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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

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DETERMINATION OF SUPERIOR BEAN GENOTYPES IN COOKING AND PHYSICAL BY MULTI-CRITERIA DECISION-MAKING METHOD

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ABSTRACT

It is aimed to examine and predict the effects of bean genotypes using cooking and physicochemical properties on seed quality index and yield in this study. The seed quality index was calculated by combining the analytical hierarchical process and standard scoring functions, which is one of the multi-criteria decision-making methods, using the linear combination technique. To determine the seed quality index, a data set was created with 11 indicators. analytical hierarchical process was used to weight importance levels of examined traits depending on the genotypes. Seed quality index of registered cultivars according to investigated characteristics of cultivars and genotypes IV. Class, 6 genotype (Bombay genotype) was found to be in class V. Obtained seed quality and physical properties by determining with seed quality index obtained in this study, estimation of seed quality in beans with analytical hierarchical process was evaluated successfully. As a result, according to seed quality index of bean cultivars and genotypes, it was determined that genotype 6 had superior characteristics in terms of productivity, in addition genotypes 8 with 9 and registered cultivars could also show superior characteristics.

Keywords: Analytical hierarchical process, Cooking characteristics, Legumes, Physical properties, Yield

INTRODUCTION

Legumes are very important for human and animal nutrition, besides providing biologically fixed nitrogen by bacteria for soil (Koivunen et al., 2015). Bean (*Phaseolus vulgaris* L.) is one of the most important edible legumes used for direct human consumption (Didani and Dumlupınar, 2022). As a matter of fact, beans contribute to the nutrition, economic and social welfare of many people in developing countries (Anderson et al., 2016; Natabirwa et al., 2018). Historically, legumes like peas and certain types of beans have been traditionally grown in Nordic countries, with the latter being mainly suitable for animal fodder if grown north of latitude 60°N (Meltzer et al., 2019). Beans are very rich in protein, energy, and dietary fibre. It is also a rich source of micronutrients such as iron, zinc, B vitamins and health-promoting bioactive compounds such as polyphenols (Blair 2013). Many dried bean varieties with different physico-chemical and sensory properties meet changing consumer preferences. In general, some of the well-known bean genotypes are black turtle beans, cranberry, borlotti beans, flageolet beans, kidney beans, pea beans, pink beans, pinto beans, and white beans, yellow beans, which are primarily grown in tropical and subtropical regions (Nicoletto et al., 2019).

According to 2019 data in the world, 33 million hectares of dry beans were harvested, and 28.9 million tons were produced. In the same year, 225 thousand tons of dry beans were produced in an area of 89 thousand hectares in Turkey. Yield for dry beans has been 874 kg ha^{-1} in the world and 2531 kg ha^{-1} in Turkey (FAO, 2019). On the other hand, production and yield amount of beans, is not at a sufficient level yet. Indeed, worldwide bean consumption is still low, estimated to be between 4-66 kg per capita per year for different countries (Blair 2013).

One of the most important goals of research programs is to increase the yield per unit area. It is necessary to develop suitable varieties for the region, to know the degree of influence of factors affecting yield and relations between each other, and to make the selections in breeding programs according to these criteria (Oner et al., 2023). The first of the main strategies for success in cultivar development programs is the breeder's understanding of interactions of characteristics with one another that constitute yield and quality of plants that are effective in regional conditions (Agarwal et al., 2013). Simple correlations are not sufficient to evaluate complex relationships between many characters related to dependent variables (MacCallum et al., 2002). It is great importance to develop new approaches to facilitate selection of superior properties genotypes together with

the determination of the characteristics that affect yield and yield in plant breeding.

Multi-criteria making decisions, multi-objective making decisions, and multidimensional making decision are frequently used by scientists (Diaz and Soares, 2022). In this context, some multivariate indexes such as the analytic hierarchy process (AHP), which is a general measurement theory, have been developed (Darko et al., 2018). AHP is frequently used in decision-making processes that use multiple criteria to estimate preference values for a particular set of features. AHP can be used for both concrete and abstract data. The method is easy to use and can help researchers combine concrete and abstract criteria and make the best decision (Ziemba 2022).

In AHP, pairwise comparison is first used to evaluate the relative importance of different alternatives for a set of criteria or attributes. Then, individual preferences are estimated using AHP and combined into group preferences using weighted goal programming (Coffey and Claudio, 2021). When groups have different opinions, weighted goal programming (WGP) based models can be used to generate consensus preference values (Dhahri et al., 2020). AHP has generally been applied to decide on a suitable site for environmental management and different purposes (Garcia 2022). The AHP approach has also been adopted by many researchers for agricultural decisions (Sengupta et al., 2022). For example, Garcia and Guitart (2022) in rice, Gebru et al. (2023) in the identification and selection of superior genotypes in tomato used AHP.

In this study, beans of different types were grown in 2019 and 2020. It aimed to determine the most productive type according to seed quality indices by weighting the yield obtained with the quality and physical properties of seeds with AHP.

MATERIALS AND METHODS

Materials

In the study, 20 bean genotypes and 2 registered cultivars were used as seed material. Some characteristics and supplied locations of bean types used in the experiment are given in Table 1.

Table 1. Seed characteristics and supplied locations of bean genotypes/varieties used in the experiment

Material code	Genotype name	Supplied location	Seed colour
G1	Bosna Hersek 1	Bosnia and Herzegovina	light brown with burgundy spots
G2	Bosna Hersek 2	Bosnia and Herzegovina	black
G3	Bosna Hersek 3	Bosnia and Herzegovina	yellow
G4	Iran	Iran	light brown with burgundy spots
G5	Taskentcity	Uzbekistan/Tashkent	light brown spots on burgundy
G6	Bombay	Turkey/Bolu	white
G7	Black Bean	Turkey/Mersin	black
G8	Dermason	Turkey/Mersin	white
G9	Bat Bean	Turkey/Mersin	white hilum and black spots at the junction
G10	Bagel Bean	Turkey/Mersin/Mut	white
G11	Claret red bean	Turkey/Mersin/Mut	claret red
G12	Purple Bean	Turkey/Mersin/Mut	purple
G13	Osmanli Bean	Turkey/Isparta	dark purple/black
G14	Ayse Kadin	Turkey/Isparta	white
G15	Isparta Bean	Turkey/Isparta	yellow
G16	Sugar Bean	Turkey/Konya	white
G17	Kirmizi-Beyaz Alaca	Kyrgyzstan	hilum and background in red color joint on white
G18	Tomanity	Kyrgyzstan	red
G19	Ryabaya	Kyrgyzstan	light brown with burgundy spots
G20	Sudan	Sudan/Al Junaynah	light brown spots on burgundy
RV1	Onceler 98*	Turkey/Isparta	white
RV2	Yunus 90*	Turkey/Isparta	white

* – registered bean varieties, G – genotype, RV – registered varieties

Setup of Experiment, Climatic and Soil Characteristics of Area

The coordinates of Isparta where the research was carried out are 37°45'59.4"N 30°33'11.8"E trial fields on (April 29) 2019 and (May 1) 2020 in Turkey. The experiment was carried out according to the randomized complete block design (RCBD) with 3 replications. The bean genotypes used in the experiment are given in Table 1. The plot size of the study was 8 m^2 (4 m x 2 m) and it was established to have 4 rows in each plot. The sowing norm was set to be 50×20 cm between and above the rows. In the study, fertilization was made with 40 kg of N and 60 kg of P_2O_5 per hectare. In the study, irrigation was done with a drip irrigation system according to the moisture condition of soil. Each genotypes was checked every other day and harvested as genotypes matured and threshed after drying in a shaded area. When climate characteristics in 2019 and 2020, when study was carried out, and long-term were examined, the temperature in both years (21.2 and 22.6°C, respectively) was higher than

the long-term data (20.2°C), and total precipitation in long-term data (140.0 mm) less than second year (162.4 mm) more than first year (131.5 mm). It was observed that average relative humidity (49.2% and 45.6%) was less than long-term data (51.2%). The research was conducted in the same region in both years. When the soils taken from this region are analyzed, the results; soil texture is a clayey-loamy structure, with a slightly alkaline reaction (mean 7.60), salt content is in the slightly salty group (mean 325 µS/cm), and it is reported to be poor in terms of organic matter content (mean 1.53).

Examined Properties

After determining the yield characteristics of seeds obtained from each plot, hundred seed weight, hydration capacity, swelling index, cooking time, dry volume, seed width and length, testa rate, geometric mean diameter, volume, bulk density, and true density properties were determined.

The study calculated the hundred-seed weight by counting 100 seeds 4 times for replication and then averaging. Hydration capacity (g seed^{-1}) is the amount of water absorbed by seed in g. Therefore, after removing the unswelled hard-shelled seeds in the samples whose 100-seed weight was determined, the hydration capacity of weighed seeds was determined according to the formula below (Eq 1). Seeds that did not absorb any water at the end of the 16-hour soaking period and did not change weight were accepted as hard-shelled seeds (Karaman 2019).

Hydration capacity (g seed^{-1})= Y−[(X−(X/100) x N2)] (Eq 1)

N1 – N2

According to the equation,

Y = Wet weight (g) after non-swelling seeds are separated,

X = Dry 100 seed weight (g),

N1= Initial number of seeds (pieces),

N2= Number of unswelled hard-shelled seeds (pieces).

If there is no swelling;

hydration capacity is calculated according to the formula (Eq 2).

Hydration capacity (g seed^{-1}) = (Wet weight-Dry weight) / 100 (Eq 2)

The swelling index was determined according to following formula (Karaman 2019; Eq 3).

Swelling index (%) = [Wet volume-100) / (Dry volume-50)] (Eq 3)

Dry volume (ml), 100 bean seeds were taken into a 100 ml measuring cylinder, 50 ml of distilled water was added, and the data obtained was recorded as dry volume (Karaman 2019). Cooking time (min) was recorded when 50 soaked samples were thrown into boiling water, and then checked every 3 minutes, When testa was peeled, the seed split into two and the white dot inside disappeared (Ozaktan 2021; Luo et al. 2023;). The testa (seed coat) ratio (%) was separated from the testa of 10 seeds from bean seeds soaked for 16 hours, and separated testa and cotyledon were dried in an oven at 65°C until their weight stabilized. Then, their individual weights were determined. It was obtained by dividing the determined dry testa weight by the total dry seed weight and multiplying it by 100. Seed width and length (mm); width and length sizes of seeds used in the study were measured with the help of a caliper.

Geometric mean diameter (Dp, mm) feature was determined by using the formula below (Eq 4). The volume (V) property is calculated using the formula below (Eq 5). The B value in formula expresses the spherical diameter of seed and determined from the formula below at Eq 6 (Taner et al., 2018). Bulk density (Pb) Eq 7 for small seeds (Unal et al., 2008); for large seeds Eq 7 calculated from formulas. True density (Pt) was determined from the formula below (Eq 8).

$Dp = (Width*Length*Thickness)^{1/3}$ (Eq 4)

$V=\pi*B2*L2 / 2a6*(2*Length-B)$ (Eq 5)

$B= (Width*Thickness)^{1/2}$ (Eq 6)

$Pb= (843.3-6.2)*Moisture\ content$ (Eq 7)

$Pt = (Dry\ weight / Dry\ volume)*10000$ (Eq 8)

Descriptive statistics of data in the study were made in the Minitab 17 statistical package program, variance analysis. The difference between the averages Tukey multiple comparison in determining differences test was used. The obtained data were subjected to path analysis, and according to data obtained from this analysis, AHP weights were created by considering the direct impact shares.

Determination of Seed Quality

In the realization of the study, 22 bean genotypes were used as material. Since each seed material has different units, it was first transformed into a unitless state by applying the standard scoring function. Then, the indicators were weighted with AHP developed by Saaty (1980) in order to determine the effect levels of seed quality indicators. Firstly, seed quality indicators were converted to unitless scores between 0.1 and 1.0 to be comparable with each other using standard scoring functions (SSF) (Keshavarzi et al., 2022). Generally, three different scoring functions (SSF) are used: 'more is better', 'less is better' and 'mid-point is optimum' (Gozukara et al., 2021). The SSF equations for parameters are listed in Table 2.

Table 2. Standard scoring functions and parameters for quantitative seed parameters

Parameters	FT*	SSF Equation**
Testa ratio	LB	$f(x)=\begin{cases}0.1 & x\le L\\ 1-0.9\times\frac{x-L}{U-L}+0.1 & L\le x\le U\\ 1 & x\ge U\end{cases}$
Cooking time	LB	
Dry weight	MB	$f(x)=\begin{cases}0.1 & x\le L\\ 0.9\times\frac{x-L}{U-L}+0.1 & L\le x\le U\\ 1 & x\ge U\end{cases}$
Dry volume	MB	
Hydration capacity	MB	
Swelling index	MB	
Hundred seed weight	MB	
Seed width	MB	
Seed length	MB	
Geometric mean diameter	MB	
Volume	MB	
Bulk density	MB	
True density	MB	

*FT: means function type; MB: means more is better; LB: means low is better; **SSF means standard scoring function; L and U are the lower and the upper threshold value, respectively.

In line with the values obtained in this study, more is better and less is better approaches were used. In more is better function, dry weight, dry volume, hydration capacity, swelling index, hundred seed weight, seed width, seed length, geometric mean diameter, volume, bulk and true density are taken, while in the 'less is better' approach, testa rate and cooking time characteristics were taken. With the AHP method, it is possible to make pairwise comparisons to determine the weights and priorities of both qualitative and quantitative factors and it has proposed a comparison that evaluates importance degree ranging from 1 to 9. Pairwise comparison is applied to criteria and sub-criteria according to expert opinions and evaluations (Rouyendegh and Savalan, 2022). The numerical values indicating the relative importance of each other according to the Saaty scale are given in Table 3.

Table 3. Saaty scale

Significant level	Explanation	Description
1	equality important	Two items are equally important
3	one less important than other	Criterion 1 is slightly more important than criterion 2
5	necessary or strongly important	1 criterion is more important than criterion 2
7	strongly important	1 criterion very important, practically dominant or demonstrable situations relative to criterion 2
9	absolutely important	1 criterion is the strongest (extremely) significant and the highest accuracy to criterion 2
2, 4, 6, 8	intermediate values	It is used when indecisive between two evaluations that are close to each other and when a compromise is needed between two values.

Considering the importance of criteria, a comparison matrix (n x n dimensions) was created between criteria (Eq 9).

$$A=\begin{bmatrix} a_{11} & a_{12} & a_{13} & a_{14} & a_{1n} \\ a_{21} & a_{22} & a_{23} & a_{24} & a_{2n} \\ a_{31} & a_{32} & a_{33} & a_{34} & a_{3n} \\ a_{41} & a_{42} & a_{43} & a_{44} & a_{4n} \\ a_{n1} & a_{52} & a_{53} & a_{54} & a_{nn} \end{bmatrix} \quad \text{(Eq 9)}$$

In this matrix created, all data must have positive values. A: pairwise comparison matrix aij: importance of element i relative to element J (i, J……n). Properties of pairwise comparison matrix;

aji = 1/aji aij>0(i, j=1, 2….,n) for pairwise comparison to be fully consistent,

aik=aji ajk (I, j, k=1, 2,….n)

If it is consistent; aij=Wi/WJ

(Wi=priority value for element i, Wj: priority value for element J)

After the comparison matrix table was created, the matrix was normalized. Normalization is done by the data in each cell is divided by the column total of that cell. The W column vector, called the priority vector, is obtained by taking the arithmetic average of sum of data in each row in the normalization table obtained from pairwise comparisons. This vector represents the percentage importance weights of the criteria (Eq 10).

$$w_i=\frac{\sum_{j=1}^{n} a_{ij}}{n} \quad \text{(Eq 10)}$$

W_i= priority vector or weight of i criterion; ai = element i in normalization table; aj = element j in normalization table; n = is number of criteria.

The pairwise comparison matrix (A) is multiplied by the priority vector (w) to get vector D (Eq 11).

$$D_i = A_i \times W_i = \begin{bmatrix} a_{11} & a_{12} & a_{13} & \cdots & a_{1n} \\ a_{21} & a_{22} & a_{23} & \cdots & a_{2n} \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ a_{n1} & a_{n2} & a_{n3} & \cdots & a_{nn} \end{bmatrix} \times \begin{bmatrix} w_1 \\ w_2 \\ \cdots \\ \cdots \\ \cdots \\ w_n \end{bmatrix} \quad \text{(Eq 11)}$$

E_i values in Eq12 are obtained by the elements (d_i) of vector D column are divided by elements (W_i) of priority vector.

$$E_i = \frac{d_i}{w_i} \quad \text{(Eq 12)}$$

The sum of E_i values is divided by a number of criteria and the arithmetic average is taken. With this process, the largest eigenvalue of matrix called λ_{max} (Eq 13) is found.

$$\lambda_{max} = \frac{\sum_{i=1}^{n} E_i}{n} \quad \text{(Eq 13)}$$

λ_{max} = Maximum eigenvalue; n = number of criteria

In order to measure consistency in comparisons, eigenvector method is used, and consistency index (CI-Consistency Index) is obtained (Eq 14).

$$CI = \lambda_{max}\text{-}n/n\text{-}1 \quad \text{(Eq 14)}$$

Consistency ratio (CR) value; As seen in the equation in Equation 15, it is obtained by dividing Consistency index (CI) by the Random index (RI) (Table 4) value (Eq 15) If CR value is less than 0.10, comparisons made by decision maker are consistent, If CR value is 0.10 greater, it indicates that the comparisons are inconsistent or there is a computational error. In this case, comparisons should be reconsidered (Saaty 1980).

$$CR = CI/RI \quad \text{(Eq 15)}$$

Table 4. Random index values (RI) depending on number of criteria

n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
RI	0.00	0.00	0.58	0.90	1.12	1.24	1.32	1.41	1.45	1.49	1.51	1.48	1.56	1.57	1.59

After features weighted with AHP were standardized with SSF, seed quality index values were determined using linear combination technique approach (Eq 16), and they were classified according to Table 5.

Table 5. Classification of seed quality index (SQI)

Class	Definition	SQI
I	Very low	< 0.40
II	Low	0.40 – 0.50
III	Moderate	0.50 – 0.60
IV	High	0.60 – 0.70
V	Very high	> 0.70

$$SQI = \sum_{i=1}^{n} (Wi.Xi) \quad \text{(Eq 16)}$$

Where SQI: Seed quality index for agricultural usage, Wi: Weighting of parameter i, Xi: Sub-criterion score of parameter i. The above formula was applied to each seed material.

RESULTS AND DISCUSSIONS

Seed Characteristics

Descriptive statistics on seed characteristics of bean genotypes and cultivars are given in Table 6 and the difference between bean and bean genotypes is shown in Table 7 and Table 8.

Yield values of bean genotypes and varieties varied between 1209.9 and 3088.9 kg ha^{-1} (Table 6). Yield in beans varies depending on the climate and soil conditions of the region where it is grown, cultural practices and genetic structure of bean varieties (Bakure et al., 2023; Çukurcalıoğlu et al., 2023). In addition, seed yield comes to the fore at the beginning of selection criteria in beans. On the other hand, dry volume of bean seeds varied between 62.00 and 95.00 ml and mean dry volume value was determined as 79.53 ml. Dry volume varies depending on the dry weight of seeds. As a matter of fact, Aydogan et al. (2020) stated that dry volume values of bean genotypes and varieties vary between 76.50-98.50 ml, and there is a positive and significant relationship between dry weight and dry volume. hydration capacity, which is one of the important selection criteria in bean, varied between 0.04-0.66 g $seed^{-1}$ with swelling index 1.14%-2.47% (Table 6). Shimelis and Rakshit (2005) determined that the hydration capacity of bean varieties varies between 0.08-0.19 g $seed^{-1}$. In addition, researchers stated that bean varieties with high hydration capacity and high hydration index require less cooking time, thus saving fuel energy. Cooking time in beans is one of the main factors used to determine cooking quality. As a matter of fact, in the study, the cooking time of bean genotypes and varieties varied between 30.00-85.00 minutes, and mean cooking time was determined as 43.38 minutes (Table 6). Longer cooking times result in nutrient loss and limit end uses. Therefore, it is of great importance to consider cooking time (Shimelis Rakshit 2005).

Table 6. Descriptive statistics of bean seeds

Variable	Min.	Max.	Mean	Std. Deviation	Skewness	Kurtosis
Seed yield (kg ha^{-1})	1209.9	3088.9	1893.7	48.08	0.40	-0.55
Dry volume (ml)	62.00	95.00	79.53	10.10	-0.08	-1.15
Hydration capacity (g seed^{-1})	0.04	0.66	0.33	0.17	-0.16	-0.79
Swelling index (ml seed^{-1})	1.14	2.47	1.69	0.32	0.45	-0.15
Cooking time (min)	30.00	85.00	43.38	11.73	2.08	4.82
Coat ratio (%)	6.19	11.31	8.68	1.22	0.12	-0.59
Hundred seed weight (g)	26.02	93.23	47.42	15.15	1.41	2.07
Seed width (mm)	6.39	11.94	8.39	1.18	1.00	1.22
Seed length (mm)	10.76	17.76	14.46	1.96	0.09	-0.92
Geometric mean diameter (mm)	7.21	11.55	9.08	0.93	0.36	0.33
Volume	22.86	58.71	36.74	7.57	0.67	0.83
Bulk density (kg m^{-3})	826.10	848.14	835.01	4.30	0.94	1.33
True density (kg m^{-3})	1005.90	1364.20	1204.20	95.80	-0.42	-0.87

Table 7. Average values of seed yield and cooking characteristics of bean genotypes as a result of two years combined analysis

Genotypes	GY	DV	HC	SI	CT	CR	HGW
G1	2361.50^{d}	84.83^{cd}	0.34^{e}	1.43^{l-n}	35.67^{k-m}	8.90^{b-h}	47.47^{e-h}
G2	1747.79^{hı}	65.00^{j}	0.10^{gh}	1.67^{f-k}	36.00^{k-m}	7.07^{h}	45.53$^{g-ı}$
G3	1249.89^{m}	62.67^{j}	0.08^{h}	1.90^{c-e}	31.00^{o}	8.60^{c-h}	37.84^{jk}
G4	1890.83^{g}	84.17^{cd}	0.35^{e}	1.23^{no}	38.00$^{ı-k}$	9.52^{a-d}	81.21^{b}
G5	2161.50^{e}	71.17^{ı}	0.18^{fg}	1.85^{d-g}	63.00^{b}	8.07^{d-h}	46.12^{f-h}
G6	2998.90^{a}	87.17^{bc}	0.45^{b-d}	2.04^{b-d}	84.00^{a}	9.49^{a-e}	91.21^{a}
G7	1574.09^{k}	70.83^{ı}	0.24^{f}	1.47^{j-m}	47.00^{d}	10.76^{ab}	26.88^{m}
G8	1754.41^{h}	94.33^{a}	0.64^{a}	1.68^{e-j}	43.33^{e-g}	10.29^{a-c}	53.09^{de}
G9	1985.59^{f}	94.33^{a}	0.52^{b}	1.65^{g-l}	40.00^{hı}	7.46^{f-h}	50.41^{d-g}
G10	1400.71^{l}	77.17^{fg}	0.37^{de}	2.12^{bc}	35.00^{lm}	8.09^{d-h}	40.01$^{ı-k}$
G11	1665.59^{ıj}	76.17^{f-h}	0.35^{e}	1.46^{j-n}	39.00^{h-j}	9.22^{a-g}	30.38^{lm}
G12	1259.61^{m}	65.33^{j}	0.05^{h}	1.73$^{e-ı}$	39.00^{h-j}	8.35^{c-h}	38.92^{jk}
G13	2321.81^{d}	90.00^{b}	0.33^{e}	1.27^{m-o}	55.00^{c}	8.51^{c-h}	50.92^{d-g}
G14	1579.29^{k}	79.67^{ef}	0.40^{c-e}	1.61^{h-l}	44.67^{de}	10.96^{a}	60.65^{c}
G15	1642.99^{jk}	82.50^{de}	0.43^{cd}	1.15^{o}	34.00^{mn}	7.36^{gh}	35.90^{j-l}
G16	1267.01^{m}	78.33^{fg}	0.41^{c-e}	1.89^{d-f}	37.00^{j-l}	8.00^{d-h}	36.99^{jk}
G17	2033.31^{f}	75.67^{gh}	0.34^{e}	2.24^{ab}	52.33^{c}	7.27^{gh}	35.11^{kl}
G18	2588.90^{b}	87.67^{bc}	0.46^{bc}	1.64^{g-l}	41.33^{f-h}	9.47^{a-f}	34.10^{kl}
G19	2311.10^{d}	73.00^{hu}	0.10^{gh}	1.44^{k-n}	44.00^{ef}	8.36^{c-h}	52.00^{d-f}
G20	1254.41^{m}	65.17^{j}	0.05^{h}	1.55$^{ı-l}$	43.00^{e-g}	9.49^{a-e}	41.87^{h-j}
RV1	2448.29^{c}	94.33^{a}	0.43^{cd}	1.81^{e-h}	31.33^{no}	7.50^{e-h}	54.47^{cd}
RV2	2164.09^{e}	90.17^{b}	0.61^{a}	2.43^{a}	40.67$^{g-ı}$	8.23^{d-h}	52.00^{d-f}
F-Value	972.48**	215.00**	144.38**	59.31**	493.25**	8.92**	170.35**
CV	1.43	1.52	7.60	10.47	2.14	7.42	4.28

** Significant at P<0.01, Means followed by the same letter in the columns are not significantly different at P ≤ 0.05, GY: Seed Yield; HGW: Hundred Seed Weight; H: Hydration Capacity; CT: Cooking Time; SI: Swelling Index; DV: Dry Volume; CR: Coat Ratio

The coat rate of bean genotypes and varieties varied between 6.19% and 11.31%. As a matter of fact, Sozen and Karadavut (2020) stated that coat rate in bean seeds is an important quality criterion and that it can show differences in changing environmental conditions. The 100 seeds weight varied between 26.02-93.23 g, and the mean 100 seed weight was determined as 47.42 g. The 100-seed weight of beans is an important feature affecting yield, and large-sized bean varieties are preferred in terms of market value. The mean seed width and length of bean genotypes and cultivars were determined as 8.39 and 14.46 mm, respectively. Bean seed width and length are important in terms of marketing and vary depending on cultural practices, region where it is grown and the genetic structure of variety. Cirka and Ciftci (2018) determined that seed length varies between 11.15-16.40 mm, seed width 6.41-10.26 mm in amplectant bean types, and seed length 12.24-13.18 mm and seed width 6.34-8.45 mm in dwarf types. The mean geometric mean diameter of bean genotypes and varieties was 9.08 mm, the mean volume was 36.74, the mean bulk density was 835.01 kg m^{-3}, and the mean true density was 1204.20 kg m^{-3}. The normal distribution is symmetrical. The degree of deterioration of symmetry in a normal distribution is called skewness. The distribution is called right (positive) skewed if it is long-tailed to the right and skewed to the left (negative) if it is

long-tailed to the left. The degree of sharpness or roundness of the normal distribution curve is called kurtosis. Except for the skewness coefficients, dry volume and hydration capacity of the bean seeds, it was determined that the other properties showed positive distributions away from the normal distribution. It is seen that true density, dry volume and hydration capacity values are seed traits with negative skewness among all examined traits (Table 6). The trait with the furthest distribution from normal distribution was determined as cooking time, and cooking time values were generally lower than mean values. The characteristics that show the closest distribution to normal are dry volume, hydration capacity, coat ratio and seed size.

