıral-97	58.0	52.73	181.9	5.41	12.11
Konevi	68.0	73.53	200.5	5.79	23.94
Larende	42.0	68.80	158.4	4.24	13.42
Lord	62.0	63.53	118.5	3.86	7.76
Manava	58.0	59.00	104.9	4.29	14.27
Martı	56.0	65.20	126.0	4.82	14.20
Meriç	74.0	63.53	131.1	4.69	14.42
Olgun	54.0	58.93	255.8	4.36	37.79
Oliver	58.0	57.93	205.2	5.20	17.33
Orza 96	64.0	57.67	158.1	5.50	16.59
Özdemir-05	38.0	55.73	166.3	4.94	16.38
Özen	44.0	59.73	162.4	4.65	12.63
Premium	54.0	45.47	123.7	5.50	15.74
Ramata	58.0	67.47	215.2	4.08	7.35
Samyeli	56.0	47.47	153.3	4.08	11.97
Sancak	50.0	62.47	132.0	3.88	19.53
Scarpia	76.0	56.27	123.0	4.12	13.54
Seymen	54.0	61.33	104.6	4.14	12.43
Sladoran	42.0	54.07	192.5	3.28	11.61
Sultan	28.0	63.73	157.0	4.98	17.46
Sur-93	14.0	55.47	174.1	3.86	11.00
Şahin-91	16.0	68.27	205.3	4.51	13.01
Tarm-92	8.0	68.53	112.7	6.22	23.99
Tokak 157/37	10.0	59.26	214.9	5.31	15.17
Toprak	24.0	67.93	175.2	6.63	14.87
Ünver	34.0	63.80	148.4	5.54	15.59
Vamıkhoca 98	38.0	55.40	158.8	5.68	10.50
Yalın	30.0	58.00	155.4	4.51	17.06
Yerçil-147	54.0	50.53	216.8	4.49	18.00
Yesevi 93	66.0	50.40	122.2	4.69	21.32
Yıldız	68.0	54.53	142.0	4.73	21.48
Zeus	58.0	57.60	176.0	4.66	22.44
Zeynel Ağa	60.0	57.40	162.0	4.58	17.17
Mean	51.4	63.76	151.7	4.97	12.00
F value (Variety)	90.23**	9.07**	5993.6**	115.71**	29.67**
LSD (0.05)	6.00	8.02	1.4	0.26	3.21
CV (%)	6.35	9.03	0.7	3.72	19.18

F values marked with ** are significant at the probability level of 0.01.

Cell membrane damage

The cell membrane is one of the first targets of many stresses, including drought, and therefore, the stability of the cell membrane is crucial. Cell membrane stability is considered one of the best physiological indicators of drought stress tolerance (Bandurska, 2004). The difference between barley varieties in terms of cell membrane damage was significant ($P < 0.05$) (Table 2). The cell membrane damage rates of the varieties ranged from 2.34%

to 37.79%, and the mean rate was 12.00%. The Olgun variety had the highest cell membrane damage rate, while the lowest rate of cell membrane damage was found in the Akdane variety (Table 2). Kocheva & Georgiev (2003) reported that the cell membrane damage rate of both varieties was 20% in their study of two barley varieties. Ozturk et al. (2016) reported that cell membrane damage ranged from 0.16-47.26% in their study of 64 bread wheat genotypes. Wang & Huang (2004) reported that barley genotypes with high cell membrane stability (71-80%) performed better under drought conditions. Therefore, the Akdane, Akhisar 98, Aydanhanım, Çumra 2001 and Çetin 2000 varieties, which have a low rate of cell membrane damage, may perform better in drought-prone regions.

Germination at Different Osmotic Potentials

Germination rate

The difference between the varieties and their osmotic potential treatments was significant in terms of the germination rate, and the "variety x treatment" interaction was important due to the different reactions of the varieties to their osmotic potential treatment (Table 3). In the control treatment, the germination rates of the varieties ranged from 89.0% to 100.0%. The highest germination rates were obtained for the Arcanda, Bilgi-91, Burakbey, Çıldır 02, Erciyes and Sultan varieties. The lowest germination rate was observed for Vamıkhoca 98.

In this study, the germination rates of varieties with an osmotic potential of -4 bar ranged from 18.0-100.0%. The Arcanda, Bolayır and Fahrettinbey varieties had the highest germination rates, while the Akhisar 98 variety had the low germination rate (Table 3). In this study, the germination rates of varieties with an osmotic potential of -6 bar ranged between 2.0-100.0%. The Çetin 2000 variety had the highest germination rate, while the Sladoran variety had the lowest. (Table 3). In our study, the germination rate decreased as the osmotic potential increased. The average germination rate, which was 95.96% in the control treatment, decreased to 78.10% at -4 bar osmotic potential and 56.88% at -6 bar osmotic potential. Balkan & Genctan (2013) reported that the germination rate of bread wheat decreased with the treatment of osmotic stress, and the germination rate, which was 96.72% on average in the control treatment, was 86.25% at -0.5 MPa and 27.50% at -1.0 MPa. Oukarroum et al. (2005) reported that the germination rate of barley varieties relative to the average decreases as osmotic stress increases to 100% at 0.0 MPa, 87.8% at -0.5 MPa, 78.5% at -1.0 MPa, 59.7% at -1.5 MPa, and 51.4% at -2.0 MPa. Karami & Sepehri (2017) reported that the germination rate was 82.0% in the control treatment, 68.7% of an osmotic potential of -0.3 bars, and 60.6% in an osmotic potential of -0.6 bar and that the drought created by PEG 6000 caused a decrease in the germination rate of barley. Ozturk et al. (2016), who measured the germination rate of bread wheat genotypes under osmotic stress, reported that the germination percentage of genotypes ranged between 85.3-99.3% in the control treatment and 38.7-90.7% at -5 bar osmotic potential.

Root length

The difference between the varieties and their osmotic potential treatment was significant in terms of root length, and the "variety x treatment" interaction was important due to the different reactions of the varieties to osmotic potential treatments (Table 3). In the control treatment, the lengths of the roots of the varieties ranged from 5.57-17.31 cm. The Kırıl-97 variety had the greatest root length, while the Şahin-91 variety had the shortest. In this study, the lengths of the roots of the cultivars with an osmotic potential of -4 bar ranged from 3.00-9.09 cm. The Bolayır variety had the longest root length, while the Hilal variety had the shortest. In this study, the lengths of the embryonal roots of the varieties with an osmotic potential of -6 bar ranged from 0.63-8.13 cm. The Cervoise variety had the longest length of the roots, while the Sladoran variety had the shortest (Table 3). In our study, it was determined that root length decreased as osmotic potential increased. The average shoot length, which was 10.21 cm in the control treatment, decreased to 6.16 cm at -4 bar osmotic potential and 5.27 cm at -6 bar osmotic potential. Balkan & Genctan (2013) reported that the root length, which was 137.71 mm on average in the control treatment, decreased to 83.64 mm at -0.5 MPa and 9.47 mm at -1.0 MPa. Karami & Sepehri (2017) reported that the root length was 9.20 cm in the control treatment, 8.00 cm at an osmotic potential of -0.3 bar, and 6.00 cm at an osmotic potential of -0.6 bar and that the drought created by PEG 6000 caused a decrease in root length in barley. Ozturk et al. (2016), who measured the embryonal root length of bread wheat genotypes under osmotic stress, reported that the embryonal root length of the genotypes ranged between 14.84 and 38.13 cm in the control treatment and between 2.91 and 13.92 cm at an osmotic potential of -5 bar.

Table 3. Germination Rates and Embryonal Root Lengths and Variance Analysis Results of Barley Varieties According to Different Opposing Potential Treatments

Cultivars	Germination Rate				Root Length			
	Control	-4 Bar	-6 Bar	Mean	Control	-4 Bar	-6 Bar	Mean
Akar	98.67	99.00	93.00	96.89	12.57	6.43	6.37	8.46
Akdane	93.33	96.00	92.00	93.78	12.04	7.80	7.03	8.96
Akhisar 98	94.67	18.00	7.00	39.89	11.81	3.86	7.84	7.84
Altıkat	96.00	87.00	96.00	93.00	9.29	5.57	5.93	6.93
Arcanda	100.00	100.00	96.00	98.67	11.44	6.27	7.47	8.39
Atılır	93.33	55.00	77.00	75.11	10.51	3.87	5.27	6.55
Avcı-2002	97.33	96.00	99.00	97.44	10.31	6.19	6.57	7.69
Aydanhanım	97.33	87.00	71.00	85.11	11.59	6.17	5.83	7.86
Balkan 96 (Igri)	97.33	96.00	97.00	96.78	9.43	7.97	6.83	8.08
Barış	94.67	96.00	87.00	92.56	11.21	7.61	7.20	8.67
Başgül	96.00	94.67	60.00	83.56	10.84	7.03	6.37	8.08
Bayrak	97.33	18.67	49.00	55.00	14.12	5.73	6.93	8.93
Beyşehir	98.67	94.67	92.00	95.11	12.59	6.51	6.13	8.41
Bilgi-91	100.00	88.00	93.00	93.67	13.19	5.75	7.03	8.66
Bolayır	98.67	100.00	97.00	98.56	9.12	9.03	7.33	8.50
Burakbey	100.00	93.33	84.00	92.44	10.24	5.56	4.93	6.91
Bülbül 89	94.67	96.00	91.00	93.89	11.18	5.27	5.20	7.22
Cervoise	94.67	91.00	73.00	86.22	8.71	7.57	8.13	8.14
Clarica	97.33	95.00	82.67	91.67	12.50	7.43	7.60	9.18
Cumhuriyet 50	96.00	99.00	80.00	91.67	11.57	6.77	7.20	8.51
Çatalhöyük 2001	97.33	97.00	99.00	97.78	11.95	7.13	7.40	8.83
Çetin 2000	93.33	96.00	100.00	96.44	12.08	7.67	6.47	8.74
Çıldır 02	100.00	98.00	88.00	95.33	12.57	7.03	7.10	8.90
Çumra 2001	93.33	92.00	87.00	90.78	9.21	5.17	6.73	7.04
Durusu	93.33	97.00	97.00	95.78	11.31	7.40	7.60	8.77
Efes 98	96.00	72.00	77.00	81.67	12.67	5.33	4.73	7.58
Emon	98.67	55.00	61.00	71.56	12.11	5.00	5.40	7.50
Erciyes	100.00	34.00	14.00	49.33	12.59	6.33	4.72	7.88
Erginel 90	98.67	82.00	91.00	90.56	8.69	5.17	6.43	6.76
Escadre	98.67	84.00	57.00	79.89	14.97	6.33	5.57	8.96
Fahrettinbey	98.67	100.00	88.00	95.56	5.87	5.33	5.10	5.44
Fırat	98.67	60.00	80.00	79.56	11.23	3.83	5.17	6.74
Gazda	93.00	60.00	68.00	73.67	9.72	5.50	3.83	6.35
Harman	97.00	60.00	90.00	82.33	12.09	6.83	5.57	8.16
Hasat	92.00	56.00	68.00	72.00	11.54	5.83	6.54	7.97
Hilal	94.00	49.00	6.00	49.67	9.01	3.00	1.58	4.53
İnce-04	95.00	45.00	81.33	73.78	11.50	7.00	6.27	8.26
Kalaycı-97	93.00	68.00	24.00	61.67	12.52	6.50	2.53	7.18
Karatay 94	98.00	51.00	64.00	71.00	13.97	5.17	5.13	8.09
Kendal	97.00	61.00	51.00	69.67	13.95	5.50	4.90	8.12
Keser	96.00	59.00	65.00	73.33	14.09	7.33	5.40	8.94
Kıral-97	92.00	84.00	63.00	79.67	17.31	6.67	5.37	9.78
Konevi	98.00	60.00	81.00	79.67	11.21	7.33	6.77	8.44
Larende	96.00	93.00	51.00	80.00	11.93	6.33	4.90	7.72
Lord	97.00	82.00	88.00	89.00	12.83	8.17	6.23	9.08
Manava	94.00	78.00	27.00	66.33	12.35	6.17	3.54	7.35
Martı	97.00	82.00	85.00	88.00	11.03	7.67	6.57	8.42
Meriç	96.00	87.00	82.67	88.56	10.19	6.17	5.40	7.25
Olgun	96.00	67.00	57.00	73.33	9.20	6.83	7.20	7.74
Oliver	92.00	74.00	88.00	84.67	8.19	6.00	6.27	6.82
Orza 96	96.00	82.67	26.67	68.44	7.22	5.83	3.92	5.66
Özdemir-05	95.00	76.00	49.33	73.44	7.77	6.60	4.87	6.41
Özen	92.00	87.33	53.33	77.56	9.01	5.87	3.63	6.17
Premium	94.00	94.67	4.00	64.22	8.51	5.87	2.17	5.52
Ramata	95.00	96.00	21.33	70.78	8.28	7.33	3.41	6.34
Samyeli	96.00	85.33	6.67	62.67	7.64	5.47	3.06	5.39

Table 3. (continued)

Sancak	94.00	61.33	32.00	62.44	7.97	6.40	4.73	6.37
Scarpia	94.00	80.00	10.67	61.56	6.57	6.40	4.71	5.89
Seymen	89.33	77.33	6.67	57.78	9.37	6.00	3.25	6.21
Sladoran	94.00	81.33	2.00	59.11	6.97	5.57	0.63	4.39
Sultan	100.00	93.33	33.33	75.56	6.53	5.17	4.40	5.37
Sur-93	94.67	88.00	25.33	69.33	7.07	7.70	4.97	6.58
Şahin-91	92.00	82.67	28.00	67.56	5.57	4.90	3.30	4.59
Tarm-92	94.67	88.00	32.00	71.56	6.70	6.07	4.50	5.76
Tokak 157/37	92.00	98.00	12.00	67.33	10.37	6.87	4.47	7.24
Toprak	94.67	88.00	21.00	67.89	6.37	5.97	4.47	5.60
Ünver	96.00	60.00	8.00	54.67	5.63	4.43	3.00	4.36
Vamıkhoca 98	89.00	31.00	16.00	45.33	8.77	6.60	3.52	6.30
Yalın	98.00	85.00	44.00	75.67	7.80	3.63	2.03	4.49
Yerçil-147	94.67	32.00	10.00	45.56	6.13	5.87	4.25	5.42
Yesevi 93	93.33	64.00	10.00	55.78	8.20	5.20	6.39	6.60
Yıldız	97.00	88.00	29.00	71.33	8.90	6.43	3.90	6.41
Zeus	94.67	93.00	16.00	67.89	6.10	5.60	3.01	4.90
Zeynel Ağa	93.33	83.00	16.00	64.11	9.87	6.27	3.94	6.69
Mean	95.66	78.10	56.88	76.88	10.21	6.16	5.27	7.22
F value (Variety)	2.99**	17.14**	41.82**	39.30**	10.3**	5.87**	14.69**	16.36**
F value (Treatment)	-	-	-	1611.12**	-	-	-	1449.43**
F value (V x T)	-	-	-	23.43**	-	-	-	6.83**
LSD (0.05)	4.10	13.71	14.11	6.67	2.21	1.29	1.19	0.94
CV (%)	3.07	12.60	17.80	10.83	15.56	15.01	16.25	16.23

F values marked with ** are significant at the probability level of 0.01.

Shoot length

The difference between the varieties and their osmotic potential treatment was significant in terms of shoot length, and the "variety × treatment" interaction was important due to the different reactions of the varieties to osmotic potential treatment (Table 4). In the control treatment, the shoot lengths of the varieties varied between 8.92 and 17.31 cm. The Kırıl-97 variety had the longest shoot, while the Özen variety had the shortest. In this study, the shoot lengths of the varieties with an osmotic potential of -4 bar ranged between 2.88 and 10.20 cm. The Bolayır variety had the longest shoot, while the Yalın variety had the shortest. The shoot lengths of the varieties with an osmotic potential of -6 bar ranged between 0.50 and 9.17 cm. The Durusu variety had the longest shoot length, while the Sladoran variety had the shortest shoot length (Table 4). In our study, the shoot length decreased as the osmotic potential increased. The average shoot length, which was 12.64 cm in the control treatment, decreased to 6.04 cm at -4 bar osmotic potential and to 4.31 cm at -6 bar osmotic potential. Szira et al. (2008) reported that osmotic stress caused a decrease in shoot length and that the average shoot length was 28.89 cm in the control treatment and 18.16 cm in the stress treatment. Balkan & Genctan (2013) reported that the average shoot length, which was 136.76 mm in the control treatment, decreased to 114.92 mm at -0.5 MPa and reached -1.0 MPa, but there was no seedling development. Karami & Sepehri (2017) reported that the shoot length was 10.22 cm, 8.0 cm at -0.3 bar osmotic potential and 6.39 cm at -0.6 bar osmotic potential in the control treatment and that the drought created by PEG 6000 caused a decrease in shoot length in barley. Ozturk et al. (2016) who measured the shoot length of bread wheat genotypes under osmotic stress, reported that the shoot length ranged between 7.99-19.55 cm in the control treatment and 0.07-4.73 cm at -5 bar osmotic potential according to the genotype.

Seed vigor index

The difference between the varieties and their osmotic potential treatments was significant in terms of the seed vigor index, and the "variety × treatment" interaction was important due to the different reactions of the varieties to the osmotic potential treatments (Table 4). In the control treatment, the seed vigor indices of the varieties ranged from 1520.6 to 3198.0. The Kırıl-97 variety had the highest seed vigor index, while the Şahin-91 variety had the lowest seed vigor index. The seed vigor indices of the varieties with an osmotic potential of -4 bar ranged from 202.5 to 1923.3. The Bolayır variety had the highest seed vigor index, while the Akhisar 98 variety had the lowest seed vigor index. In this study, the seed vigor indices of the varieties with an osmotic potential of -6 bar ranged from 9.0 to 1623.9. The Durusu variety had the highest seed vigor index, while the Sladoran variety had the lowest seed vigor index (Table 4). In our study, the seed vigor index decreased as the osmotic potential increased. The average seed vigor index, which was 2186.5 in the control treatment, decreased to 984.0 at -4 bar osmotic potential

and to 648.7 at -6 bar osmotic potential. Dhanda et al. (2004) reported that the seed vigor index varies between 1313.1-3400.3 under normal conditions and between 146.2-585.6 under arid conditions to determine the differences in various characteristics of wheat under osmotic stress conditions and the interactions between them. Karami & Sepehri (2017) reported that the seed vigor index was 1558.10 in the control treatment, 1154.21 at an osmotic potential of -0.3 bar, and 802.59 at an osmotic potential of -0.6 bar and that the drought created by PEG 6000 caused a decrease in the seed vigor index in barley. Ozturk et al. (2016), who measured the seed vigor index of bread wheat genotypes under osmotic stress, reported that the seed vigor index of the genotypes ranged between 2331.1-5028.2 in the control treatment and 210.1-1666.8 at an osmotic potential of -5 bar.