Table 8. Average values of seed physical characteristics of bean genotypes as a result of two years combined analysis

Genotypes	SW	SL	GMD	V	BD	TD
G1	8.17^{f-j}	13.94^{d-g}	8.97$^{f-ı}$	35.34$^{f-ı}$	841.17^{ab}	1266.68^{a-c}
G2	8.22$^{f-ı}$	11.66^{ı}	8.44^{ıj}	31.66^{ıj}	832.28^{de}	1074.51^{de}
G3	6.67^{k}	13.14^{gh}	7.63^{kl}	25.68^{kl}	832.45^{de}	1028.56^{e}
G4	8.35$^{f-ı}$	13.73^{e-g}	9.11^{e-h}	36.56^{e-h}	846.98^{a}	1252.89^{a-c}
G5	8.88^{d-f}	15.74^{c}	9.32^{d-g}	38.28^{d-g}	840.55^{b}	1134.32^{c-e}
G6	11.67^{a}	17.32^{a}	11.42 ^{a}	57.40^{a}	836.07^{b-d}	1242.34^{a-c}
G7	6.62^{k}	11.72^{ı}	7.35^{l}	23.75^{l}	835.54^{b-d}	1139.73^{c-e}
G8	9.27^{c-e}	17.52^{a}	9.85^{b-d}	42.94^{b-d}	836.02^{b-d}	1350.80^{a}
G9	8.45^{f-h}	16.12^{bc}	9.85^{b-d}	42.71^{b-d}	832.06^{de}	1301.97^{ab}
G10	7.40^{jk}	13.58^{f-h}	8.22^{jk}	29.75^{jk}	831.66^{de}	1190.31^{b-d}
G11	7.37^{jk}	14.80^{d}	8.20^{jk}	29.81^{jk}	833.70^{c-e}	1213.24^{bc}
G12	7.62^{ıj}	14.50^{d-f}	8.75^{g-j}	33.66^{g-j}	832.41^{de}	1068.36^{de}
G13	10.16^{b}	13.50^{gh}	9.59^{b-e}	40.77^{b-e}	837.36^{b-d}	1308.78^{ab}
G14	7.86^{g-j}	13.07^{gh}	8.61^{h-j}	32.63^{h-j}	838.48^{bc}	1238.00^{a-c}
G15	7.91^{g-j}	12.74^{h}	8.59^{h-j}	32.55^{h-j}	836.51^{b-d}	1258.47^{a-c}
G16	7.88^{g-j}	10.88^{ı}	8.30^{j}	30.98^{ıj}	835.35^{b-d}	1232.63^{a-c}
G17	9.67^{b-d}	14.79^{d}	10.16^{b}	45.54^{b}	833.58^{c-e}	1185.29^{b-d}
G18	8.29$^{f-ı}$	17.38^{a}	9.48^{c-f}	39.90^{c-f}	828.92^{e}	1278.05^{ab}
G19	8.63^{e-g}	16.94^{ab}	9.86^{b-d}	42.87^{b-d}	831.60^{de}	1143.24^{c-e}
G20	7.72^{h-j}	13.44^{gh}	8.60 $^{h-j}$	32.57^{h-j}	833.10^{c-e}	1056.77^{de}
RV1	9.85^{bc}	14.62^{de}	9.93^{bc}	43.48^{bc}	831.81^{de}	1217.03^{a-c}
RV2	7.87^{g-j}	17.05^{ab}	9.43^{c-f}	39.39^{c-f}	832.59^{de}	1309.81^{ab}
F-Value	62.82**	131.60**	70.81**	71.48**	14.03**	13.47**
CV	3.06	2.06	2.11	4.23	0.23	3.55

** Significant at P<0.01, Means followed by same letter in the columns are not significantly different at P ≤ 0.05, SW: Seed Width; SL: Seed Length; GMD: Geometric Mean Diameter; V: Volume; BD: Bulk Density; TD: True Density

The direct effects and contribution margins of characteristics examined in the study on yield are given in Table 9.

Yield is a quantitative trait that is under the influence of more than one genetic factor. Some characteristics affect the yield directly and some indirectly (Pushkarnath et al., 2022). Since correlation coefficients do not provide sufficient information by the breeders, path coefficient, which is accepted as the standard partial regression coefficient, which allows the separation of direct and indirect effects of correlation coefficients into their components, is used (Khan et al., 2022). With this method, it gives a clear idea about effect of a feature on yield or other features. It is especially important to reveal the selection criteria. When Table 9 is examined, the highest direct effect on yield was determined in volume property (42.07%), and the lowest in the seed width with 0.97% contribution margin. The volume property is calculated by formulating the seed size (length, width, and thickness). The higher volume feature, the higher yield efficiency.

Table 9. Path coefficients and contribution margins related to direct effects of seed characteristics on seed yield in beans

Characteristic	Direct impact %	Characteristic	Direct impact %
Dry volume	17.59	Seed width	0.97
Hydration capacity	16.81	Seed length	5.61
Swelling index	1.63	Geometric mean diameter	39.89
Cooking time	4.07	Volume	42.07
Coat ratio	8.75	Bulk density	25.40
100 seed weight	2.31	True density	1.13

AHP Weighting

AHP is applied in different areas such as planning, choosing the best alternative, resource allocation, conflict resolution, optimisation and numerical extensions of AHP, and there are many studies for this purpose. With the AHP method, purpose-oriented priorities and important criteria are revealed, especially in breeding studies. In this study, bean genotypes as a result of evaluation of seed quality with physical characteristics and yield with weights of AHP are given in Figure 1. The contribution weight of the seed parameters calculated by AHP to seed quality was determined as the highest yield with 0.176, and the lowest for true density with 0.020. Among characteristics examined, the highest yield feature, following hundred seed weight of 0.134 and hydration capacity of 0.131 take part in. An important indicator in seed yield is the hundred seed weight, and in seed quality is hydration capacity. The higher hundred seed weight of a variety, the higher yield is parallel to this feature. Another important quality feature is its hydration capacity, and it has been stated by many researchers that the more seed absorbs water, the shorter the cooking time of seed (Karaman 2019, Aydogan et al., 2020). This feature is especially important in marketing. There are also important environmental and genetic factors that affect both hundred seed weight and hydration capacity.

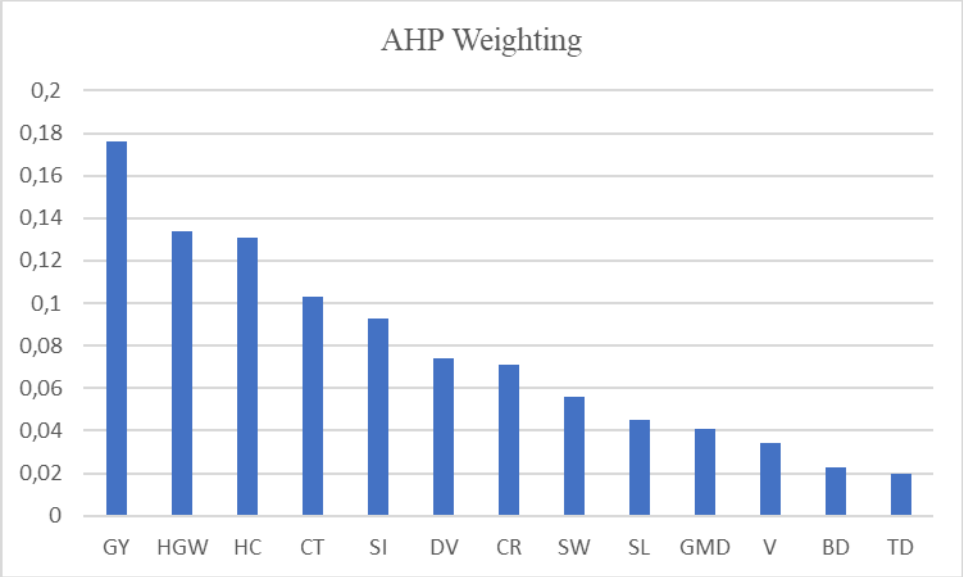

Figure 1. Contribution weight of seed parameters to seed quality calculated by the AHP

Weighting of the total data set; GY: Seed Yield; HGW: Hundred Seed Weight; H: Hydration Capacity; CT: Cooking Time; SI: Swelling Index; DV: Dry Volume; CR: Coat Ratio; SW: Seed Width; SL: Seed Length; GMD: Geometric Mean Diameter; V: Volume; BD: Bulk Density; TD: True Density

According to the analytical hierarchy analysis, the quality of the seeds obtained, including physical properties and yield values, varied depending on the genotypes. The seed quality index (SQI) values obtained from the evaluation of the scores obtained as a result of AHP weights and standard scoring functions with the linear combination technique are given in Table 10.

Table 10. Seed quality index (SQI) values and classes of bean genotypes and varieties

SQI	Genotype	Class	SQI	Genotype	Class
0.279	G7	1	0.500	G21	3
0.298	G22	1	0.545	G13	3
0.322	G3	1	0.548	G1	3
0.338	G12	1	0.550	G4	3
0.403	G11	2	0.556	G19	3
0.410	G2	2	0.585	G20	3
0.432	G18	2	0.605	G8	4
0.446	G15	2	0.625	G9	4
0.463	G10	2	0.685	Yunus 90	4
0.463	G17	2	0.687	Onceler 98	4
0.481	G5	2	0.751	G6	5

According to the obtained quality index values, genotypes 3, 7, 12, and 22 were Class I quality 'very low' genotypes G2, G5, G10, G11, G15, G17 and G18 were II. Quality ‘low’; genotypes G1, G4, G13, G19, G20 and G21 III. class ‘moderate’, G8, G9, G14 and G16 genotypes were IV. Class 'high' and G6 genotype was classified as Class V 'very high'. The highest value in SQI obtained by using 11 indicators was determined in G6 genotype.

CONCLUSION

As a result of the evaluation of obtained datas, the yield values and seed quality index were correlated, and productivity estimates of the genotypes were made. Among the indicators in obtained data, it was determined that a hundred seeds weight contributed the most effectively to yield. Among all materials, it was determined that the seed quality of registered cultivars was high (IV class), and genotypes varied between low and very high. In the study, it was determined that seed quality of genotype 6 was higher (V class) than the registered varieties. It was determined that genotype 6, with high seed quality, also had high productivity. With this study, it has been concluded that a successful evaluation will be made in terms of productivity by creating weighting with AHP in the evaluation of seed quality. Many features examined in the studies were weighted with AHP, and the seed quality index was calculated and predicted with high accuracy with these estimation models. These prediction models are significant for the selection stages of plant breeders. In this way, it is thought that it will save breeders less labour and time. As a result, according to the seed quality index of bean varieties and genotypes with different characteristics, it was determined that genotype 6 had superior characteristics in terms of productivity. In addition 8 with 9 genotypes and registered varieties could also show superior characteristics.

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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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FORAGE YIELD AND QUALITY OF INTERCROPPED ANNUAL RYEGRASS WITH BERSEEM CLOVER SOWN AT DIFFERENT SEED MIXTURE RATIOS UNDER RAINFALL CONDITIONS OF MEDITERRANEAN CLIMATE

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ABSTRACT

This research was carried out under rainfall conditions of the Mediterranean climate to study the effects of different seed mixture ratios (Berseem clover (BC100), annual ryegrass (AR100), BC80-AR20, BC60-AR40, BC40-AR60, BC20-AR80) on forage yield and quality characteristics of mixture. The experiments were conducted in a randomized complete block design with three replications during the growing season of 2020-2021 and 2021-2022. Annual ryegrass sown as pure produced higher green forage yield than berseem clover sown as pure and the mixtures. In the mixture of berseem clover at 20% seed rate and pure annual ryegrass, higher hay yields were determined compared to the other mixtures. In terms of total LER, BC80-AR20 and BC20-AR80 mixtures were the two most advantageous mixtures, while the aggressivity value of annual ryegrass showed a positive value and became the dominant species. Pure sown berseem clover had lower ADF, NDF and higher crude protein content than pure sown annual ryegrass and other mixtures tested. According to these results, it can be said that berseem clover can be included in the mixture at low seed rates for high forage yield under rainfall conditions, while berseem clover can be grown as pure for high forage quality.

Keywords: Legume-grass mixture, seed mixture rate, yield and quality

INTRODUCTION

Intercropping systems, known as low-cost production systems, not only produce more biomass, but also provide a more balanced nutrient supply to ruminants than pure legume or grass sowings (Tahir et al., 2022; De Silva et al., 2023). This system, which is widely applied in Mediterranean countries (Salama, 2020; Ertekin and Yilmaz, 2022), serves the ecosystem by reducing the use of chemicals (pesticides, herbicides, fertilizers) and ensures the sustainability of agriculture due to climate change (Lithourgidis et al., 2011; Brooker et al., 2015; Erkovan, 2022). The fact that legume-grass species within this system have different responses to diseases, pests, soil and climatic conditions and different abilities to use limited resources minimises the risks in terms of crop production (Atis et al., 2012). Indeed, in legume-grass mixtures, legumes reduce the need for nitrogen fertiliser by fixing nitrogen (Giambalvo et al., 2011; Raza et al., 2023) and increase the quality of the mixture forage with high protein content (Solomon et al., 2011; Rajab et al., 2021). On the other hand, grasses reduce legume-induced risks such as bloat (Vasilakoglou and Dhima, 2008), bone disorders (Hall et al., 1991), provide structural support and also minimize harvest losses (Salama and Badry, 2015). In addition, it should not be forgotten that the intercropping system will reduce the grazing pressure on meadow-pasture areas, which are dependent on natural rainfall, especially in countries such as Turkey, which is located in the arid zone, and will meet the quality roughage needs of livestock by contributing to the utilisation of lands left vacant in the winter period in barren areas (Kavut et al., 2014).

Although intercropping systems have many advantages mentioned above, it is quite difficult to establish mixtures due to the difference in agricultural practices of the species included in the mixture compared to pure sowing. Sowing times, fertiliser and water requirements, phenology and harvesting times may be different for species grown in mixed cropping (Droushiotis, 1989). In addition, species in the mixture may compete with each other in terms of light, water and nutrients, leading to yield losses (Chen et al., 2004). In this context, it is necessary to select legume and grass species suitable for the ecological conditions in which the crop will be established and to adjust the seed rates of these species correctly in order to obtain forage with high

yield and quality and to ensure the sustainability of the mixed crop (Osman and Nersoyan, 1986). Indeed, Caballero et al. (1995) reported that oat had a competitive advantage over vetch, and that oat sown pure gave higher herbage yield than vetch-oat mixtures. Hatipoglu et al. (2005) reported that in mixtures of Persian clover and annual ryegrass at different seeding rates, pure Persian clover and different mixture treatments gave higher quality herbage yields than pure annual ryegrass. El-Karamany et al. (2014) reported that in mixtures of berseem clover and barley at different seeding rates, pure sown berseem clover gave higher forage yield than the mixtures, but had lower dry matter ratio than the other mixtures. Salama (2020) studied the forage yield of berseem clover and mixtures of some grass species at different seeding rates and found that the highest forage yield was recorded in the mixture of berseem clover and triticale (75%:25%).

The aim of this study was to determine the effect of seed mixture ratios on the forage yield and quality of a mixture of berseem clover and annual ryegrass, which can be grown as an alternative crop for the crop rotation of wheat under rainfall conditions.

MATERIALS AND METHODS

Site Description

This study was conducted for two years (2020-2021 and 2021-2022) at the research and application field of Kozan Vocational School of Cukurova University (37°27'57"N, 35°48'12"E, altitude: 151 m). According to the results of the analysis of the soil samples taken from the experimental area, the soil had a pH of 7.50, a high lime content (26.9%), a low phosphorus content (47.7 kg ha^{-1}), a high potassium content (922.7 kg ha^{-1}) and a clayey texture. The daily average precipitation and temperature values of Kozan district, where the research was conducted, obtained from the 6th Regional Directorate of Meteorology, and the cumulative precipitation values for the growing seasons and long years are shown in Figure 1. As seen in Figure 1, the cumulative rainfall realised in 2020/2021 (390.4 mm) and 2021/2022 (492.0 mm) was below the long-term average cumulative rainfall (542.6 mm). In addition, the average monthly temperature in the 2020-2021 and 2021-2022 growing seasons was 13.8 °C and 12.2 °C, respectively.

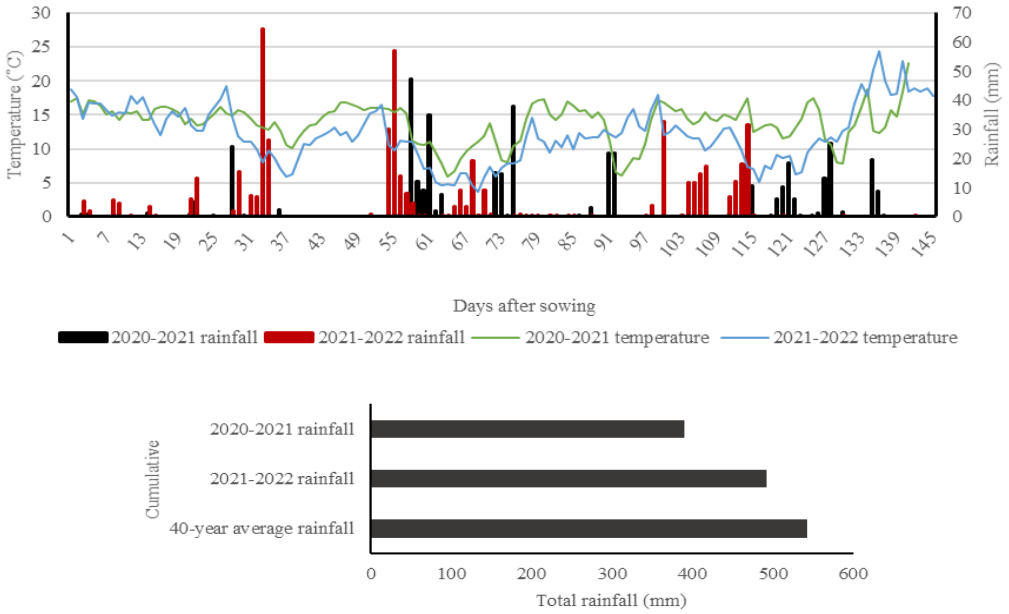

Figure 1. Some climatical values of experimental area for the experiment periods and long term averages

Experimental Design and Treatments

The experiment was conducted in a complete randomized block design with three replications. Treatments were; berseem clover (*Trifolium alexandrinum* L. cv Derya) in pure stand (BC); annual ryegrass (*Lolium multiflorum* Lam. cv Elif) in pure stand (AR); and their mixture with different seeding ratios (BC80-AR20; BC60-AR40; BC40-AR60; BC20-AR80). Pure stands were sown at a sowing ratio of 25 kg ha^{-1} for berseem clover and 20 kg ha^{-1} for annual ryegrass (Acikgoz, 2001).

Agricultural Practices

In both years of the study, the experimental plots were left fallow during the summer and prepared for sowing in early autumn after ploughing and levelling. In the experiment, each plot consisted of 6 rows of 5 m length. The row spacing was 20 cm. In both growing seasons, mixed and pure stands were sown by hand on November 18. The seeds of the species in the mixture were mixed and sown in the same row. Diammonium phosphate (18-46-0) fertilizer (100 kg ha^{-1}) was applied to the area where the experiment was conducted. There was no irrigation

during the study years. No herbicide treatments were used; all plots were kept free of weeds by hand-weeding.

Sampling and Measurements

In both years of the study, harvesting was carried out on 07.04.2021 and 12.04.2022, during the full flowering period of berseem clover (Yucel et al., 2018a). At harvest time, three 60x60 cm wooden frames were randomly placed in each plot and clipped with sickle at a stubble height of 5 cm. In each plot, green forage weight of the plot was calculated by taking the average of the green weights determined in three sampling areas, and green forage yield per hectare was calculated by making the necessary conversions. The mixture species harvested from the three sampling sites were manually separated and each species was placed in different paper bags and dried in dry oven at 70 °C for 48 hours. The dried samples were allowed to reach room temperature and the dry weights of each species were determined. The dry weights of berseem clover and annual ryegrass were collected for each sampling area and the dry weight of the plot was determined by averaging the total dry weights of the species in the three sampling areas. Then, hay yield per hectare was calculated by making the necessary conversions. The dry weight of berseem clover determined in each sampling area was proportioned to the total dry weight and the proportion of berseem clover in the botanical composition by weight was calculated by taking the average of the values determined from the three sampling areas. The dried samples of each species were ground to pass through a 1 mm screen. Crude protein was determined by the Kjeldahl procedure (N concentration x 6.25) (Kacar and Inal, 2010). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were sequentially determined as described by Van Soest et al. (1991) using the semiautomatic ANKOM^{220} Fiber Analyzer (ANKOM Technology, Macedon, NY, USA). Relative feed value (RFV) was calculated using the equations described by Sheaffer et al. (1995);

Digestible dry matter (DDM, % of body weight) = 88.9 - (0.779 x ADF, % of DM) (Eq. 1)

Dry matter intake (DMI, % of DM) = 120 / NDF, % of DM (Eq. 2)

Relative feed value (RFV) = DDM (% of body weight) x DMI (% of DM) / 1.29 (Eq. 3)

The land equivalent ratio (LER) was used as index for mixed stand advantage for both legume (berseem clover) and grass (annual ryegrass) (Willey, 1979). LER values were calculated as follow;

$$LER = LER_{annual\ ryegrass} + LER_{berseem\ clover}, \text{ (Eq. 4)}$$

$$LER_{annual\ ryegrass} = \frac{Y_{ab}}{Y_a}, \ LER_{berseem\ clover} = \frac{Y_{ba}}{Y_b} \text{ (Eq. 5)}$$

Where Y_b and Y_a were the yields of berseem clover and annual ryegrass as sole crop, respectively, and Y_{ab} and Y_{ba} were yields of berseem clover and annual ryegrass in the mixture, respectively.

Aggressivity (A) was calculated using the equations described by McGilchrist (1965);

$$A_{annual\ ryegrass} = \frac{Y_{ab}}{Y_a \times Z_{ab}} - \frac{Y_{ba}}{Y_b \times Z_{ba}} \text{ (Eq. 6)}$$

$$A_{berseem\ clover} = \frac{Y_{ba}}{Y_b \times Z_{ba}} - \frac{Y_{ab}}{Y_a \times Z_{ab}} \text{ (Eq. 7)}$$

Where, Z_{ba} and Z_{ab} were the seed rates of berseem clover and annual ryegrass in the seed mixture.

Competitive ratio (CR) is another index used to determine the competition between species in a mixture. The CR index was formulated as follows (Atis et al., 2012);

$$CR_{annual\ ryegrass} = \frac{LER_{annual\ ryegrass}}{LER_{berseem\ clover}} \times \frac{Z_{ab}}{Z_{ba}} \text{ (Eq. 8)}$$

$$CR_{berseem\ clover} = \frac{LER_{berseem\ clover}}{LER_{annual\ ryegrass}} \times \frac{Z_{ba}}{Z_{ab}} \text{ (Eq. 9)}$$

Statistical Analyses

The yield and quality values obtained from the research were analysed by analysis of variance (ANOVA) in the MSTAT-C (Michigan State University V. 2.10) statistical package program. As a result of the analysis of variance, treatment means for the statistically significant were compared using the Duncan test at a significance level of P≤0.05 (Steel and Torrie, 1980).

RESULTS

Green Forage Yield

In the research carried out for two years, year and mixture factors and year x mixture interaction made a statistically significant (P≤0.01) difference in green forage yield (Table 1).

Table 1. Mean squares for traits in different mixture treatments

Measurements	Year (Y)	Mixture (M)	Y x M
Green forage yield (t ha^{-1})	116.10**	70.36**	21.71**
Hay yield (t ha^{-1})	16.30**	19.55**	2.98**
Berseem clover ratio (%)	1142.39**	2612.00**	12.59ns
LER value of berseem clover	0.01ns	0.36**	0.04**
LER value of annual ryegrass	0.01ns	0.32**	0.01ns
Total LER value	0.03ns	0.03*	0.03ns
Aggressivity value of berseem clover	0.09ns	0.58**	0.23*
Aggressivity value of annual ryegrass	0.09ns	0.58**	0.23*
Competitive ratio of berseem clover	0.13*	0.50**	0.24**
Competitive ratio of annual ryegrass	0.02ns	0.99**	0.22ns
NDF (DM%)	109.69**	35.40*	20.59ns
ADF (DM%)	136.45**	24.32*	15.17ns
Crude protein (DM%)	61.91**	52.57**	13.05**
Relative feed value	871.74**	60.46ns	136.03ns
Degrees of Freedom (df)	1	5^{+}	5^{+}

*:P≤0.05, **: P≤0.01, ns: not significant, $^{+}$3 for Berseem clover ratio, LER values, Aggressivity values and Competitive ratios

In the first year of the research, the averaged green forage yield was 17.0 t ha^{-1}, while in the second year of the research; the averaged green forage yield was significantly higher than the first year of the research and was determined as 20.6 t ha^{-1}. According to the two-year mean values, the average green forage yield ranged from 15.6 t ha^{-1} (BC80-AR20) to 24.2 t ha^{-1} (AR100) in different mixture treatments (Table 2).

Table 2. Means of green forage yield, hay yield and berseem clover ratio determined in different mixture treatments.

Mixtures	Green forage yield (t ha^{-1})			Hay yield (t ha^{-1})			Berseem clover ratio (%)		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
BC100	10.8 h*	21.5 c	16.2 d	1.6 f	3.8 cd	2.7 d	-	-	-
BC80-AR20	13.6 g	17.6 e	15.6 d	2.6 e	3.7 d	3.2 c	57.3	67.6	62.5 a
BC60-AR40	14.7 f	17.8 e	16.2 d	2.7 e	3.9 cd	3.3 c	22.8	39.2	31.0 b
BC40-AR60	19.7 d	19.3 d	19.5 c	3.5 d	4.3 bc	3.9 b	15.0	28.1	21.5 c
BC20-AR80	20.3 d	21.5 c	20.9 b	3.9 cd	4.6 ab	4.3 a	7.5	23.3	15.4 d
AR100	22.7 b	25.8 a	24.2 a	4.3 bc	4.9 a	4.6 a	-	-	-
Mean	17.0 b[1]	20.6 a		3.1 b	4.2 a		25.7 b	39.6 a	

*) There is no statistically significant difference between the means shown with similar letters in the same column according to the Duncan test at P≤0.05 level of significance.
1) There is no statistically significant difference between the means of the years shown with similar letters at P≤0.05 level of significance

The fact that the year x mixture interaction was found to be significant indicates that the effects of the years on green forage yield differed significantly depending on the mixture treatments. As a matter of fact, while the average green forage yield did not differ significantly in BC40-AR60 mixture, the average green herbage yield in the other mixtures tested showed a significantly higher value in the second year compared to the first year (Table 2).

Hay Yield

The results of variance analysis revealed that year (P≤0.01), mixture (P≤0.01) and year x mixture (P≤0.01) interactions had a statistically significant effect on hay yield (Table 1).

In parallel with the green forage yield, the averaged hay yield was significantly higher in the second year (4.2 t ha^{-1}) compared to the first year (3.1 t ha^{-1}) of the study (Table 2). According to the two-year averages, the mean hay yield varied between 2.7 t ha^{-1} (BC100) and 4.6 t ha^{-1} (AR100) in different mixture treatments (Table 2).

In the 2020-2021 growing season, the average hay yield was between 1.6 t ha^{-1} (BC100) and 4.3 t ha^{-1} (AR100), while in the 2021-2022 growing season, the average hay yield was between 3.8 t ha^{-1} (BC100) and 4.9 t ha^{-1} (AR100) in different mixture treatments (Table 2).

Berseem Clover Ratio

According to the results of the analysis of variance, the effect of year (P≤0.01) and mixture (P≤0.01) factors on the rate of berseem clover was statistically significant, while the year x mixture interaction did not make a statistically significant difference on the rate of berseem clover (Table 1).

In the first year of the research, averaged berseem clover ratio was 25.7%, while in the second year of the research, averaged berseem clover ratio showed a

statistically significant higher value and was determined as 39.6%. According to the two-year average values, the ratio of berseem clover in different mixture treatments varied between 15.4% and 62.5%. The highest proportion of berseem clover was found in BC80-AR20 mixture, while the lowest proportion of berseem clover was found in BC20-AR80 mixture (Table 2).

Land Equivalent Ratio (LER)

According to the results of variance analysis, the mixture factor significantly affected the LER value of berseem clover, the LER value of annual ryegrass and total LER value. Also, it was found that year x mixture interaction made a significant difference in the LER value of berseem clover (Table 1).

According to the two-year average values, the averaged LER value of berseem clover ranged between 0.24 and 0.80, and the average LER value of annual ryegrass ranged between 0.25 and 0.78. The highest total LER value average was found in the mixture of BC80-AR20 (1.05), while the mixtures of BC20-AR80 (1.02) and BC40-AR60 (0.98) were in the same statistical group in terms of LER value average (Table 3).

Table 3. Means of LER value of species in mixtures and total LER value determined in different mixture treatments.

Mixtures	LER value of berseem clover			LER value of annual ryegrass			Total LER value		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
BC80-AR20	0.92 a*	0.67 b	0.80 a	0.26	0.25	0.25 d	1.18	0.91	1.05 a
BC60-AR40	0.39 cd	0.41 c	0.40 b	0.48	0.49	0.48 c	0.86	0.90	0.88 b
BC40-AR60	0.32 cd	0.32 cd	0.32 c	0.68	0.63	0.66 b	1.00	0.95	0.98 ab
BC20-AR80	0.18 e	0.29 d	0.24 d	0.84	0.73	0.78 a	1.02	1.02	1.02 a
Mean	0.45	0.42		0.56	0.52		1.02	0.95	

*) There is no statistically significant difference between the means shown with similar letters in the same column according to the Duncan test at P≤0.05 level of significance.

In the study, berseem clover LER value did not show a statistically significant difference in BC60-AR40 and BC40-AR60 mixtures according to growing seasons, whereas in BC80-AR20 mixture, berseem clover LER value showed a significantly lower value in the second year compared to the first year. The LER value of berseem clover in the BC20-AR80 mixture showed a significantly higher value in the second year compared to the first year (Table 3).

Aggressivity (A)

The results of the analysis of variance showed that the effect of the year factor on the aggressivity value was statistically insignificant, while the mixture factor and year x mixture interaction made a significant difference on the aggressivity values of the species in the mixture (Table 1).

According to the two-year average values, the mean of berseem clover aggressivity value varied between -0.54 (BC60-AR40) and 0.20 (BC20-AR80) in different mixture treatments (Table 4).

Table 4. Means of aggressivity value and competitive ratio of species determined in different mixture treatments.

Mixtures	Aggressivity value of berseem clover			Aggressivity value of annual ryegrass			Competitive ratio of berseem clover			Competitive ratio of annual ryegrass		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
BC80-AR20	-0.14 b*	-0.40 b	-0.27 b	0.14 a	0.40 a	0.27 a	0.92 b	0.70 bc	0.81 b	1.13	1.53	1.33 b
BC60-AR40	-0.55 b	-0.54 b	-0.54 b	0.55 a	0.54 a	0.54 a	0.54 c	0.57 c	0.56 c	1.90	1.88	1.89 a
BC40-AR60	-0.33 b	-0.25 b	-0.29 b	0.33 a	0.25 a	0.29 a	0.72 bc	0.78 bc	0.75 bc	1.46	1.38	1.42 b
BC20-AR80	-0.13 b	0.54 a	0.20 a	0.13 a	-0.54 b	-0.20 b	0.88 b	1.60 a	1.24 a	1.16	0.64	0.90 c
Mean	-0.29	-0.16		0.29	0.16		0.77 b[1]	0.91 a		1.41	1.36	

*) There is no statistically significant difference between the means shown with similar letters in the same column according to the Duncan test at P≤0.05 level of significance.
1) There is no statistically significant difference between the means of the years shown with similar letters at P≤0.05 level of significance

Effects of the year on the aggressivity values of berseem clover did significantly change depending on the mixture. In the second year, agressivity value of berseem clover in the mixture of BC20-AR80 was significantly higher than that in the first year while it did not significantly vary in the other mixtures depending on the year (Table 4).

Competitive Ratio (CR)

The results of variance analysis revealed that the effects of year (P≤0.05), mixture (P≤0.01) and year x mixture interaction (P≤0.01) on berseem clover competition rate were statistically significant, while the

effect of mixture treatments on annual ryegrass competition rate was significant at 1% level (Table 1).

In the first year of the study, the average competition ratio of berseem clover was 0.77, while in the second year of the study, the average competition ratio of berseem clover showed a significantly higher value compared to the first year of the study and was determined as 0.91. Berseem clover competition ratio varied between 0.56 (BC60-AR40) and 1.24 (BC20-AR80), while annual ryegrass competition ratio varied between 0.90 (BC20-AR80) and 1.89 (BC60-AR40) in different mixture treatments (Table 4).

In the study, the significant effect of year x mixture interaction on the competition rate of berseem clover showed that the effect of the years on the competition rate of berseem clover differed significantly depending on the mixtures. In fact, while the competition rate of berseem clover in the BC20-AR80 mixture showed a significantly higher value in the second year compared to the first year, the competition rate of berseem clover in the other mixtures tested did not differ significantly between years (Table 4).

NDF Ratio

The results of the analysis of variance showed that the effect of year (P≤0.01) and mixtures (P≤0.05) on the NDF ratio made a statistically significant difference (Table 1).

In the first year of the research, the average NDF ratio was determined as 61.9%, while in the second year of the research, the average NDF ratio was significantly lower than the first year of the research and was determined as 58.4%. According to the two-year average values, the average NDF ratio varied between 56.6% (BC100) and 63.3% (BC20-AR80) in different mixture treatments (Table 5).

Table 5. Means of NDF, ADF, crude protein ratio and relative feed value determined in different mixture treatments.

Mixtures	NDF Ratio (DM%)			ADF Ratio (DM%)			Crude protein ratio (DM%)			RFV		
	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean	2020-2021	2021-2022	Mean
BC100	55.0	58.2	56.6 c*	37.4	33.7	35.5 b	22.1 a	19.7 b	20.9 a	98.0	90.5	94.2
BC80-AR20	62.1	55.2	58.7 bc	40.2	35.3	37.8 ab	16.7 de	19.5 bc	18.1 b	83.5	103.5	93.5
BC60-AR40	65.1	59.5	62.3 ab	37.3	34.0	35.7 b	12.7 f	18.2 b-d	15.5 c	81.5	96.6	89.0
BC40-AR60	61.3	58.8	60.0 a-c	41.0	35.0	38.0 ab	12.0 f	17.7 c-e	14.8 c	90.9	98.8	94.9
BC20-AR80	66.3	60.4	63.3 a	42.6	35.2	38.9 ab	13.2 f	16.0 e	14.6 c	81.1	95.0	88.0
AR100	61.8	58.5	60.1 a-c	39.9	41.8	40.8 a	11.9 f	13.3 f	12.6 d	90.7	100.3	95.5
Mean	61.9 a[1]	58.4 b		39.7 a	35.8 b		14.8 b	17.4 a		87.6 b	97.5 a	

*) There is no statistically significant difference between the means shown with similar letters in the same column according to the Duncan test at P≤0.05 level of significance.
1) There is no statistically significant difference between the means of the years shown with similar letters at P≤0.05 level of significance

ADF Ratio

The results of the analysis of variance showed that the factors year (P≤0.01) and mixture (P≤0.05) had a statistically significant difference on the ADF ratio (Table 1).

While the average ADF rate was 39.7% in the first year of the study, the average ADF rate in the second year of the study was significantly lower than the first year of the study and was determined as 35.8%. In different mixture treatments of berseem clover and annual ryegrass, the lowest ADF ratio average was 35.5% in the pure sowing of berseem clover, while the highest ADF ratio average was 40.8% in the pure sowing of annual ryegrass (Table 5).

Crude Protein Ratio

According to the results of variance analysis, the effect of year (P≤0.01) and mixture (P≤0.01) factors and year x mixture interaction (P≤0.01) on crude protein ratio was statistically significant (Table 1).

While the average crude protein ratio was 14.8% in the first year of the research, the average crude protein ratio in the second year of the research was significantly higher than the first year of the research and was determined as 17.4%. According to the two-year averages, the mean crude protein ratio was significantly higher in pure sown berseem clover (20.9%) than in the other mixture treatments tested, while the mean crude protein ratio was significantly lower in pure sown annual ryegrass (12.6%) than in the other mixture treatments tested (Table 5).

In the study, the significant effect of the year x mixture interaction on crude protein ratio revealed that the effect of years on crude protein ratio differed significantly depending on the mixtures. As a matter of fact, while the average crude protein ratio of pure sown berseem clover showed a significantly lower value in the second year compared to the first year, the average crude protein ratio of pure sown annual ryegrass did not show significantly different values depending on the years. On the other hand, the average crude protein ratio of the mixture treatments including both species showed a significantly higher value in the second year compared to the first year.

Relative Feed Value (RFV)

The results of the analysis of variance showed that the effect of years (P≤0.01) on RFV was statistically significant, while the effect of mixture and the interaction of year x mixture was not statistically significant (Table 1).

In the first year of the study, RFV was 87.6, while in the second year of the study, RFV was significantly higher

than the first year of the study and was found to be 97.5 (Table 5).

DISCUSSION

This study showed that pure sowings and mixtures of annual ryegrass and berseem clover at different seeding rates significantly affected forage yield and some quality characteristics under rainfall conditions. However, the yield values (green forage yield, hay yield) obtained from pure sowings and mixtures of annual ryegrass and berseem clover, and the beseem clover ratio differed depending on the years in which the trials were conducted (2020-2021, 2021-2022). This can be explained by the fact that the first year of the study (2020-2021) was warmer and drier than the second year of the study (2021-2022), which was conducted under rainfall conditions without irrigation (Figure 1). Osman and Nersoyan (1986) reported that the effect of drought on the species in the mixture varied and the herbage yield in the mixture showed lower values in drought years. However, the authors stated that the legume species in the mixture were less affected by drought than the grass species and the reason for this was that the ground water was high in the experimental area where the research was carried out, and the legumes benefited more from this water thanks to their deep roots. In our study, contrary to the researchers, the fact that our experimental area was not in the bottom land and that the legumes could not fully benefit from the limited rainfall in the first experimental year can be considered as the reasons for the lower rate of legume mixture participation in the yield in the first year compared to the second year. (Yucel et al., 2018b) reported that in legume-grass mixtures, the drought resistance of legumes was lower than that of grass and the rate of legume participation in the mixture yield decreased in the drought year. Pedraza et al. (2017) reported that yield losses in legume species in annual forage mixtures grown under different environmental conditions in the dry season (<300 mm) and in grass species in the wet season (>630 mm). On the other hand, the quality of the mixed diets (NDF, ADF, crude protein ratio, RFV) varied according to the year of the study. The higher proportion of berseem clover, an annual legume forage crop, in the mixture yield in the second year compared to the first year and the difference in climatic conditions between years can be considered as reasons for the higher quality of the mixture herbage in the second year compared to the first year. Solomon et al. (2011) reported that the use of legumes alone or in mixtures had a positive effect on crude protein ratio compared to grass forage crops, Thompson et al. (1992) reported that herbage quality increased in mixtures with legumes. In another study, Negesse et al. (2010) reported that low rainfall and high temperature accelerated plant maturation, causing excessive accumulation of structural carbohydrates in the plant and decreased herbage quality.

In addition to the significant effect of years on the forage yield and some quality values obtained from the research, the effect of pure sowing of berseem clover and annual ryegrass and their mixtures at different seed rates were also significant. As a matter of fact, according to the two-year average values, pure sown annual ryegrass gave higher forage yield and hay yield than the mixtures of the two species at different seed rates (except of BC20-AR80) and pure sown berseem clover. This shows that the species in the mixture compete with each other in terms of light, water and nutrients, while intra-specific competition is lower in pure sowings (Caballero et al., 1995). Although many researchers (Osman and Nersoyan, 1986; Giambalvo et al., 2011; Rajab et al., 2021; Meza et al., 2022) reported that legume-grass mixtures gave higher herbage yields than pure sowing of species, Pedraza et al. (2017) reported that under rainfall conditions, there was no significant difference in yield between pure sowing and mixtures of species depending on soil structure, rainfall and frost event during the year. Similarly, Vasilakoglou and Dhima (2008) reported that there was no difference in herbage yield between pure berseem clover and berseem clover-barley mixtures. Lithourgidis et al. (2006) reported that the herbage yields of legume-grass mixtures were lower than those of the pure sowings of the species due to the competition between the mixture species. On the other hand, there was a linear increase in herbage yield as the proportion of annual ryegrass seed in the mixture increased (Ross et al., 2003), while the proportion of berseem clover in the botanical composition decreased linearly. Yucel et al. (2018a) reported that legume species are less competitive than grass species and herbage yield increased as the seed rate of legumes in the mixture decreased.

The results of the study revealed that the mixtures of berseem clover and annual ryegrass at different seed rates had both complementary and competitive effects. Different indexes are used to determine the effect of competition between species in the mixture and the advantage of the mixture (Anil et al., 1998). One of these indexes, LER, shows the efficiency of mixed sowings of species in utilising environmental resources in comparison to pure sowings of species (Mead and Willey, 1980). According to the two-year average values in our study, as the proportion of berseem clover in the botanical composition decreased, the LER value of annual ryegrass increased, while the LER value of beseem clover decreased (Dhima et al., 2007). In general, in different mixtures, the LER value of berseem clover was less than 0.5, while the LER value of annual ryegrass was greater than 0.5. In this case, it shows that the intercropping system is an advantage for annual grass and a disadvantage for berseem clover. In terms of total LER, BC80-AR20 (1.05) and BC20-AR80 (1.02) were the two most advantageous mixtures (Table 3). This result indicates that between 2 and 5 per cent more land is needed to obtain the same yields from the mixture species than from the pure sowing (Ofori and Stern, 1987). Another index used to determine the advantage of a mixture is the aggressivity value, which expresses how much the relative yield increase of one species in the mixture exceeds that of the other species in the mixture (McGilchrist, 1965). In the study, except for the BC20-AR80 mixture, the aggressivity value of annual ryegrass

in the mixtures showed a positive value and it was the dominant species. Saia et al. (2016) reported that in legume-grass mixtures, grasses generally had higher aggressivity values and grasses were more dominant species than legumes. On the other hand, the competition ratio is another way of assessing the competition between different species in the mixture. In the study, except for the mixture BC20-AR80, the competitive species in the mixtures was annual ryegrass (Table 4).

In the study, lower NDF, ADF ratio and higher crude protein ratio were determined in pure sown berseem clover compared to pure sown annual ryegrass and different mixture treatments. In addition, there was a linear decrease in the crude protein ratio as the seed rate of berseem clover in the mixture decreased (Salama and Badry, 2015). De Santis et al. (2004) reported that berseem clover leaves were soft textured and had high digestible carbohydrates and protein. In another study, Solomon et al. (2011) reported that the use of legumes in pure or mixed cropping had a positive effect on crude protein ratio compared to grasses. El-Karamany et al. (2014) reported that pure sown legume species gave higher crude protein than grass species and legume-grass species and legume-wheatgrass mixtures. Salama (2020) reported that while the inclusion of berseem clover in the mixtures increased the crude protein content of the mixtures as compared to the pure grasses, there was a lower crude protein content in the mixtures compared to the pure berseem clover.

In the light of these results, it can be said that in regions with low rainfall, it would be more economical for growers who prefer high-yielding forage as an alternative crop for the crop rotation of wheat to establish pure annual ryegrass or to include a low proportion of berseem clover in the mixture. On the other hand, if high quality forage is preferred, either a low proportion of annual ryegrass should be included in the mixture or berseem clover should be established as pure. However, it should be kept in mind that pure berseem clover may cause bloating in animals and may cause bone disorders in animals due to Ca and P imbalance.

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MOLECULAR CHARACTERIZATION OF MUTATION REGIONS IN HERBICIDE-RESISTANT QUINOA (*Chenopodium quinoa Willd.*) MUTANT LINES

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ABSCTRACT

Quinoa (*Chenopodium quinoa* Willd.) is a viable alternative crop due to its adaptability to unfavorable climate and soil conditions, and its seeds are nutritionally rich. However, the lack of selective herbicides for weed control in quinoa fields poses a significant challenge for cultivation. Consequently, developing herbicide-resistant quinoa lines is essential.

In this study, the Titicaca variety of quinoa was used. Sodium azide at a concentration of 1.5 mM was employed for mutagenesis. Herbicide-resistant plants were identified by applying herbicides from the imazamox to the M_3 generation. The resistant lines were designated as ET-6, ET-7, OT-11, and T-103. Among the four mutant lines obtained through seed mutagenesis, the OT-11 line exhibited a cytosine to adenine (C→A) substitution in the ALS gene, while the ET-6 line showed a thymine to guanine (T→G) substitution. These mutations in the OT-11 and ET-6 genotypes were classified as transversion-type mutations. A transition-type mutation was observed in the T-103 mutant line, involving a thymine to cytosine (T→C) substitution at nucleotide 1114.

The findings suggest that effective weed control in quinoa cultivation can be achieved by developing varieties resistant to IMI group herbicides. Continued research on herbicide resistance should focus on the ET-6, OT-11, and T-103 lines in subsequent generations.

Keywords: *Chenopodium quinoa,* Herbicide resistance, Imazamox, Mutation, Sodium azide.

INTRODUCTION

With the global population on the rise, the demand for food is also increasing. Projections indicate that the world's population will reach 9 billion by the 2050s, leading to a 70% increase in the demand for animal feed and nutritional fiber resources (Langyan et al., 2022). Urbanization and climatic conditions negatively impact agricultural fields. Additionally, improper practices in irrigation, fertilization, and land management contribute to a decline in agricultural production. To sustain soil fertility, agricultural practices must be adapted to changing climatic conditions. This adaptation includes identifying, developing and incorporating into production high-yielding and high-quality plant varieties that can thrive in adverse climatic and saline soil conditions (Gungor et al., 2022). Quinoa (*Chenopodium quinoa Willd.*), a pseudocereal originating from South America, demonstrates remarkable adaptability, growing in environments ranging from high-altitude regions to deserts and tropical climates, with temperatures between -8°C and 40°C and relative humidity around 88% (Tapia, 2015). It can grow in soils with pH levels from 4.5 to 9.06, making it suitable for sodic and alkaline soils (Jacobsen, 2003). Quinoa's adaptability positions it as a viable alternative to traditional crops under adverse climatic conditions (Sosa-Zuniga et al., 2017). The seeds of quinoa are nutrient-rich, containing an average of 12% protein with balanced amino acids and a substantial mineral content, surpassing that of wheat and rice. The seeds provide K (927 mg/100 g), Ca (149 mg/100 g), Mg (250 mg/100 g), P (384 mg/100 g), S (150–220 mg/100 g), Fe (13.2 mg/100 g), and Zn (4.4 mg/100 g) (Konisi et al., 2004). This nutritional profile enables quinoa to meet essential human dietary needs (Ocampo et al., 2023).

Quinoa cultivation has expanded to over 100 countries, with Peru and Bolivia accounting for over 90% of the global production. Global quinoa production is approximately 160.000 tonnes, with an average yield of 0.93 tonnes per hectare. The United States is the largest consumer and importer of quinoa (Patan et al., 2024; FAO, 2024). Although quinoa exhibits slow initial growth, it rapidly develops in subsequent stages, reaching harvest maturity 3 to 3.5 months after planting, depending on the variety. The plant's sufficient dry matter content makes it suitable for silage production (Carpıcı et al., 2023). Its short

growing period allows it to be used as a second crop in suitable climates. With proper genotype selection, quinoa can yield up to 10 tonnes per hectare under dry conditions and 20 tonnes per hectare under irrigated conditions (Tan and Temel, 2018). Despite increased cultivation fields in the past decade, quinoa yield has not proportionately increased. One of the major challenges to improving quinoa yield is weed competition, which can cause up to 34% yield losses (Basaran, 2021). Effective weed control is hindered by the lack of quinoa-specific herbicides. Herbicides that disrupt plant functions such as photosynthesis and amino acid synthesis can also harm quinoa due to the similarity of these functions between cultivated plants and weeds (Basaran, 2021). Developing herbicide-resistant quinoa through biotechnological methods, such as Clearfield® technology, can enhance yield and reduce production costs. Herbicides targeting the AHAS genes, like Sulfonylurea (SU), imidazolinone (IMI), triazolopyrimidine (TP), pyrimidinyl-thiobenzoates (PTB), and sulfonyl-aminocarbonyl-triazolinone (SCT), inhibit the synthesis of essential amino acids, leading to plant death. Clearfield® technology aims to confer herbicide resistance to plants through various biotechnological applications, thereby improving weed control and increasing crop yields (Rizwan et al., 2015).

Genetic diversity of plants and increasing this diversity is very important in plant breeding (Chuchert et al., 2022). This study aims to develop herbicide resistant quinoa lines (resistant to Imazamethabenz-methyl, Imazamox, Imazapic, Imazapyr, Imazaquin and imazethapyr) using classical breeding methods. These herbicide resistant lines can serve as genetic resources for variety development.

MATERIAL AND METHOD

Material

In this study, the early and dwarf Titicaca variety of quinoa was utilized, which has been deemed suitable for forage and seed yield in our country (Yazar and Kaya, 2014; Tan and Temel, 2018). Earliness is a desirable trait for high-altitude fields such as Erzurum, which have a short growing season. Titicaca is a Danish variety, also known as Q-52, developed from Peruvian quinoa.

Study Duration and Location

The study was conducted from 2019 to 2020 in field and greenhouse experiments at the plant production field of Atatürk University, Faculty of Agriculture. The experimental details are provided under the following sub-headings.

Seed Preparation

This phase was completed in 2018, following the method used for developing imidazolinone (IMI) herbicide-resistant wheat in the USA (Newhouse et al., 1992). Initially, 400 g of quinoa seeds were soaked in cold tap water for 24 hours. For sterilization, the seeds were treated with 1.5% sodium hypochlorite (NaOCl) for 20 minutes and rinsed three times with sterile distilled water. Subsequently, the seeds were treated with ethanol (EtOH) three times at 5-minute intervals, followed by three rinses with sterile distilled water. The rinsed seeds were then treated with 1.5 mM sodium azide at room temperature for 3 hours in closed bottles with magnetic stirrers. After treatment, the seeds were dried on filter papers at room temperature for 24 hours and prepared for use in field and greenhouse studies.

M_1 Generation

Chemically mutagen-treated quinoa seeds were planted in irrigated experimental fields of the Atatürk University Plants Production and Research Center in May 2018. Sowing was done manually in prepared rows. Based on soil analysis, fertilizer was applied at 125 kg N ha^{-1} and 80 kg P_2O_5 ha^{-1} (Geren, 2015). All phosphorus was supplied during planting; 75 kg ha^{-1} of nitrogen was applied at planting, with the remaining 50 kg ha^{-1} applied when the plants reached 30-40 cm in height. Weeds were manually removed, and the plants were irrigated as needed. A total of 166.7 mm of rainfall occurred between May and September, with an average temperature of 16.075°C during the growing period (Mgm, 2020). M1 generation seeds were planted in the fields, and Imazamox was applied to the green parts of the plants at a rate of 30 g ai ha^{-1} when they had three leaves. Harvesting occurred in August-September when the seeds matured. All plants were bulked together, and M_2 seeds were collected for herbicide tolerance screening.