Table 4. Shoot Lengths and Seed Vigor Indices and Variance Analysis Results of Barley Varieties According to Different Osmotic Potential Treatments

Cultivars	Shoot Length				Seed Vigor Index			
	Control	-4 Bar	-6 Bar	Mean	Control	-4 Bar	-6 Bar	Mean
Akar	11.44	6.73	6.13	8.10	2368.4	1303.4	1162.7	1611.5
Akdane	14.22	7.05	6.33	9.20	2448.8	1425.9	1228.7	1701.2
Akhisar 98	15.47	4.45	6.65	8.86	2584.3	202.5	108.9	965.2
Altıkat	12.67	5.95	6.10	8.24	2108.1	998.7	1155.2	1420.7
Arcanda	13.11	7.60	7.70	9.47	2454.7	1386.7	1455.0	1765.4
Atılır	12.57	3.68	4.37	6.87	2150.4	569.3	747.2	1155.6
Avcı-2002	12.70	8.83	9.03	10.19	2236.8	1442.6	1543.4	1740.9
Aydanhanım	13.51	5.36	4.73	7.87	2441.5	1001.5	789.3	1410.8
Balkan 96 (Igri)	11.87	9.87	8.05	9.93	2073.3	1712.7	1448.6	1744.9
Barış	14.15	8.09	6.30	9.51	2400.7	1506.6	1172.2	1693.2
Başgül	13.35	5.65	4.21	7.74	2322.6	1203.1	671.4	1399.0
Bayrak	13.73	5.25	5.47	8.15	2715.5	209.0	609.7	1178.0
Beyşehir	11.35	8.07	6.17	8.53	2366.3	1381.2	1134.6	1627.4
Bilgi-91	10.47	6.03	7.40	7.96	2366.0	1035.3	1343.7	1581.6
Bolayır	9.60	10.20	7.97	9.26	1848.9	1923.3	1485.1	1752.4
Burakbey	11.51	6.25	6.37	8.04	2174.7	1102.5	949.2	1408.8
Bülbül 89	12.61	7.01	6.20	8.61	2253.0	1178.5	1036.1	1489.2
Cervoise	14.41	7.49	8.27	10.06	2185.6	1368.8	1208.6	1587.7
Clarica	14.84	5.72	6.77	9.11	2667.7	1249.2	1185.6	1700.8
Cumhuriyet 50	12.89	7.68	7.47	9.34	2348.9	1429.0	1206.3	1661.4
Çatalhöyük 2001	14.09	7.91	8.90	10.30	2532.1	1456.2	1615.2	1867.8
Çetin 2000	12.99	8.42	7.50	9.64	2340.4	1544.9	1396.7	1760.7
Çıldır 02	12.65	8.53	7.03	9.40	2522.0	1525.0	1243.7	1763.6
Çumra 2001	12.85	7.68	8.47	9.66	2058.2	1182.0	1321.8	1520.7
Durusu	13.07	9.40	9.17	10.54	2276.0	1628.8	1623.9	1842.9
Efes 98	13.53	6.25	3.60	7.80	2515.2	927.6	642.6	1361.8
Emon	13.46	3.74	3.91	7.04	2523.7	513.6	635.9	1224.4
Erciyes	13.99	6.99	1.90	7.63	2658.7	455.4	93.0	1069.0
Erginel 90	10.93	6.62	5.63	7.73	1937.4	971.4	1095.7	1334.8
Escadre	12.20	5.75	3.95	7.30	2681.1	1032.0	558.8	1424.0
Fahrettinbey	11.01	7.31	4.07	7.46	1668.8	1264.7	807.2	1246.9
Fırat	11.68	4.83	4.23	6.91	2260.9	525.5	753.7	1180.0
Gazda	9.72	5.22	2.97	5.97	1811.4	654.5	461.0	975.7
Harman	12.09	4.40	4.90	7.13	2347.7	702.3	948.4	1332.8
Hasat	11.54	6.33	5.93	7.94	2120.0	666.4	874.2	1220.2
Hilal	9.01	3.63	2.17	4.94	1695.2	326.1	30.0	683.8
İnce-04	11.50	3.31	4.67	6.49	2185.0	498.4	889.4	1190.9
Kalaycı-97	12.52	5.23	2.30	6.68	2328.0	812.8	116.3	1085.7
Karatay 94	13.97	3.73	2.60	6.77	2736.6	474.0	526.0	1245.5
Kendal	13.95	4.23	2.33	6.84	2709.2	609.9	380.6	1233.2
Keser	14.09	4.87	4.13	7.70	2705.9	722.9	619.1	1349.3
Kıral-97	17.31	7.57	3.93	9.61	3198.0	1202.6	584.5	1661.7
Konevi	11.21	5.20	5.53	7.32	2198.1	764.7	1003.0	1321.9
Larende	11.93	5.79	2.33	6.69	2290.3	1127.2	380.6	1266.1
Lord	12.83	4.66	4.77	7.42	2489.5	1046.6	967.5	1501.2
Manava	12.35	4.29	2.23	6.29	2319.5	815.8	160.0	1098.4

Table 4. (continued)

Martı	14.47	6.83	3.93	8.41	2471.3	1190.8	895.6	1519.2
Meriç	10.19	5.74	3.90	6.61	1957.1	1036.3	771.4	1254.9
Olgun	11.54	5.52	4.80	7.29	1991.0	828.4	713.7	1177.7
Oliver	10.81	5.59	4.75	7.05	1747.3	857.1	970.9	1191.7
Orza 96	10.75	5.20	2.03	6.00	1722.7	917.8	172.2	937.6
Özdemir-05	9.53	5.50	2.53	5.86	1643.9	923.1	383.5	983.5
Özen	8.92	5.73	2.40	5.68	1648.8	1013.2	322.2	994.7
Premium	11.55	6.57	1.50	6.54	1886.7	1176.8	21.7	1028.4
Ramata	12.77	7.10	2.22	7.36	2000.3	1389.6	129.8	1173.2
Samyeli	12.52	4.77	1.00	6.10	1932.1	876.7	37.3	948.7
Sancak	13.92	6.63	2.40	7.65	2056.4	805.2	232.1	1031.2
Scarpia	12.93	6.03	2.33	7.10	1831.2	997.9	72.8	967.3
Seymen	14.17	5.53	1.58	7.09	2102.4	891.5	42.1	1012.0
Sladoran	15.60	5.75	0.50	7.28	2122.0	924.1	9.0	1018.4
Sultan	13.20	6.93	3.63	7.92	1973.3	1133.5	268.9	1125.3
Sur-93	12.37	5.57	2.62	6.85	1832.7	1167.1	186.1	1062.0
Şahin-91	10.93	6.57	2.53	6.68	1520.6	958.6	164.9	881.4
Tarm-92	14.57	5.20	2.13	7.30	2011.9	995.9	212.3	1073.3
Tokak 157/37	15.79	6.63	2.07	8.16	2408.2	1323.2	80.1	1270.5
Toprak	14.17	5.90	2.30	7.46	1947.7	1044.3	142.6	1044.9
Ünver	13.03	3.17	1.75	5.98	1792.0	458.6	38.0	762.9
Vamıkhoca 98	13.43	7.82	1.90	7.72	1969.0	449.8	86.2	835.0
Yalın	12.70	2.88	1.83	5.80	2008.6	403.2	170.6	860.8
Yerçil-147	12.83	4.73	2.50	6.69	1796.9	353.9	66.3	739.0
Yesevi 93	12.63	3.70	3.44	6.59	1942.3	570.0	99.5	870.6
Yıldız	11.70	5.10	1.75	6.18	1995.6	1019.5	163.8	1059.6
Zeus	12.17	4.73	1.84	6.25	1729.6	962.1	77.4	923.0
Zeynel Ağa	13.01	6.60	2.23	7.28	2137.6	1068.7	100.2	1102.2
Mean	12.64	6.04	4.31	7.66	2186.5	984.0	648.7	1273.1
F value (Variety)	5.55**	9.00**	27.51**	15.84**	6.62**	16.45	39.13**	26.59**
F value (Treatment)	-	-	-	4636.18**	-	-	-	4669.70**
F value (V x T)	-	-	-	8.68**	-	-	-	10.59**
LSD (0.05)	1.89	1.47	1.21	0.89	356.1	255.7	222.1	163.2
CV (%)	10.72	17.41	20.14	14.48	11.69	18.64	24.57	15.99

F values marked with ** are significant at the probability level of 0.01.

Correlations between early drought selection criteria

The correlation coefficients between the early selection criteria are given in Table 5. According to the correlation table, there was a positive and significant correlation between coleoptile length and germination rate ($r=0.261^*$), shoot length ($r=0.246^*$) and seed vigor index ($r=0.252^*$); a positive and highly significant correlation between specific leaf area and cell membrane damage ($r=0.367^{**}$); a negative and highly significant correlation between specific leaf area and shoot length ($r=-0.345^{**}$); a negative and significant correlation between specific leaf area and seed vigor index ($r=-0.278^*$); a positive and significant correlation between first leaf width and germination rate ($r=0.263^*$), root length ($r=0.228^*$) and seed vigor index ($r=0.278^*$); a positive and highly significant correlation between first leaf width and shoot length ($r=0.325^{**}$); a negative and highly significant correlation between cell membrane damage and germination rate ($r=-0.410^{**}$), root length ($r=-0.387^{**}$), shoot length ($r=-0.547^{**}$) and the seed vigor index ($r=-0.507^{**}$); a positive and highly significant correlation between germination rate and root length ($r=0.717^{**}$), shoot length ($r=0.807^{**}$) and the seed vigor index ($r=0.943^{**}$); a positive and highly significant correlation between root length and shoot length ($r=0.849^{**}$) and the seed vigor index ($r=0.797^{**}$); a positive and highly significant correlation between shoot length and the seed vigor index ($r=0.932^{**}$).

Table 5. Simple Correlation Coefficients between Early Drought Selection Criteria

	SSAD	CL	SLA	LW	CMD	GR	RL	SL
CL	0.071							
SLA	-0.167	-0.197						
LW	0.054	0.017	-0.108					
CMD	-0.027	-0.201	0.367**	-0.049				
GR	0.197	0.261*	-0.174	0.263*	-0.410**			
RL	0.123	0.157	-0.189	0.228*	-0.387**	0.717**		
SL	0.162	0.246*	-0.345**	0.325**	-0.547**	0.807**	0.849**	
SVI	0.200	0.252*	-0.278*	0.278*	-0.507**	0.943**	0.797**	0.932**

SSAD Seedling survival after drought, CL Coleoptile length, SLA specific leaf area, LW first leaf width, CMD cell membrane damage, GR germination rate at -6 bar, RL root length at -6 bar, SL -6 bar shoot length, SVI: Seed vigor index at -6 bar

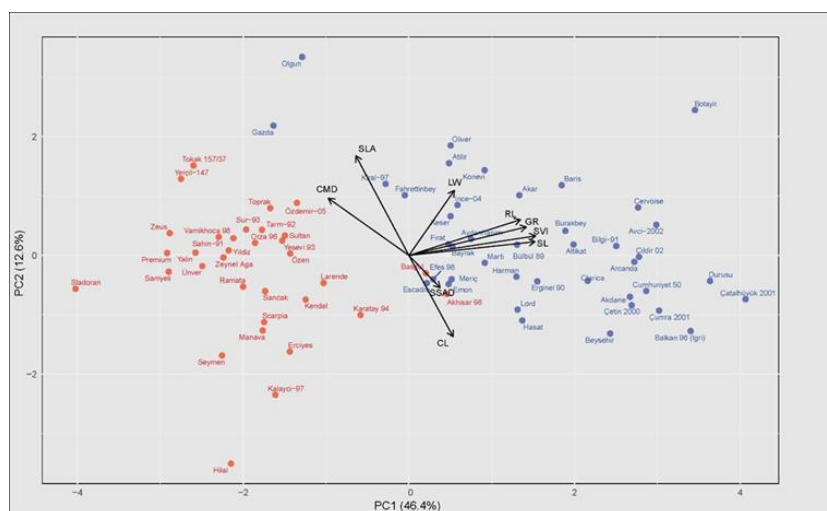
Grouping of Selection Criteria and Barley Varieties by Principal Component Analysis

The seed vigor index, shoot length, root length and germination rate selection criteria under early drought conditions were explained by principal component analysis; 46.4% of the total variance was explained by the first basic component, 59.1% of the first two basic components, and 70.2% of the first three basic components (Table 6). The highest loads for the first basic component are determined in the SVI, SL, GR and RL criteria respectively. The SVI, SL, GR and RL criteria were far from the origin of the first basic component, in the positive region and have a high correlation. They were negatively related to the SLA and CMD criteria (Figure 1). Cervoise, Avcı-2002, Bilgi-91 and Bolayır varieties, which are located in the far and positive region of origin in terms of the SVI, GR, SL and RL criteria, can be considered resistant in terms of these criteria (Figure 1). The highest loads in terms of the second component are determined in the criteria of SLA, LW, CMD, RL, GR, SVI and SL (Table 6).

Table 6. Eigenvalues of Principal Components Obtained from Early Drought Conditions Analysis Results and Selection Criteria

Selection Criteria	Principal Components					
	1	2	3	4	5	6
SSAD	0.116	-0.194	0.863	0.342	-0.140	-0.239
CL	0.164	-0.488	-0.107	0.209	0.815	-0.041
SLA	-0.197	0.599	-0.159	0.512	0.207	-0.500
LW	0.169	0.390	0.330	-0.691	0.400	-0.254
CMD	-0.299	0.343	0.316	0.163	0.313	0.739
GR	0.436	0.171	-0.008	0.203	0.057	0.050
RL	0.415	0.215	-0.082	0.130	-0.090	0.259
SL	0.468	0.082	-0.051	-0.012	-0.054	0.085
SVI	0.471	0.117	-0.028	0.118	-0.028	0.066
Eigen value	4.1778	1.1348	1.0029	0.8981	0.8452	0.5173
Variance (%)	46.4	12.6	11.1	10.0	9.4	5.8
Total variance (%)	46.4	59.1	70.2	80.2	89.5	95.3

SSAD Seedling survival after drought, CL Coleoptile length, SLA specific leaf area, LW first leaf width, CMD cell membrane damage, GR germination rate at -6 bar, RL root length at -6 bar, SL -6 bar shoot length, SVI: Seed vigor index at -6 bar

**Figure 1.** Biplot analysis of selection criteria (A) and cultivars (B) based on early drought conditions averages

The mean rank and standard deviation of the mean rank and rank sum values of the 74 barley varieties used in this study, calculated over the six selection criteria listed in Table 7. The rank sum values of the varieties varied between 17.8 and 62.8. These values were divided into four groups according to the scale in Table 6: resistant, medium resistant, medium sensitive and sensitive. Accordingly, the Konevi, İnce-04 and Fahrettinbey varieties were included in the resistant group of 26 varieties of medium resistant, with a rank total ranging from 30.1-41.0. Thirty-two variety with rank totals ranging from 41.1 to 52.1 were determined to be moderately sensitive, and 13 varieties with rank totals ranging from 52.2 to 62.8 were determined to be sensitive.

Table 7. Mean Rank, Standard Deviation of the Average Rank and Rank Sum Values of Barley Varieties and Drought Resistance Groups According to Rank Totals

Cultivars	Rank means	Standard deviation	Rank Sum	Resistant (17,8-29,0)	Medium resistant (29,1-40,3)	Medium sensitive (40,4-51,6)	Sensitive (51,7-62,8)
Akar	21.2	15.0	36.2	Konevi	Oliver	Altıkat	Ünver
Akdane	19.1	19.7	38.8	İnce-04	Erginel 90	Şahin-91	Kalaycı-97
Akhisar 98	32.4	22.1	54.6	Fahrettinbey	Çıldır 02	Çatalhüyük 2001	Manava
Altıkat	23.9	17.2	41.1		Olgun	Özdemir-05	Vamıkhoca 98
Arcanda	23.3	21.4	44.8		Fırat	Kıral-97	Yerçil-147
Atılır	24.3	18.7	43.0		Keser	Toprak	Akhisar 98
Avcı-2002	22.9	21.7	44.6		Martı	Sancak	Yesevi 93
Aydanhanım	23.8	16.2	40.0		Meriç	Atılır	Scarpia
Balkan 96 (Igri)	18.2	22.7	40.9		Bülbül 89	Lord	Hilal
Barış	24.4	21.1	45.5		Larende	Avcı-2002	Samyeli
Başgül	24.1	13.7	37.8		Akar	Bayrak	Seymen
Bayrak	28.8	15.9	44.7		Burakbey	Arcanda	Sladoran
Beyşehir	23.6	23.2	46.7		Kendal	Emon	Premium
Bilgi-91	25.3	21.8	47.1		Escadre	Cumhuriyet 50	
Bolayır	17.4	21.9	39.4		Başgül	Clarica	
Burakbey	21.7	15.1	36.8		Karatay 94	Barış	
Bülbül 89	22.7	13.1	35.8		Sultan	Orza 96	
Cervoise	24.1	23.1	47.2		Efes 98	Harman	
Clarica	25.2	20.3	45.5		Durusu	Yalın	
Cumhuriyet 50	22.4	23.0	45.5		Akdane	Sur-93	
Çatalhüyük 2001	16.8	25.0	41.8		Bolayır	Beyşehir	
Çetin 2000	26.2	24.6	50.8		Özen	Bilgi-91	
Çıldır 02	16.3	15.6	31.9		Aydanhanım	Cervoise	
Çumra 2001	24.1	24.6	48.7		Hasat	Tarm-92	
Durusu	17.4	21.2	38.7		Gazda	Çumra 2001	
Efes 98	21.8	16.9	38.6		Balkan 96 (Igri)	Ramata	
Emon	29.1	16.3	45.4			Tokak 157/37	
Erciyes	31.1	19.1	50.3			Zeynel Ağa	
Erginel 90	19.7	11.1	30.8			Erciyes	
Escadre	26.3	11.3	37.7			Çetin 2000	
Fahrettinbey	17.0	8.2	25.2			Yıldız	
Fırat	23.9	10.3	34.2			Zeus	
Gazda	24.0	16.8	40.8				
Harman	28.0	17.8	45.8				
Hasat	23.4	16.8	40.2				
Hilal	32.6	24.1	56.6				
İnce-04	17.8	5.1	22.8				
Kalaycı-97	33.9	19.0	52.9				
Karatay 94	27.0	10.8	37.8				
Kendal	28.6	9.1	37.6				
Keser	23.3	10.9	34.2				
Kıral-97	26.8	15.6	42.4				
Konevi	11.4	6.3	17.8				
Larende	27.6	8.4	36.0				
Lord	27.1	16.7	43.9				
Manava	36.3	17.1	53.5				
Martı	22.8	11.7	34.5				
Meriç	23.7	11.9	35.5				
Olgun	18.8	14.9	33.7				
Oliver	18.2	12.0	30.2				
Orza 96	30.0	15.7	45.7				
Özdemir-05	29.3	12.9	42.2				

Table 7. (continued)

Özen	30.3	9.6	39.9
Premium	40.4	22.4	62.8
Ramata	31.6	17.3	48.8
Samyeli	40.9	18.4	59.3
Sancak	29.9	12.8	42.7
Scarpia	36.9	19.6	56.5
Seymen	40.9	18.6	59.5
Sladoran	41.0	19.2	60.2
Sultan	27.9	10.1	38.0
Sur-93	34.1	12.1	46.2
Şahin-91	29.0	12.5	41.5
Tarm-92	29.1	18.9	48.0
Tokak 157/37	31.3	17.7	49.0
Toprak	27.8	14.8	42.6
Ünver	35.1	17.5	52.6
Vamıkhoca 98	36.8	16.7	53.5
Yalın	33.6	12.6	46.2
Yerçil-147	31.8	22.5	54.3
Yesevi 93	33.1	23.0	56.1
Yıldız	31.7	19.2	50.8
Zeus	31.8	20.3	52.1
Zeynel Ağa	32.3	17.0	49.4

4. CONCLUSION

Drought is the most important abiotic stress that limits agricultural production and significantly threatens the food supply worldwide. Drought resistance is a complex trait that is a common function of numerous morphological, physiological and biochemical characteristics. Drought-tolerant varieties are defined as those that can produce relatively high yields under drought conditions. The 74 barley varieties used in this study were divided into four groups, resistant, medium resistant, medium sensitive and sensitive, according to the rank total values calculated over 6 selection criteria. Accordingly, the Konevi, İnce-04 and Fahrettinbey varieties were determined to be resistant to early drought, 26 variety of medium hardiness with a rank total ranging from 29.1 to 40.3 were detected, 32 varieties with a rank total between 40.4 and 51.6 were determined to be moderately sensitive, and 13 variety with a rank total between 51.7 and 62.8 were determined to be sensitive. The Konevi, İnce-04 and Fahrettinbey varieties can be used as parents in drought resistance breeding and may also provide advantages in areas where early drought occurs.