Determination of Resistant Plants in M_2 and M3 Generations

M_2 seeds were obtained from plants identified as resistant at the end of the process. The bunches were sun-dried, and the seeds were manually separated from the husks. At the end of this period, 22 lines were identified for resistance. To verify the durability of resistance, M3 stage tests were conducted under greenhouse conditions. Fertilizer was applied at 125 kg N ha^{-1} and 80 kg P2O5 ha^{-1} (Geren, 2015), with all phosphorus supplied at planting and 75 kg ha^{-1} of nitrogen applied at planting and 50 kg ha^{-1} when the plants were 30-40 cm tall. Under greenhouse conditions, the plants were irrigated as needed. When the plants had three leaves, the herbicide was re-applied at a dose of 30 g ai ha^{-1}. It was observed that some lines initially resistant in the field were susceptible and died in the greenhouse test. Ultimately, four lines were identified as resistant.

Molecular Characterization of herbicide resistance plants

To elucidate the molecular nature of the IMI-herbicide-resistant mutant lines, the mutant region was amplified via PCR, and the type and location of the mutation were determined through sequencing. The mutant lines and the corresponding region of the non-mutated control variety were amplified by PCR and subjected to sequence analysis. The resulting DNA sequences were aligned to identify potential mutations in the gene sequence. DNA was extracted from both the mutant lines and the control quinoa

variety using a modified CTAB method (Ausubel et al., 1994).

PCR reactions were conducted in a 20 µl reaction mixture containing 50 ng DNA, 0.25 µM primers, 200 µM dNTPs, 1.5 mM MgCl2, 1x PCR buffer, and 0.5 U Taq DNA polymerase. The PCR parameters were as follows: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, 56-60°C for 30 seconds, and 72°C for 45 seconds, with a final extension at 72°C for 10 minutes. Primers specific to the AHAS gene of Chenopodium, Amaranthus, Bassia, and Salsola were used for PCR analysis (Table 1). The PCR products were analyzed in 1.5% agarose gel with 1X SB buffer at 100 V/cm for 150 min and finally were stained with ethidium bromide (0.2 µg mL-1) and visualized under UV light of Universal Hood II (Bio-Rad, Hercules, CA, USA).

Table 1. Sequences, annealing temperature of primers used

Primer Pairs	Nucleotide sequence (5′-3′)	Annealing Temperature	Genus
F1- R4	TTTTGTTTCCCGATTTAGCCC AATCAAAACAGGTCCAGGTC	58 ^{0}C	*Amaranthus* *Amaranthus*
CHALSF1 R4	GCGTCTACTTGTKCAAA AATCAAAACAGGTCCAGGTC	57 ^{0}C	*Chenopodium* *Amaranthus*
ALS1FB ALSGR1	ATCACCCCTTCTCTTCTTCAA CATCAAACCTAACCCCGAAA	58 ^{0}C	*Chenopodium* *Chenopodium*
ALSGF2 ALS1RD	TTTCGGGGTTAGGTTTGATG AGTAGTAGCAAGCAGCATGTG	58 ^{0}C	*Chenopodium* *Chenopodium*
F1 RUTH-R-3B	TTTTGTTTCCCGATTTAGCCC AACTTGTTCTTCCATCACCTTCG	58 ^{0}C	*Amaranthus* *Salsola*
CHALSF4 RUTH-R-3B	GACCTGGACCTGTTTTGATT AACTTGTTCTTCCATCACCTTCG	57 ^{0}C	*Chenopodium* *Salsola*
RUTH-F-1C RUTH-R-3B	CKGGCCGTGTKGGTGTTTG AACTTGTTCTTCCATCACCTTCG	60 ^{0}C	*S Salsola* *Salsola*

Sequence analysis of PCR products was performed using Sanger sequencing through services provided by Medsantek. The resulting DNA sequences were subjected to multiple alignment using the ClustalW module within the CLC Sequence BioEdit package (Hall et al., 1999). Following alignment, the specific nucleotides where mutations occurred and the mutation patterns were identified.

RESULTS

Molecular Characterization of herbicide resistance plants

a. DNA extraction of IMI-tolerant candidate quinoa plants

In the M_2 generation, 20,000 plants were screened under field conditions. Twenty-two plants identified as resistant were monitored until they reached harvest maturity and were harvested by cutting the clusters during the ripening period. The M_3 generation study was conducted under greenhouse conditions. At the conclusion of this stage, four of the twenty-two lines were confirmed to be resistant. These resistant lines were designated as ET-6, ET-7, OT-11, and T-103.

b. Molecular analyses of candidate mutant lines

To elucidate the molecular nature of the IMI-herbicide-resistant mutant lines, we amplified the acetolactate synthase (ALS) gene region from M3 generation mutant lines using various primer pairs. The target region, approximately 1939 base pairs in length (nucleotides 198 to 2137), was sequenced. Mutant lines OT-11, T-103, ET-6, and ET-7, along with the unmutated control quinoa cultivar, underwent PCR amplification and sequencing using ALS gene sequences obtained from a gene bank. The sequences were aligned using multi-clustalw to identify mutations in the herbicide-resistant lines.

The sequence analysis revealed specific nucleotide substitutions in the ALS gene of the mutant lines. In OT-11, cytosine was replaced by adenine (C→A) at the 935th nucleotide. In ET-6, thymine was replaced by guanine (T→G) at the 1712th nucleotide. Both OT-11 and ET-6 mutations are classified as transversion-type mutations. In T-103, a transition-type mutation was observed, with thymine replaced by cytosine (T→C) at nucleotide 1114. No mutations were detected in ET-7 within the analyzed 1939 base pair region, suggesting that the mutation might be located in the unexamined 521 kb fragment (Table 2).

Table 2. Location of mutations in AHAS gene of quinoa lines

	910	920	930	940	950
	……..|……|	……..|……..|	……..|……..|	……..|……..|	……..|……..|
Quinoa_ALS	TCTGGTAGGC	CTGGACCTGT	TTTGATTGAT	ATTCCTAAAG	ATATTCAGCA
OT-11	TCTGGTAGGC	CTGGACCTGT	TTTGATTGAT	ATTCATAAAG	ATATTCAGCA
ET-6	TCTGGTAGGC	CTGGACCTGT	TTTGATTGAT	ATTCCTAAAG	ATATTCAGCA
Et-7	TCTGGTAGGC	CTGGACCTGT	TTTGATTGAT	ATTCCTAAAG	ATATTCAGCA
T (Control)	TCTGGTAGGC	CTGGACCTGT	TTTGATTGAT	ATTCCTAAAG	ATATTCAGCA
T-103	TCTGGTAGGC	CTGGACCTGT	TTTGATTGAT	ATTCCTAAAG	ATATTCAGCA
	1110	1120	1130	1140	1150
	……..|……|	……..|……..|	……..|……..|	……..|……..|	……..|……..|
Quinoa_ALS	GGGAGGTGGG	TGTTTGAATT	CTGGTGAGGA	ATTGAGGAAA	TTCGTCGAAT
OT-11	GGGAGGTGGG	TGTTTGAATT	CTGGTGAGGA	ATTGAGGAAA	TTCGTCGAAT
ET-6	GGGAGGTGGG	TGTTTGAATT	CTGGTGAGGA	ATTGAGGAAA	TTCGTCGAAT
Et-7	GGGAGGTGGG	TGTTTGAATT	CTGGTGAGGA	ATTGAGGAAA	TTCGTCGAAT
T (Control)	GGGAGGTGGG	TGTTTGAATT	CTGGTGAGGA	ATTGAGGAAA	TTCGTCGAAT
T-103	GGGAGGTGGG	TGTCTGAATT	CTGGTGAGGA	ATTGAGGAAA	TTCGTCGAAT
	1710	1720	1730	1740	1750
	……..|……|	……..|……..|	……..|……..|	……..|……..|	……..|……..|
Quinoa_ALS	CTCAGGTGGT	TTAGGAGCCA	TGGGGTTTGG	GCTACCAGCT	GCTATTGGAG
OT-11	CTCAGGTGGT	TTAGGAGCCA	TGGGGTTTGG	GCTACCAGCT	GCTATTGGAG
ET-6	CTCAGGTGGT	TGAGGAGCCA	TGGGGTTTGG	GCTACCAGCT	GCTATTGGAG
Et-7	CTCAGGTGGT	TTAGGAGCCA	TGGGGTTTGG	GCTACCAGCT	GCTATTGGAG
T (Control)	CTCAGGTGGT	TTAGGAGCCA	TGGGGTTTGG	GCTACCAGCT	GCTATTGGAG
T-103	CTCAGGTGGT	TTAGGAGCCA	TGGGGTTTGG	GCTACCAGCT	GCTATTGGAG

Several methods are employed to develop herbicide-resistant plants, including target gene modification, altering gene expression, inhibiting target enzyme activity, detoxifying the herbicide, and preventing herbicide uptake or transport to the target gene (Tan et al., 2005). Among these, target gene modification and herbicide detoxification are widely used (Kirkwood, 2002), especially in plants resistant to amino acid synthesis inhibitors (Duke, 2005). Commercial examples include Clearfield®, Roundup Ready®, and LibertyLink® plants, which are resistant to imidazolinone, glyphosate, and glufosinate herbicides, respectively (Tan et al., 2005).

The Clearfield production system combines herbicide-resistant plants with IMI group herbicides for effective weed control. Artificial mutagenesis and the selection of desirable traits in mutant plants are key strategies for developing herbicide resistance. Chemical mutagens like NaN3 are preferred for their efficacy at inducing mutations, typically resulting in AT→GC base changes and subsequent amino acid and phenotype alterations (Al-Qurainy and Khan, 2009).

IMI group herbicides effectively control broad-spectrum weeds that other herbicides cannot, such as red rice (Oryza sativa L.), a major global rice cultivation problem. Herbicide-resistant rice varieties developed using Clearfield technology have solved this issue (Webster and Masson, 2001). Similarly, herbicide-resistant quinoa varieties can control weeds like Chenopodium album and Amaranthus retroflexus without harming the crop, improving seed quality and yield.

Clearfield technology has significantly impacted agriculture by enabling effective weed control and minimizing damage from other agricultural practices. In Kenya, for instance, the harvest index increased by 17 % in maize cultivated in Orobanche-infected soils using Clearfield technology. This technology is also effective against broomrape in sunflower cultivation.

Mutation breeding differs from GMO technology as it does not involve transferring foreign genes into the plant genome. Instead, it induces small changes within the plant's own genome, minimizing potential negative effects. This method is extensively used in corn and canola farming. In 2002, 15% of the 4.9 million tons of corn produced in the USA were from Clearfield technology seeds, and in 2000-2001, 20% of the canola grown in Canada was derived from Clearfield seeds (Tan et al., 2005; Beckie, 2004). Additionally, Clearfield technology has resolved issues caused by organophosphate insecticides in corn, which affect the ALS gene. This demonstrates that Clearfield technology not only addresses weed problems but also reduces the adverse effects of other agricultural inputs, gaining wider acceptance among producers and consumers wary of GMO plants.

CONCLUSIONS

Weed control is a major challenge in agricultural production, and chemical control remains the most effective method. However, the development of herbicide-resistant weeds and the emergence of new weed species necessitate the creation of new herbicides. Although quinoa has been cultivated since ancient times, large-scale

cultivation has only become feasible in recent decades. The presence of morphologically similar plants and the lack of plant-specific herbicides complicate weed management in large fields. Effective weed control is crucial for high-yield quinoa cultivation. Given the lengthy process of developing plant-specific herbicides, focusing on herbicide-resistant plants is more practical. IMI group herbicides are preferred for their efficacy at low doses and lower toxicity to organisms.

This study aimed to induce resistance to IMI group herbicides in the quinoa cultivar Titicaca through mutagenesis. Prior to mutagenesis, preliminary studies determined the 50 % lethal dose and application time for quinoa seeds. Based on these results, a protocol involving 1.5 mM sodium azide mutagen at room temperature was established. The durability of the resulting lines was assessed by comparing their ALS gene base sequences with those of the resistant lines, revealing amino acid changes typically observed in mutation studies.

Developing quinoa varieties resistant to IMI group herbicides can enhance weed control in agriculture. Additionally, quinoa's high forage yield and nutrient-rich seeds can contribute to animal feed. Herbicide resistance studies should continue in lines ET-6, OT-11, and T-103, advancing through several generations. Artificial mutation applications can expedite the generation of wide genetic variation, a critical and challenging aspect of breeding. Resistant quinoa varieties could be integrated into crop rotations with herbicide-resistant corn, soybean, and cotton.

In regions with high soil salinity and elevated groundwater levels, such as the 3.6 million hectares in our country affected by these conditions (Kanber et al., 2005) quinoa's known salt tolerance could be beneficial. Resolving weed issues could make these otherwise unusable lands productive through quinoa cultivation.

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EFFECT OF PACLOBUTRAZOL AND ABSCISIC ACID APPLICATION ON GROWTH AND PRODUCTIVITY OF CANOLA (*Brassica napus* L.)

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ABSTRACT

Canola (*Brassica napus* L.) is an important non-conventional oilseed crop that can be grown in diverse soil and climatic conditions. However, seed shattering after maturity is a major problem in canola production as it can cause up to 50% yield loss if harvesting is delayed due to adverse conditions. Different agronomic and physiological interventions can be opted to enhance yield stability and shattering resistance in canola. The present field study aimed at exploring the potential effects of paclobutrazol and abscisic acid on the growth and yield contributing attributes of canola. The treatments of the experiment included a control (no-spray), water spray, paclobutrazol (50 mg L^{-1}), abscisic acid (0.5 mM), and a combination of paclobutrazol+ abscisic acid (50 mg L^{-1}+0.5 mM). The current experiment examined two canola cultivars, Hybrid (45S42) and Inbred Sandal Canola. The experiment was laid out under Randomized Complete Block Design (RCBD) with a factorial arrangement having three replications. The results showed that exogenous application of paclobutrazol and abscisic acid alone or in combination significantly affected both canola cultivars. The plant height decreased significantly however, leaf area index, grain weight, number of seeds per silique, and number of branches per plant were increased compared with the control. Moreover, the combination of paclobutrazol and abscisic acid recorded a higher increase in leaf area index, dry matter accumulation, plant yield, and number of seeds per pod than their individual application. Between cultivars, the Hybrid cultivar (45S42) recorded better yield as compared to the Inbred Sandal Canola. These results thereby suggest that Hybrid (45S42) cultivar with combined application of paclobutrazol and abscisic acid can be a suitable option for gaining high yield and productivity.

Keywords: Growth retardants, Canola, Plant height, Seed Shattering, Production

INTRODUCTION

The increase in human population has changed the dietary habits of mankind along with increased demands for food and fuel. In the future, providing a balanced diet to the rising population will be a major challenge for global agriculturists. Fats and oils are an integral part of each daily diet (Karp and Richter, 2011). Pakistan has become the world's third-largest edible oil importer. Pakistan has been lacking in producing edible oil and spends a giant amount of budget on the import of edible oil and oilseeds to fulfil our necessities (Shahzad, 2003).

Rapeseed (*Brassica napus* L.) is an annual crop that ranks among the major five oil-seed crops cultivated worldwide (Khattab and Laila, 2002 and Liersch et al., 2013). The production potential of many crops in developing countries is underexplored mainly because of inadequate management and nutrition-related constraints (Gosh et al., 2006; Safdarı-monfared et al., 2020 and Sher et al., 2023). Among oilseed crops, rapeseed and mustard is the second main source in the globe which provides up to 13% of oil in the world. In Pakistan, canola was introduced in 1995 to replace low-quality conventional rapeseed and mustard crops. Seed of canola contains 40% oil and 30-35% protein. However, the average yield is very low compared to other developed countries of the world (Shahzad, 2003 and Saleem, 2018). Several factors such as, storage of seeds, agronomic practices, weed population, pod shattering, and lower harvest index contribute to a decrease in the productivity of canola

(Madani, 2016). Plant height has a great effect on the production of canola because greater height boosts the chances of lodging (Gan et al., 2008). In canola production, lodging is a major contributor of decreased yield under favorable climatic conditions (Kumar et al., 2012 and Wu and Ma, 2018).

Different chemical substances can be applied to control various aspects of development and growth in plants. Paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)-pentan-3-ol] is a member of the triazole family, and can alter the levels of key plant hormones, including gibberellins, abscisic acid, and cytokinins (Kum and Gebeloglu, 2024). Paclobutrazol impacts the isoprenoid pathway and modifies plant hormone levels by inhibiting gibberellin synthesis, increasing cytokinin levels, and consequently reducing stem elongation. Application of paclobutrazol also reduced the stem height in *Solanum tuberosum* L., (Mabvongwe et al., 2016), Mango (*Mangifera indica* L.), (Kumar et al., 2020), *Cymbidium hybridum* L. (Li et al., 2020), and ornamental tomatoes (de Moraes et al., 2005). Paclobutrazol application has also been reported to improve canola yield (Zhou and Xi, 1993).

However, several studies have reported the role of paclobutrazol and abscisic acid in reducing shattering losses and crop lodging (Kumar et al., 2012). However, the combined exogenous application of paclobutrazol and abscisic acid has not been studied and needs to be further explored. Furthermore, the application of these growth retardants has not been studied yet in canola cultivars. The objective of the current study was to evaluate the vital role of the above-mentioned growth retardants on the morphological and yield attributes of canola cultivars. It has been hypothesized that exogenous application of paclobutrazol and abscisic acid may suppress the plant height and improve the productivity of two canola cultivars Hybrid Canola (45S42) and Inbred Sandal Canola.

MATERIALS AND METHODS

Experimental Location, Setup, Design, and Treatments

The field experiment was conducted during the winter season of 2019-2020. The investigational area is at 31° North latitude, 73° East longitude, at an elevation of 186 meters from sea level (Agronomy Farm, University of Agriculture, Faisalabad). The experimental design was randomized complete block design (RCBD) under factorial arrangement and each treatment was replicated thrice. The treatments of the experiment included a) control, b) water spray (WS), c) paclobutrazol (50 mgL^{-1}), d) abscisic acid (0.5 mM), and e) paclobutrazol (Pbz=50 mg L^{-1}) + abscisic acid (ABA=0.5mM). The exogenous application of these growth retardants was done separately and combined on the canola cultivars Hybrid 45S42 and Inbred Sandal Canola at the bud initiation stage only once. The seedbed was prepared with a cultivator followed by planking. The net plot size of the trial plot was 4 m × 1.8 m. Seeds of both cultivars were sown in mid of October by using a 4.5 kg ha^{-1} seed rate with the help of drill at the row spacing of 45 cm. The nitrogen and phosphorus fertilizer doses at 90:60 kg ha^{-1} were applied to fulfil the nutrient requirement. Three irrigations were applied throughout the experiment. Standard plant protection measures were adopted to protect crops from insects and diseases. For weed control, hand weeding was carried out after 35 days.

Soil Analysis

Before the sowing of the crop, soil samples were collected from the experimental site by using an auger to different depths from 0 to 30 cm. The samples were air-dried, ground, and passed through a 2 mm strainer and were analyzed (Table 1).

Table 1. Physicochemical properties of experimental soil

Sand (%)	60
Silt (%)	14
Clay (%)	26
Textural Class	Sandy clay Loam
EC (dSm^{-1})	1.59
pH	7.9
Organic Matter	0.72
Total Nitrogen	0.051
Available P (ppm)	5.08
Available K (ppm)	172

Morphological Attributes

The morphological attributes were determined according to standard procedures. The leaf area index was calculated by using a leaf area meter having model number (LI-3100). The stem diameter was measured with the help of a Vernier caliper. Plant height was recorded by using measuring tape from the soil level to the growing tip of the plant. Five plants were used from every plot randomly for the number of branches per plant counted physically. For seed yield, one line of 1 m was harvested at maturity from each block, then threshed and weighted out then converted into kg ha^{-1} by using the unitary method.

Yield Contributing Attributes

Five plants were used from every plot randomly for the number of siliques per plant. Ten siliques opened in the tray which were taken randomly from each plot and No. of seeds per silique were physically counted. The electrical weight balance was used for thousand-grain weight. Two lines from each plot are harvested and weighed out and then converted into kg ha^{-1}. The seed yield is determined by the harvested two lines from all experimental blocks then threshed and weighted out then converted into kg ha^{-1} by using the unitary method.

The Harvest index was calculated with the help of the flowing formula,

$$Harvest\ Index\ \% = economic\ yield / Biomass \times 100$$

Statistical Analysis

A two-way ANOVA analysis was carried out to find the influence of treatments and cultivars on the growth, and yield-related traits of canola. Tukey's HSD test was used to test the treatments' difference at 5% probability level (Steel et al., 1997). The clustered heat map and correlation matrix were done by the use of R-Studio and Origin software respectively.

RESULTS

Morphological attributes

Morphological attributes were significantly affected by the exogenous application of growth retardants in both cultivars of canola (Tables 2 and 3). The interaction between the cultivars × exogenous application was significant for total dry matter, stem diameter and plant height while non-significant for other morphological attributes (Table 5). Average across cultivars, exogenous application of paclobutrazol and abscisic acid increased the maximum leaf area index, stem diameter, number of branches, and total dry matter while causing a reduction in the plant height as compared to control conditions. When we used them separately their effect was less significant in most of the cases. However, the combined application of paclobutrazol and abscisic acid showed a significant improvement in these morphological attributes such as maximum leaf area index and total dry matter increased by 14% and 10% respectively, while suppressing the plant height by 17% as compared to control conditions. However, among the cultivars the Hybrid 45S42 showed better performance in the above-mentioned morphological attributes as compared to the Inbred Sandal Canola.

Table 2. Effect of exogenous application of abscisic acid and paclobutrazol on the morphological attributes of canola cultivars.

Canola Cultivars	Treatments	LAI	Stem Diameter (cm)	Plant Height (cm)	TDM (g m^{-2})
Hybrid (45S42)	Control	3.03 e	6.43 c	212.11 a	500.19 c
	WS	3.26 bc	5.37 ef	213.14 a	526.35 b
	Pbz	3.15 d	7.26 b	190.33 b	530.63 b
	ABA	3.35 ab	7.31 b	188.54 b	526.64 b
	Pbz+ABA	3.43 a	9.39 a	166.31 d	536.90 a
Inbred Sandal Canola	Control	2.85 f	4.73 f	190.74 b	421.31 h
	WS	2.95 ef	5.66 de	192.10 b	431.17 g
	Pbz	2.96 e	6.13 cd	181.35 c	464.75 e
	ABA	3.21 cd	5.90 cde	180.47 c	457.23 f
	Pbz+ABA	3.27 bc	7.71 b	164.74 d	472.01 d
HSD Value		0.18	1.68	8.77	19.05

WS (Water spray) Pbz (Paclobutrazol), ABA (Abscisic acid), and Pbz + ABA, Difference among values (means of three replicates) show a significant difference across the mean at $p<0.05$ according to the Tukey's HSD test.

Table 3. Effect of exogenous application of abscisic acid and paclobutrazol on the morphological and yield contributing attributes of canola cultivars.

Canola Cultivars	Treatments	Number of Branches/ Plant	Number of Seeds/Silique	Number of Siliques/Plant
Hybrid (45S42)	Control	7.33 de	24 cde	246.67 f
	WS	8.33 d	25 bcd	250.67 ef
	Pbz	9.33 b	26 abc	266.67 bc
	ABA	10.00 c	28 a	270.33 b
	Pbz+ABA	13.33 a	28 a	294.33 a
Inbred Sandal Canola	Control	4.66 f	22 e	231.67 g
	WS	6.66 e	24 de	227.67 g
	Pbz	7.67 d	23 de	249.00 ef
	ABA	8.33 de	25 bcd	256.00 de
	Pbz+ABA	10.67 c	27 ab	261.00 cd
HSD Value		3.20	3.85	12.11

WS (Water spray) Pbz (Paclobutrazol), ABA (Abscisic acid), and Pbz + ABA, Difference among values (means of three replicates) show a significant difference across the mean at $p<0.05$ according to the Tukey's HSD test.

Yield contributing attributes

Yield-contributing attributes were significantly affected by the exogenous application of paclobutrazol and abscisic acid in both cultivars of canola (Tables 3 and 4). The interaction among the cultivars × exogenous application was significant for number of siliques per plant and thousand-grain weight while non-significant for other morphological attributes (Table 5). Average across cultivars, exogenous application of paclobutrazol and abscisic acid increased the number of siliques per plant, number of seeds per silique, thousand grain weight, seed yield, biological yield, and harvest index as compared to control conditions. When we used them separately their effect was less significant in most of the cases. However,

the combined application of paclobutrazol + abscisic acid showed a significant increase in these yield-contributing attributes such as the number of siliques per plant and biological yield increased by 16% and 4% respectively as compared to control conditions. However, among the cultivars the Hybrid 45S42 showed better performance in the above-mentioned yield-contributing attributes as compared to the Inbred Sandal Canola.

Table 4. Effect of exogenous application of abscisic acid and paclobutrazol on the yield contributing attributes of canola cultivars.

Canola Cultivars	Treatments	1000 grain weight (g)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
Hybrid (45S42)	Control	3.93 d	1965 bc	7694.00 abcd	24.92 a
	WS	4.02 d	1973 bc	7744.70 abc	24.97 a
	Pbz	6.65 b	2013 b	7845.00 ab	25.62 a
	ABA	6.77 b	2060 ab	7995.70 a	26.36 a
	Pbz+ABA	9.03 a	2160 a	8071.30 a	26.88 a
Inbred Sandal Canola	Control	1.89 f	1760 e	7141.00 e	24.19 a
	WS	2.70 e	1792 de	7204.00 de	24.40 a
	Pbz	4.99 c	1840 de	7354.30 cde	24.76 a
	ABA	6.44 b	1847 de	7385.00 bcde	24.74 a
	Pbz+ABA	7.11 b	1870 cd	7432.30 bcde	25.14 a
HSD Value		0.79	109.99	490.17	4.73

WS (Water spray) Pbz (Paclobutrazol), ABA (Abscisic acid), and Pbz + ABA, Difference among values (means of three replicates) show a significant difference across the mean at $p<0.05$ according to the Tukey's HSD test.

Correlation matrix and heat map

The morphological and yield-contributing attributes show strong positive and negative correlations under the influence of the exogenous application of growth retardants and control conditions according to Pearson's correlation analysis as shown in (Fig 1). The maximum leaf area, stem diameter, total dry matter, and the number of branches showed a strong positive correlation with the yield-contributing attributes in both cultivars of canola. However, these aforementioned indices showed a strong negative correlation with the plant height of both cultivars of canola.

Heat map analysis was done among the morphological and yield contributing attributes in both cultivars of canola. The interaction strength between the recorded aforementioned attributes and the treatments was shown by variation in the color of boxes of the heat map. The colors from dark blue (positive strongly) to dark red (negative strongly) in the scale of the heat map closely resemble the boxes colors. The highest enhancement in the morphological and yield contributing while reduction in the plant height was observed in the treatment V1T4 followed by the treatment V2T4. The treatment V2T0 showed a significant reduction in these attributes (Fig. 2).

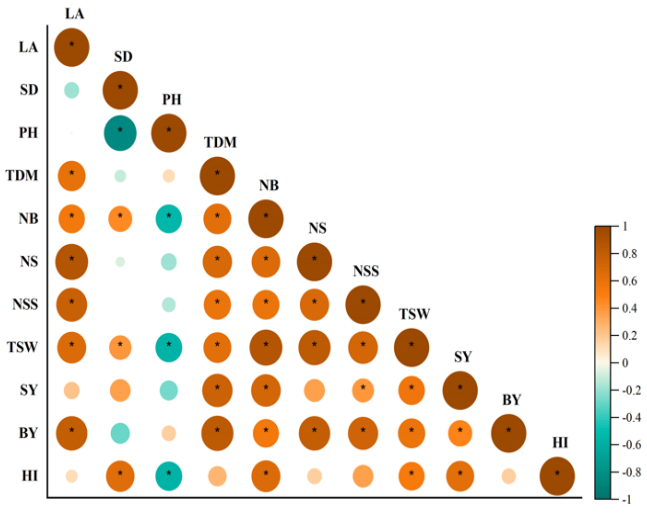

Figure 1. Pearson correlation analysis among the morphological and yield contributing indices of both cultivars of canola. The abbreviations of the attributes are leaf area index (LAI), stem diameter (SD), number of branches per plant (NB), number of silique per plant (NS), number of seeds per silique (NSS), thousand grain weight (TGW), seed yield (SY), biological yield (BY), and harvest index (HI).

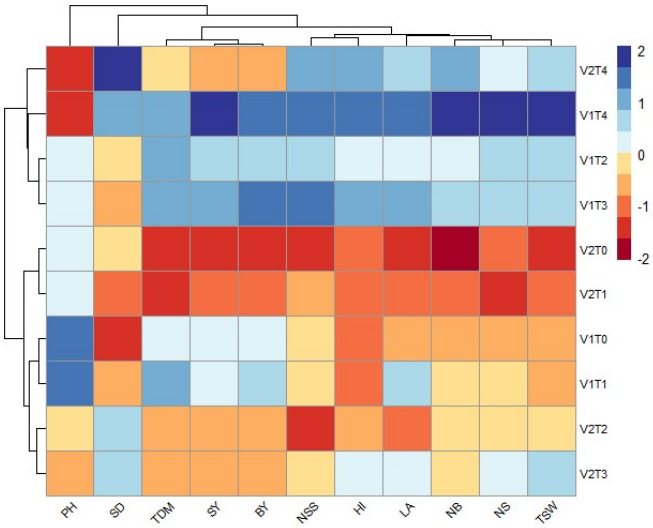

Figure 2. Heat map analysis showed the morphological and yield contributing indices of both cultivars of canola. Please see the caption of Fig. 2 for abbreviations of the attributes while in the group of treatment T0 (Control), T1 (Water spray), T2 (Paclobutrazol), T3 (Abscisic acid), and T4 (Paclobutrazol + Abscisic acid). The canola cultivars are represented by V1 (Hybrid 45S42) and V2 (Inbred Sandal Canola).

Table 5. Analysis of variance regarding the effect of exogenous application of abscisic acid and paclobutrazolon the yield contributing attributes of canola cultivars.

Attributes	Cultivar	Treatment	Cultivar × Treatment
LAI	0.284***	0.166***	0.006^{ns}
No. of Branches	32.03***	29.78***	0.45^{ns}
No. of seeds/silique	34.13***	14.63***	2.13^{ns}
No. of siliques/plant	3203.33***	1633.63***	92.67**
Plant Height	1117.28***	1374.23***	113.53***
Stem Diameter	9.50***	9.04***	1.02***
TDM	41716.30***	1810.60***	245.50**
1000 seed weight	16.13***	28.93***	0.72***
Seed Yield	338553***	21585***	3470^{ns}
Biological Yield	2409467***	119148**	5178^{ns}
Harvest Index	5.04^{ns}	12.09**	0.59^{ns}

ns=non-significant, significant at p≤0.05*, p≤0.01**, p≤0.001***

DISCUSSION

The morphological attributes such as maximum leaf area index, stem diameter, number of branches, and total dry matter were significantly increased while plant height was reduced with the exogenous application of growth retardants (paclobutrazol, abscisic acid, and their combinations) in both cultivars of canola as compared to control conditions (Table 2 and 3) as reported in the previous studies (Hazar and Bora, 2018 and Desta and Amare, 2021). These findings are in line with Li et al. (2020), who reported that the use of plant growth retardants can decrease plant height. The decline in plant height is primarily due to a decrease in the elongation of the internodes. It is also observed that when growth retardant was applied, the upper internodes were shortened (Syaputra et al., 2013 and Tesfahun and Menzir, 2018). By preventing stem expansion, growth retardants in rapeseed promote lateral growth and fruit/flower development, producing bushier plants with more flowers (Ijaz et al., 2015). Our research also indicated that the hybrid canola cultivar (45S42) showed maximum values of these traits than the inbred canola cultivar (Sandal). These results are similar to Fernandez et al. (2023) who reported that hybrids have the potential advantage to perform better as compared to inbred cultivars.

In both cultivars of canola yield attributes such as the number of seeds per silique, 1000 grain weight, seed yield, biological yield, and harvest index significantly increased with the exogenous application of growth retardants (paclobutrazol, abscisic acid, and their combination) as compared with control conditions (Table 3 and 4) as found in the previous investigations (Qian-Mei et al., 2023). Ali et al. (2024) also reported that the use of foliar application of paclobutrazol (a compound present in triazole fungicide) increased the yield of canola (*Brassica napus* L.). The number of branches and pods per plant increased because of the growth retardant treatment. Furthermore, there was a significant increase in seed yield because when a plant does not use all of its energy to elongate its stems, it can use that energy to produce more flowers, fruits or seeds, and the parts of the plant that humans typically harvest for yield (Ahmad et al., 2023 and Sima et al., 2024). It is also reported that the application of growth regulators can be used to increase stem diameter, number of branches per plant, number of siliques per plant, number of seeds per silique, and grain weight. These findings were similar to the results of Mccaskill et al. (2019) who reported that the use of plant growth retardants can reduce plant height and increase crop yield by reducing lodging and shattering losses.

CONCLUSION

The present study revealed that the use of plant growth retardants such as paclobutrazol and abscisic acid had beneficial effects on plant growth and yield contributing attributes leading to improved yield of canola Furthermore, overly large canopies were efficiently handled via the use of plant growth retardants, which reduced plant height and promoted the formation of seeds and productivity. Better results were obtained when paclobutrazol and abscisic acid were applied in combination, as compared to their alone application. Overall, foliar Pbz and ABA treatment shows potential for increasing canola yields in a sustainable manner. Future studies should examine their processes, the best times to apply them, and the approaches that work best for various cultivars and environments. These tools have complicated and situation-specific impacts, but they may be useful parts of climate-smart canola production systems.

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TRANSFORMER NETWORKS TO CLASSIFY WEEDS AND CROPS IN HIGH-RESOLUTION AERIAL IMAGES FROM NORTH-EAST SERBIA

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ABSTRACT

The intricate backgrounds present in crop and field images, coupled with the minimal contrast between weed-infested areas and the background, can lead to considerable ambiguity. This, in turn, poses a significant challenge to the resilience and precision of crop identification models. Identifying and mapping weeds are pivotal stages in weed control, essential for maintaining crop health. A multitude of research efforts underscore the significance of leveraging remote sensing technologies and sophisticated machine learning algorithms to enhance weed management strategies. Deep learning techniques have demonstrated impressive effectiveness in a range of agricultural remote sensing applications, including plant classification and disease detection. High-resolution imagery was collected using a UAV equipped with a high-resolution camera, which was strategically deployed over weed, sunflower, tobacco and maize fields to collect data. The VIT models achieved commendable levels of accuracy, with test accuracies of 92.97% and 90.98% in their respective evaluations. According to the experimental results, transformers not only excel in crop classification accuracy, but also achieve higher accuracy with a smaller sample size. Swin-B16 achieved an accuracy of 91.65% on both the training and test datasets. Compared to the other two ViT models, the loss value is significantly lower by half, at 0.6450.

Keywords: agriculture; drone; image classification; multi-head attention; remote sensing; vision transformers.

Abbreviations: unmanned aerial vehicles (UAV), deep learning (DL), natural language processing (NLP), vision transformers (ViT), convolutional neural networks (CNN), multilayer perceptron (MLP).

INTRODUCTION

In an ever-changing and progressive industrial landscape, agriculture plays a significant role in overcoming numerous challenges to achieve high yields while maintaining plant growth and quality standards to meet the demands of both society and the market. Yet, the age-old problem persists in modern agriculture: an over-reliance on pesticide interventions to boost production capacity, enhance quality, and combat unwanted plant growth, especially weeds (Grammatikis et al. 2020; Ustuner et al. 2020). Weeds compete with primary crops for vital development resources such as water, nutrients and sunlight. They pose a significant challenge to the outlook for agricultural production. The widespread use of herbicides in sprayed fields increases environmental damage such as air, water and soil pollution. Some weed species develop resistance to these chemicals. This continuing trend could threaten crop yields if weed resistance is fully realized. Site-specific weed and crop control management needs to be developed as an area of research (Iqbal et al. 2019; Vrbničanin et al. 2017).

One effective solution is the use of automated crop monitoring and inspection systems which offer promising environmental and economic benefits. The advantage of using robotic technology is that it reduces labour costs and minimises the use of herbicides. In addition, weeds often have similar colour, texture and shape characteristics as crops. Automated weed control systems face the challenge of identifying and mapping weeds in the field (Iqbal et al. 2019; Wu et al. 2020). Unmanned Aerial Vehicles (UAV) utilise RGB and additional multispectral imagery to map weed density in fields. UAVs capture images as they fly over fields at different altitudes (Huang et al. 2018a, 2020b, 2018c). It uses learning algorithms to distinguish and classify weeds from crops by segmenting these large images into smaller, regular frames for effective analysis (dos Santos Ferreira et al. 2017a, 2019b).

Unlike conventional machine learning methods, which heavily depend on meticulous feature engineering, deep learning (DL) techniques autonomously extract features from images, yielding a wealth of detailed information. This results in notably enhanced performance, particularly on larger and more diverse datasets. DL has emerged as a

transformative force across numerous domains, including agriculture object detection and recognition (Hasan et al. 2021; Lecun et al. 2015). Convolutional neural networks have achieved superiority and success in tasks by extracting features from images in object detection and image classification processes through convolution filters by utilizing principles such as local connectivity, weight sharing and translation equivalence (Lecun et al. 2015; He et al. 2016). In particular, convolution-based architectural networks, including frequently used models such as VGG-16, GoogLeNet, ResNet-50, ResNet-101, AlexNet and Inception-v3, have been widely used for weed detection or classification (Madsen et al. 2020; Szegedy et al. 2016; Niu et al. 2021).

Attention mechanisms, developed primarily for natural language processing (NLP), have made significant advances and have shown significant performance improvements compared to previous versions (Niu et al. 2021; Vaswani et al. 2017). However, its adaptation to vision-related tasks has limited the significant computational demands that correspond to the higher number of pixels in images compared to NLP word studies, making traditional attention models unsuitable for use (Hasan et al. 2021; Lu et al. 2020). A significant increase in the use of transformer-attention models can be seen in computer vision with the advent of the sign-relative transformer (Li et al., 2022). Unlike CNN-based methods operating at the pixel level, ViT treats image patches as distinct units of information during training, utilizing self-attention modules to discern their interrelations. ViT has demonstrated superior image classification accuracy over CNNs when ample training data and computational resources are available (Beyer et al., 2022). Nevertheless, the application of vision transformer models for tasks such as weed and crop classification using high-resolution UAV images remains largely unexplored.

In our study, we introduce an innovative methodology for automatically identifying weeds and crops in multispectral images captured by drones, strengthening the vision transformer approach. Our research setup involves a drone equipped with a high-resolution camera, facilitating image acquisition across diverse crop plots under real-world conditions, encompassing tobacco, sunflower, maize, and weed varieties (Czymmek et al., 2019). Our primary aim is to investigate the viability of transformer architectures for specialized tasks like plant recognition in UAV imagery, given the scarcity of labeled data. To address this challenge, we employ data augmentation and transfer learning techniques, supplemented by an evaluation of the self-attention mechanism using vision transformers across varying proportions of training and testing data within a cross-validation framework. Our contributions encompass the integration of low-altitude aerial imagery from UAVs with self-attention algorithms for crop management, pioneering exploration of transformer models for weed and crop image classification, and the assessment of deep learning algorithm generalization capabilities in crop plant classification across different model variations (Alzahrani et al., 2023).

MATERIALS AND METHODS

Study Sites

This study focused on the cultivation of corn, sunflower, and tobacco crops. Drone imagery was obtained from multiple plots in the Kuzmin village of the Sremska Mitrovica region in Serbia during May and June of 2023 (45.0223 N, 19.4052 E) (Figure 1). Table 1 presents the geographical details and agricultural configurations for the crops. An experiment spanning multiple sites was conducted to assess the system's resilience across various ecological zones, aiming to comprehend its adaptability through the diversity of crops and fields involved in the study. The research focuses on industrial crops, with each station subjected to distinct treatment methodologies (Culpan, 2023).

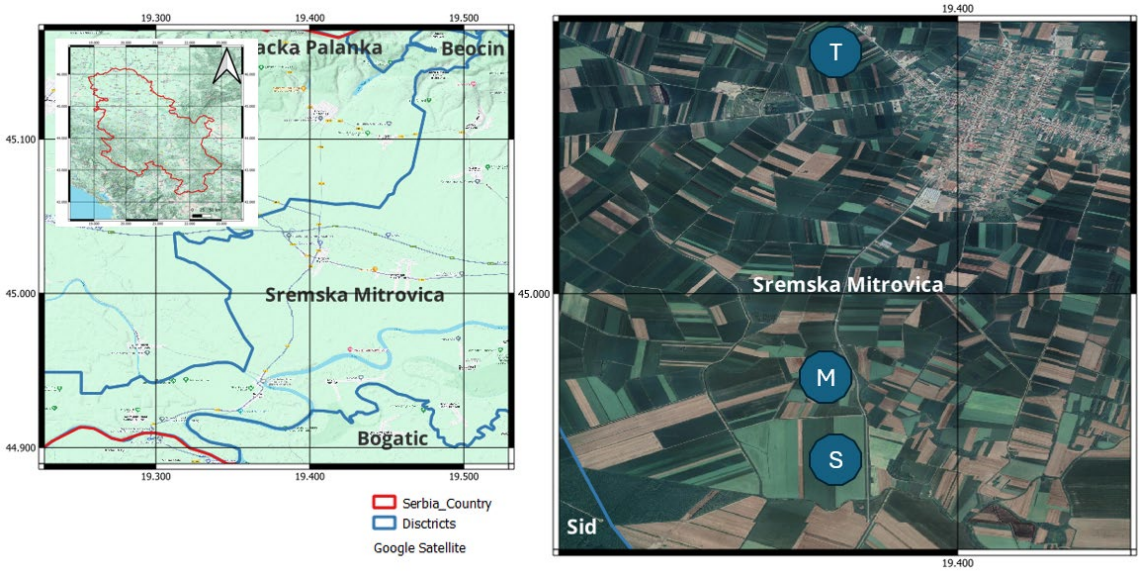

Figure 1. Geographical position of the research area. (S-Sunflower, M-Maize and T-Tobacco, base image from Google Earth)

Table 1. Performance comparison with the state-of-the-models.

	Train Loss	Train Accuracy	Test Loss	Test Accuracy
Swin-B	0.6450	0.9165	0.7256	0.8733
Vit-B16	1.2252	0.9331	1.2516	0.9297
Vit-B32	1.3532	0.9133	1.2878	0.9098

The sunflower images were captured on the 12th (2-leaf stage) and the 14th (4-leaf stage) of June 2023. Photographs of the maize and tobacco were taken during the 3-leaf phase, and on the 8-leaf phase. The density of planting ranges from 33,000 to 45,000 plants per hectare. Soils with excellent filtration capabilities were identified. Additionally, irrigation facilities were available for 95% of the plots, allowing for regulated water conditions (Kayin et al., 2024).

UAV data collection

In this research, imagery was sourced from fields of tobacco, sunflower, and maize, which were sown with inter-row spacings of 60-70 cm. The photographs were taken using a camera mounted on the DJI Mavic 3 multispectral drone. A corpus of 350 RGB photographs was compiled, each with a resolution of 5472 x 3648 pixels and a color depth of 24 bits (Louargant et al., 2017). The environmental conditions during the acquisition of these images were obtained with air temperatures ranging from 24.0 to 26.0 °C and relative humidity levels ranging from 55% to 65%. The drone was equipped with a camera stabilized with a 3-axis brushless gimbal to maintain consistent camera alignment even in strong wind conditions. Flight heights were deliberately set at 10 meters for sunflower plots and 12 meters for maize and tobacco plots. These heights have been optimally chosen to ensure high quality image capture while reducing the duration of drone flights. For maize fields, the increased height was necessary due to more mature plant growth and wing winds. The program of aerial imagery acquisition in different fields was planned to be done at an early stage based on the review and assessment of weed infestation levels in the field. This approach, which spread the imagery over multiple days, resulted in a range of variability and shadow elements in the images, with the tobacco field photographed in the afternoon light conditions, and the sunflower and maize fields photographed in the midday light conditions with the sun at its full perpendicular position.

Prior to launch, the flight path of the UAV was meticulously planned, setting the flight velocity at 2 meters per second. To enhance the quality of image stitching, it was imperative that the overlap of image footprints exceeded 80% both longitudinally and laterally along the flight path. Positional accuracy and altitude control were rigorously maintained within a tolerance of 1 meter and 0.2 meter, respectively, utilizing the Global Positioning System (GPS) and a barometric sensor. Consistent resolution of 0.33 centimeters per pixel was upheld across three different sites, with adjustments in flight elevation compensating for variations in camera pixel sizes to ensure uniform resolution across all imagery.

Image Pre-Processing

For the purposes of this analysis, the model required a comprehensive aerial photograph of the area under investigation. Consequently, prior to conducting any model-based analysis, it was necessary to merge the UAV captured images into a singular, cohesive site map. This image integration process was facilitated using the Pix4Dmapper software (Reedha et al., 2022). All images captured by the UAV were uploaded into the Pix4D software, where the image coordinate system, geolocation data, camera specifications, and other pertinent details were calibrated in alignment with the specifications of the UAV and its camera system (refer to Figure 2).

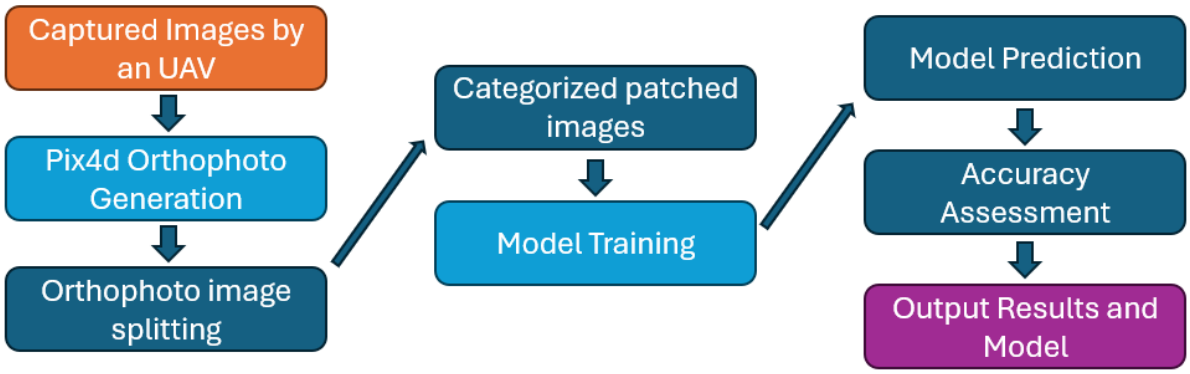

Figure 2. Sequence of steps for image preprocessing and subsequent model forecasting.

From the assembled orthophotos, we extracted specific image segments representing both crops and weeds. These segments were then adjusted to a uniform size of 96x96 pixels. The rationale behind this specific size selection stems from the fact that the dimensions gravitated towards a median of 96 pixels, suggesting a possible proportional relationship between the UAV's operational altitude and the physical scale of the crops documented in the research plots. Weeds were entered into the algorithm by creating classes for weeds, soil and three different agricultural crops.

Vision Transformers (ViT)

Image transducers use the operating principles of transducer models for NLP tasks. It has pioneered a groundbreaking change in deep learning methodology by demonstrating the ingenuity of computer vision. The traditional dominance of convolutional neural networks has been challenged by the success of transformative models in visual data analysis. NLP-based image transformers are attracting the interest of researchers by providing a breakthrough architecture for image classification. The versatility of transformer models also marks a paradigm shift beyond traditional CNN frameworks (Alzahrani et al., 2023).

As described in Vaswani et al's groundbreaking 2017 study, "Attention is All You Need" the core principles of transformers revolve around self-attention mechanisms (Vaswani et al., 2017). As NLP endeavors to demonstrate competence in managing sequential data, the level of importance such mechanisms skillfully assign to different parts of the input data will have increased. These ground-breaking applications of vision transformers apply the self-attention perspective to image inputs and conceptualize them not as conventional pixel grids but as hierarchical arrays of patches, similar to the processing of words in sequential sentences.

Given an image of dimensions H x W x C, the ViT model proceeds by segmenting the images into patches of uniform size, where H represents height, W width and C colour channels, and each patch size is set to have dimensions PxPxC. The total number of patches generated from an image (N) is determined by dividing the total image area by the area of a single patch and is calculated as N = (H/P) x (W/P). For example, for images to be fed at 224x224 with a selected model's patch size of 16, the formula [(224/16) x (224/16)] = [14 x 14] results in a total of 196 patch or array tokens (Xia et al. 2024; Kang et al. 2021).

The way ViT works starts with segmenting images into uniform patches. These patches are then flattened, linearly transformed and enriched with spatial and positional embeddings to preserve spatial and positional context and encoded in a manner similar to the text string processing in transducers. The subsequent stage entails passing the resultant sequence of image patch embeddings through a canonical transform coder architecture (Zhai et al., 2021). The encoder logic consists of multiple layers of multi-head self-attention and feed-forward neural networks, allowing the model to selectively focus on and interpret different regions of the image, thereby distinguishing complex relationships between patches (refer to Figure 3). The output of the transform encoder typically completes the image classification task by generating predictions based on the embedded representations, provided that the output of the transform encoder is fed to the classification head, which typically comprises a linear layer.

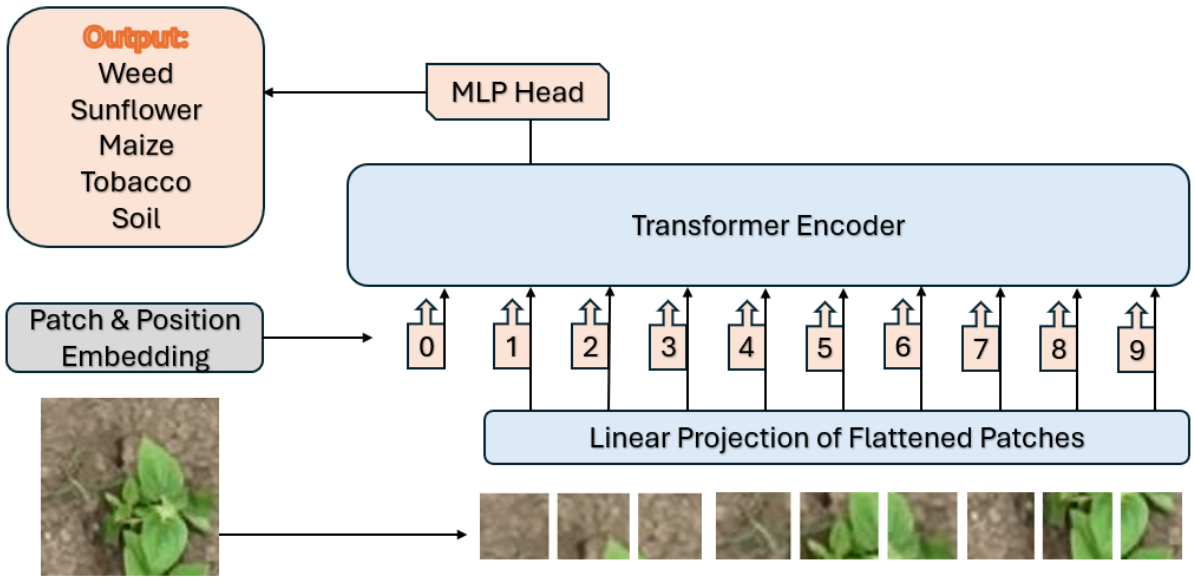

Figure 3. The vision transformer architecture flow shows patch process.

After segmenting the images into patches, the next step is to convert these patches into a one-dimensional format. This transformation can be described mathematically as H*W*C = N*P*P*C. In general, for one-dimensional basis

vector scenarios, the channel value is set to C=1. The flattened patches are mapped to a space equation using a linear transformation layer to produce vectors of size D (Khan et al., 2021). The 'classification token' is created at this stage and passed to the next stage as a class token. The element used as the class token is concatenated with each of the linearly transformed one-dimensional patch vectors and continues, retaining the classification information in the array. The transformer network architecture processes the patches and the class token along with the initial positioning in the array. The learnable embedding created in dimension D is evaluated for use in classification. The methodology of this work reflects the natural language processing strategy of the BERT architecture for image classification within the ViT framework (Bazi et al., 2021).