ACKNOWLEDGEMENT

This article was produced from the doctoral thesis titled "Adaptation of Barley Varieties to Erzurum Dry Agricultural Conditions, Characterization and Genetic Diversity in Terms of Early Drought Resistance" in the Department of Field Crops, Institute of Science and Technology, Atatürk University, under the supervision of Prof. Dr. Kamil HALILOĞLU.

DISCLOSURE STATEMENT

The authors declare that they have no known competing personal relationships that could have appeared to influence the work reported in this paper.

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Transferability of Genomic SSR Markers in *Lens* Species for Genus Characterization and Lentil (*Lens culinaris* Medik.) Breeding

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ARTICLE INFO

Research Article

Received: 20 January 2025

Accepted: 13 June 2025

Published: 23 June 2025

Keywords:

Crop improvement

Genetic diversity

Lentil

SSR

Transferability

ABSTRACT

Simple sequence repeats (SSRs) are pivotal for germplasm characterization and crop improvement. However, lentil breeding faces challenges due to the limited availability of markers. This study assessed the transferability of genomic SSR markers developed in cultivated lentils to wild *Lens* species. A total of 100 SSR loci were analyzed in 21 accessions representing all the *Lens* genus using PowerMarker software v3.25, which identified 41 polymorphic loci. These loci generated 219 alleles, with an average of 5.34 per locus and a polymorphic information content (PIC) value of 0.61. Notably, 52.2% of the polymorphic loci were shared among all *Lens* species. Transferability rates varied across species: 41% for *Lens nigricans*, 40% for *Lens culinaris* subsp. *tomentosus*, 39% for *Lens culinaris* subsp. *orientalis*, 34% for *Lens culinaris* subsp. *odemensis*, 34% for *Lens ervoides*, and 31% for *Lens lamottei*. The identified polymorphic markers provide a valuable resource for exploring the genetic diversity of wild lentil relatives and the breeding of beneficial agronomic traits into new lentil cultivars.

Citation: Koboyi, B.W., Keklik, M., Doganay, O., & Bakır, M. (2025). Transferability of Genomic SSR Markers in *Lens* Species for Genus Characterization and Lentil (*Lens culinaris* Medik.) Breeding. *Turkish Journal of Field Crops*, 30(1), 223-234. <https://doi.org/10.17557/tjfc.1623413>

1. INTRODUCTION

Lentil (*Lens culinaris* Medik.) is a widely cultivated legume crop, classified within the galegoid clade of the *Fabaceae* (*Leguminosae*), which is the third largest plant family (Konda & Annapragada, 2024). This autogamous diploid legume, with a chromosome number of $2n = 14$ and a large genome of approximately 4 Gbp (Bett & Cook, 2016; Ates, 2019), is cultivated over 5.01 million hectares worldwide, yielding around 6.53 million tons annually. The leading producers include Canada, India, Australia, Türkiye, the USA, and Nepal (FAOSTAT 2023). Besides its role in soil fertility management by nitrogen fixation, lentil is an essential, affordable dietary staple. It is rich in carbohydrates (63.4%), protein (24.5%), essential minerals, vitamins, and dietary fiber, which is also beneficial for livestock nutrition (Dhull et al., 2022).

Despite ongoing debates regarding the classification of the *Lens* genus, the widely accepted taxonomy includes four species: *L. culinaris*, with its four subspecies (subsp. *culinaris*, subsp. *orientalis*, subsp. *odemensis*, and subsp. *tomentosus*), *L. lamottei*, *L. ervoides*, and *L. nigricans* (Wong et al., 2015; Koul et al., 2017). Phylogenetic analysis using Genotyping-by-Sequencing (GBS) has further grouped these species into distinct genetic pools. The primary gene pool encompasses *L. culinaris*, *L. orientalis*, and *L. tomentosus*; the secondary pool includes *L. lamottei* and *L. odemensis*, while the tertiary and quaternary pools consist of *L. ervoides* and *L. nigricans*, respectively. Among these, *L. culinaris* subsp. *orientalis* is considered the putative progenitor of cultivated lentils, whereas *L. nigricans* represents the most distant relative (Wong et al., 2015; Koul et al., 2017; Dissanayake et al., 2020; Liber et al., 2021).

The domestication of lentils that dates back to the Pre-Pottery Neolithic resulted in the co-occurrence of most *Lens* wild relatives in Southwestern Türkiye, the Mediterranean, and the Aegean regions (Ferguson et al., 1998). Although domestication contributed to the acquisition and/or loss of novel genes due to varying environmental stimuli, global warming has exacerbated the negative effects of stress on lentil cultivation. This trend has prompted the development of innovations to enhance yields on farmlands to counteract food scarcity (Caracuta et al., 2017).

Molecular markers and characterization of wild gene pools with extensive genetic variability are crucial to introduce target genes into cultivated lentil varieties. Precise identification of wild genetic resources increases the success of gene introgression into crops, and marker-assisted selection (MAS) using DNA markers such as simple sequence repeats (SSRs) can improve the efficiency of lentil breeding (Rubiales & Fondevilla, 2012; Dissanayake et al., 2020; Koboyi & Bakir, 2024). Simple Sequence Repeats (SSRs) are widely recognized as ideal molecular markers due to their utility in mapping and genetic diversity analyses (Saidou et al., 2016). Their effectiveness has been demonstrated across various legumes, including lentil (Gutierrez-Gonzalez et al., 2022; Sharma et al., 2023), marama bean (Li, 2023), chickpea (Ningwal et al., 2023), mungbean (Mehandi et al., 2023), peanut (Daudi et al., 2021), Medicago (Zhao et al., 2024), and common bean (Ozkan et al., 2022). Although the development of genomic SSRs requires significant cost and labor, their numerous advantages include widespread occurrence throughout the genome, high polymorphism, locus specificity, co-dominant multi-allelic nature, high information content, reproducibility, and transferability (Cevik et al., 2015; Begna & Yesuf, 2021).

The conserved flanking regions of SSRs across related species or genera enable the assessment of their polymorphic potential and transferability, which is crucial for understanding phylogeny and genetic diversity (Datta et al., 2013). This transferability has been observed within the same legume species, as seen in *Lens* (Dikshit et al., 2015) and *Cicer* (Choudhary et al., 2009), as well as across species (Reddy et al., 2010; Datta et al., 2010; Gupta et al., 2012; Jingade et al., 2014; Verma et al., 2014; Bakir, 2019; Singh et al., 2020). Although relatively few sets of genomic SSR markers have been developed in lentil specifically, their polymorphism offers an invaluable resource for crop improvement. These markers hold significant potential for enhancing lentil breeding programs through marker-assisted selection. (Hamwieh et al., 2009; Verma et al., 2014; Andeden et al., 2015; Bakir & Kahraman, 2019; Demir & Bakir, 2022; Bakir et al., 2024).

This study aims to evaluate the transferability of genomic SSR markers developed in cultivated lentil to its wild relatives. The transferable SSR markers identified in this study will be invaluable for advancing lentil molecular research programs, providing a robust tool for understanding genetic variation, improving marker-assisted selection (MAS) strategies, and accelerating the development of enhanced lentil varieties. Furthermore, this research expands the limited genomic resources available for lentil improvement by uncovering additional genetic diversity within the *Lens* genus. It facilitates the identification of both intra- and interspecies candidate genes for future breeding efforts.

2. MATERIALS AND METHODS

Plant Materials and DNA Extraction

The plant material comprised 18 accessions of wild lentil species and subspecies obtained from Akdeniz University, Faculty of Agriculture, Department of Field Crops, and three cultivated cultivars of *L. culinaris* subsp. *culinaris* sourced from the GAP International Agricultural Research Center (Table 1). The seeds were grown in a growth chamber for three weeks at the Erciyes University Genome and Stem Cell Center, Türkiye, and the five pooled seedlings were used for DNA extraction. The genomic DNA isolation from the 21 samples was done using the CTAB method protocol of Lefort et al. (1998). The quantification and determination of the quality of the extracted DNA were performed using the NanoDrop®ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and agarose gel electrophoresis (1%).

Table 1. List of the 21 genotypes used in the Transferability Analysis

No	Species	Accession number	Origin
1	<i>L. nigricans</i>	IG72545	ICARDA-Syria
2	<i>L. nigricans</i>	IG136636	ICARDA-Syria
3	<i>L. nigricans</i>	IG136639	ICARDA-Syria
4	<i>L. lamottei</i>	IG116009	ICARDA-Syria
5	<i>L. lamottei</i>	IG110812	ICARDA-Syria
6	<i>L. lamottei</i>	IG110811	ICARDA-Syria
7	<i>L. ervoides</i>	IG72862	ICARDA-Syria
8	<i>L. ervoides</i>	IG72914	ICARDA-Syria
9	<i>L. ervoides</i>	IG72920	ICARDA-Syria
10	<i>L. culinaris</i> subsp. <i>tomentosus</i>	IG72644	ICARDA-Syria
11	<i>L. culinaris</i> subsp. <i>tomentosus</i>	IG72830	ICARDA-Syria
12	<i>L. culinaris</i> subsp. <i>tomentosus</i>	IG72643	ICARDA-Syria
13	<i>L. culinaris</i> subsp. <i>orientalis</i>	IG135399	ICARDA-Syria
14	<i>L. culinaris</i> subsp. <i>orientalis</i>	IG136679	ICARDA-Syria
15	<i>L. culinaris</i> subsp. <i>orientalis</i>	IG136670	ICARDA-Syria
16	<i>L. culinaris</i> subsp. <i>odemensis</i>	IG116047	ICARDA-Syria
17	<i>L. culinaris</i> subsp. <i>odemensis</i>	IG136653	ICARDA-Syria
18	<i>L. culinaris</i> subsp. <i>odemensis</i>	IG116049	ICARDA-Syria
19	<i>L. culinaris</i> subsp. <i>culinaris</i>	Firat-87	GAPUTEM-Turkiye
20	<i>L. culinaris</i> subsp. <i>culinaris</i>	Tigris	GAPUTEM-Turkiye
21	<i>L. culinaris</i> subsp. <i>culinaris</i>	Seyran-96	GAPUTEM-Turkiye

SSR Amplification and Genetic Diversity Analysis

One hundred genomic SSRs were developed by Bakir and Kahraman (2019), Demir and Bakir (2022), and Bakir et al. (2024), and were used for amplification. This validation was performed in a total reaction volume of 15 µl, comprising 200 ng of genomic DNA, 1X DreamTaq Green Buffer (includes 2 mM of MgCl) (Thermo Scientific, Waltham, MA, USA), dNTPs (0.2 mM of each), 10 µM of each forward and reverse primer, DreamTaq DNA polymerase (0.5 u) (Thermo Scientific, Waltham, USA), and PCR water. The reactions were performed in a Bio-Rad MyCycler US device by an initial step of 3 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 50-66 °C, 2 min at 72°C, and a final extension of 10 min at 72°C. The amplified fragments were electrophoresed in 4% MetaPhor™ agarose for 4 hours and visualized in the Gel-Documentation Unit (Bio-Rad, Hercules, CA, USA). Markers exhibiting clear bands in the 100-500 bp range were selected. Genetic diversity analysis according to Scheulke (2000) to ascertain polymorphism in the 21 genotypes using an M13 tailed primer (M13 universal sequence (– 21), TGT AAA ACG ACG GCC AGT) attached to the 5' of the forward primers and tagged with fluorophores ROX, HEX, or 6-FAM at the 5' end to enable multiplexing was performed in a Bio-Rad Mycycler US device. The final reaction mixture of 15 µl consisted of 15 ng of genomic DNA, 1X DreamTaq Green Buffer (includes two mM of MgCl₂) (Thermo Scientific, Waltham, MA, USA), dNTPs (0.2 mM of each), 0.1 µM of each primer (forward and reverse), M13 labeled (-21) universal primer of 0.1 µM, DreamTaq DNA polymerase (Thermo, 0.5 U - Thermo Scientific, Waltham, MA, USA), and PCR water. The amplification program consisted of an initial step of 3 min at 94°C, 35 cycles of 1 min at 94°C and 1 min at 50-66 °C, 2 min at 72 °C, followed by eight cycles of 1 min at 94°C, 1 min at 53°C, 2 min at 72°C, and a final extension of 10 min at 72°C. The amplicons were grouped in threes in a ratio of 1:1:2 for HEX, 6-FAM, or ROX fluorescent dyes, respectively, in a mixture with 0.5 µl GeneScan-600 LIZ size standards (Applied Bio-systems, Foster City, CA, USA) and 9.5 µl Hi-DiTM formamide (Applied Bio-systems, Foster City, CA, USA). The products were denatured at 95 °C for 5 min, immediately chilled on ice, and placed in an ABI

PRISM® 3500 Genetic Analyzer (Applied Bio-systems, Foster City, CA, USA) for capillary electrophoresis. The GENEMAPPER software v5.0 (Applied Bio-systems, Foster City, CA, USA) was used for fragment size detection.

Data Analysis

Genetic diversity analysis was carried out at the locus level for each species. Statistical analysis was implemented using PowerMarker software V3.25 (Liu and Muse, 2005) by calculating the genetic parameters; the polymorphism information content ($PIC=1-\sum p_i^2-\sum \sum 2p_i^2p_j^2$) for each SSR marker according to Botstein et al. (1980), observed heterozygosity (H_o), expected heterozygosity ($H_e=1-\sum p_i^2$) (Nei, 1973), and allele number (n) per locus. The dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) from the genetic similarity matrix by using the MEGA6 (Tamura et al., 2007), and bootstrap analyses over the marker loci were performed and a consensus tree obtained using the consensus program in the Phylip package (Felsenstein, 1993).

3. RESULTS AND DISCUSSION

Transferability of SSR Markers to Wild Lentil Species

Wild species are an invaluable resource that harbors most of the gene repertoire for resistance/tolerance to biotic and abiotic stresses. Therefore, interspecies transferability using genomic SSRs offers great opportunities to identify and utilize this diversity for crop improvement. In the current study, the inter-specific amplification to wild *Lens* species yielded 31% in *L. lamottei*, 34% in *L. ervoides*, and 34% in *L. culinaris* subsp. *odemensis*, 39% in *L. culinaris* subsp. *orientalis*, 40% in *L. culinaris* subsp. *tomentosus*, and 41% in *L. nigricans*. In comparison to the former, these rates are considerably lower. Verma et al. (2014) reported 100% amplification of genomic DNA for all the subspecies of *L. culinaris*, while cross-species amplification ranged from 80.72% in *L. ervoides* to 87.88% in *L. lamottei* for 33 polymorphic SSRs. Singh et al. (2016) also reported successful amplification in *Lens* wild species, with transferability rates of 94.82% in *L. culinaris* subsp. *orientalis*, 94.82% in *L. culinaris* subsp. *tomentosus*, 95.4% in *L. nigricans*, 96.55% in *L. ervoides*, and 98.81% in *L. culinaris* subsp. *odemensis*. A cross-transferability study by Singh et al. (2020) using 46 polymorphic markers revealed an amplification of 58% in *L. nigricans*, 60% in *L. lamottei*, 69% in *L. odemensis*, and 71% in *L. ervoides*. Compared to our results, this variation could be attributed to the type of SSRs used in these studies. EST-SSRs are known to be highly transferable compared to genomic SSRs because they originate from conserved coding regions that control specific traits (Varshney et al., 2005). High cross-species transferability of EST-SSRs has been reported in other crops such as chickpea (Choudhary et al., 2009) and peanut (Oliveira et al., 2023).

The lentil cultivars Firat-87, Seyran-96, and Tigris were positive controls in PCR reactions for all 100 tested markers. A total of 41 SSRs showed polymorphism, with 21(51%) of these being commonly amplified in all the wild species (Table 2). This could imply that these markers originate from conserved genes across wild and cultivated lentil species. In addition, the transferability of these SSRs suggests that the flanking regions of amplified SSR motifs are conserved across *Lens* spp. (Tiwari et al., 2021). Whereas 23 of the 100 markers were unamplified (23%) and 19 were registered as monomorphic (19%), 17 markers produced unspecified bands (Table 2). The polymorphism rates in the tested wild lentil species were 31% (*L. lamottei*), 34% (*L. ervoides*), 34% (*L. culinaris* subsp. *odemensis*), 39% (*L. culinaris* subsp. *orientalis*), 40% (*L. culinaris* subsp. *tomentosus*), and 41% (*L. nigricans*). These 41 SSRs detected a total of 219 alleles in 21 genotypes, with an average of 5.34 alleles per locus (Table 3). The alleles ranged from 2 to 12, with the marker Lc_MCu56 detecting the lowest allele number and the Lc_MCu70 detecting the highest. While the H_e ratio of the polymorphic markers ranged from 0.216 (Lc_MCu26) to 0.897 (Lc_MCu70) with an average of 0.657, the H_o value range was from 0.000 for multiple markers to 1.000 (Lc_MCu56) with an average of 0.140. The PIC value varied between 0.20 for Lc_MCu26 and 0.88 for Lc_MCu70, with an average value of 0.61.

Table 2. Transferability of 41 genomic SSR markers across five wild *Lens* species.

Locus	<i>L. nigricans</i>	<i>L. culinaris</i> subsp. <i>tomentosus</i>	<i>L. culinaris</i> subsp. <i>orientalis</i>	<i>L. culinaris</i> subsp. <i>odemensis</i>	<i>L. ervoides</i>	<i>L. lamottei</i>
Lc_MCu1	+	+	+	-	+	-
Lc_MCu2*	+	+	+	+	+	+
Lc_MCu4	+	+	+	-	+	-
Lc_MCu5	+	+	+	+	+	-
Lc_MCu6	+	-	+	+	-	-
Lc_MCu9*	+	+	+	+	+	+
Lc_MCu10*	+	+	+	+	+	+
Lc_MCu15*	+	+	+	+	+	+
Lc_MCu17*	+	+	+	+	+	+
Lc_MCu18*	+	+	+	+	+	+
Lc_MCu19	+	+	+	+	-	+
Lc_MCu20	+	+	+	-	+	+
Lc_MCu21	+	+	+	+	-	+
Lc_MCu24	+	+	-	+	+	+
Lc_MCu26*	+	+	+	+	+	+
Lc_MCu28*	+	+	+	+	+	+
Lc_MCu31	+	+	+	-	+	+
Lc_MCu32*	+	+	+	+	+	+
Lc_MCu35*	+	+	+	+	+	+
Lc_MCu45	+	+	+	-	+	+
Lc_MCu47*	+	+	+	+	+	+
Lc_MCu49	+	+	+	+	-	+
Lc_MCu50	+	+	+	+	-	+
Lc_MCu53*	+	+	+	+	+	+
Lc_MCu56	+	+	+	+	+	-
Lc_MCu58	+	+	+	+	-	+
Lc_MCu62*	+	+	+	+	+	+
Lc_MCu69	+	+	+	+	+	-
Lc_MCu70*	+	+	+	+	+	+
Lc_MCu74	+	+	+	-	+	-
Lc_MCu79*	+	+	+	+	+	+
Lc_MCu80	+	+	-	+	+	+
Lc_MCu81*	+	+	+	+	+	+
Lc_MCu85*	+	+	+	+	+	+
Lc_MCu87*	+	+	+	+	+	+
Lc_MCu89*	+	+	+	+	+	+
Lc_MCu91*	+	+	+	+	+	+
Lc_MCu94	+	+	+	+	-	-
Lc_MCu96*	+	+	+	+	+	+
Lc_MCu98	+	+	+	-	+	-
Lc_MCu100	+	+	+	+	+	-

The primers marked with an asterisk (*) in the table successfully amplified across all wild species

Table 3. Number of alleles (n), expected heterozygosity (*He*), observed heterozygosity (*Ho*), and polymorphic information content (PIC) values obtained after polymorphism analysis of 21 genotypes at 41 SSR loci.

Locus	n	<i>He</i>	<i>Ho</i>	PIC
Lc_MCu1	4	0.685	0.142	0.62
Lc_MCu2*	7	0.761	0.000	0.73
Lc_MCu4	4	0.685	0.142	0.62
Lc_MCu5	9	0.713	0.047	0.68
Lc_MCu6	4	0.577	0.047	0.51
Lc_MCu9*	4	0.500	0.142	0.44
Lc_MCu10*	9	0.800	0.095	0.77
Lc_MCu15*	3	0.391	0.238	0.35
Lc_MCu17*	7	0.789	0.000	0.76
Lc_MCu18*	7	0.770	0.000	0.74
Lc_MCu19	7	0.712	0.000	0.68
Lc_MCu20	4	0.693	0.000	0.64
Lc_MCu21	3	0.580	0.000	0.51
Lc_MCu24	6	0.780	0.000	0.74
Lc_MCu26*	3	0.216	0.238	0.20
Lc_MCu28*	3	0.321	0.285	0.29
Lc_MCu31	4	0.704	0.190	0.64
Lc_MCu32*	5	0.591	0.190	0.55
Lc_MCu35*	7	0.780	0.000	0.75
Lc_MCu45	8	0.784	0.523	0.75
Lc_MCu47*	6	0.693	0.000	0.65
Lc_MCu49	3	0.648	0.000	0.57
Lc_MCu50	4	0.507	0.000	0.46
Lc_MCu53*	3	0.471	0.000	0.39
Lc_MCu56	2	0.500	1.000	0.37
Lc_MCu58	3	0.571	0.190	0.48
Lc_MCu62*	4	0.607	0.000	0.56
Lc_MCu69	5	0.748	0.285	0.71
Lc_MCu70*	12	0.897	0.000	0.88
Lc_MCu74	7	0.718	0.238	0.67
Lc_MCu79*	6	0.739	0.000	0.69
Lc_MCu80	6	0.798	0.000	0.76
Lc_MCu81*	10	0.851	0.333	0.83
Lc_MCu85*	4	0.651	0.047	0.58
Lc_MCu87*	5	0.719	0.285	0.67
Lc_MCu89*	3	0.430	0.000	0.37
Lc_MCu91*	8	0.839	0.000	0.81
Lc_MCu94	5	0.647	0.285	0.60
Lc_MCu96*	5	0.729	0.428	0.68
Lc_MCu98	4	0.658	0.333	0.59
Lc_MCu100	6	0.667	0.047	0.61
Average	5.34	0.657	0.140	0.61
Total	219	26.94	5.761	25.0

*Polymorphic markers are commonly amplified in all the wild lentil species.

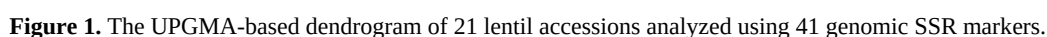
The low polymorphism of lentils among different molecular markers is mainly owed to its autogamous behavior. However, SSRs are frequently used to detect higher polymorphism because they are abundant throughout the genome, easy to detect with PCR, multi-allelic, and codominant (Saidi et al., 2022). Verma et al. (2014) reported a 100% polymorphism of all the tested loci, with an average PIC value of 0.66. Singh et al. (2020) validated 50 EST-SSRs to achieve 92% polymorphism with an average PIC value of 0.44. Out of 106 markers, 29 (27.4%) polymorphic markers were reported by Singh et al. (2016), with an average PIC value of 0.436. Our study observed a 41% polymorphic rate and an average PIC value of 0.61 from the 100 genomic SSR markers tested on wild lentil species. This result is similar to the findings by Dikshit et al. (2015), in which 75 EST-SSRs and 27 genomic SSRs were tested on 86 *Lens* genotypes to produce 42.2% polymorphism with 31 EST-SSRs (41.3%) and 12 (44.4%) genomic SSRs. Reddy et al. (2010) also reported a 43% polymorphism in the 30 lentil microsatellites tested on a wide range of genotypes, including wild subspecies. Our finding is, however, higher than 32.72% by Singh et al. (2019). Although polymorphism could be

attributed to the level of genetic variation among the tested individuals, the kind of SSRs utilized is also influential, as it has been reported that genomic SSRs are often more polymorphic than EST-SSRs. This is because genomic SSRs widely spread throughout the genome belong to the non-coding regions with greater variation in the flanking regions. On the other hand, EST-SSRs originate from conserved coding regions that control specific traits and are therefore under high selection pressure that limits the number of mutations (Varshney et al., 2005; Choudhary et al., 2009; Dikshit et al., 2015). The PIC is indispensable for SSR selection for gene tagging and diversity studies; the higher the value, the more informative and better for phylogenetic analyses. The current research exhibited high polymorphism with a high average number of alleles per locus (5.34) and a high PIC value (0.61) greater than 0.5. This indicates their effectiveness in detecting variation and relatedness among accessions (Peng & Lapitan, 2005; Rajendra et al., 2019; Saxena et al., 2019). Additionally, mostly dinucleotide repeats among the tested SSRs could have influenced the polymorphism. Dinucleotide repeats are located outside gene coding regions that are prone to mutations, making them highly polymorphic (Odeny et al., 2007; Oliveira et al., 2023; Topu et al., 2023)

Genetic Diversity Analysis among Different Lens Species

The UPGMA dendrogram was constructed based on the genetic similarity matrix using 41 SSR markers, which grouped the 21 *Lens* genotypes into two major groups that bifurcated into subclusters. Group I is split into subcluster I, comprising all *L. ervoides* accessions and *L. tomentosus* accessions except *L. tomentosus* (IG72830), and subcluster II, comprising all *L. odemensis* accessions, all *L. lamottei* accessions, two *L. nigricans* accessions (IG136639 and IG72545), and one *L. tomentosus* accession (IG72830). Group II was further grouped into two subclusters, with *L. culinaris* subsp. *culinaris* (Firat87, Seyran96, Tigris) in subcluster I, while subcluster II comprised all accessions of *L. orientalis* and one *L. nigricans* (IG36636). Pairwise genetic similarities to cultivated *L. culinaris* subsp. *culinaris* accessions were highest with *L. culinaris* subsp. *orientalis* and lowest between *L. ervoides* (IG72862) and *L. culinaris* subsp. *culinaris* (Tigris) (6%). However, among wild species *L. culinaris* subsp. *orientalis* and *L. nigricans* (81%) showed the highest similarity, while the lowest similarity was observed between *L. ervoides* and *L. nigricans* (10%).

Introgression of target traits into cultivated varieties is actualized by accurate evaluation of crop wild relatives (CRW) as a prerequisite to enhance genetic gains (Rajpal et al., 2023). In our study, the cultivars of *L. culinaris* subsp. *culinaris* (Firat-87, Seyran-96, and Tigris) clustered in Group II with all *L. culinaris* subsp. *orientalis* and *L. nigricans* (IG136636). And the genetic similarity of all wild subspecies/species to the cultivated subspecies showed *L. culinaris* subsp. *orientalis* as the closest. Our results are similar to Dissanayake et al. (2020), who, consistent with Wong et al. (2015), found *L. orientalis* to be the closest to *L. culinaris*, with *L. odemensis*/*L. Lamottei*, *L. ervoides*, and *L. nigricans*, respectively. Liber et al. (2021), using principal component analysis (PCA), discriminant analysis of principal components (DAPC), and phylogeny, also grouped *L. orientalis* and *L. culinaris* together from the four major *Lens* genera identified in concurrence with Dikshit et al. (2015). Additionally, Gutierrez-Gonzalez et al. (2022), using transcriptome analysis, identified *L. orientalis* as the most similar to *L. culinaris* and *L. nigricans* as the least similar. Similarly, Hamwieh et al. (2009) and Verma et al. (2014) reported the clustering of cultivated germplasm with *L. culinaris* subsp. *orientalis*. This indicates *L. culinaris* subsp. *orientalis* as the progenitor of cultivated lentils. In support of this, our findings align well with those reported by Reddy et al. (2010), Wong et al. (2015), Dikshit et al. (2015), and Liber et al. (2021), which also characterized wild *Lens* species and subspecies using molecular markers. Consistent with Sharma et al. (1995, 1996) and Hamwieh et al. (2009), our results affirm the close genetic relationship between *L. culinaris* and *L. orientalis*. The clustering of *L. odemensis* and *L. lamottei* observed in our dendrogram corresponds with their assignment to the secondary gene pool by Wong et al. (2015). Moreover, our finding that *L. tomentosus* grouped with *L. ervoides*, rather than *L. orientalis* and *L. culinaris*, aligns with Liber et al. (2021) but contrasts with the primary gene pool classification by Wong et al. (2015). Notably, *L. nigricans* accession IG136636 clustered away from the other *L. nigricans* lines, as also reported in studies by Wong et al. (2015) and Liber et al. (2021), which suggested this species belongs in a quaternary gene pool due to its divergence and hybridization limitations. The lowest similarity (10%) between *L. ervoides* and *L. nigricans* in our study matches Reddy et al. (2010) and Wong et al. (2015), confirming the high interspecific variation between these species. These comparisons further validate the utility of the SSR markers used and reinforce the reliability of the phylogenetic relationships presented in this study. Our phylogenetic analysis placed *L. odemensis* quite far from *L. culinaris* (Figure 1). Despite the controversy from crosses made between *L. odemensis* and *L. culinaris*, similar results showing the separation of these two species (Wong et al., 2015; Polanco et al., 2019; Dissanayake et al., 2020).



An evaluation of the genetic relationships among wild subspecies/species revealed the highest similarity between *L. culinaris* subsp. *orientalis* and *L. nigricans* (81%), while the lowest was observed between *L. ervoides* and *L. nigricans*.

(10%). The finding of *L. culinaris* subsp. *orientalis* and *L. nigricans* (81%) similarity could be specific to the accessions used, with an explanation from the refuted findings of Renfrew (1973) that had suggested *L. nigricans* as the progenitor of *L. culinaris*. The inferences from the electrophoretic studies of seed protein profiles found *L. culinaris*, *L. orientalis*, and *L. nigricans* to be closely related. Furthermore, reports from cytoplasmic studies of *Lens* showed three chromosome interchanges between the cultivated *L. culinaris* and *L. nigricans* and only one between the cultivated species and *L. orientalis* (Hoffman, 1988). These studies showed the evolutionary relatedness of these *Lens* species and provided more evidence for *L. orientalis* as a putative progenitor of cultivated lentils.

Ahmad et al. (1997) and Wong et al. (2015) reported results similar to this study, which found the highest genetic dissimilarity between *L. ervoides* and *L. nigricans*, indicating the highest interspecific variation among these wild species. Based on phylogenetic analyses and population genetics, Liber et al. (2021) observed *L. nigricans* and *L. ervoides* to be very different from the cultivated genus. Additionally, *L. c.* subsp. *odemensis*, referred to as the sister clade of *L. ervoides*, is also quite distinct from *L. nigricans* (Dissanayake et al., 2020). Intraspecific variations reflected by the scattered placement of *L. nigricans* and *L. tomentosus* accessions were also observed. Although Reddy et al. (2010) clustered cultivated lentil accessions with *L. orientalis* (ILWL 1) and *L. nigricans* (ILWL 14) in group I, the result contradicts the grouping according to gene pools that placed *L. culinaris*, *L. orientalis*, and *L. tomentosus* species together, while *L. nigricans* was placed in a distinct quaternary gene pool (Wong et al., 2015). Furthermore, the structure and phylogenetic analyses showed that *L. nigricans* is the most distant relative of cultivated lentils owing to the unsuccessful development of interspecific offspring with *L. culinaris* past the F1 generation (Wong et al., 2015). This within-species genetic variation could be a result of continuous genetic transmissions, since high genetic diversity at the species level has been reported in *Lens* (Sharma et al., 1996; Hamwieh et al., 2009; Reddy et al., 2010; Kumar et al., 2014). In addition, the observations of the genetic similarity among these accessions could be attributed to the limited number of markers, their dispersion in the genome, and the general genetic composition of the tested plant material (Tantasawat et al., 2011).

4. CONCLUSION

Conclusively, 41 genomic SSRs developed in cultivated lentils were successfully transferable to wild *Lens* and used to characterize the genus by assessing their genetic variation and phylogeny. These polymorphic SSRs are a valuable addition to lentils' limited genetic resources for further characterization of *Lens* germplasm to point out genetic relationships that would permit the discovery of novel genes and their utilization in agronomical advancements. Additionally, these markers will contribute to evolutionary studies, comparative mapping, linkage mapping, and quantitative trait loci (QTL) identification. Based on these findings, the assayed accessions could be utilized in breeding programs to enable the introgression of genes into cultivated lentils for yield improvement and/or tolerance to abiotic and biotic stresses.

ACKNOWLEDGEMENT

This study was supported by Erciyes University Scientific Research Unit (Project No: FCD-2021-10106). We gratefully acknowledge Prof. Dr. Cengiz Toker and the Department of Field Crops, Faculty of Agriculture, Akdeniz University, for providing the plant material used in this study. We also thank Betül-Ziya Eren Genome and Stem Cell Centre at Erciyes University for providing the laboratory facilities essential for the successful completion of this research.

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Co-Inoculation of Bradyrhizobium and Arbuscular Mycorrhizal Fungus Alleviates the Effects of Drought Stress in Soybean (*Glycine max* L.)

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ARTICLE INFO

Research Article

Received: 21 July 2024

Accepted: 18 April 2025

Published: 23 June 2025

Keywords:

Antioxidant enzymes

Drought stress

Mycorrhiza

Oil quality

Soybean

ABSTRACT

Legumes are sensitive to drought stress, which adversely affects their seed yield, protein and oil content. This was investigated in a two-year field experiment conducted using a split-plot design with three replications in the Mughan plain, Ardabil. The experimental factors included drought stress as the main plot at three levels (60, 100, and 140 mm of evaporation from a class A pan) and the co-inoculation of soybean symbiotic bacteria and arbuscular mycorrhizal fungus species across eight treatments (*Bradyrhizobium japonicum*, *Funneliformis mosseae*, *Rhizophagus irregularis*, *Glomus fasciculatum*, *B. japonicum* + *F. mosseae*, *B. japonicum* + *R. irregularis*, *B. japonicum* + *G. fasciculatum*, and control) as the subplot. The results revealed that heightened drought stress led to a reduction in plant dry weight, pod number, seed number per plant, and seed yield in all treatments in both study years. However, this reduction was less pronounced in some treatments, especially those involving co-inoculation with *B. japonicum* + *R. irregularis* and *B. japonicum* + *G. fasciculatum*. Conversely, all treatments exhibited an increase in stomatal resistance, chlorophyll *a* concentration, soluble sugars, malondialdehyde (MDA), peroxidase (POD), and superoxide dismutase (SOD) under drought conditions (100 and 140 mm) compared to the normal irrigation conditions (I_{60}). The saturated fatty acids (palmitic and stearic acids) declined in inoculated plants compared to the control, while the trend was the opposite for unsaturated fatty acids (linoleic, linoleic, and oleic acids). Drought stress increased palmitic acid content by up to 32.4% and reduced linolenic acid content by up to 13.4%. Among the treatments, co-inoculation with *B. japonicum* + *R. irregularis* and *B. japonicum* + *G. fasciculatum* demonstrated a more significant improvement in the soybean's drought tolerance compared to the others. Given these results, inoculating soybean plants with rhizobial bacteria and *R. irregularis* mycorrhizae can be recommended as a strategy to enhance their drought resistance and improve their seed yield and oil quality.

Citation: Zirak-Qoturbulagh, M. A., Mehri, S., Soleimanzadeh, H. & Ansari, M. H. (2025). Co-inoculation of bradyrhizobium and arbuscular mycorrhizal fungus alleviates the effects of drought stress in soybean (*Glycine max* L.). *Turkish Journal of Field Crops*, 30(1), 235-248. <https://doi.org/10.17557/tjfc.1519684>

1. INTRODUCTION

Over the past two decades, climate change has emerged as a significant environmental concern, manifested as rising air temperatures and reduced rainfall in Iran, leading to diminished agricultural yields. Among the affected crops, soy production has notably declined. Soybeans are a vital source of protein and oil whose significance is underscored by their high unsaturated fatty acid content, oil digestibility, meal quality, ability to fix nitrogen through symbiosis with *Rhizobium* bacteria, and contribution to soil fertility enhancement (Carter & Tegeder, 2016; Israilov et al., 2023). Soybean oil is composed of 49% linoleic acid and 25% oleic acid. Soybeans contain more protein than other oilseeds (Ran et al., 2024). The global significance of soybeans is attributed to their high protein content, ranking them third in value within the typical human diet (Semba et al., 2021). Drought stress during the soybean growth cycle, particularly in the reproductive phase, adversely affects its quantity and quality. In this regard, water scarcity has been reported to reduce the number of pods and seeds per plant, thousand-seed weight, seed yield, and harvest index (Mondani et al., 2019). In addition, drought stress alters the synthesis pathways of various compounds and secondary metabolites by inducing secondary stresses like oxidative stress (Hasanuzzaman et al., 2022).

The application of cost-effective, stable, and eco-friendly agents, such as mycorrhizal fungi, is a beneficial mechanism to enhance plant tolerance to drought stress (Wang et al., 2017; Samsami et al., 2019). These fungi form symbiotic relationships with plant roots, thereby improving the uptake efficiency of macronutrients and even micronutrients. Legumes can also form symbiotic associations with mycorrhiza. This symbiosis aids the plant in adapting to environmental changes (Ashwin et al., 2022). In addition to mycorrhiza, a variety of soil microorganisms in the rhizosphere, known as plant growth-promoting rhizobacteria (PGPR), can bolster plant growth and yield through mechanisms like biological N_2 fixation, the synthesis of phytohormones (auxins, cytokinins, and gibberellins), the increased solubility of insoluble compounds like phosphorus and potassium through synthesizing organic acids, the production of siderophores, and the higher availability of micronutrients, especially Fe (Pathania et al., 2020). Also, they have particularly been interesting due to their ability to colonize the root area and produce a wide range of enzymes and metabolites, which can be beneficial in water-deficient conditions (Elabed et al., 2019). The colonization of mycorrhizal helper bacteria, such as rhizobial symbiotic bacteria, on the root area strengthens the symbiotic relationship and confers numerous advantages to the host plant (Bencherif et al., 2019; Gough et al., 2021).