To preserve the spatial relationships of the original image during the positional encoding process, positional encodings are sequentially added to the patch embeddings. These encodings contain no information about the 2D spatial arrangement of the patches and require the model to learn the spatial relationships between patches from scratch. The transform encoder is fed to the encoder by adding the concatenated patch and position embedding sequence. In the presence of the encoder, the sequence is transformed, allowing the class token introduced at the beginning of the sequence to focus on and assimilate important features from the patches. This process allows a comprehensive embedding process to be learnt, particularly for classification purposes. After the encoder, the class token integrated with the residual information is used to generate a prediction vector by multiplying it with the output of a multilayer perceptron (Han et al. 2020; Suh et al. 2018). After the normalisation layer, the prediction vector becomes capable of image classification using the softmax function and results in a probability distribution. Each image frame is transferred by adding a layer of adaptability and complexity to the architecture of Vision Transformer models at different scales, such as basic and tiny models. The architecture of ViT models, the size of patches, the size multiplicity of embeddings and the self-attention mechanism are often referred to as the width of the model. The depth of the encoder layers, the number of attention heads and the dimensions of the MLP block are called the MLP width and define several basic parameters. These variables allow the ViT model to be customised and optimised for specific task definitions and data sets (Suravarapu et al. 2023; Zhao et al. 2023).

Attention Mechanism

The basic architecture of the converters has enabled significant advances in image analysis through the implementation of query, key and value vectors that are central to their operation. Scaled Dot Product Attention is the core component of this architecture. It allows dynamic weighting of the importance of different parts of the input data. The mathematical formula is defined below as equation (1). Calculates the dot products of queries by scaling them with keys and adding their attention scores. This process improves the model's ability to focus on relevant parts of the data, providing an innovative approach to understanding both textual and visual information (Ma et al. 2023; Mauricio et al. 2023).

$$\text{Self Attention (Q,K,V)} = \text{softmax}\left(\frac{QK^T}{\sqrt{d_k}}\right)*V \quad (1)$$

In the scaled dot attention mechanism, the variables Q, K and V represent the query vector, transformed key vector and value vector. The scaling process (d_k) is very important for the dimensionality of the key vector. This scaling is done to smooth the dot products and ensure that they remain within the appropriate range. This facilitates a steady gradient decay during the training of deep learning models. The purpose of using (d_k) is to help reduce the potential problem of overly large dot product values that can lead to decreasing derivatives (Shin et al. 2023; Abdalla et al. 2019; Thakur et al. 2023). Preservation of model sensitivity to input subtleties. This balancing act is the basis of attention scoring. This determines how each element in the sequence should manage attention among all the other elements. It increases the model's ability to use relevant information when constructing representations.

In the self-attention process, for each element in the array, the mechanism computes the dot product between the query representation and the key representations of all other entries. The data normalised by applying the softmax operation is transferred to the result set with the attention score, which measures the amount of focus each item should have on all other items in the array.

Since the transformer architecture model assimilates the inputs in a sequential manner, it is through spatial embeddings that parallel data processing is allowed. In encoding the sequence information of the input, embeddings are very important in encoding the sequence information for the transducer to understand and send to the next step (Zhao et al. 2023; Mauricio et al. 2023). The transformer uses its own attention to collectively assimilate the information from each element of the input. It must preserve the order of the data, which is crucial for the operations of the converter, and spatial embeddings must be explicitly added to the input. The positionally enriched inputs are structured into an array with an integrated class embedding based on positional indices to help categorise the input data after the self-attention change (Suravarapu et al. 2023; Ma et al. 2023).

Self-attention works by mapping a set of input vectors onto a set of output vectors, and independently assessing the importance of inputs relative to others. It highlights the importance of context in the process by allowing the model to focus on appropriate aspects of the input. The results of the self-attention module are the sum of aggregated attention scores covering contextual relevance across the sequence. The transformer framework is fundamentally built around these attention mechanisms, often utilizing a multi-head approach to expand the model's capability to focus on various parts of the input simultaneously (Thakur et al., 2023). The scaled dot-product attention and its extension into multi-head attention are pivotal components of this model, which are further elucidated in Figure 4 of the referenced work.

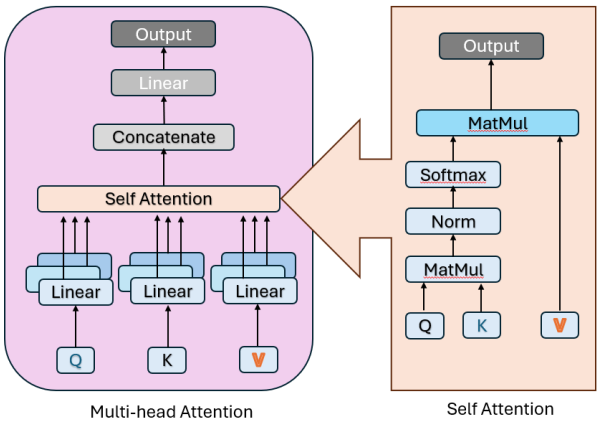

Figure 4. Attention mechanism (Niu et al. 2021).

Evaluation Metrics

We utilized the latest classification methods of Swin B16, ViT-B16 and ViT-B32 models in our project. The research utilized the method of cross-validation to validate the robustness and precision of the proposed models. Cross-validation, which evaluates the performance of a model on test data not used during training, is widely used because of its robustness. It is a method that is attracting attention as a resampling strategy because of its low bias rate. As the classes in our dataset are evenly distributed, we also applied a layered k-fold approach. Ensure that all classes are represented in the validation phase of each fold (Huang et al. 2018; Reedha et al. 2022).

Deep learning models are evaluated by comparing their performance against a benchmark of excellence, known as the gold standard. The accuracy metric is a measure of the model's predictive ability and is calculated as the ratio of correct predictions to the total number of predictions made. (Alzahrani et al. 2023).

$$Accuracy = \frac{(TP+TN)}{(TP+TN+FP+FN)} \quad (2)$$

$$Precision = \frac{TP}{(TP+FP)} \quad (3)$$

Precision is often used to evaluate the performance of deep learning classification models. Precision indicators are calculated as the ratio of true positive predictions to the sum of true positive and false positive predictions. Precision indicates the model's ability to correctly identify positive specimens among all specimens it classifies as positive (Sunil et al., 2023).

$$Recall = \frac{TP}{(TP+FN)} \quad (4)$$

$$F1 \text{ score} = 2*\frac{(Precision*Recall)}{(Precision+Recall)} \quad (5)$$

Recall measures the proportion of true positives in the dataset that the model successfully identifies as positive. Simplified, it measures the proportion of true positive predictions made by the model from all positive samples in the dataset. Recall is critical to understanding how effectively the model is able to identify and classify all relevant examples. The F1 score is a widely accepted benchmark for evaluating performance classification scenarios (Hand et al. 2009; Ozcift et al. 2011). It is a method of harmonising and balancing the trade-off between precision and recall by calculating harmonic averages. By integrating both metrics, the F1 score provides a nuanced view of model performance beyond just accuracy.

RESULTS

Each model was trained using a leave-one-out technique with triple cross-validation. The model based on the basic architecture was trained and cross-validated with triple folds and achieved the highest average accuracy of 93.31%. Table 1 illustrates all experimental outcomes related to crop weed classification across the three ViT models. This approach involved training the models on 3150 samples (70%), validating them with 900 samples (20%), and testing them with 450 image samples (10%).

Analysis of the experimental results reveals that the Swin-B16 model surpasses the Vision Transformer models. The Swin-B model achieved the highest accuracy of 91.65% and 0.6450 loss, while the ViT-B16 model closely trailed with a 93.31% accuracy and a minimal loss of 1.2252. All network families exhibit impressive accuracy and F1-score, with the vision transformer models demonstrating the most effective prediction performance in classifying crop and weed images (shown Table 2). The table illustrates the data, revealing notably high recall metric values for each specified category within the dataset. The Swin-B16 model underwent training with batch sizes set at 32, utilizing the SGD optimizer over 20 epochs. Impressively, the model attained an accuracy of 91.65% on both the training and testing datasets. Compared to the other two models, the loss value is notably lower by half, measuring at 0.6450.

The Swin-B16 model exhibited remarkable performance across the first two experiments, displaying consistently high recall and F1-score values. Notably, its performance peaked in the first fold, indicating its efficacy in accurately identifying various classes within the dataset. At the third fold the model showed a decrease in sensitivity, particularly evident in the maize class where sensitivity dropped to 54%. Despite this, the model was successful in discriminating between weed classes and demonstrated the ability to effectively discriminate between different vegetation types.

The ViT-B16 produced consistently impressive results on all three folds. Remarkably, the sensitivity values for the soil class remained high in every experiment, highlighting the robustness of the system in correctly classifying this category. However, the model showed relatively lower sensitivity in the sunflower class compared to other categories. This points to potential difficulties in accurately identifying this crop. It was also observed that the recall for weed classification was relatively low. This indicates some limitations in the accurate detection of weed samples.

Table 2. Evaluated the performance of the models derived from a three-fold cross-validation.

	Experiment 1			Experiment 2			Experiment 3		
Swin-B16	*Precision*	*Recall*	*F1-Score*	*Precision*	*Recall*	*F1-Score*	*Precision*	*Recall*	*F1-Score*
Maize	0.9700	1.0000	0.9800	0.8900	1.0000	0.9400	0.5400	0.8200	0.6500
Soil	0.9700	1.0000	0.9800	0.9900	0.9400	0.9600	0.6100	0.3000	0.4000
Sunflower	0.9800	0.9200	0.9500	0.9900	0.9600	0.9700	0.7600	0.9900	0.8600
Tobacco	0.9300	0.9700	0.9500	0.9900	0.9800	0.9800	0.7700	0.9400	0.8500
Weed	0.9400	0.8900	0.9100	0.9500	0.9100	0.9300	0.9800	0.4700	0.6300
Vit-B16	*Precision*	*Recall*	*F1-Score*	*Precision*	*Recall*	*F1-Score*	*Precision*	*Recall*	*F1-Score*
Maize	0.9064	0.9936	0.9480	0.9873	0.9936	0.9904	0.7635	0.9936	0.8635
Soil	1.0000	0.8782	0.9352	1.0000	0.9295	0.9635	0.9923	0.8200	0.9021
Sunflower	0.7919	1.0000	0.8839	0.9286	1.0000	0.9630	0.9398	1.0000	0.9689
Tobacco	0.9792	0.9038	0.9400	1.0000	1.0000	1.0000	1.0000	0.9808	0.9903
Weed	0.9160	0.7692	0.8362	0.9416	0.9295	0.9355	0.9141	0.7500	0.8239
Vit-B32	*Precision*	*Recall*	*F1-Score*	*Precision*	*Recall*	*F1-Score*	*Precision*	*Recall*	*F1-Score*
Maize	0.8864	1.0000	0.9398	0.8211	1.0000	0.9017	0.9176	1.0000	0.9571
Soil	0.9430	0.6731	0.7749	0.9922	0.8205	0.8982	1.0000	0.8782	0.9352
Sunflower	0.8254	1.0000	0.9043	0.9722	0.8974	0.9333	0.9689	1.0000	0.9842
Tobacco	0.9810	0.9936	0.9873	0.9017	1.0000	0.9483	0.9809	0.9872	0.9840
Weed	0.7465	0.6795	0.7114	0.8889	0.8205	0.8533	0.9032	0.8974	0.9003

As can be seen in the Table 3, the ViT-B32 model showed different sensitivity values in the three folds. On the first fold, the precision values indicate a relatively high classification accuracy, ranging from 74% to 98%. However, in subsequent folds, the precision values varied between 82% and 100% and different values were observed for each class. In particular, the maize and sunflower classes showed excellent recall in all trials. The effectiveness of the model in accurately identifying these specific product types is highlighted.

Table 3. Location and crop planting settings.

Crop Type	Sunflower	Tobacco	Maize
Latitude	19.3873	19.3846	19.3873
Longitude	44.9829	45.0335	44.9829
Date	26/05/2023	22/06/2023	27/05/2023
Flight Height (m)	10	12	12
Row Spacing (m)	0.70	0.65	0.60
Plant Spacing (m)	0.25	0.30	0.25

DISCUSSION

The production of maize and sunflowers is of global importance, with high economic and commercial nutritional value. However, they are susceptible to various diseases and weather events that pose a significant threat to both yield and quality. Early and accurate detection and diagnosis of weed infestations is essential for implementing field-based control strategies and preventing potential losses. This study reaffirms the efficacy of the proposed methods for distinguishing between crops and weeds, offering valuable insights into the performance of various models. The findings of this research demonstrate notable accuracy, laying a solid foundation for the development of automated systems capable of detecting and managing areas affected by weeds in their early stages. Ultimately, such advancements are poised to enhance the efficiency and sustainability of cultivated crop production.

All models underwent training and validation using identical crop samples, encompassing all classes from the same agricultural field. Our investigation reveals that when applied to our agricultural dataset featuring five classes for weed identification, the ViT B-16 architecture, pretrained on the ImageNet dataset, surpasses other architectures and exhibits enhanced resilience to fluctuations in dataset size. Employing ViT for weed classification yields promising outcomes, particularly when dealing with a limited array of classes. In forthcoming experiments, we intend to broaden the dataset by incorporating additional classes to encompass a wider spectrum of crop types. Introducing supplementary classes may potentially lower the classification top-1 score, especially when categorizing plants with analogous shapes and colors. Nevertheless, the ViT is anticipated to yield superior results compared to the Swin-B16 model, given its demonstrated robustness. Although the loss value of the Swin model is lower it cannot provide high precision, recall and F1 scores (Reedha et al. 2022; Wang et al. 2023).

During training, the increments should be applied in such a way as to cover different environmental variations, such as variations in outdoor brightness. The use of augmentations plays an important role in promoting model

convergence and generalisation by changing the examples. This increases the ability of the model to generalise effectively by facilitating the representation of differences in the dataset. If the image acquisition conditions are significantly different, the model's performance may degrade. For example, capturing images of plants after rainfall may result in a change in vibrancy and shape compared to those captured in sunlight. To address these inconsistencies, additional image acquisition is planned for the coming season to ensure robust performance under changing environmental conditions.

CONCLUSIONS

The evolving agricultural environment requires the development of new systems that can accurately identify weeds and crops in different environmental conditions. The solution we used in the classification study overcomes this challenge by exploiting the latest advances in deep learning, pioneered in NLP and now proving useful in computer vision. ViT models have demonstrated superior performance accuracy in a wide range of applications. The model run on the ViT-B16 model using 3 different folding techniques emerges as the best performing model, achieving a test accuracy of 92.97%. Our results also show that the use of smaller patches contributes to improved accuracy. Looking ahead, we aim to develop a hybrid approach to address the complex challenges of crop-weed separation and transformer model design with convolution integration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

Publicly available datasets were analyzed in this study. This data can be found here: **[https://drive.google.com/file/d/1P8L0V_4-szEByJkODL3TuoNY-H-QxlFx/view?usp=sharing]**.

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EFFECT OF SOIL CONDITIONERS APPLIED TO SEED ON GRAIN YIELD AND YIELD CHARACTERISTICS IN WHEAT

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ABSTRACT

This study, researching the effect of seed treatment with liquid soil conditioners on yield properties of bread wheat varieties, was carried out during 2017 and 2018 years in the experimental field of Tekirdag Namik Kemal University Faculty of Agriculture, Department of Field Crops. The experiments were conducted with 3 bread wheat varieties and 4 soil amendments (control + 3 different liquid soil amendments) in 3 repetitions. In the experiment, 4 different treatments including 3 different soil regulators and 1 control (T1: Control; T2: 13-5-8+glycine betaine; T3: 15% organic matter, 15% humic and fulvic acid+0.03% potassium and T4: 25% organic matter + 65% humic acid + 6% potassium (T4) were made. Seeds treated with a spray and then dried were sown as split plot experimental design. In the study, the variations in the plant height (PH), spike length (SL), number of spikelets per spike (NSS), number of grains per spike (NGS), grain weight per spike (GWS), spike fertility index (SFI), harvest index (HI) and grain yield (GY) parameters were investigated for the bread wheat varieties. According to the research results, all soil conditioners applied to seeds were determined to affect the investigated characters at a statistically significant level. For the PH parameter, T3 treatment caused a significant increase, while for the SFI parameter, T2 treatment caused a significant increase. For the HI parameter, treatments T2 and T3 had the highest effect. Spike characteristics like SL, NSS and NGS increased compared to controls with all soil conditioner treatments, while parameters like GWS and SFI differed according to variety. Grain yield, the most important parameter for wheat, provided the highest results in different soil conditioner treatments depending on the varieties. T4 treatment caused clear increases in the SL, NSS, NGS, GWS and GY parameters. According to the data obtained was evaluated, soil conditioner applications caused a significant increase in the parameters examined in wheat. T4, which contains 25% organic matter + 65% humic acid + 6% potassium, was determined as the most effective soil conditioner for many parameters.

Keywords: Humic substance, soil conditioner, *Triticum aestivum* L., yield

INTRODUCTION

The yield properties of agricultural soils are weakening from year to year due to reasons such as temperature, mistaken agricultural practices and erosion, which make sustainable agricultural production impossible. Increasing the yield of agricultural land by using appropriate cultivation techniques is an important requirement for sustainable agriculture. Within the sustainable agriculture approach, organic farming techniques and organic fertilizer have an important place (Turhan, 2005). The basic principle in organic farming is to use organic fertilizers instead of chemical fertilizers. With the use of organic fertilizer, the organic matter content of soils increases and thus, the yield capacity of soils increases with enhanced physical, chemical and biological traits (Oksel et.al., 2022). Producers aware of the importance of organic matter use a variety of organic materials, sold commercially and called soil conditioners, for this purpose. The effects of organic soil conditioners used in agricultural soils are proposed to largely come from the humic substances (HS) they contain (Liu and Cooper, 2000).

HS greatly affect soil quality due to its role in several complex chemical and biochemical reactions in soil and has vital importance in preserving soil yield. Humic substances are basic components of the terrestrial ecosystem and humic substances comprise 60% of soil organic matter (Shah et al., 2018). While HS may reach 5-7% in soils rich in terms of humus, it may exceed 80% in materials like leonardite (Bezuglova and Klimenko, 2022). The main fraction of humic substances is humus and it typically

occurs in organic waste, agricultural by-products, fresh plant and animal organic matter and from fermentation of coal. Fermentation of these substances under conditions with controlled temperature, water, aeration and time comprise the soil biota (Canellas and Olivares, 2014). Weber et. al. (2018) reported that HS have bio-stimulating effects on plant growth and interest in HS has increased with this awareness. Canellas and Olivares, (2014) stated that HS primarily affects root development and growth dynamics. HS increases the development of plant roots and hence is known as a supporter of plant growth on a broad scale. Generally, one of the expected traits of HS may be said to be that they will increase intake by the plant by increasing the bioavailability of macro and micronutrients (Garcia et al., 2016; Shah et al., 2018). Additionally, among their important traits are the improvement in the physical, chemical and biological traits of soil (Weber et al. 2018; Senesi et al., 1996) and mineral nutrition that encourage root and shoot growth (Shah et al., 2018; Ramos et al., 2015). It was determined that glycine betaine also had a positive effect on the plant traits examined in canola (Safdari-Monfared et al., 2020).

Wheat is the agricultural product with the highest production in Turkiye and additionally the highest consumption of 179.3 kg per person annually according to 2021 data (TUIK, 2023). Due to reasons such as the high yield linked to suitable climate and soil structure, cultivation being suitable for mechanized farming and hence lack of excess labour needs, wheat cultivation is performed in large areas of Thrace (Turkiye) (Ozturk et al., 2009). According to 2021 data, a total of 445,042 ha area was farmed for wheat in Thrace (Tekirdag, Kırklareli, Edirne) and 2,208,211 tons of wheat were produced (TUIK, 2023). Producers in this region implement intensive fertilization programs to be able to obtain maximum product from the agricultural area. With the conceptualization of the importance of organic farming in recent periods and additionally the use of support from the Ministry of Agriculture, the importance given to organic fertilization within this fertilization program has rapidly increased. With this aim, the use of organic soil conditioners sold commercially and containing HS has been popularized. In a variety of studies about the wheat plant, the use of humic substances for wheat was shown to have a positive impact on plant development and product amounts (Arduc et al., 2020; El-Hashash et al., 2022; Pacuta et al., 2021). Humic substances may be classified as humic acids, fulvic acids and humins according to their solubility in water at different pH levels. Humic acids (HA) do not dissolve in water under acid conditions (pH <2); however, they dissolve at highly alkaline pH. Fulvic acid is soluble in water at all pH levels, while humins are humic substances that are not water soluble at any pH (Zavarzina et al., 2021). Humic substances (especially humic acids) may be applied to the plant from soil with irrigation and from the leaves. In recent periods, the use of HS applied to seeds before planting has become more common in the Thrace region. However, research on this topic is limited in our country. As a result, this study was aimed to investigate the effect on yield and yield properties of different liquid soil conditioners, commonly used in wheat-growing areas of the Thrace region and containing HS, applied to seeds of different wheat varieties before sowing.

MATERIALS AND METHODS

The research was completed as a 2-year field experiment in 2016-2017 and 2017-2018 growing years at Tekirdag Namik Kemal University, Faculty of Agriculture, Department of Field Crops. The soil properties of the experiment area are given in Table 1.

Table 1. Soil characteristics of the experimental area

Dept	O.M.	pH	EC	$CaCO_3$	Texture (%)				Available elements (ppm)				
(cm)	(%)		(dSm^{-1})	(%)	Clay	Silt	Sand	T. Class	P	K	Fe	Zn	Mn
0-20	1,08	6.25	0,33	0.01	42,50	24,2	33,30	C	16	169	27	0,32	25
20-40	1,11	6.52	0,30	0.01	43,00	24,9	32,10	C	15	164	25	0,41	20

O.M: Organic matter (%); EC: Electrical conductivity ($dS.m^{-1}$)

The soils in the experiment area have low organic matter, mildly acid-neutral pH, no salt, low lime, class clay (C) texture, adequate available P, adequate available K, high available Fe, low available Zn and adequate available Mn.

Soils in the research area had organic matter (%) determined with the modified Walkley-Black method (Jackson, 1979), and pH and soil salinity (EC) identified in saturation paste (Soil Survey Lab. Staff, 1992). The amount of $CaCO_3$ (%) was determined with the Scheibler Calcimetry Method (Soil Survey Lab. Staff, 1992), available P was extracted according to Olsen and Sommers (1982), and detected with ICP-OES and available K was extracted in solution with 1 N ammonium acetate and detected with ICP-OES (Soil Survey Lab. Staff, 1992). Microelements (Fe, Zn and Mn) were extracted in solution with DTPA and detected with ICP-OES (Lindsay and Norvell, 1978). For sand (%), silt (%) and clay (%) proportions (texture), the Bouyoucos hydrometer method was used (Gee and Bauder, 1986). Assessment of soil analysis results used classifications stated in Schlichting and Blume (1966) for organic matter and lime, Richards (1954) for pH and EC, FAO (1990) for available P and K, and Lindsay and Norvel (1969) and Follett (1969) for Fe, Zn and Mn.

In Tekirdag, where the experiment area is located, is dominated by the Mediterranean climate in coastal regions; however, different snowfall may be observed. Interior sections have a continental climate with hot summers and cold winters. According to long-term data (1970-2023), the

mean temperature is 14.1 ℃ (maximum 18.1 ℃, minimum 8.9 ℃) and the mean annual rainfall is 583.5 mm. (MGM, 2023).

The average temperature and rainfall during the wheat growing period are highly effective on grain yield. The amount of precipitation and its distribution, especially in April and May, which include the heading and grain filling periods, significantly affect quality and yield. While the total precipitation received during the growing period of the wheat in 2016 was 395 mm, the total precipitation in April-May was 53.5 mm. The total amount of precipitation received in the 2017 growing season was 478.8 mm, and the total precipitation in April-May was 77.8 mm. In 2017, the rainfall during the growing period was 83.8 mm and 24.3 mm higher in April-May. While the average temperature was 16.5 ºC in the 2016-2017 growing period, when the experiment was conducted, it was 15.7 ºC in the 2017-2018 growing period. When these values are compared with long-term averages, they were 2.4 ºC higher in 2016-2017, while the temperature was 1.6 ºC higher in the 2017-2018 growing period. Plant material in the experiment was used Flamura 85, Selimiye and Esperia bread wheat varieties commonly sown in Thrace. Flamura 85 is a variety resistant to cold, with winter planting, good tillering capacity, moderate or high yield potential (350-600 kg da^{-1}), red hard-semi hard grains with large size and good bread quality. Selimiye is a variety recommended for sowing in a variety of regions and soil structures, are used for winter sowing due to good resistance to cold. Tillering capacity is good and yield potential is moderate or high (350-600 kg da^{-1}). Grains have red color, with hard and large structure and good bread quality. Esperia is a quality variety of winter wheat that can resist cold, and with high yield in excess tillering and wet conditions.

The experiment was performed with 3 replications according to the split plot experimental design, with main parcels assigned to varieties and soil conditioners applied to sub-parcels. Three different, commonly sold soil conditioners were used in the experiment. Attention was paid to the manufacturer's instructions relating to the amounts of soil conditioners applied to the varieties. The conditioners, contents and amounts used are given in Table 2.

Table 2. Soil conditioner treatments and contents for seeds

Treatments	Soil conditioner (amount per 1 kg seed)	Soil Conditioners
T1 (Control)	0 (Control)	
T2	3 ml/1 liter	13-5-8+glycine betaine
T3	5 ml/1 liter	15% organic matter, 15% humic and fulvic acid+0.03% potassium
T4	2 g/1 liter	25% organic matter+65% humic acid+6% potassium

In the experiment, 4 different treatments including 3 different soil regulators and 1 control (T1: Control; T2: 13-5-8+glycine betaine; T3: 15% organic matter, 15% humic and fulvic acid+0.03% potassium and T4: 25% organic matter + 65% humic acid + 6% potassium (T4) were made. Soil regulators were dissolved in 1 liter of distilled water in the amounts given in Table 2. As soil conditioners in the research, T2 was used 3 ml kg-1 seed, T3 5 ml kg-1 seed and T4 2 g kg-1 seed and sprayed on all seeds in a container. In the control application, the seeds were soaked in only 1 liter of distilled water. Seeds were left in the laboratory environment at room temperature to dry. Seeds treated with soil conditioner and dried were sown in 5 m rows, 17 cm apart in 6 rows with 500 seeds m^{-2} using a seeder. Additionally, all parcels had 20 kg da^{-1} 20.20.0 composite fertilizer applied during sowing, 17 kg da^{-1} urea applied during the tillering period and 20 kg da^{-1} ammonium nitrate (26%) applied in the bolting period. To prevent weed development, insecticide was applied with no intervention performed for diseases and pests. The second year phase of the experiment was carried out on the same land, in a different parcel, by performing the same procedures as in the first year. The plants were harvested with a HEGE-160 parcel combine harvester with analyses performed for plant height (PH), spike length (SL), number of spikelets per spike (NSS), number of grains per spike (NGS), grain weight per spike (GWS), spike fertility index (SFI), harvest index (HI) and grain yield (GY).