Inoculating soybean plants with bacteria like *Bradyrhizobium* was reported to increase seed yield by up to 28%, oil content by up to 18%, and seed protein level by up to 37% under drought conditions compared to non-inoculated plants (Israilov et al., 2023). On the other hand, it has been reported that dual inoculation with mycorrhiza and *Bradyrhizobium* can mitigate drought stress damage by reducing lipid peroxidation and membrane permeability and boosting the accumulation of osmosis-regulating compounds and antioxidant enzyme activity (Mohammadi et al., 2019; Samsami et al., 2019). In addition, symbiotic N-fixing bacteria can synthesize and secrete biologically active compounds in the root zone, thereby contributing to the root system's development and mycorrhizal colonization. On the other hand, by producing biologically active compounds and providing nutrients, mycorrhizal fungi help plant growth-stimulating bacteria survive and multiply (Egamberdieva et al., 2015). The synergy escalates plant growth by producing beneficial compounds, improving nutrient availability, and inducing plant resistance to various stresses, including nutrient deficiency, salinity, drought, acidity, and soil temperature (Musyoka et al., 2020).

The present study investigated the effects of the combined application of *Bradyrhizobium* bacteria and mycorrhizal fungi on the ecophysiological traits of soybeans under drought stress in field conditions over two years in the Mughan plain, northwest Iran.

2. MATERIALS AND METHODS

The present field research was conducted in 2022 and 2023 as a split plot experiment based on a randomized complete block design with three replications in Parsabad, Mughan, Iran (longitude 47°30' E., latitude 39°20' N., elevation 78 m above sea level). Figure 1 depicts the average rainfall and monthly temperature for both years during the crop growth period. The study was implemented as a second crop following the wheat harvest. A soil sample was collected from a depth of 0-30 cm to ascertain the texture and chemical properties of the soil at the experimental site. The results are displayed in Table 1.

The experimental treatments included drought stress as the main plot, gauged by the amount of evaporation (mm) from a Class A pan, with three levels: normal irrigation (60 mm evaporation, I_{60}), moderate drought stress (100 mm evaporation, I_{100}), and severe drought stress (140 mm evaporation, I_{140}). The subplot was assigned to the combined inoculation of soybean symbiotic bacteria and mycorrhizal fungi at eight levels: *Bradyrhizobium japonicum*, *Funneliformis mosseae*, *Rhizopagus irregularis*, *Glomus fasciculatum*, *B. japonicum* + *F. mosseae*, *B. japonicum* + *R. irregularis*, *B. japonicum* + *G. fasciculatum*, and a control treatment.

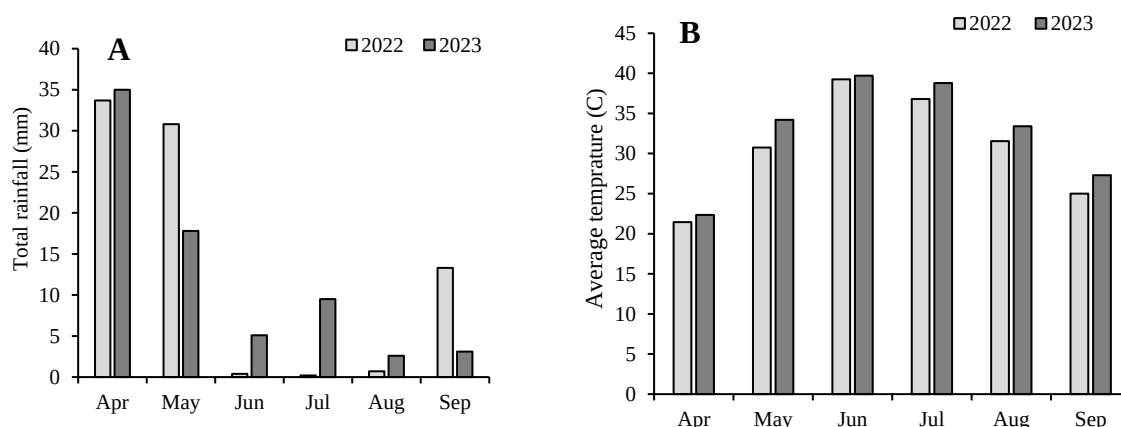


Figure 1. Total monthly rainfall (A) and average monthly temperature (B) during the soybean growth (Parsabad Weather Station).

Table 1. Results of physio-chemical analysis of the experimental soil

Year	Sand	Silt	Clay	EC (dS m ⁻¹)	pH	OC (%)	N (%)	P	K
		(%)						(mg kg ⁻¹)	
2017	17.3	37.5	45.2	0.678	7.18	0.92	0.218	19.8	234
2018	18.4	37.0	44.6	0.509	6.91	0.88	0.241	17.5	251

Following the wheat harvest, the land was prepared by plowing and discing. Each experimental plot consisted of four 5 m long rows with a planting density of 8×30 cm. After plot preparation, seeds were coated with gum arabic and inoculated with bacteria (7 g of inoculum per kg of seeds, containing 10^7 live bacterial cells g⁻¹). The mycorrhizal fungi were applied to the seedbed. Then, the seeds were sown by hand. Soil moisture was adjusted to field capacity for each irrigation event. Irrigation for all treatments was uniform until the plants were fully established, indicated by the formation of 4-5 nodes on the main stem (V₄ growths stage). At the 50% flowering stage, three plants were randomly selected from the central rows of each plot and transported to the laboratory in a liquid nitrogen container to analyze their biochemical traits.

Chlorophyll extraction and measurement

Chlorophyll *a*, *b*, and total were quantified by Arnon's (1949) method. 100 mg (0.1 g) of fresh leaf tissue was ground in a mortar with 10 ml of 80% acetone. The resulting extract was then centrifuged at 6000 rpm for 10 min. The supernatant was transferred to a 25 ml volumetric flask, and its volume was made up to 25 ml with 80% acetone. Pigments were measured spectrophotometrically using a UV 160A SHIMADZU device, for which the absorbance of the solutions was read at 645 and 663 nm. Finally, considering a final volume of 25 ml, chlorophyll concentrations were expressed in mg g⁻¹ of leaf tissue, and the amounts of chlorophyll *a*, *b*, and total were calculated.

Stomatal resistance

It was measured at the flowering stage on the youngest fully developed leaves between 06:00 and 08:00 AM before irrigation using an AP4 Delta-T Prometer device.

Concentration of soluble sugars

The concentration of soluble sugars was measured according to Irigoyen et al. (1992). So, 0.2 g of leaf samples were ground with 2 ml of sodium phosphate buffer (pH=7) and then centrifuged at 10000 rpm at 4 °C. Next, 990 µl of distilled water was added to 10 µl of the resulting supernatant. Thereafter, 5% phenolphthalein and 2.5 ml of 98% sulfuric acid were added to 0.5 ml of the resulting solution, and the absorbance was read at 490 nm.

Soluble protein

It was measured using Li's (2000) procedure. Fresh leaf samples (0.1 g) were weighed, a small amount of quartz sand was added to the homogenates, and 1 ml of distilled water was added to each before centrifuging at 12,000 rpm for 10 min. The sample extracts (0.2 ml) were placed in test tubes (two repeat tubes) and added with 1 ml of Coomassie brilliant blue G-250 reagent. The mixture was shaken vigorously, and after 2 min, absorbance was measured at 595 nm. The soluble protein content was calculated with the corresponding standard curve obtained using bovine serum albumin (BSA).

Proline

The proline content in leaf tissues was measured via reaction with ninhydrin (Bates et al., 1973). For colorimetric determinations, a solution of proline, ninhydrin acid, and glacial acetic acid (1:1:1) was incubated at 90 °C for 1 h. The chromophore was extracted using 2 ml of toluene, and its absorbance was read at 520 nm using a BioMate spectrophotometer (Shimadzu UV-160, Japan).

Malondialdehyde (MDA)

The MDA content, which is indicative of lipid peroxidation, was assessed following Boominathan & Doran's (2002) protocol. Leaf tissue was extracted in a 0.1% trichloroacetic acid (TCA) solution and centrifuged at 10,000 rpm for 5 min. The supernatant was mixed with 20% TCA solution containing 0.5% thiobarbituric acid at a ratio of 1:4, and then, it was placed in a water bath at 95 °C for 30 min. The tubes were then rapidly cooled in ice and centrifuged at 10,000 rpm for 15 min. Alongside the leaf extracts, standard solutions ranging from 0 to 100 nmol of 1,1,3,3-tetraethoxypropane were prepared, and the absorbance of the samples was read at 532 nm using a spectrophotometer. Finally, the MDA concentration in the samples was calculated in nmol g⁻¹ FW.

Enzyme extraction and assay

Frozen leaves were ground in liquid nitrogen using mortar and pestle as much as possible. Then, 0.5 g of the ground sample was transferred to a 2 ml microtube and vortexed by adding 1 ml of phosphate buffer. It was then centrifuged at 14000 rpm at 4 °C for 15 min. After that, the supernatant was transferred to a 1.5 ml microtube using a micro-sampler and re-centrifuged at the same conditions for another 10 min. The resulting supernatant was transferred to another microtube of the same size. The microtubes containing residues of the sample ground and centrifuged were transferred to -80 °C for probable future measurements (Sairam et al., 1998).

Catalase (CAT) activity

The CAT activity was assayed according to Aebi (1971) by monitoring the rate of decomposition of H₂O₂ at 240 nm in the reaction mixture consisting of 50 mM potassium phosphate buffer, 10 mM H₂O₂, and the crude enzyme solution. The H₂O₂ content was determined according to Loreto and Velikova (2001).

Superoxide dismutase (SOD) activity

The SOD activity was determined by its capacity to inhibit the photoreduction of nitroblue tetrazolium chloride (NBT) using Giannopolitis & Ries's (1977) method. The reaction mixture, totaling 3 ml, contained 50 mM potassium phosphate buffer (pH = 7.8), 13 mM methionine, 75 µM NBT, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 360 µM riboflavin, and 30 µL of the crude enzyme extract. After thorough mixing, the spectrophotometer cells were exposed to light from a 15-W fluorescent lamp placed 35 cm away for 10 min. The reaction was halted by switching off the lamp, and the absorbance of the mixture was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of the NBT photoreduction.

Peroxidase (POD) activity

The POD activity was assessed following the method described by Plewa et al. (1991). To this end, 900 µL of 50 mM phosphate buffer (pH = 7.0) containing 0.1 mM EDTA, 0.1 mM H₂O₂, and 10 mM guaiacol (2-methoxyphenol) was mixed with 50 µL of the enzyme extract. The increase in absorbance due to tetraguaiacol formation caused by POD activity was monitored at 470 nm over 2 min. One unit of enzyme activity was defined as an increase of 0.01 in absorbance at 470 nm per minute.

Yield and yield components

Eight plants were randomly selected and harvested from each plot at the maturity phase, taking care of the margins. The number of pods, seeds per pod, seeds per plant, and 100-seed weight were recorded. Seed yield, adjusted to 14% moisture content, was calculated from a 5 m² area at the center of each plot. Seed moisture content was determined using a digital moisture meter (Model GMK-303R5, Korea). To ascertain shoot dry weight, plants harvested from a 1-m² area were enclosed in paper bags and oven-dried at 72 °C for 48 h, and the result was recorded as the biological yield per ha.

Oil determination

Seed oil was extracted using rotary evaporators based on Folch et al.'s (1957) protocol. The extracted fatty acids were transformed to their methyl esters (FAME) using Metcalf et al.'s (1966) method and were determined using a gas chromatography (Unicam 4600) equipped with an FID detector.

Data analysis

All measured traits were consistent across both years, except for fatty acid composition, which was only assessed in the second year. The results recorded for the comparison of means represent the average of the three replicates per

treatment. Data were analyzed using SAS software (version 9.2), and the means were compared using the LSD test. When a significant interaction was observed between traits, the means were compared for the interactive effects using the slice method and the procedure of L.S.Means.

3. RESULTS

Leaf soluble sugar and protein contents

The analysis of variance (ANOVA) revealed that the interaction of year $\times$ drought stress $\times$ microorganisms significantly influenced the soluble sugar and protein contents (Table 2). It was revealed that as drought stress intensity was increased from I₆₀ to I₁₄₀, the concentration of soluble sugars in the leaves rose by 13-45% in the first year and by 10-34% in the second year in most treatments. The control and single inoculation treatments exhibited higher soluble sugar levels than the co-inoculation treatments (Table 4). The changes in leaf soluble proteins with increasing drought stress varied among the treatments. However, in both years and across all drought stress levels, the plants treated with *B. japonicum* + *F. mosseae* and those treated with *B. japonicum* + *R. irregularis* displayed higher soluble protein levels than most other treatments (Table 4).

Table 2. ANOVA of the effect of drought stress and microorganisms on content of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Total chl), Stomatal resistance, Soluble sugars and Soluble proteins of soybean

Source of variance	Mean Square (MS)					
	Chl a	Chl b	Total chl	Stomatal resistance	Soluble sugars	Soluble proteins
Y	5.900**	2.34**	15.70**	1562**	372069**	89.4**
Y(R)	0.171	0.059	0.244	32.90	895	28.6
D	4.950**	1.636**	12.16**	3720**	52864**	17.2ns
Y $\times$ I	6.238**	2.188**	15.80**	1041**	5650*	13.2ns
D(Y $\times$ R)	0.069	0.015	0.084	32.30	1430	10.2
M	0.032ns	0.004ns	0.039ns	16.64ns	5471**	23.9*
Y $\times$ F	0.023ns	0.010ns	0.034ns	32.56ns	6456**	14.5ns
D $\times$ M	0.067**	0.007ns	0.072*	20.23ns	12667**	24.9**
Y $\times$ D $\times$ M	0.066**	0.014ns	0.093**	40.49ns	18763**	25.1**
Error	0.020	0.017	0.035	27.70	1727	9.3
CV (%)	5.75	8.53	4.67	16.8	21.27	20.4

** Significant in 0.01, * significant in 0.05, and ns: non-significant. Y: Year, R: Replication, D: Drought stress, M: Microorganisms

Table 3. ANOVA of the effect of drought stress and microorganisms on proline and MDA content and activity of SOD, CAT, and POD of soybean

Source of variance	Mean Square (MS)				
	Proline ($\mu\text{g g}^{-1}$)	MDA (nmol g^{-1})	SOD ($\text{U g}^{-1} \text{FW min}^{-1}$)	CAT ($\text{U g}^{-1} \text{FW min}^{-1}$)	POD ($\text{U g}^{-1} \text{FW min}^{-1}$)
Y	45966**	152828**	202740**	529.7**	4.05*
Y(R)	6235ns	67.3ns	1115ns	8.74ns	1.32ns
D	5199ns	13326**	224503**	2861**	10.31**
Y $\times$ I	38105**	57103**	28557*	124.0ns	0.197ns
D(Y $\times$ R)	10241ns	1182ns	13981*	44.61ns	0.728ns
M	7094ns	24820**	27431**	213.4**	2.86**
Y $\times$ F	23832**	11756**	20125**	77.1ns	1.425ns
D $\times$ M	16068**	22317**	46734**	36.6ns	2.24**
Y $\times$ D $\times$ M	22422**	12333**	46574**	45.2ns	3.21**
Error	6255	865.9	6263	52.86	0.808
CV (%)	23.2	20.92	19.86	16.46	23.31

** Significant in 0.01, * significant in 0.05, and ns: non-significant. Y: Year, R: Replication, D: Drought stress, M: Microorganisms

Stomatal resistance

Based on the results of ANOVA, stomatal resistance was influenced by the interactive effects of year $\times$ drought stress (Table 2). The comparison of means for this trait showed an increase from 11.3% to 34.6% in the first year and from 7.73% to 20.5% in the second year as drought stress intensified from I₆₀ to I₁₄₀. However, no significant difference was observed between I₁₀₀ and I₁₄₀ in the first year (Figure 2A).

Leaf chlorophyll

The results demonstrated that the chlorophyll *a* level was significantly impacted by the interaction of year $\times$ drought stress $\times$ microorganisms, and the chlorophyll *b* content was influenced by the interaction of year $\times$ drought stress (Table 2). The comparison of means for chlorophyll *a* revealed a decrease in all treatments with increasing water deficit. The

decline was 17-31% in the first year and 20-33% in the second year at the 100 mm level, and it was 28-49% in the first year and 31-43% in the second year at the 140 mm level compared to the 60 mm level (no stress). Among the treatments, the co-inoculated plants with *B. japonicum* + *R. Irregularis* and *B. japonicum* + *G. fasciculatum* generally exhibited higher chlorophyll *a* levels under most water-deficit conditions (Table 4). The comparison of means for the chlorophyll *b* level indicated that, in both years, the 90 mm drought level exhibited the highest chlorophyll *b* content, while there was no significant difference between the 60 mm and 140 mm levels (Figure 2B). Moreover, the total chlorophyll content was affected by the interaction of year $\times$ drought stress and the simple effect of microorganisms (Table 2). As depicted in Figure 2C, the total chlorophyll content decreased with escalating stress intensity. Among the microorganisms, the inoculation treatments resulted in higher total chlorophyll content, with co-inoculation treatments (*B. japonicum* + *R. Irregularis* and *B. japonicum* + *G. fasciculatum*) outperforming other treatments (Figure 2D).

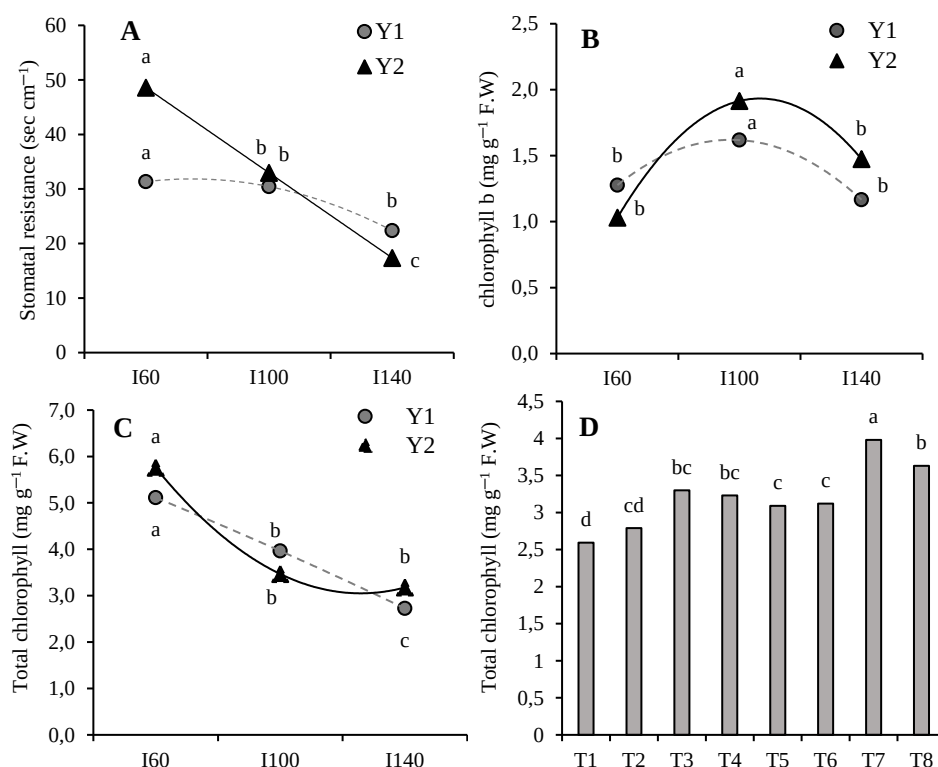


Figure 2. Mean comparison of the interaction effect of year $\times$ drought stress on stomatal resistance (A), chlorophyll *b* (B), and total chlorophyll (C), as well as the main effect of microorganism on total chlorophyll (D). I60, I100 and I140 (60, 100 and 140 mm evaporation from a class A pan, respectively). T1: control, T2: *B. japonicum*, T3: *F. mosseae*, T4: *R. irregularis*, T5: *G. fasciculatum*, T6: *B. japonicum* + *F. mosseae*, T7: *B. japonicum* + *R. irregularis*, T8: *B. japonicum* + *G. fasciculatum*.

Leaf proline and MDA contents

As the results of ANOVA indicated, the interaction between year, drought stress, and microorganisms was significant for the proline and MDA contents (Table 3). The comparison of means revealed that with increasing drought intensity, the proline and MDA contents in all treatments rose significantly in both years. Notably, plants with co-inoculation had higher proline levels and lower MDA levels than the control plants. Among the treatments, the plants treated with *B. japonicum* + *R. irregularis* produced the highest proline level under severe drought stress (I140) (510 and 580 $\mu\text{g g}^{-1}$ in the first and second years, respectively). Conversely, the highest MDA levels were found in the control treatment, with 143 and 202 nmol g^{-1} in the first and second years, respectively (Table 4).

SOD, CAT, and POD activity

The results of ANOVA demonstrated that the interaction of year, drought stress, and microorganisms had a significant impact on SOD and POD activity (Table 3). Based on the comparison of means for SOD activity, it was higher at greater drought severity in both years. Specifically, SOD activity at I₁₀₀ increased by 14.1-43.7% in the first year and 11.3-36.5% in the second year, while at I₁₄₀, it increased by 38.6-69.7% in the first year and 26.2-57.3% in the second year, compared to I₆₀. Although the highest SOD activity was observed in the control treatment in the first year, no significant difference was found in the second year compared to mycorrhizal treatments without *B. japonicum*, and SOD activity was lower in the co-inoculation treatments. In most conditions, the plants treated with *B. japonicum* + *R. irregularis* and *B. japonicum*

+ *F. mosseae* exhibited the lowest SOD activity (Table 4). POD activity patterns were similar to those of SOD. Based on the results, POD activity increased under I₁₀₀ and I₁₄₀ compared to I₆₀ by 31.3-56.4% in the first year and 26.1-68.5% in the second year. Co-inoculated plants showed lower POD activity than the control and *B. japonicum* treatment (Table 4). However, CAT activity increased from I₆₀ to I₁₀₀ and then decreased from I₁₀₀ to I₁₄₀ (Figure 3A). The comparison of the treatments revealed that *B. japonicum* + *R. irregularis* and *B. japonicum* + *G. fasciculatum* had significantly higher CAT activity than the other treatments (Figure 3B).

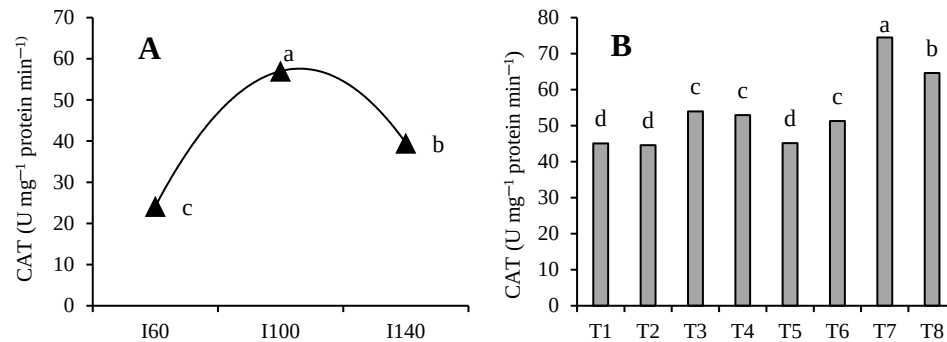


Figure 3. Mean comparison of the main effect of drought stress (A) and microorganisms (B) on CAT activity. I60, I100 and I140 (60, 100 and 140 mm evaporation). T1: control, T2: *B. japonicum*, T3: *F. mosseae*, T4: *R. irregularis*, T5: *G. fasciculatum*, T6: *B. japonicum* + *F. mosseae*, T7: *B. japonicum* + *R. irregularis*, T8: *B. japonicum* + *G. fasciculatum*.

Table 4: Mean comparison of the interaction effect of year × drought stress × microorganisms on chlorophyll a, soluble sugar, soluble protein, proline, and MDA contents and activity of SOD, and POD of soybean

Year	Drought stress	Microorganisms	chlorophyll a	Soluble sugar (mg g ⁻¹ FW)	Soluble protein	Proline (μg g ⁻¹)	MDA (nmol g ⁻¹)	SOD (U mg ⁻¹ protein min ⁻¹)	POD (U mg ⁻¹ protein min ⁻¹)
2021	I60	T1	4.45d	221a	15.7ab	208e	44.0a	257ab	2.761a
		T2	4.94a-d	182b	14.43ab	271b	33.1b	251b	2.73a
		T3	4.57cd	140b-d	14.3ab	220d	29.0b	283ab	2.511ab
		T4	4.97a-c	103d	12.01b	253bc	41.0a	287a	2.328b
		T5	4.68cd	172bc	14.10ab	268b	30.4b	188b	2.672a
		T6	4.91b-d	101d	14.76ab	256bc	31.7b	261ab	2.561ab
		T7	5.52a	111d	16.5a	235cd	33.0b	166cd	2.370b
		T8	5.33ab	124cd	15.4ab	310a	28.0b	143d	2.411b
	I100	T1	2.76d	329a	11.86b	301bc	142a	449a	4.16a
		T2	2.98a-d	299ab	12.81ab	273c	130bc	291e	4.188a
		T3	2.86a-d	180cd	11.93b	295bc	121d	358bc	3.473ab
		T4	2.83b-d	135cd	12.76ab	288bc	126cd	334cd	3.473ab
		T5	3.08a-c	112d	13.16ab	322ab	119d	367b	3.926a
		T6	2.81cd	174cd	15.41a	335ab	136ab	356bc	3.173b
		T7	3.11ab	119d	15.83a	324ab	108e	295e	3.436ab
		T8	3.14a	225bc	13.8ab	366a	94.1f	316de	3.466ab
	I140	T1	2.063c	338a	11.90c	346d	154a	665a	6.43a
		T2	2.25a-c	314a	13.23bc	340d	138b	611ab	5.72ab
		T3	2.136bc	274a	12.73bc	358c	135b	567b	5.193ab
		T4	2.386a	290a	14.05a-c	373bc	132b	468cd	5.42ab
		T5	2.273ab	302a	13.11bc	376bc	131b	472c	5.46ab
		T6	2.350a	159b	18.06a	398b	129b	454cd	5.50ab
		T7	2.374a	278a	16.58ab	510a	122b	407d	4.613b
		T8	2.285ab	162b	14.9a-c	410b	105c	451cd	4.776b
2022	I60	T1	3.52b	155a	13.43bc	238c	86.0b	377a	3.15a
		T2	4.07ab	140ab	13.75bc	278ab	84.7b	362a	3.05ab
		T3	4.15ab	132bc	12.46c	241c	118a	314a-c	3.083a
		T4	3.94ab	125bc	12.58c	266ab	70.3c	347ab	2.81a-c
		T5	4.17ab	127bc	17.0ab	237c	54.1de	250c	2.58bc
		T6	3.95ab	104d	14.75b	281ab	45.7e	323ab	2.46bc
		T7	4.36a	118cd	18.89a	286ab	55.4d	291bc	2.40bc
		T8	4.32a	116cd	17.52ab	296a	72.7c	265c	2.03c
	I100	T1	2.63d	242a	9.98c	284c	130b-d	472a	4.13a
		T2	2.91c	233a	10.25c	308bc	144a	471a	4.066a
		T3	2.74cd	127c	14.25bc	326b	140ab	391b	3.482bc
		T4	2.96c	162b	14.88bc	336b	127cd	423b	3.80ab
		T5	2.91c	246a	17.52ab	276c	136a-c	420b	3.68ab
		T6	2.95c	120c	16.57ab	332b	132a-d	418b	3.666ab
		T7	3.35b	124c	22.85a	395a	134a-d	412b	3.23c
		T8	3.63a	82.6d	16.81ab	374ab	125d	398b	3.44c
	I140	T1	2.096b	313a	10.27d	453b	203a	632a	6.072a
		T2	2.28ab	328a	14.10c	513ab	198a	624a	5.647ab
		T3	2.24ab	254a-c	15.71bc	383c	182b	511cd	5.345ab
		T4	2.31ab	213bc	15.90bc	463b	152c	540bc	5.912a
		T5	2.201ab	259a-c	21.56a	479b	170b	572b	5.758ab
		T6	2.243ab	316a	22.08a	449b	177b	468e	5.216ab
		T7	2.43a	170c	21.45a	580a	150c	495de	5.180ab
		T8	2.57a	303ab	17.65b	566a	143c	507cd	4.759b

Similar letter(s) in each column show insignificance of the difference based on the LSD test at the P < 0.05 level.

I60, I100 and I140 (60, 100 and 140 mm evaporation from a class A pan, respectively). T1: control, T2: *B. japonicum*, T3: *F. mosseae*, T4: *R. irregularis*, T5: *G. fasciculatum*, T6: *B. japonicum* + *F. mosseae*, T7: *B. japonicum* + *R. irregularis*, T8: *B. japonicum* + *G. fasciculatum*.

Table 5. ANOVA of the effect of drought stress and microorganisms on pod number, grain number, grain yield, plant biomass, oil content, and oil yield of soybean (combined analysis of the two years)

Source of variance	MS (mean square)					
	Pod number	Grain number	Grain yield (g m ⁻²)	Plant biomass (g plant ⁻¹)	Oil content (%)	Oil yield (g m ⁻¹)
Y	18.21*	42.1ns	395661**	414**	2405**	0.653ns
Y(R)	2.55	102	7772ns	3.52	91.2	313**
D	524.2**	3006**	2181007**	1711**	2687**	2432**
Y × I	5.033 ns	66.7 ns	191249**	395**	2547**	224 ns
D(Y × R)	2.094 ns	49.1 ns	5982ns	2.47ns	48.13	237
M	4.26 ns	88.7 ns	39732**	2.53ns	21.1ns	428**
Y × F	6.89 ns	140.1**	64769**	6.71*	16.6ns	102ns
D × M	6.25 ns	73.2 ns	27296**	2.48ns	470**	244**
Y × D × M	15.07**	98.6*	42690**	6.34*	619**	55.9ns
Error	4.36	48.8	5500	3.339	20.6	83.9
CV (%)	25.9	35.6	22.5	10.5	9.35	20.3

** Significant in 0.01, * significant in 0.05, and ns: non-significant. Y: Year, R: Replication, D: Drought stress, M: Microorganisms

Table 6. Mean comparison of the interaction effect of year × drought stress × microorganisms on pod number, grain number, grain yield and plant biomass

Irrigation	Microorganism	Pod number		Grain number		Grain yield (g m ⁻²)		Plant biomass (g plant ⁻¹)	
		2021	2022	2021	2022	2021	2022	2021	2022
I60	T1	19.6e	19.7e	42.7d	19.7e	147d	163d	203d	198e
	T2	23.1c-e	16.4f	62.0bc	16.4f	211cd	227c	233cd	287c-e
	T3	25.5b-d	26.4bc	58.9bc	26.4bc	205cd	241bc	266c	260de
	T4	21.8de	23.2d	46.5cd	23.2d	189cd	259bc	261c	311cd
	T5	22.3de	24.1cd	47.1cd	24.1cd	195cd	239bc	265c	316cd
	T6	27.4bc	23.1d	68.6b	23.1d	235bc	268a-c	263c	350bc
	T7	33.7a	29.2a	84.9a	29.2a	309a	308a	435a	517a
	T8	29.1ab	26.7b	72.2ab	26.7b	281ab	271ab	348b	416b
I100	T1	14.6bc	10.36e	286.1c	10.36e	102c	84.1b	102d	127e
	T2	16.2b	14.57bc	31.8bc	14.57bc	109c	109.5ab	116d	134e
	T3	16.37b	13.07cd	33.6bc	13.07cd	107c	107ab	139cd	131e
	T4	13.12c	16.55a	33.4bc	16.55a	114bc	119.3a	132cd	153cd
	T5	13.70c	15.03ab	34.8bc	15.03ab	125bc	106.4ab	150b-d	162bc
	T6	14.92bc	13.23c	38.9b	13.23c	146ab	122.6a	187a-c	140de
	T7	18.65a	11.57de	52.0a	11.57de	162a	128.2a	228a	189a
	T8	19.60a	13.29c	48.1a	13.29c	173a	121a	199ab	170b
I140	T1	6.06b	7.46c	11.23d	7.46c	24.0e	30.55e	35.6c	32.2c
	T2	7.01b	9.03a-c	11.91d	9.03c	25.6de	37.0c-e	43.3bc	50.0b
	T3	7.63b	9.51a-c	19.12c	9.51c	43.4bc	50.1a-c	38.0bc	37.2c
	T4	8.12b	9.76a-c	18.16c	9.76c	37.1cd	47.6a-d	58.3c	51.6b
	T5	33.7b	8.35bc	17.04c	8.35bc	32.5de	35.1de	50.3bc	59.6b
	T6	11.76a	11.467a	28.0a	11.467a	57.3a	59.7a	58.0c	54.0b
	T7	11.0a	11.23ab	22.86b	11.23ab	49.3ab	51.3ab	80.3a	89.3a
	T8	11.2a	10.33ab	22.47b	10.33ab	46.0bc	39.2b-e	61.3ab	79.0a

Similar letter(s) in each column shows insignificance of the difference based on the LSD test at the P < 0.05 level.

I60, I100 and I140 (60, 100 and 140 mm evaporation from a class A pan, respectively). T1: control, T2: *B. japonicum*, T3: *F. mosseae*, T4: *R. irregularis*, T5: *G. fasciculatum*, T6: *B. japonicum* + *F. mosseae*, T7: *B. japonicum* + *R. irregularis*, T8: *B. japonicum* + *G. fasciculatum*.

Seed oil content

ANOVA indicated a significant interaction between drought stress and microorganisms for both seed oil content and oil yield (Table 5). The comparison of means for the seed oil content showed that, except for the control, *B. japonicum* + *R. irregularis*, and *B. japonicum* + *G. fasciculatum* treatments, the seed oil content decreased as stress intensity increased. The highest oil content was obtained from the *B. japonicum* + *F. mosseae* treatment (29.6%) under normal irrigation conditions (I₆₀) and from the *B. japonicum* + *R. irregularis* treatment under I₁₀₀ and I₁₄₀ (Figure 4A). Oil yield, which is a function of seed yield and seed oil concentration, was the highest in all treatments at the I₆₀ level. However, drought stress (I₁₀₀ and I₁₄₀) resulted in a reduction in oil yield, ranging from 13.13% to 60.59%, in all treatments. At all drought levels, the maximum oil yield was achieved with the co-inoculation treatments *B. japonicum* + *R. irregularis*, and *B.*

japonicum + *G. fasciculatum*, which resulted in an increase in oil yield by 34.3-61.7% and 29.2-52.3%, respectively, compared to the control (Figure 4B).

Seed oil quality

The results indicated that drought stress significantly affected the PA and LINLA levels (Table 7). As drought stress intensified, the PA level increased by 10.2-12.4% (Figure 4C), whereas the LINLA level decreased by 7.14-8.09% (Figure 4D). Microorganisms also had a significant impact on fatty acids (Table 7). The comparison of means revealed that the PA and SA levels were lower in mycorrhizal plants than in the control. However, the OA, LILA, and LINLA levels were higher in most inoculated plants than in the control. Co-inoculated plants showed a marked improvement over both the control and *B. rhizobium*. Overall, the combination of *B. japonicum* + *F. mosseae* exhibited the highest levels of OA and LA, while *B. japonicum* + *G. fasciculatum* had the highest LINLA levels (Table 8).

Table 7. ANOVA of the effect of drought stress and microorganisms on content of palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LILA), and linolenic acid (LINLA)

Source of variance	MS (mean square)				
	PA (%)	SA (%)	OA (%)	LILA (%)	LINLA (%)
R	0.014ns	0.026ns	1.21ns	18.3**	0.881**
D	2.75**	0.0131ns	1.077ns	2.16ns	2.18**
Error1	0.0406	0.077	0.891	0.371	0.211
M	0.613*	0.151*	2.64**	10.6**	0.429**
D × M	0.073ns	0.005ns	0.049ns	0.8281ns	0.212ns
Error2	0.238	0.044	0.689	0.862	0.109
CV (%)	4.37	5.43	3.52	1.78	8.51

** Significant in 0.01, * significant in 0.05, and ns: non-significant. Y: Year, R: Replication, D: Drought stress, M: Microorganisms.

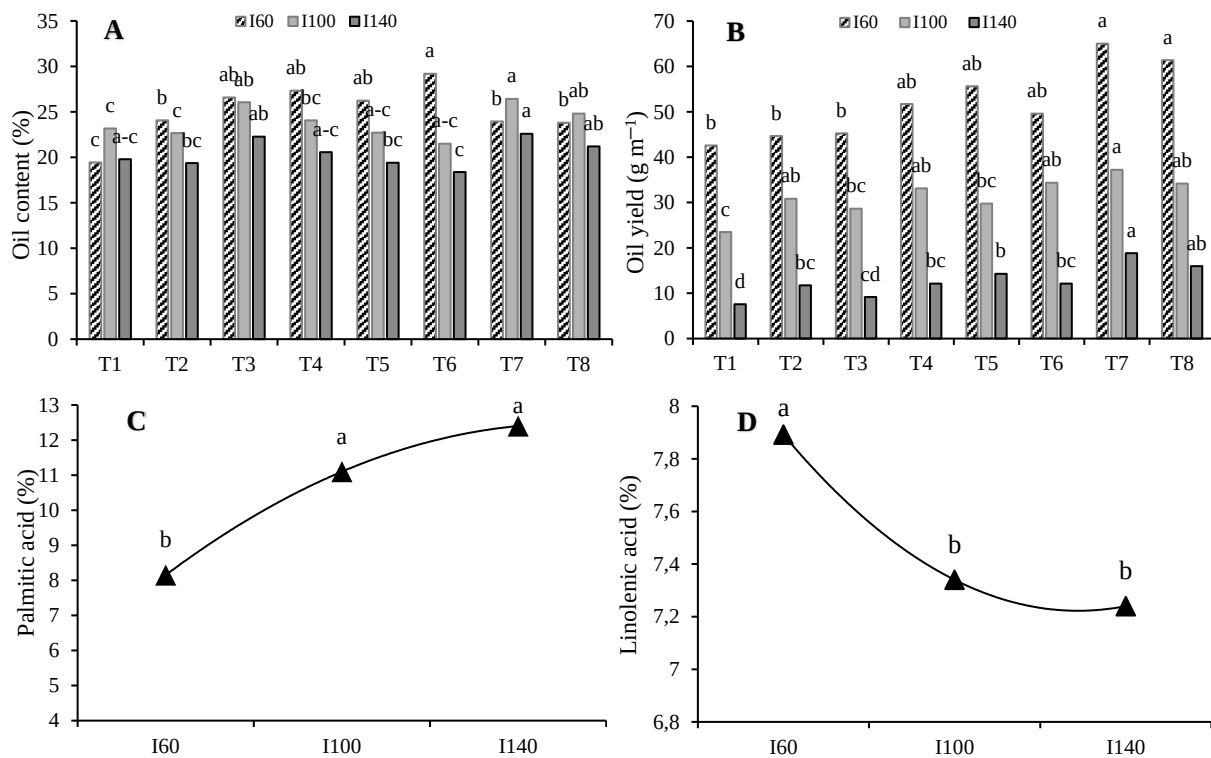


Figure 4. Mean comparison of the intraction effect of main effect of drought stress × microorganisms on oil content (A) and oil yield (B); main effect of drought stress on palmitic acid (A) and linolenic acid (B). I60, I100 and I140 (60, 100 and 140 mm evaporation from a class A pan, respectively). T1: control, T2: *B. japonicum*, T3: *F. mosseae*, T4: *R. irregularis*, T5: *G. fasciculatum*, T6: *B. japonicum* + *F. mosseae*, T7: *B. japonicum* + *R. irregularis*, T8: *B. japonicum* + *G. fasciculatum*.