Analysis of Data: Data obtained from the research in the split plot experimental design had variance analysis performed with the JUMP 5.0 statistical package. Significance levels of differences between the means were determined with the minimum least significant difference (LSD) test.

RESULTS AND DISCUSSION

The effects of soil conditioners applied to seeds in the research on PH, SL, NSS, NGS, GWS, SFI, HI and GY of the bread wheat varieties were investigated. The results obtained in the study and statistically evaluated were presented below.

According to variance analysis of the data obtained in the research (Table 3), the effects of soil conditioner treatments on PH were statistically significant at 0.01 level for varieties (V), year x variety interaction (YxV), variety x treatment interaction (VxT) and year x variety x treatment interaction (YxVxT), while the effect was statistically significant at 0.05 level in terms of treatment (T). For SL, the Y, V, YxV interaction and T were significant at 0.01 level, while the VxT interaction and YxVxT interaction were significant at 0.05 level. The soil conditioners had a significant effect on NSS in terms of Y, V, YxV interaction,

T and VxT interaction at 0.01 level. For NGS, the V, YxV interaction, T, YxT interaction and YxVxT interaction were significant at 0.01 level, while Y was significant at 0.05 level. When the temperature and precipitation values obtained in the second year of the experiment were examined, the statistical difference between the years was caused by the higher average temperature and total precipitation in the months of April-May, which are important for both the growing period and the product yield and quality in the second year.

Table 3. Mean squares related to grain yield and some yield characters

Variation resource	PH (cm)	SL (cm)	NSS (unit)	NGS (unit)	GWS (g)	SFI (%)	HI (%)	GY (kg ha^{-1})
Block	1.470	0.036	0.032	1.150	0.059	0.136	0.584	1074.764
Year (Y)	233.460	38.749**	526.501**	110.509*	3.516**	3217.223**	1053.023**	1795512.5**
Error$_1$	18.946	0.003	0.195	4.858	0.036	1.365	0.957	2244.042
Variety (V)	1095.723**	2.555**	3.565**	411.202**	0.304**	503.672**	53.623**	38446.181**
Y x V	14.862**	1.725**	24.708**	157.032**	0.110*	94.685**	23.230**	28519.292**
Error$_2$	1.435	0.032	0.222	1.612	0.024	1.197	0.938	327.382
Treatment (T)	10.469*	0.694**	3.668**	9.029**	0.019	301.440**	16.608**	2915.111**
Y x T	2.561	0.097	0.417*	13.806**	0.020	128.885**	12.257**	855.315
V x T	27.201**	0.178*	0.578**	1.304	0.038	96.700**	8.109**	5515.292**
Y x V x T	22.899**	0.151*	0.156	6.901**	0.025	125.610**	3.245**	4944.662**
Error	2.945	0.055	0.097	1.325	0.018	3.463	0.807	397.278

PH: Plant high (cm); SL: spike length; NSS: Number of spikelets per spike (unit); NGS: Number of grains per spike (unit); GWS: Grain weight per spike (g); SFI: Spike fertility index (%); HI: Harvest index (%); GY: Grain yield (kg ha^{-1})

For GWS, Y and V were significant at 0.01 level, while the YxV interaction was significant at 0.05 level. For SFI, HI and GY parameters, the Y, V, YxV interaction, T, YxT interaction, VxT interaction and YxVxT interaction were significant at 0.01 level (apart from YxT for GY).

Significance was tested for characteristics that were statistically significant as a result of variance analysis of the obtained data. Mean values and significance groups are given in Table 4.

Table 4. Treatment and variety mean values related to grain yield and some yield traits

Treatment	PH (cm)	SL (cm)	NSS (unit)	NGS (unit)	GWS (g)	SFI (%)	HI (%)	GY (t ha^{-1})
T1 (Control)	90.15 b	9.47 c	20.82 b	45.18 c	1.94	68.47 d	42.59 c	7.37 c
T2	91.21 ab	9.71 b	20.98 b	45.65 bc	1.99	78.28 a	44.82 a	7.57 ab
T3	91.83 a	9.67 b	20.97 b	46.21 ab	2.00	71.62 c	44.27 a	7.54 b
T4	90.43 b	9.95 a	21.81 a	46.82 a	2.01	73.05 b	43.60 b	7.68 a
LSD$_{0.05}$	1.160	0.159	0.210	0.778		1.258	0.607	13.474
Variety								
Flamura 85	96.77 a	10.05 a	20.73 b	47.97 a	2.12 a	76.38 a	43.72 b	7.64 b
Selimiye	92.43 b	9.64 b	21.21 a	41.20 b	1.91 b	67.68 c	42.38 c	7.10 c
Esperia	83.51 c	9.41 c	21.49 a	48.72 a	1.94 b	74.52 b	45.36 a	7.88 a
LSD$_{0.05}$	1.160	0.119	0.314	0.845	0.103	0.728	0.645	12.045

PH: Plant high (cm); SL: spike length; NSS: Number of spikelets per spike (unit); NGS: Number of grains per spike (unit); GWS: Grain weight per spike (g); SFI: Spike fertility index (%); HI: Harvest index (%); GY: Grain yield (kg ha^{-1})

According to the research results, the treatment of seeds with soil conditioners had statistically significant effects on yield and all yield parameters (apart from GWS) in terms of both variety and treatment.

The highest effect for the plant PH parameter was for Flamura 85 variety (96.77 cm) on a variety basis, followed by Selimiye and Esperia varieties. On a treatment basis, the highest value was obtained with the T3 treatment (91.83 cm), followed by the T2 treatment in the same statistical group. The T4 treatment and T1 (control) treatment had close results and were included in the same group statistically.

For the SL values, Flamura 85 variety was in first place with a value of 10.05 cm, followed by Selimiye in second place and Esperia in third place. On a treatment basis, T4 treatment had the highest effect with a value of 9.95 cm. This was followed by T2 and T3 treatments. The control dose was in last place.

On a variety basis, the NSS parameter was highest for the Selimiye and Esperia varieties (21.21 and 21.49) with the lowest effect for Flamura 85. On a treatment basis, the highest value was found with the T4 treatment (21.81), while the other treatments were included in a different statistical group with similar effect.

For the NGS parameter, the Flamura 85 and Esperia varieties (47.97 unit and 48.72 unit) had the highest effect in the same statistical group, while the Selimiye variety was in last place. Among treatments, the T4 treatment had the highest effect with a value of 46.82 unit, and this was followed by the T3 treatment in the same statistical group. The control treatment was in last place.

For the GWS parameter, data obtained on a treatment basis were not statistically significantly different, with the highest value for T4 (2.01 g) followed by T3. On a variety basis, the highest value was for Flamura 85 (2.12 g), followed by Esperia and Selimiye varieties.

For the SFI parameter, the Flamura 85 variety was in first place at 76.38% with the most statistically significant effect, followed by the Esperia variety. On a treatment basis, the T2 treatment (78.28%) was very different to the other treatments with the most significant statistical effect. The T2 treatment was followed by T4 treatment with the control treatment in last place.

For the HI parameter, the Esperia variety had the highest effect of 45.36%, followed by the Flamura 85 variety. Among treatments, T2 (44.82%) and T3 (44.27%) treatments were in the same statistical group with the highest results and the control treatment was in last place.

The highest effect for the GY parameter was 7.88 t ha^{-1} for the Esperia variety, followed by Flamura 85 variety with a value of 7.64 t ha^{-1} included in a different statistical group. Among treatments, T4 treatment provided the highest result of 7.68 kg ha^{-1}, as with several parameters, followed by T2 treatment with a value of 7.57 kg ha^{-1} included in the same statistical group. The lowest value in terms of grain yield was obtained from the control treatment which did not apply soil conditioners.

The V x T interactions and mean values for grain yield and some yield characteristics are given in Table 5. As can be understood from investigating the table showing the impact rates of varieties from treatments, the highest and most significant effect for the PH parameter was for the Flamura 85 variety with the T4 treatment (99.13 cm). This was followed by the Flamura 85 variety and T2 treatment (96.78 cm) in the same statistical group. For the SL parameter, the V x T interaction was highest for the Flamura 85 variety with the T4 treatment (10.48 cm), followed by the T2 treatment (10.01 cm).

Table 5. Variety x treatment interaction means and significance groups related to yield traits

Var.	Treat.	PH (cm)	SL (cm)	NSS (unit)	NGS (unit)	GWS (g)	SFI (%)	HI (%)	GY (t ha^{-1})
Flamura85	T1	95.09 bc	9.88 bc	20.22 g	47.15 c	2.13 ab	65.87 g	42.96 fg	7.23 ef
	T2	96.78 ab	10.01 b	20.90 de	47.50 bc	2.07 abc	83.73 a	43.65 e	7.57 cde
	T3	96.12 b	9.83 bc	20.47 fg	48.80 a	2.20 a	77.66 cd	45.70 b	8.06 a
	T4	99.13 a	10.48 a	21.33 bc	48.42 abc	2.07 abc	78.25 bc	42.58 gh	7.698 cd
Selimiye	T1	93.96 bcd	9.48 de	21.30 bcd	40.27 e	1.80 e	68.84 f	40.56 ı	7.21 ef
	T2	92.00 de	9.63 cde	20.73 ef	40.68 e	1.92 cde	70.88 ef	43.54 e	6.98 gh
	T3	94.18 bcd	9.76 bcd	21.10 cde	41.23 de	1.88 de	65.60 g	42.19 h	6.80 h
	T4	89.57 e	9.67 cde	21.70 b	42.63 d	2.04 a-d	65.40 g	43.23 ef	7.410 def
Esperia	T1	81.45 g	9.04 f	20.93 cde	48.12 abc	1.90 cde	70.71 ef	44.25 d	7.67 cd
	T2	84.84 f	9.48 de	21.30 bcd	48.77 ab	2.00 bcd	80.25 b	47.28 a	8.15 a
	T3	85.18 f	9.42 e	21.33 bc	48.60 abc	1.93 cde	71.61 e	44.94 c	7.76 bc
	T4	82.58 g	9.68 cde	22.40 a	49.40 a	1.93 cde	75.50 d	44.98 c	7.93 ab
$LSD_{0.05}$		2.204	0.301	0.400	1.478	0,172	2.390	0.489	25.59

Var.: Variety, Treat.: Treatment.
PH: Plant high (cm); SL: spike length; NSS: Number of spikelets per spike (unit); NGS: Number of grains per spike (unit); GWS: Grain weight per spike (g); SFI: Spike fertility index (%); HI: Harvest index (%); GY: Grain yield (kg ha^{-1})

The highest effect of soil conditioner treatment on NSS was for the Esperia variety with the T4 treatment (22.40 unit), followed by the Selimiye variety with the T4 treatment (21.70 unit). When the obtained data are investigated, for the NSS parameter, the T4 treatment provided the highest values for all varieties. This situation shows the positive effect of the T4 treatment on NSS.

For NGS results, the highest value was for the Esperia variety with the T4 treatment (49.40 unit) and the Flamura 85 variety with the T3 treatment (48.80 unit). These were followed by the T2 treatment of Esperia variety, T4 treatment of Flamura 85 variety and T3 treatment of Esperia variety in the same statistical group. According to the data obtained, the T4 and T3 treatments had higher values for the NGS parameter.

When the effect of soil conditioners on the GWS parameter is investigated, the highest effect was for the Flamura 85 variety with T3 treatment (2.20 g), followed by Flamura 85 variety with T1 treatment (2.13 g) in second place. These were followed by T2 and T4 treatments of Flamura 85 variety and T4 treatment of Selimiye variety in the same statistical group. In terms of the GWS characteristics, the T3 treatment of Flamura 85 variety, T4 treatment of Selimiye variety and T2 treatment of Esperia variety provided the highest values, showing that the

varieties displayed different positive reactions to this treatment.

For the SFI parameter, the highest effect of soil conditioners applied to seeds was for Flamura 85 variety with T2 treatment (83.73%). This was followed by Esperia variety with T2 treatment (80.25%). For the SFI parameter, T2 treatment provided the highest results for all bread wheat varieties.

For HI results, the highest value among the three varieties was for Esperia with T2 treatment (47.28%), with Flamura 85 variety and T3 treatment (45.70%) in second place. T4 treatment of Flamura 85 variety (42.58%) provided lower results compared to the T1 (control) treatment. The highest harvest index values for the Selimiye and Esperia varieties were obtained with the T2 treatment, while it was highest with the T3 treatment for the Flamura 85 variety. This reveals that for this parameter, the Flamura 85 variety gave a different result compared to the other treatments.

GY, which is the most important parameter among the yield characteristics of bread wheat, gave high results in three varieties in different treatments. The highest result was obtained in the Esperia variety (8.15 t ha^{-1}) applied to T2, followed by the Flamura 85 variety to which T3 was applied, which is in the same statistical group (8.06 t ha-1). The highest value for the Selimiye variety was found with the T4 treatment (7.41 t ha^{-1}).

According to the results obtained, soil conditioner applications generally gave statistically higher results than the control application for the parameters. While Flamura 85 gives the highest result among the varieties, T4 gave the highest result in soil regulators.

Among soil conditioner treatments, T4 treatment provided the highest results for 5 parameters and was in 2nd place for 3 parameters. The T2 and T3 treatments had the highest effect for two parameters each. The soil conditioners used in the research had different contents. The T3 and T4 treatments contained more organic material, while the T2 treatment contained mineral matter and glycine betaine. Though the content of the T3 and T4 treatments were similar, T4 contained more OM and humic substance concentrations. Accordingly, T4 treatment with the highest organic matter and humic substance content can be said to have a positive effect on the yield parameters. Humic substances affect the root development of plants and increase the growth of the above-soil portions, support nutrient element intake and increase the yield of agricultural products by an average of 30-90% linked to the fertilizer given (Bezuglova and Klimenko, 2022). The treatment dose of humic acid may be important in terms of yield characteristics. Studies about this topic observed that just as humic acid treatment with different proportions provided similar results, it also provided different results. The researchers stated that humic substances had stimulating effects on plants (Weber 2018, Chen and Aviad, 1990), while the increase in humic substance concentration reduced this effect (Chen and Aviad 1990). In a study of the yield characteristics of wheat, Kaya et al., (2005) reported that humic acid treatment caused a reduction in PH. As a result, it is important to pay attention to the concentration of HS contained within the soil conditioners.

According to the mean values obtained in the research (Table 4), high results related to spike traits like SL, NSS, NGS and GWS were found for the T4 treatment and generally the Flamura 85 variety. However, while the SFI parameter was highest for the Flamura 85 variety, similar to the other varieties, on a treatment basis high values were found with T2 treatment. The reason for the high SFI value with the T2 treatment, different to the other spike traits, maybe that SFI shows the general traits of the spike and is based on general traits like spikelet number, grain number and grain size on the spike. Ozen and Akman (2014) reported that the number of spikelets on the spike positively affected the number of grains and hence spike grain yield. When the variety x soil conditioner application interaction was examined in grain yield, the highest yield was found in Esperia (T2) and Flamura (T3) varieties, which are in the same statistical group. These were followed by T4 application in the Esperia variety. The lowest data were determined in T2 and T3 applications in Selimiye variety. These data obtained showed that the response of the varieties to the soil conditioner application was different, while the response of the Esperia variety was good, the response of the Selimiye variety was low.

The spike length and grain yield had parallel increases in the study and the T4 treatment provided the highest results. According to researchers, spike length is generally linked to the genetic structure of the variety (Bilgin and Korkut, 2005) and there is a positive and significant correlation between spike length with grain yield. Due to the excess spike length of cereals and the dense arrangement of spikelets on the spike axis, in the grain-filling period, there is the opportunity for grains to fill out more easily and grain weight to increase. Some researchers reported that spike length is a trait increasing grain yield (Bilgin and Korkut, 2005; Ozen and Akman, 2014).

In the research, there was generally a negative variation between PH and GY results (Table 4). Generally, treatments with high PH values had lower GY results. For the PH parameter, the T4 treatment provided lower results compared to the T2 and T3 treatments, while it provided higher results for the GY parameter. When assessed in terms of varieties, linked to genetic traits, the Flamura 85 variety had the highest values in terms of PH, while the Esperia variety had the lowest values. However, in terms of GY, the highest results were obtained with the shortest variety of Esperia. The reason for this is that the higher PH value leads to more expenditure of nutrient elements produced as a result of photosynthesis on plant height development, and as a result, fewer photosynthesis products are available for yield and yield traits. Some studies have reported results showing a negative correlation between PH and GY (Korkut and Bilgin, 2005; Naneli et al., 2015). As a result, in plant cultivation studies performed for grain yield, tall plant height should not be chosen. This is because tall plant height causes excess consumption of

photosynthesis products as well as causing more lodging and disease problems in plants.

CONCLUSION

Wheat cultivation is performed in a large area of the Thrace region and this is a region with intense commercial fertilizer use. Tekirdag is in first place within this region. The amount of organic fertilization in fertilization programs implemented in the region has increased over time. Producers beginning organic fertilization to increase yield apply soil conditioners with organic sources and a variety of content to the soil. In recent periods, for wheat agriculture, planting after wetting seeds with liquid soil conditioners has become popular. However, research on this topic is limited. In this study, performed with this aim, the seeds of the Flamura 85, Selimiye and Esperia bread wheat varieties were treated with 3 different soil conditioners commonly used in Thrace. According to the mean treatment and variety values obtained from the research results (Table 4), all soil conditioners applied to seeds generally caused differences compared to control. The T4 treatment especially caused a pronounced positive effect on the SL, NSS, NGS, GWS and GY parameters. For the PH parameter, T3 caused a significant effect, while for the SFI parameter, the T2 treatment provided a significant effect. For the HI parameter, T2 and T3 treatments both had the highest effect. According to the mean variety x treatment interaction mean values related to yield traits (Table 5), the T4 treatment for the Flamura 85 variety increased plant height, reduced plant height for the Selimiye variety and was not statistically significant for the Esperia variety. For all treatments, spike traits like SL, NSS and NGS increased compared to controls, while suitable soil conditioner treatment caused variation according to a variety of parameters like GWS and SFI. For the HI results, the T2 treatment of the Selimiye and Esperia varieties and the T3 treatment of the Flamura 85 variety provided highest results. Grain yield, one of the most important parameters in wheat, had the highest results with different treatments linked to variety, with the most pronounced effect for Esperia with T2 treatment, Flamura 85 with T3 and Selimiye with T4. When the data obtained was evaluated, soil conditioner applications caused a significant increase in the parameters examined in wheat. T4, which contains 25% organic matter + 65% humic acid + 6% potassium, was determined as the most effective soil conditioner for many parameters

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THE EFFECT OF CHEMICAL FERTILIZERS ON THE QUANTITATIVE AND QUALITATIVE TRAITS OF OREGANO (*Origanum vulgare* L) PLANT

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ABSTRACT

This experiment was conducted to investigate the effects of NPK fertilizer on oregano (*Origanum vulgare* L.) plant for two years (2017 and 2018). Various levels of nitrogen (0, 50 and 100 kg ha^{-1}), potash (0, 40 and 80 kg ha^{-1}), and phosphorus (0 and 30 kg ha^{-1}) fertilizers were applied. The results revealed that the use of NPK fertilizers significantly increased the dry weight of the oregano plant's aerial parts (leaves, inflorescences, and whole aerial parts). In the first year, the dry weight of the whole shoot increased by 82 to 124 g m^{-2}, and in the second year, it increased by 82 to 129 g m^{-2}. Moreover, regarding essential oil concentration, this parameter in inflorescence ranged from 2.38% to 3.66% in the first year, while in the second year, it ranged from 3.02% to 4.14%. Notably, the inflorescence had a higher essential oil concentration compared to the leaves. The study also found that the use of NPK fertilizer at a ratio of 100:20:40 kg ha^{-1}resulted in the highest percentage of essential oil in the aerial. Conversely, the control treatment led to a decrease in essential oil yield. Among the essential oil compounds, Carvacrol and p-Cymene were the predominant components, with concentrations ranging from 49.36% to 60.32% and 2.03% to 4.56%, respectively, in various oregano plant organs.

Keywords: Dry weight, Essential oil, Inflorescence, Oregano, Nitrogen.

INTRODUCTION

Oregano (*Origanum vulgare* L.) is one of the most selled culinary and medicinal herb throughout the world. Flowering aerial parts and leaves of oregano have been used as a popular flavoring of food stuffs and as an antioxidant agent in cosmetics (Morshedloo et al, 2018). One of the most important issues in the field of agriculture and medical sciences and even global trade is attention to the production, processing and use of medicinal plants (Pirzad et al. 2006). The importance of medicinal plants is due to the production of effective substances that play an important role in pharmaceutical, cosmetic, perfumery, dyeing and flavoring industries (Kim et al., 2005). Oregano medicinal plant belongs to the lamiaceae family and has been registered as a plant used in traditional medicine as well as an effective plant in the world's authoritative pharmacopoeias (Skoula and Harborne, 2002; Azizi et al., 2009).

The study of different aspects of crop plants, including the quantitative and qualitative changes of these plants in response to different nutritional sources, is of particular importance. Proper nutrition of medicinal plants along with compliance with the principles of organic production, while preserving the environment, increases the quantitative and qualitative yield of effective substances in these plants (Sahoo, 2001).

In addition to the fact that nutrients should be easily used by the plant, the balance between their amounts is also very important. Nitrogen is the most important food element that is needed in large quantities by higher plants. Nitrogen plays a role in the formation of proteins, nucleic acids, chlorophyll, coenzymes, photosynthesis and plant secondary metabolism (Lynch et al., 2012). The production of hydrocarbon materials increases with the increase of nitrogen, and its consumption for protein fuel and the production of secondary metabolites increases (Qranjik and Galeshi, 2001). Sotiropoulou and Karamanos (2010) showed by examining four nitrogen levels (zero, 40, 80 and 120 kg ha^{-1}) that with increasing nitrogen fertilization, vegetative yield and essential oil yield of oregano also increased. Yazdani Bioki et al. (2014) investigated the effect of urea fertilizer and azocompost on marjoram plant, and their results showed that the use of 120 kg ha^{-1}of nitrogen will result in the highest economic yield in marjoram plant.

Phosphorus is one of the essential elements involved in many vital processes of plants. Phosphorus, one of the components of ATP, plays an important role in energy transfer. Phosphorus helps to absorb nutrients by increasing

the growth of roots, so the accumulation of dry matter in the plant increases (Nahar et al., 2014). In addition, phosphorus plays an important role in energy transfer and regulation of enzyme activity and signal transmission (Sarker et al., 2015). Optimum nutrition of plants with potassium can have a positive role in improving water relations, chlorophyll level, increasing leaf area, increasing root growth and increasing plant photosynthetic activity and thus increasing plant yield. Also, the potassium in the fertilizer improves the yield and quality of the product (Hasanuzzaman et al., 2018). The studies conducted so far on medicinal plants show that the use of chemical fertilizers improves the growth and absorption of elements in these plants (Kumawat et al., 2006). Also, studies showed that a number of physical and chemical characteristics of soil affect the concentration and composition of oil in Greek oregano and other related species (Panagopoulos, 2012).

To produce effective medicinal substances, medicinal plants need a suitable nutritional system including all kinds of nutrients, and by increasing soil fertility, the effectiveness of inputs can be increased. Considering the increasing need for medicinal plants and the effect of fertilizers on their quality and quantity, it is necessary to identify the most suitable amount of fertilizers to determine the plant's needs in the conditions of the region. On the other hand, in the west of Mazandaran, every year, many fields are cultivated with oregano, and due to the lack of information on the amount of chemical fertilizers used in this plant, there was a difference between the farmers in the region in terms of the amount of fertilization. Also, the effect of using chemical fertilizers on the amount of essential oil and components of essential oil has been paid less attention. Therefore, according to the environmental conditions of the region, providing optimal plant needs and environmental risks in excessive use of chemical fertilizers, this study has been conducted to determine the most suitable amount of NPK fertilizers in the yield and essential oil of oregano plant.

MATERIALS AND METHODS

Location and geographic location of the experiment

The experiment was conducted at a research farm at Islamic Azad University, Chalous Branch, located at 82 40° 58′ N and 53° 69′ E with an altitude of 3 m above sea level, during the 2017-18 crop year. Also, oregano seedlings were produced by seed propagation in a greenhouse and cultivated in a research field

Characteristics of the soil and weather of the experiment site

To determine the soil characteristics, samples were taken from a depth of 0-30 cm before cultivation (Table 1). The mean temperature, precipitation and relative humidity of the experimental years are shown in Table 2 (Prepared from the meteorological office of Mazandaran Province).

Table 1. Physical and chemical properties of soil

Texture	Organic matter (%)	active cation elements (%)	N (%)	K (ppm)	P (ppm)	pH	Ec (ds m^{-1})
Sandy Clay Loam	0.92	14.24	0.13	76	14.36	7.4	0.52

Table 2. Meteorological parameters for the field sites during experiment

Months	Mean temperature (°C)		Relative humidity (%)		Precipitation (mm)	
	2017	2018	2017	2018	2017	2018
March	9.85	8.25	83.00	86.50	99.70	119.20
April	14.20	11.65	82.50	80.00	62.60	114.70
May	18.00	15.35	78.50	83.30	21.20	51.20
June	24.90	21.50	73.00	78.00	10.30	29.30
July	28.30	26.00	71.00	75.00	7.10	15.30

Treatments and experimental design

This experiment was conducted as a factorial design in the form of a randomized complete block design in 3 replications. Nitrogen fertilizer was added to the plots at three levels (0, 50 and 100 kg ha^{-1}) in the form of pure urea (45%) and in two stages before planting and 2 weeks after planting and rooting. Potash at three levels (0, 40 and 80 kg ha^{-1}) as K_2O 20% and phosphorus at two levels (0 and 30 kg ha^{-1}) before planting were added to the plots.