Table 8: Mean comparison of the interaction effect of drought stress $\times$ microorganisms on content of palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LILA), and linolenic acid (LINLA)

Microorganisms	PA (%)	SA (%)	OA (%)	LILA (%)	LINLA (%)
Control	11.6a	4.544a	22.13c	48.63c	7.295c
<i>Brady rhizobium japonicum</i>	11.56ab	4.453ab	23.03bc	50.4c	7.321c
<i>Funneliformis mosseae</i>	11.00b-d	4.484ab	23.95ab	51.8b	7.53bc
<i>Rhizophagus irregularis</i>	11.09a-d	4.378ab	23.78ab	52.58ab	7.558bc
<i>Glomus fasciculatum</i>	10.89cd	4.474ab	23.7ab	52.01b	7.63bc
<i>B. japonicum</i> + <i>F. mosseae</i>	11.33a-c	4.043c	24.21a	53.65a	7.498c
<i>B. japonicum</i> + <i>R. irregularis</i>	11.06a-d	4.055c	24.02a	52.88ab	7.918ab
<i>B. japonicum</i> + <i>G. fasciculatum</i>	10.69d	4.35ab	23.6ab	52.53ab	8.16a

Similar letter(s) in each column shows insignificance of the difference based on the LSD test at the $P < 0.05$ level.

4. DISCUSSION

The application of plant growth-stimulating microorganisms has recently emerged as an eco-friendly and cost-effective method for sustainable crop production in various conditions. Our findings indicated that inoculating soybean plants with certain mycorrhizal species and *Bradyrhizobium* enhanced their physiological traits, such as leaf chlorophyll, proline, MDA, and CAT, POD, and SOD activities, thereby increasing their drought tolerance, in both study years. It was observed that co-inoculation with *R. irregularis*, *G. fasciculatum*, and *B. japonicum* increased chlorophyll *a* content by 4.7-17.3% under I_{100} and 6.34-22.5% under I_{140} conditions compared to the control. Also, it increased the proline content by 13.2-39.4% in the I_{100} conditions and 6.8-19.5% in the I_{140} conditions versus the control. However, most treatments exhibited lower SOD activity than the control. The plants co-inoculated with *B. japonicum* + *R. irregularis* and *B. japonicum* + *F. mosseae* showed the least SOD activity under most conditions. These results are consistent with the findings of Musyoka et al. (2020) and Dabré et al. (2022). Gough et al. (2021) attributed the rise in leaf chlorophyll content and oxidative enzyme activity in mycorrhizal plants to enhanced water uptake by mycorrhizal fungi because the hyphae of the mycorrhizal fungi extended root reach in the soil and increased water availability (Mohammadi et al., 2019). The increase in SOD activity as a general response to water stress (Hasanuzzaman et al., 2022) could be due to inhibited enzyme synthesis, altered subunit enzyme assembly under stress (Fazeli et al., 2015), degradation by induced peroxisomal proteases, or photoinactivation of the enzyme (Liu et al., 2008). Severe drought stress leads to a reduction in chlorophyll *a*, *b*, and total, as well as carotenoids, due to increased production of oxygen free radicals, which cause lipid peroxidation and subsequent pigment breakdown (Jiménez et al., 2021). Under stress conditions, the moderate increase in leaf SOD activity in plants inoculated with *Rhizobium* and mycorrhiza suggests that inoculation can enhance this enzyme's activity to combat oxidative damage arising from water scarcity. Microorganisms can, therefore, modulate oxidative reactions and antioxidant defenses (Ortiz et al., 2015) such that co-inoculation with mycorrhizal fungi and *Bradyrhizobium* boosts antioxidant production, reducing reactive oxygen species (ROS) under stress and protecting cells from oxidative damage (Samsami et al., 2019). Andrade et al. (2010) reported increased SOD and POD activity in bean plants inoculated with mycorrhiza. Salloum et al. (2018) state that increased root colonization extends the host plant's root system, which allows for greater soil penetration by fungal hyphae, thus expanding the root's access to soil and improving water and nutrient uptake efficiency. Proline and MDA levels rose with increasing drought severity in all treatments. However, co-inoculated plants showed higher proline and lower MDA levels than the control. In this regard, Nath et al. (2016) asserted that high MDA concentrations in leaves under water stress could be accompanied by elevated H_2O_2 accumulation in plants, indicating extensive membrane lipid peroxidation. Nonetheless, inoculated plants exhibited lower MDA levels than non-inoculated control plants, suggesting that both microorganisms played a role in ROS metabolism (Ashwin et al., 2022). Water stress has been shown to decrease MDA concentrations in mycorrhizal species compared to the control, although the extent varies among symbiotic fungal species (Basyal & Walker, 2023).

The accumulation of proline and soluble proteins under stress may, on the other hand, result from reduced proline oxidation, its enhanced synthesis from glutamate, or increased protease activity (Jiménez et al., 2021). Proline serves to protect cytosolic enzymes (e.g., carboxylase) and cell structures, so it accumulates in cells during stress (Fang & Xiong, 2015). Water deficit conditions trigger an increase in proline within the plant by upregulating proline-synthesizing enzymes and reducing the activity of proline-degrading enzymes (Serraj & Sinclair, 2002). Mycorrhizal plants, benefiting from improved water relations and nutrition, can better withstand drought stress than non-mycorrhizal plants, so their proline content increases slightly versus non-mycorrhizal plants (Hashem et al., 2019; Oliveira et al., 2022). However, lower soluble sugar content in the leaves of mycorrhizal plants, compared to non-mycorrhizal ones, may be due to the increased translocation of these sugars to the roots (Garg & Cheema, 2021; Basyal & Walker, 2023).

The number of pods, a key determinant of legume yield, is highly reliant on the plant's nutrient supply (Carter & Tegeder, 2016; Ilker et al., 2018; Semba et al., 2021). Our findings indicated that while drought stress reduced yield and its components, microorganisms bolstered plant tolerance and increased the number of pods, seeds, and grain yield. This

enhancement is likely due to a gradual nutrient supply and improved physiological growth conditions. It can be inferred that the chemical and physical properties of humic acid surrounding mycorrhizae (Wang et al., 2017) may contribute to increasing the accumulation of nutrients required by the plant and enhancing the number of auxiliary branches, pods, and seed yield per plant (Al-Karaki & Williams, 2021) by improving water retention, nutrient storage capacity, growth-regulating hormones, and the activity of microorganisms (Mondani et al., 2019). Beneficial microorganisms, particularly mycorrhizal fungi, can boost plant biomass and biological yield by synthesizing phytohormones, enhancing nutrient availability, facilitating nutrient uptake, reducing heavy metal toxicity in the plant, deterring pathogens, and inducing systemic resistance to pathogens (Grümberg et al., 2015; Wang et al., 2017). Similarly, Samsami et al. (2019) observed a 43.8% increase in biological yield due to soybean seeds inoculated with *Rhizobium* and mycorrhiza, attributing this boost to the production of indole-3-acetic acid (IAA) by these microorganisms. On the other hand, Bharti et al. (2018) found that soybean plants inoculated with rhizobium exhibited a significant increase in auxiliary branches, seeds, and pods and ascribed this finding to the increase in nitrogen fixation, nutrient uptake, and the production of growth-stimulating hormones. In the same vein, Dabré et al. (2022) and Egamberdieva et al. (2015) reported that the rise in seed and pod numbers in plants might stem from the increased nutrient availability provided by mycorrhizae and *Bradyrhizobium*, which supports our findings. Musyoka et al. (2022) assessed the role of mycorrhiza in mung bean root development. They found that mycorrhiza enhanced nutrient uptake from the soil by boosting photosynthetic capacity and improving plant water relations. Some researchers believe that the combination of humic acid or amino acids as carbon sources can increase the performance of mycorrhizal fungi, so Torun & Toprak (2020) concluded that the combination of AMF and K-humate enhanced the antioxidant defense system by increasing antioxidant enzymes and antioxidant capacity. Basak et al. (2019) also reported that under salinity stress applying mycorrhiza showed a positive relationship to stem height, stem and root wet weight, and root amino acids.

The synergistic interaction between mycorrhizae and *Bradyrhizobium* with soybean has been reported as a crucial factor in helping plants protect themselves from various stresses. Gholinezhad et al., (2020) studied the impact of mycorrhizal species on the seed oil and yield of sesame cultivars and reported their positive influence on seed yield and oil quality under drought conditions. Furthermore, Rahimzadeh & Pirzad (2019) discovered that the seeds of mycorrhizal plants yielded a higher oil percentage and synthesized more α -linolenic, linoleic, and oleic acids. Our research revealed that drought stress led to an increase in palmitic acid (a saturated fatty acid) and a decrease in linolenic acid (an unsaturated fatty acid). Thus, it can be inferred that water stress significantly reduces unsaturated fatty acid content while increasing saturated fatty acid content. According to Tohidi Moghadam et al. (2011), water stress diminishes the sink capacity by shortening the growth period, thereby curtailing the synthesis time for unsaturated fatty acids in seeds. However, mycorrhizal inoculation, particularly along with *Bradyrhizobium*, reduced palmitic and stearic acids (saturated fatty acids) and elevated oleic, linoleic, and linolenic acids (unsaturated fatty acids). Based on Ghasemi et al. (2023), mycorrhiza application enhanced seed oil quality by lowering erucic acid and increasing linoleic acid in sesame seeds. *R. irregularis* and *G. fasciculatum* outperformed the other two species in most evaluated traits. Indeed, various studies on different plants across various regions of Iran, particularly in arid zones, have consistently identified *F. mosseae* and *R. irregularis* as dominant species (Zardak et al., 2018; Bazgir et al., 2020). However, in our study, *F. mosseae* had a lesser impact on the measured traits than the other two species, possibly due to its lower adaptability to the regional environmental and soil conditions or the host plant species and variety. Augé (2001) argues that the extent of colonization by a fungal species is influenced by the plant species, fungus type, and even isolates of the same species collected from different locations. Therefore, the symbiotic compatibility of fungal species with the host plant depends on the plant type, species, physiological state, soil conditions (e.g., pH and EC), and altitude (Wang et al., 2017).

5. CONCLUSION

The data indicated that both yield and yield components declined as the intensity of drought stress increased. The application of *R. irregularis* and *G. fasciculatum* mycorrhizae, particularly to the plants inoculated with *B. japonicum*, showed positive effects. This combination helped mitigate oxidative damage from drought, as evidenced by lower MDA levels. It also increased soluble proteins, proline, and POD and SOD activities, contributing to better drought tolerance and improved seed yield, oil content, and quality. Consequently, our findings suggest that symbiotic relationships of *R. irregularis* and *G. fasciculatum* mycorrhizae, along with *B. japonicum* bacteria, can alleviate drought stress damage by enhancing certain physiological characteristics of the soybean plant, thereby bolstering its stress tolerance. For subsequent research, it is recommended to assess the impact of drought stress at various growth stages of soybean inoculated with diverse mycorrhizal species and growth-promoting bacteria on physiological and agronomic traits.

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The Potato Clones Selected for High and Low Plant Yield in the F₁ Generation of Atlantic × 101 (Nif) Cross

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ARTICLE INFO

Research Article

Received: 26 May 2025

Accepted: 22 June 2025

Published: 23 June 2025

Keywords:

Highest (Q₁) quartile

Lowest (Q₄) quartile

Normal distribution

Potato clonal selection

Progeny testing

Selection differential

Truncated selection

ABSTRACT

The study aimed to compare the performances of clones selected for high low single plant yield in the greenhouse growing in the progeny test run in seedbed growing. A total of 104 plants were randomly selected among 1104 F₁ plants and tuber yield of each plant was measured and the tubers were kept as clone. Assuming a normal distribution, the truncated selection was applied at the highest (Q₁) and lowest (Q₄) quartiles of the normal distribution at the s = 0.05; 0.10; 0.15; 0.20 and 0.25 selection pressures. Clones selected were grown in a seedbed nursery as progeny testing. The results can be summarized as follows: The progeny population could be separated into two distinct groups with mean values of 5.16 g and 1.35 g, at the s = 0.25 selection pressure. In the greenhouse the highest quartile at the s = 0.05 selection pressure had the highest mean of 6.40 g and reduced to 5.16 g at the 0.25 level. In the lowest quartile the lowest mean was 0.60 g at the s = 0.05 level and increased up to 1.35 g in the s = 0.25 level. The highest quartile (Q₁) had the mean 49.51 g at s = 0.05 and reduced to 35.53 g in the s = 0.25. The lowest mean was obtained in the lowest quartile (Q₄) at the s = 0.05 with a value of 24.30 g and increased up to 33.85 g at the s = 0.25 level. The highest amount of the difference between the highest and the lowest selection was obtained as 25.21 g at the s = 0.05 pressure level in the seedbeds.

Citation: Aydin, M. A., Ozturk, G., & Yildirim, M. B. (2025). The potato clones selected for high and low plant yield in the F₁ generation of Atlantic × 101 (Nif) cross. *Turkish Journal of Field Crops*, 30(1), 249-256. <https://doi.org/10.17557/tjfc.1706800>

1. INTRODUCTION

In potato breeding, first stage of selection of individuals starts in the F_1 generation grown in the greenhouse in contrast to Pedigree breeding of cereals which starts in the F_2 population. This is a shortcut in potato breeding due to insufficient Seed production in the first generation. A clone is the tubers of a single plant F_1 plants carries the identical copy complete genetic structure. Therefore, the potato clone obtained from the tubers of the F_1 plant represent whole genetic structure. Thus, in potato breeding; a breeder can select high yielding clones as early as possible to be tested in the field growing. Since the first selection applied in the F_1 generation grown in the small pots in the greenhouse the success of selection could not be evaluated. Therefore, the progeny of the selected clones should be grown under field conditions to evaluate yielding capacity of clones (Mullin et al., 1966). In practice seeds are increased in the seedbeds or in the laboratory using meristem culture.

The success of the selection could be controlled in the progeny testing of the clones selected in the greenhouse by growing them in the seedbeds or in the field (Yildirim, 1972; 1981; Ozturk and Yildirim, 2020; Yildirim and Ozturk, 2021). The success in a potato breeding depends on the direction and duration of selection during the field testing. The success of selection and the possibility of obtaining high yielding potato genotype depends on the genetic variation of the starting clones in the F_1 generation (Konstantinova and Fadeev, 2020).

In potato breeding, yield components and other components should be combined in a potato clone before submitting it to the releasing process. Since a breeding program aims high yield the lower yielding clones have been eliminated in the first cycle of selection process (Brown et al., 1987; Brown et al., 1988; Gopal et al., 1992; Ozturk and Yildirim, 2014).

Selection theory assumes a normal distribution of individuals grown in the base population. If base population where the plant selection starts, should be assumed as a normal distribution. The success of selection applied for a quantitative trait could be evaluated by using the parameters and characteristics of a normal distribution such as mean and variance.

The F_1 plants grown in the greenhouse are ranked by their phenotypical values of some quantitative traits and certain percentages is selected. This method is called a truncated selection for quantitative traits. The difference between the means of selected individuals and the population mean ($\bar{x}_s - \bar{x}_p$) are defined as selection differential (S). The difference between the means of the progeny testing population and the mean of starting population ($\bar{x}_p$) is defined as genetic advance (GA) and was estimated by the $\Delta Z = h^2 \times S$ formula (Lush, 1945; Kelly, 2008; 2011). The widely used formula for genetic advance is $GA = i\sigma_p h^2$ where i = *Selection intensity*, σ_p = *phenotypic standard deviation* and h^2 = *heritability in narrow sense* (Lush, 1945; Falconer, 1964; Yildirim and Ozturk, 2021).

The F_1 generation of the Clone 6/7 x 101 (Nif) crossings were grown in the greenhouse and the single plant yield of F_1 plants were evaluated and selection was applied at such as $s = 0.05; 0.10; 0.15; 0.20$ and 0.25 . The success of the selection was controlled in the seedbed growing. The results indicated that selection at high and moderate selection pressures was effective for single plant yield and tuber number. Lower selection pressure were found to be more effective for single tuber weight. Selection applied at medium level ($s = 0.15$) resulted in high single plant yield (Aydin and Ozturk (2022).

Selection differentials were estimated by selecting individuals with the highest (Q_1) and the lowest quartiles (Q_4) at different selection pressures (s) ($0.05; 0.10; 0.15; 0.20; 0.25$) in the greenhouse growing. The selected groups were compared with each other by conducting progeny control in the following seedbed nursery. As a result of the comparison, means of single plant yields for high and low value individuals were 24.02 g ($s = 0.25$) and 18.19 g ($s = 0.05$), respectively (Yilmaz, 2023).

The purpose of this study was to compare the performance of the F_1 clones selected for the highest and the lowest ends of a normal distribution for single plant yield in the greenhouse by growing them in the progeny testing stage grown in the seedbed nursery.

2. MATERIALS AND METHODS

The study was conducted in the laboratory, greenhouse and seedbeds of the Department of the Field Crops of the Ege University in 2022 and 2023. The temperature and precipitation values of Bornova in 2022 and 2023 are given in Figure 1 (Anonymous, 2023).

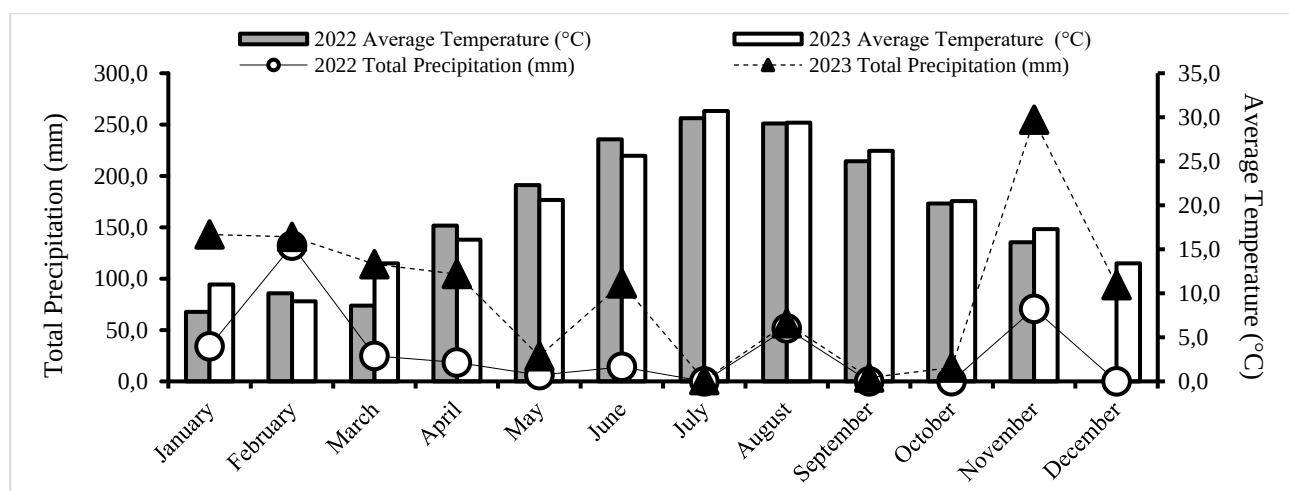


Figure 1. Average monthly temperature (°C) and total monthly precipitation amounts (mm) for the 2022 and 2023 growing seasons at the trial site (Anonymous, 2023)

True potato seeds (TPS) obtained from the cross of Atlantic x 101 (Nif) were used in the study certain characteristics of parents and single plant yield, tuber number and single tuber weight of the parents are shown in Table 1 and Table 2.

Table 1. Certain characteristics of parents

Genotype	Parentage	Characteristics
Atlantic	Wauseon x Lenape	Medium-late, medium dormancy, abundant flowering (light lavender), high-quality pollen, oval-round tubers, white skin and flesh color, medium shallow tuber eye depth, high dry matter content, low total glycol alkaloid level (Webb et al., 1978; ECPD, 2025)
101 (Nif)	Cosima x R.143	Medium early, productive, oval tuber, 23.4% dry matter and 16.8% starch, high flower and fruit production (Ozturk and Yildirim, 2020)

Table 2. Mean of the tuber number, the single tuber weight (g), and the plant yield (g) of parents measured in pots and seedbeds

Parents	GREENHOUSE GROWING			SEEDBED GROWING		
	Tuber number	Single tuber weight (g)	Single Plant yield (g)	Tuber number	Single tuber weight (g)	Single Plant yield (g)
Atlantic	1.80	1.80	3.24	1.80	9.30	16.74
101 (Nif)	2.00	2.20	4.40	2.20	11.50	25.30

Crossing and growing the True Potato Seeds (TPS) in the greenhouse

The crossings of Atlantic x 101 (Nif) were completed in the spring period of 2022 in the seed laboratory of Department of Field Crops at Ege University.

Fruits harvested were squeezed and 2510 TPS were obtained. The TPS of the cross and the parents were grown in 10x20 cm pots in November 2022 and 1104 plantlets were grown and the seedlings were transferred to plastic pots containing mixture of soil and turf in 2: 1 ratio. Each individual pot contained one single potato plant.

A total of 104 plants were randomly selected and harvested separately and their tuber were recorded and saved as clone. The clones were measured for the single plant yield, tuber number and single tuber weight.

The selected clones were grown in seedbeds in September, 2023. Plot size was a single row in 1.5 m length. Planting was done in 30 x 50 cm. Normal growing procedures were applied and the seedbeds were harvested by hand on December 26, 2023. The single plant yield, tuber number and single tuber weight was recorded.

Statistical evaluation of clonal data obtained in the greenhouse

The population of 104 clones assumed to be in normal distribution and the following parameters were estimated as given by Yildirim and Ozturk (2021).

n: number of individual clones

$\bar{x}$: average

$$\bar{x} = \sum \frac{x_i}{n}$$

$$\text{Variance } (s^2) = \frac{\sum x_i^2 - \frac{(\sum x_i)^2}{n}}{n - 1}$$

$$\text{Standard deviation } (s) = \sqrt{s^2}$$

$$\text{Standard error of mean } (s_{\bar{x}}) = \sqrt{\frac{s^2}{n}}$$

$$\text{Selection Differential} = \bar{x}_{\text{selected}} - \bar{x}_{\text{base population}}$$

$$z = \frac{\bar{x}_{\text{selected}} - \bar{x}_{\text{base population}}}{s}$$

$$i = \frac{z}{p} \text{ where } p = 1 - s \text{ (s: selection ratio applied such as 0.05; 0.10; 0.15; 0.20 and 0.25)}$$

3. RESULTS AND DISCUSSION

The distribution of the single plant yields of the selected 104 clones of Atlantic x 101 (Nif) cross grown in the greenhouse is shown in Figure 2.

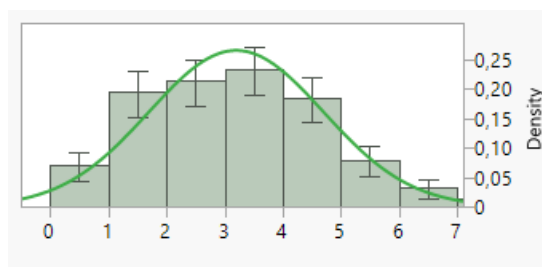


Figure 2. Distribution of the single plant yield of randomly selected potato clones in the greenhouse

It could be seen in Figure 2 that the distribution of single plant yield of clones could be in a normal distribution in the greenhouse.

The results of simple statistical evaluation of data for the single plant yield in the greenhouse and seedbed nursery growing are shown in Table 3.

Table 3. Estimates of simple statistics of the normal distribution for 104 potato clones grown in the greenhouse (base) and progeny testing in the seedbeds

Characteristics	Number of individuals (n)		Range (min.-max. value)		Mean ($\bar{x}$)		Standard deviation (s)	
	G1	SB	G1	SB	G1	SB	G1	SB
Single plant yield (g)	104	104	0.33-7.02	1.51-110.62	3.20 ± 0.15	35.86 ± 2.65	1.50	27.07
Single tuber weight(g)	104	104	0.33-2.82	1.51-37.34	1.39 ± 0.05	15.70 ± 0.93	0.51	9.45
Tuber number	104	104	1-4	1-4	2.34 ± 0.09	2.21 ± 0.10	0.91	1.00

G1: Greenhouse Generations, SB: Seedbed Growing

It could be observed in the Table 3 that the single plant yields of potato clones in the greenhouse ranged as 0.33 g – 7.02 g; single tuber weight ranged in 0.33 g – 2.82 g and tuber number ranged as 1 - 4 interval. Similarly, ranges were 1.51 g – 110.62 g, 1.51 g – 37.34 g and 1 – 4 for single plant yield, single tuber weight and tuber number respectively in the seedbed growing. Since the single plant yield is accepted as a yield component and the other two traits are correlated with single plant yield we will focus on comparing the single plant yield of two opposite selections applied at the values varying of the selection pressure in the greenhouse with their means in the seedbed growing.

The population mean and the selection differentials estimated for the highest and the lowest quartiles for clone yields in the greenhouse and seedbed growing are shown in Table 4 and Table 5.

Table 4. Means of selected groups and selection differentials estimated for the highest and the lowest quartiles in the greenhouse growing at different selection pressure(s)

Selection pressure (s)	Selection intensity (i)	Population mean ($\bar{x}$) (g)	Highest (Q_1)		Lowest (Q_4)	
			Mean of selected (g)	Selection differential (SD) (g)	Mean of selected (g)	Selection differential (SD) (g)
0.05	2.06	3.20	6.40 ± 0.17	3.20	0.60 ± 0.08	-2.60
0.10	1.83	3.20	6.01 ± 0.16	2.81	0.84 ± 0.10	-2.36
0.15	1.69	3.20	5.64 ± 0.16	2.44	1.08 ± 0.10	-2.12
0.20	1.61	3.20	5.38 ± 0.16	2.18	1.24 ± 0.10	-1.96
0.25	1.53	3.20	5.16 ± 0.16	1.96	1.35 ± 0.09	-1.85

It could be seen in Table 4 that the highest selection differential (SD) was 3.20 g in the highest quartile (Q_1) at the $s = 0.05$ level and the lowest selection differential in the lowest quartile (Q_4) was |-2.60| g. The values of selection differentials for $s = 0.10$ (2.81 g), $s = 0.15$ (2.44 g), $s = 0.20$ (2.18 g) and $s = 0.25$ (1.96 g) in the selection pressure from the highest level and |-2.36|, |-2.12|, |-1.96| and |-1.85| in the selection pressure in the lowest level respectively.

The highest mean for selected clones at the $s = 0.05$ was 6.40 g in the highest quartile and 1.35 g at the $s = 0.25$ selection pressure in the lowest quartile.

The selection differentials observed in Table 4 were as expected. At the $s = 0.05$ level, the highest group exhibited a selection differential of 3.20 g, while the lowest group showed |-1.85| g. At the $s = 0.25$ level, these differentials decreased from 3.20 to 1.96 g and increased to |-2.60| to |-1.85| g, selected in the Q_1 and Q_4 quartiles respectively.

The mean of single plant yield obtained in the greenhouse growing and its progeny testing results of following stage at various selection pressure were compared in Table 5. The increases in the mean of selected group in the seedbed growing were positive and descending order from $s = 0.05$ to $s = 0.25$, except for $s = 0.10$. Such as 6.40 g and 49.51 g at the $s = 0.05$ level but 52.46 at the $s = 0.10$ selection pressure level.

Table 5. Selection differentials obtained for the highest (Q_1) and lowest (Q_4) quartiles in the greenhouse and their mean values obtained in the seedbed growing conditions

Selection pressure (s)	Selection intensity (i)	Highest (Q_1)				Lowest (Q_4)			
		Selection differential (greenhouse) (g)	Mean of selected (greenhouse) ($\bar{x}_{G1}$) (g)	Mean of seedbed ($\bar{x}_{SB}$) (g)	Gain (g)	Selection differential (greenhouse) (g)	Mean of selected (greenhouse) ($\bar{x}_{G1}$) (g)	Mean of seedbed ($\bar{x}_{SB}$) (g)	Gain (g)
0.05	2.06	3.20	6.40	49.51 ± 16.13	46.31	-2.60	0.60	24.30 ± 4.97	21.70
0.10	1.83	2.81	6.01	52.46 ± 10.72	49.65	-2.36	0.84	31.32 ± 4.80	28.96
0.15	1.69	2.44	5.64	42.46 ± 7.89	40.02	-2.12	1.08	32.89 ± 6.33	30.77
0.20	1.61	2.18	5.38	35.85 ± 6.56	33.67	-1.96	1.24	34.05 ± 5.40	32.09
0.25	1.53	1.96	5.16	35.53 ± 5.93	33.57	-1.85	1.35	33.85 ± 5.10	32.00

The similar conclusion could be valid in the lowest side selection such as the mean was 0.60 g and 24.30 g in the greenhouse and seedbed growing at the 0.05 level, respectively. Means of selected groups were in decreasing order except from $s = 0.20$ to $s = 0.25$ on the contrary, it was from low to high in the seedbed growing.

There is a lack of information based on actual study on comparing lowest and highest selection pressures in the literature related to potatoes. We can give some agreed results with $s = 0.25$ in Yildirim (1980) wheat, Cagiran (1989) barley and Kelly (2011) yellow monkeyflower (*Mimulus guttatus*).

Aydin and Ozturk (2022) reported that the highest level of selection in the greenhouse was 7.70 g at a $s = 0.05$ selection pressure for high side. Yilmaz (2023) reported that the highest mean of the single plant yield was 11.14 g at $s = 0.05$ level and lowest single plant yield at the same pressure level was 1.76 g. In this study the discrepancies were found at the $s = 0.10$ level in the highest and $s = 0.25$ level. These discrepancies supported Bradshaw et al. (1998), who reported that the clonal selection could not succeed without additional selection application in the progeny testing stage and the following selection periods.

Another comparison of high and low level selection of clones in the base and progeny population are shown in Table 6. This table indicates a clear increase in the means of a single plant yield during seedbed growing.

In Table 6 we could also observe certain discrepancies in the high side and low side selection groups such as clone 40-133 had 5.74 g in the greenhouse but had 100.30 g single plant yield in the seedbed growing. The single clone has caused 52.46 g in the $s = 0.10$ in Table 5.

In general, we expect means reduced in accord with selection pressure from 0.05 to 0.25; at the highest quartile we also expect increased means from $s = 0.05$ to 0.25 in the lowest selection side, but the single plant yields had a variable trend in two selection procedures.

Table 6. Single plant yields of the clones at 0.05; 0.10; 0.15; 0.20 and 0.25 selection pressure for the highest (Q_1) and the lowest (Q_4) selection groups grown in the greenhouse and in the seedbeds

HIGH LEVEL (Q_1)				LOW LEVEL (Q_4)		
No	Clone	Greenhouse (g)	Seedbeds (g)	Clone	Greenhouse (g)	Seedbeds (g)
1	40-105	7.02	71.78	40-183	0.33	36.92
2	40-89	6.47	44.57	40-261	0.50	9.76
3	40-223	6.33	97.11	40-19	0.66	16.17
4	40-17	6.21	4.54	40-356	0.74	31.12
5	40-342	5.99	29.57	40-241	0.75	27.53
6	40-250	5.80	3.80	40-27	0.82	54.57
7	40-133	5.74	100.30	40-341	0.95	46.24
8	40-92	5.56	45.40	40-262	1.14	11.77
9	40-252	5.51	55.70	40-13	1.23	44.72
10	40-361	5.45	71.87	40-171	1.30	34.41
11	40-103	5.38	8.64	40-162	1.30	34.90
12	40-215	5.00	51.04	40-156	1.45	110.62
13	40-369	4.98	33.43	40-111	1.47	3.32
14	40-333	4.96	44.39	40-50	1.54	15.51
15	40-357	4.91	6.19	40-104	1.55	32.62
16	40-66	4.91	11.01	40-275	1.61	16.04
17	40-57	4.67	14.63	40-65	1.69	60.20
18	40-126	4.60	6.50	40-35	1.70	12.76
19	40-338	4.54	18.50	40-135	1.72	57.17
20	40-326	4.53	10.64	40-52	1.75	8.00
21	40-263	4.39	23.34	40-240	1.78	50.78
22	40-214	4.35	71.68	40-244	1.80	10.73
23	40-8	4.29	4.26	40-101	1.80	71.08
24	40-119	4.25	72.39	40-43	1.81	11.07
25	40-90	4.23	14.13	40-3	1.84	3.09
26	40-29	4.17	8.43	40-173	1.85	68.94

In plant breeding individual plant selection was applied assuming a normal distribution and high individuals with high value are selected following a truncated selection procedure. The success of selection depends on the additive variation and could be controlled by growing the selected individual in the following stage, such as progeny testing. In this study the clones of lowest section of the base population (greenhouse) were also chosen to compare the success probability of selection applied at two reverse directions.

This type of selection could help increase the genotypic variation and could be beneficial in the field evaluation to control some unexpected variation. For example, clones 40-156 (110.62 g) have surpassed the highest plant yield, 40-133 (100.30 g), indicating some possibilities for increasing genetic variation. This type of differences could not be explained

easily. Some experimental deficiencies as well as some epigenetic factors could be effective during the growing period (Bradshaw et al., 1998).

The mean of single plant yields of the highest and the lowest selection ratios are shown in Figure 3. Also, the difference between the highest and the lowest levels of selection at the base and progeny populations are shown in Figure 4.

The difference between selection at the $s = 0.05$ pressure level of the highest side and selection of the lowest side was 25.21 g. This could be accepted a clear cut difference obtained in the progeny control.

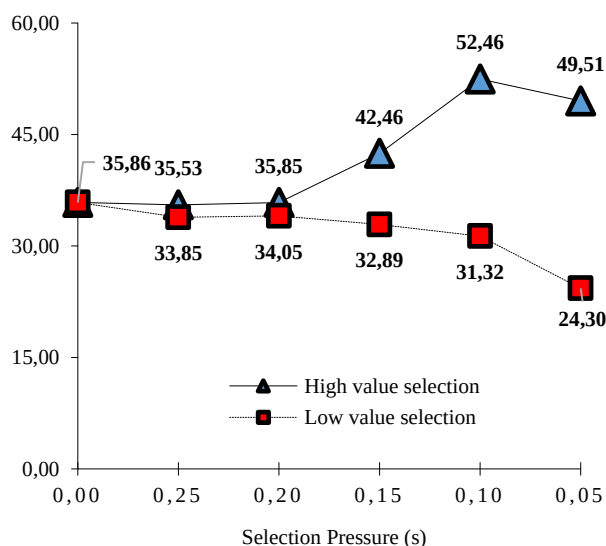


Figure 3. The single plant yield of the clone selected at high side and low side selection in the seedbeds

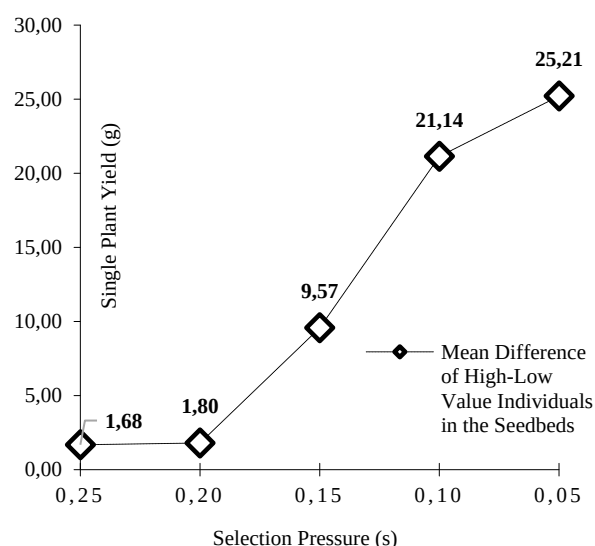


Figure 4. The differences between the means of selected groups at highest and lowest sides in increasing order from $s = 0.05$ to $s = 0.25$ level

4. CONCLUSION

Based on the comparison of the mean performance of selected single plants from the highest and lowest quartiles in the F_1 generation and their corresponding means in the seedbed nursery, the following conclusions can be drawn: Selection pressure at the $s = 0.05$ level applied for single plant yield in the greenhouse growing resulted in high means in the seedbed growing stage (49.51 g). Similarly, the lowest selection pressure applied at the same level in the following stage resulted in low mean in the seedbed control, such as 33.85 g. It could be generalized that mean of the selected groups from the 0.05 to the 0.25 level pressure decreased in the highest side of the application (6.40 g - 5.16 g) on the contrary, the mean of the selected groups increased from low to high in the lowest side (Q_4) such as (0.60 g - 1.35 g).

The apparent difference between the effect of low and high level selection for single plant yield resulted in the clear-cut differences at the $s = 0.05$ pressure level, such as 6.40 g and 0.60 g as expected.

Some discrepancies encountered in the selection procedure include high plant yields in the high and low pressure groups such as 100.30 g in the highest part and 110.62 g in the lowest part.

To cope with this kind of unexpected results, selection pressure could be reduced down to moderate levels such as $s = 0.10 - 0.15$ in practical breeding procedure. In this study the difference between the highest and the lowest selected groups has resulted in as expected levels. If the aim is to establish a successful potato breeding program, applying moderate selection pressures—such as $s = 0.20 - 0.25$ can be advisable during the greenhouse selection stage.

ACKNOWLEDGEMENT

We would like to extend our heartfelt thanks to the Department of Field Crops, Faculty of Agriculture, Ege University, for providing us with the academic environment, resources, and steady support which greatly contributed to the progress and completion of this study.

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