Preparation of seedlings

Oregano seeds were obtained from Karaj Agricultural Research, Education and Development Institute. The seeds were planted in wooden boxes in the greenhouse in early March. First, the seed bed was wet, then the seeds were planted on the bed and covered with 0.5 cm of light soil. The seeds were irrigated with a geyser in the form of misting. After growing and at the five-leaf stage, the seedlings were transferred to the field.

Land preparation and cultivation

The desired land was plowed at the end of November using a reversible iron bull to a depth of 25-30 cm and then it was plowed twice. Then the plotting of the land was done in early April. The total test plots were 54 plots and each test block consisted of 18 plots measuring 2.50 x 3.50 m.

The distance between the blocks was 3 m and the distance between the plots was 1 m. Transplanting was done manually in early the late April. The distance between rows of crops was 50 cm and the distance between plants was 30 cm. The plots were watered regularly and every week. Weed control was done manually. Harvesting was done at the stage of full bloom (the highest amount of essential oil in the aerial parts of the oregano plant is at the time of full inflorescences).

Measurements

After harvesting, 1 m^2 of each plot was selected after removing the margins and the wet parts of the plant were determined, and after drying in natural conditions (shade and dry air), the dry weight of leaves, inflorescences and the whole plant was measured.

The extraction of essential oil was done in the flowering stage using a clevenger apparatus for 4 hours and by water distillation method (Said-Al Ahl et al., 2009). First, 10 grams of the dried samples were poured into a 1000 ml flask and about 100 ml of distilled water was added to it, and extraction and essential oil extraction was done. The essential oil yield of the plant organs was calculated from the product of the essential oil content and its total dry weight.

The analysis of all essential oils was performed using a Hewlett Packard 5890 II GC (single injector (split/split less or Packed) single FID detector), equipped with a HP-5 capillary column (30 m, 0.25 mm i.d., 0.25 m film thickness) and a mass spectrometer HP 5972 as detector (Agilent Brand). The carrier gas was helium, at a flow rate of 1 ml min^{-1}. The column temperature was initially 55 ◦C for 3 min, then gradually increased to 200 ◦C at 3 ◦C/min and finally increased to 220 ◦C at 5 ◦C min^{-1}. For GC–MS detection, an electron ionization system was used with an ionization energy of 70 eV. The extracts were diluted 1:100 (v/v) with acetone and 1 l of the diluted samples was injected automatically in spitless mode. Injector and detector temperatures were set at 220 and 290 ◦C, respectively.

Statistical analysis

Analysis of data variance was done with SAS v.3 software and Duncan's multiple range tests were used at a probability level of 5% to compare the mean of the desired traits (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Dry weight

Results of variance analysis of the effect of chemical fertilizers on the dry weight of oregano is shown in Appendix Table 1. The results showed that the interaction effect of year, nitrogen, phosphorus and potassium fertilizer on dry weight of leaf, inflorescences and whole plant was significant. With the increase in nitrogen, phosphorus and potash fertilizer consumption, the dry weight of leaves has increased. The average increase in leaf dry weight was significantly between 30.38 and 43.75 g m^{-2} in the first year and between 32.18 and 45.25 g m^{-2} in the second year. The increase in leaf dry weight in the second year was higher than in the first year. The maximum dry weight of leaves with the application of $N_{100}P_{30}K_{80}$, $N_{100}P_{30}K_{40}$ and $N_{100}P_0K_{80}$ in the second year of the experiment was 45.25, 45.21 and 44.90 g m^{-2}, respectively. The lowest dry weight of leaves was observed in the condition of no application of chemical fertilizers (Figure 1). It has been reported that nitrogen deficiency causes leaf fall from the lower part of the plant (Hopper, 1996). Nitrogen plays an effective role in the development of new cells and increases vegetative growth and the number of secondary shoots in the plant. By increasing root growth, phosphorus helps the plant absorb nutrients, so there is a greater accumulation of dry matter (Nahar et al., 2014). The use of phosphorus fertilizers can increase plants' uptake of phosphorus and lead to improved plant yield (Sumer et al., 2023). Plants that are fertilized with low levels of nitrogen usually have short height, few foliage and yellow leaves. They have thin and weak plant cover, which ultimately leads to a decrease in plant yield (Diepen Brock, 2000). In this study, it was observed that the lack of application of chemical fertilizers, especially nitrogen, has led to the lack of development of leaves and the fall of leaves in oregano.

Appendix Table 1. The results of analysis of variance of the effect of NPK on the dry weight of different organs of oregano plant

S.O.V	df	Leave dry weight	inflorescences dry weight	Whole dry weight
Year	1	80.46*	203.97**	524.79*
Block(Year)	4	6.07	1.52	55.28
N	2	936.84**	944.96*	12549.23**
K	2	45.95*	32.60	415.74*
P	1	6.43*	10.82*	57.25*
Year*N	2	0.85	31.40	130.96
Year*K	2	1.02	3.25	19.25
Year*P	1	0.01	0.02	0.20
N*K	4	4.11	1.62	12.24
Year*N*K	4	1.63	2.06	7.85
N*P	2	6.41	10.04*	1.63
Year*N*P	2	2.01	0.32	2.47
K*P	2	1.12	1.54	4.71
Year*K*P	2	6.08	0.26	0.90
N*K*P	4	2.07	0.28	3.41
Year*N*K*P	4	43.08*	51.25*	460.70**
Error	68	15.56	15.12	120.43

*, ** and ns: significant at the level 0.05, 0.01

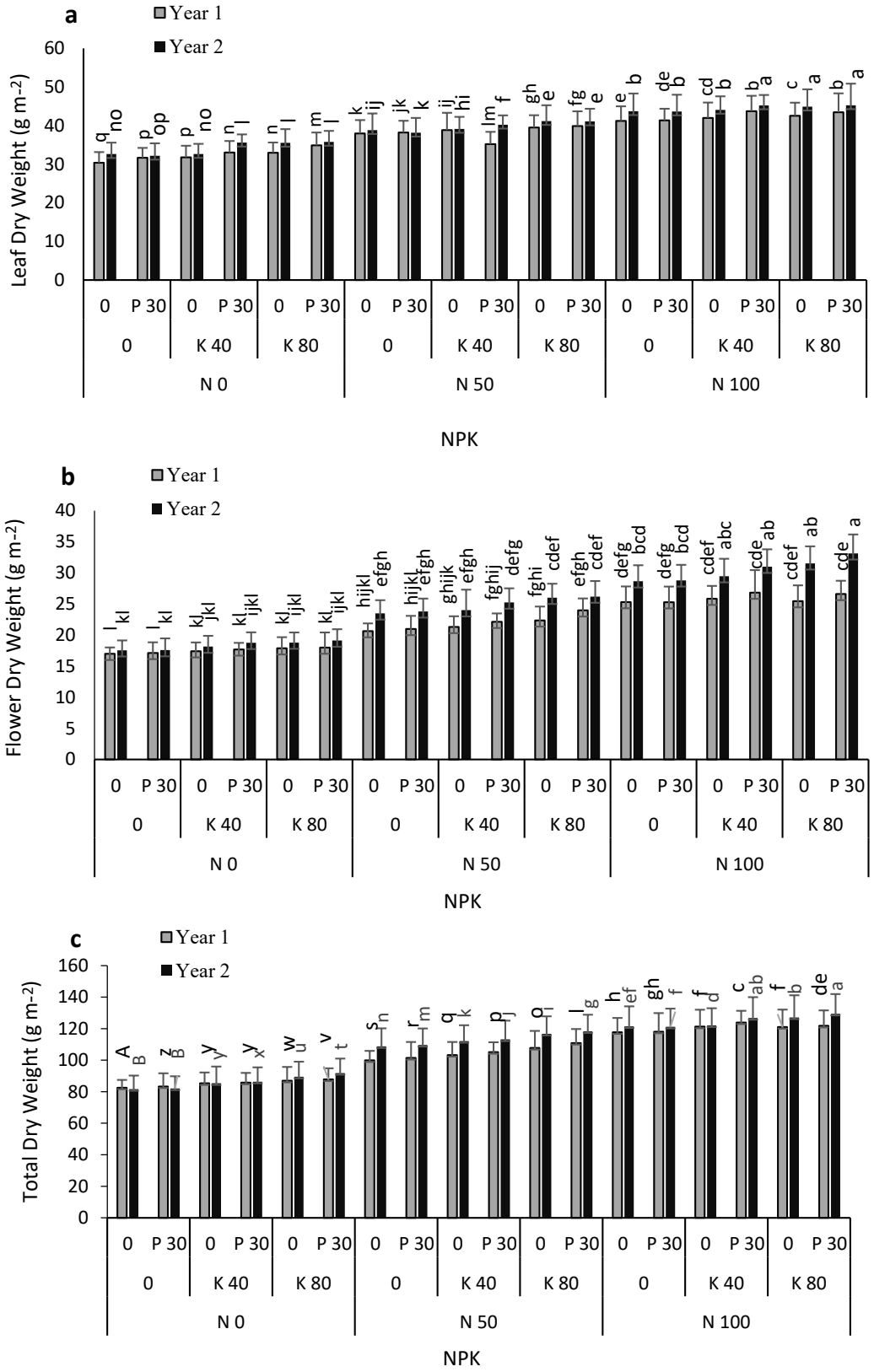

Figure 1. Application of NPK fertilizers in different amounts on leaf dry weight (a), inflorescence dry weight (b) and total dry weight (c) during the years 2017 and 2018. Values represent means ± S.E. Significant differences among treatments were measured by the least significant difference (LSD) at $P < 0.05$ and indicated by different letters.

The dry weight of inflorescences increased significantly with the increase in the use of chemical fertilizers. The average increase in dry weight of inflorescences was significantly about 16.98 to 26.60 g m^{-2} in the first year and between 17.55 to 33.13 g m^{-2} in the second year. The dry weight increase of inflorescences in the second year was

higher than the first year (Figure 1b). The results showed that the highest dry weight of inflorescences was observed in the $N_{100}P_{30}K_{80}$ treatment in the second year of the experiment at the rate of 33.13 g m^{-2}. In Figure 1b, the role of nitrogen fertilizer in increasing the dry weight of oregano inflorescences is well shown, so that in the absence of nitrogen fertilizer, the dry weight of inflorescences did not increase significantly with the use of potash and phosphorus fertilizers, but the application of 50 and 100 kg $ha^{-1}$of nitrogen fertilizer especially in the second year, it has increased the number of inflorescences. The use of nitrogen fertilizer has made the absorption of nutrients potassium and phosphorus better. Some researchers attributed the reduction of nutrients due to nitrogen deficiency as premature aging of leaves, reduction of chlorophyll and photosynthesis and therefore reduction of transfer of microbes to seeds, lack of root growth and development in the soil and therefore reduction of the ability to absorb elements from the soil (Uauy et al. , 2006; Yuan et al., 2005).

Increasing the consumption of nitrogen, phosphorus and potash fertilizers has increased the dry weight of the whole plant. The average increase in dry weight of the whole shoot was significantly around 82 to 124 g m^{-2} in the first year and between 82 and 129 g m^{-2} in the second year. The role of nitrogen in increasing the total dry weight can be seen in Figure 1c. The absence of nitrogen application despite the use of phosphorus and potash fertilizers did not significantly increase the dry weight of the oregano plant, but the use of nitrogen fertilizer significantly increased the dry weight of the whole plant. The highest total dry weight was obtained in $N_{100}P_{30}K_{80}$ at the rate of 129.4 g m^{-2}, then $N_{100}P_{30}K_{40}$ treatment in the second year of the experiment. The increase in total dry weight and inflorescence in the second year compared to the first year was probably due to the better temperature conditions for the growth and development of the oregano plant. The lowest dry weight of the whole plant was observed in the condition of no application of chemical fertilizer (Figure 1c). The lack of nutrients by affecting the photosynthetic activity of the plant and increasing respiration leads to the decrease of hydrocarbon substances as a result of the decrease in growth and yield. The increase in dry weight of the whole plant in the second year was higher than in the first year. Pal et al. (2016) showed yield generally increase in accordance with the increases in N, P and K fertilizer rates. Sotiropoulou and Karamanos (2010) showed that the application of nitrogen fertilizer increases the vegetative yield of oregano plant, which is consistent with the results of this study. The yield and quality of the oregano were influenced by nitrogen, potassium and phosphorus (Matłok et al, 2020). In the early stages of plant growth, phosphorus increases nitrogen absorption (Naomi et al, 2021). Studies have shown that there is a close relationship between the supply of nutrients and the increase of plant dry matter (Malakooti and Nafisi, 1995; Kumawat et al., 2006). In case of nitrate deficiency, the level of cytokinin hormone in the plant is reduced, and as a result, growth is reduced by affecting cell proliferation and expansion (Rahayu et al., 2005). On the other hand, the use of nutrients in optimal concentrations increases the rate of plant photosynthesis and the absorption of nutrients, which leads to the development of the leaf area and the number of secondary branches. As a result, the dry weight of plants treated with chemical fertilizers has increased.

Concentration of essential oil

The results showed that the interaction effect of year, nitrogen, phosphorus and potassium fertilizer on concentration of essential oil of leaf, inflorescences and whole plant was significant (Appendix Table 2). The effect of using chemical fertilizers on the concentration of essential oil in different organs of oregano plant is shown in Figure 2. The results showed that with the increase in the use of chemical fertilizers, the percentage of essential oil in oregano organs has increased. The average increase in leaf essential oil percentage was about 0.98% to 1.22% in the first year and between 1.00% and 1.23% in the second year (Figure 2a). The concentration of essential oil in inflorescences varied between 2.38 and 3.66% in the first year and between 3.02 and 4.14% in the second year (Figure 2b). Also, the concentration of essential oil in all parts of the plant was measured between 1.50 and 2.35% in the first year and between 1.66 and 2.58% in the second year. When fertilizer was applied at 50 and 100 kg ha^{-1}, oregano aerial parts had higher levels of essential oil than when nitrogen was not applied. (Figure 3c). The use of NPK in the order of 100:20:40 kg ha^{-1}had the highest percentage of essential oil in aerial parts. In the presence of low or non-use of chemical fertilizers, oregano leaves had a decreased percentage of essential oil. Essential oils' content and composition are affected by a number of factors, including genetic makeup and cultivation conditions, including climate, habitat, harvesting time, water stress, and fertilizer use. An increase in the essential oil content of plants with an increase in the concentration of nutrients has also been reported in previous studies (Sifola and Babieri, 2006). Nitrogen is one of the essential nutrients in plants, which is used for the synthesis of many organic compounds in plants, such as amino acids, proteins, enzymes, and nucleic acids. Since enzymes and amino acids play an important role in the biosynthesis of plant essential oils (Koeduka et al., 2006). The presence of nitrogen as a key factor can affect the production of essential oils in aromatic plants (Briat et al., 2015; Gedik, and Akgul., 2023). It is well known that P and K is an essential element in reproductive and vegetative growth can increase by the increased P and K applications. also, Phosphorus has many other cellular functions in plants and affects the primary and secondary metabolites (Mengel and Kirkby, 2001). In this study, it seems that the use of chemical fertilizers, especially nitrogen, has increased the essential oil by continuing the activity of the leaf area, participating in the structure of chlorophyll and enzymes involved in photosynthetic carbon metabolism.

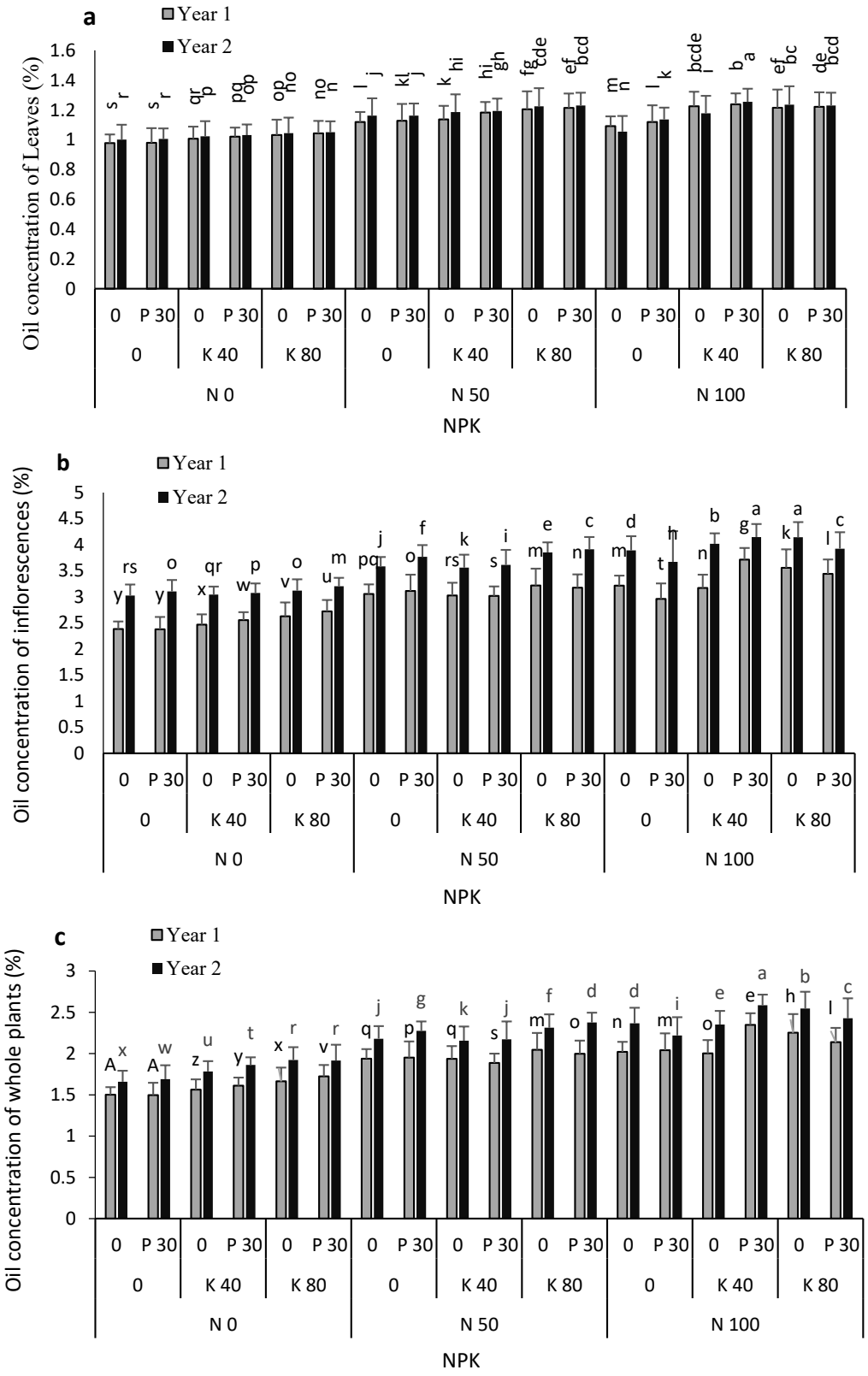

Figure 2. The application of NPK fertilizers in different amounts on the content of leaf essential oil (a), the inflorescences (b) and the whole plants (c) during the years 2017 and 2018. Values represent means ± S.E. Significant differences among treatments were measured by the least significant difference (LSD) at $P < 0.05$ and indicated by different letters.

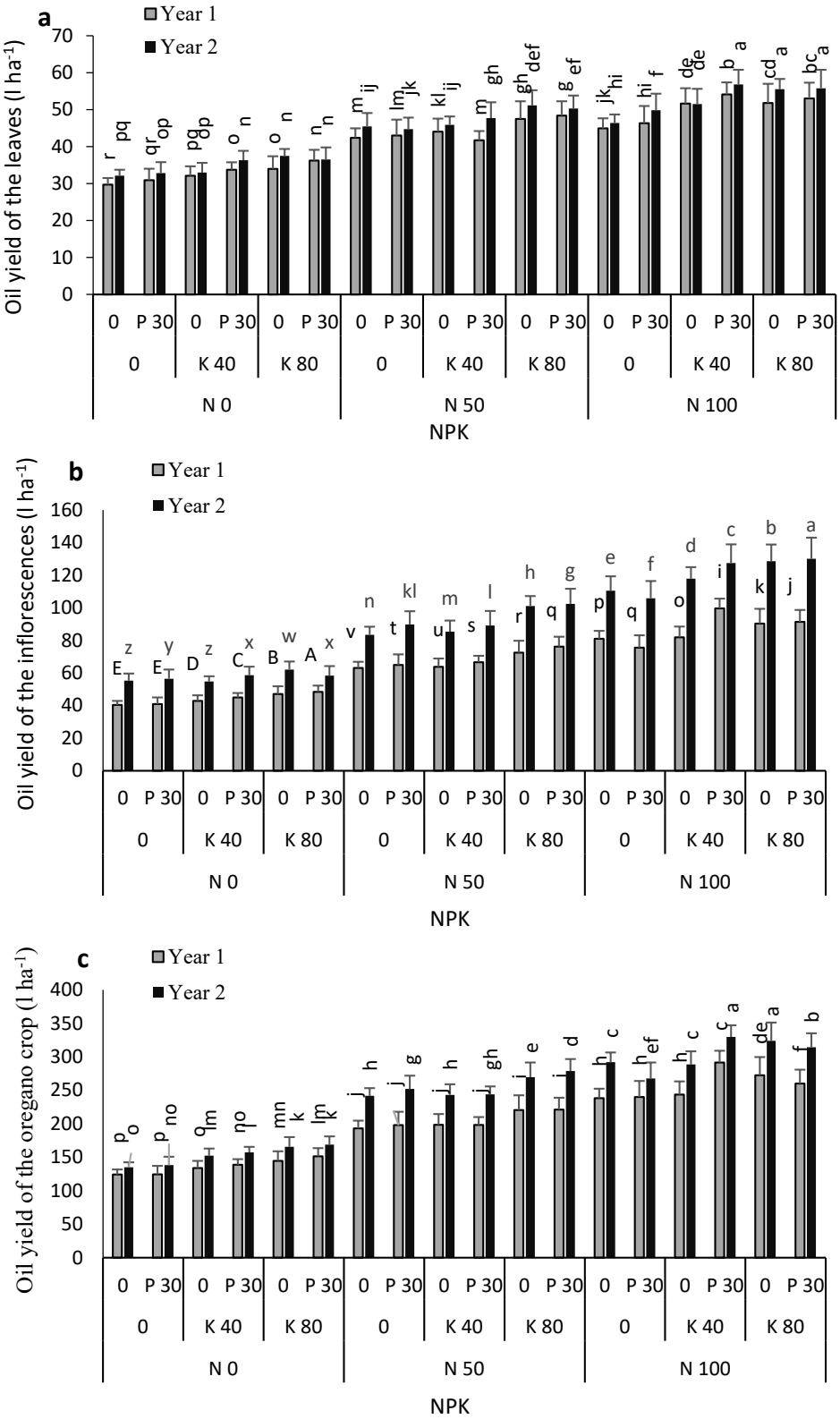

Figure 3. Application of NPK fertilizers in different amounts on essential oil yield of leaf (a), inflorescence (b) and whole plants (c) during the years 2017 and 2018. Values represent means ± S.E. Significant differences among treatments were measured by the least significant difference (LSD) at $P < 0.05$ and indicated by different letters.

Appendix Table 2. The results of analysis of variance of the effect of NPK on the essential oil and oil yield of different organs of oregano plant

S.O.V	df	concentration of essential oil leave	concentration of essential oil inflorescences	concentration of essential oil whole plant	Essential oil yield of the leaves	Essential oil of the inflorescences	Essential oil of the whole plant
Year	1	0.0052	9.81**	1.824	158.28*	15084.30**	37364.63
Block(Year)	4	0.0019	0.13	1.230	11.27	102.94	17059.34
N	2	0.3183**	6.81**	3.140**	2968.01**	24898.87**	169138.56**
K	2	0.0681**	0.47*	0.248**	299.75**	1250.88*	7502.17*
P	1	0.0086	0.02	0.022	39.10	169.98	725.44
Year*N	2	0.0024	0.01	0.016	2.79	895.46	2901.57
Year*K	2	0.0002	0.02	0.004	0.31	41.57	143.44
Year*P	1	0.0002	0.002	0.0043	0.69	3.39	20.99
N*K	4	0.0093*	0.13*	0.040*	28.95*	206.88*	711.42**
Year*N*K	4	0.0006	0.02	0.002	2.33	25.82	46.02
N*P	2	0.0017	0.02	0.001	13.72	17.31	31.61
Year*N*P	2	0.0017	0.04	0.018	2.16	6.39	134.53
K*P	2	0.0011	0.08	0.049	5.57	118.20	871.25
Year*K*P	2	0.0003	0.03	0.0005	13.39	12.56	42.25
N*K*P	4	0.0009	0.11	0.051	2.41	83.89	1139.33
Year*N*K*P	4	0.30**	0.81*	0.24*	196.15*	792.69*	6569.52**
Error	68	0.04	0.29	0.09	71.04	287.60	1316.03

*, ** and ns: significant at the level 0.05, 0.01

Essential Oil yield

The results showed that the interaction effect of year, nitrogen, phosphorus and potassium fertilizer on the essential oil yield of leaf, inflorescences and whole plant was significant (Appendix Table 2). The effect of using chemical fertilizers on the yield of essential oil in different organs of oregano plant is shown in Figure 3. The results showed that with the increase in the use of chemical fertilizers, the yield of essential oil in oregano organs has increased. The average yield of leaf essential oil increased from 29.72 to 54.14 l ha^{-1} in the first year and between 32.15 to 56.79 l ha^{-1} in the second year (Figure 3a). The yield of essential oil in inflorescences varied between 40.41 and 99.66 l ha^{-1} in the first year and between 55.26 and 130.07 l ha^{-1} in the second year (Figure 3b). Also, the yield of essential oil in all plant organs was measured between 124.21 and 291.51 l ha^{-1} in the first year and between 134.99 and 329.51 l ha^{-1} in the second year (Figure 3c). A significant difference was observed in the yield of essential oil between fertilizer treatments, so that the application of 50 and 100 kg $ha^{-1}$of nitrogen fertilizer has increased the yield of essential oil of the aerial parts of oregano compared to the absence of nitrogen application. The use of NPK in the order of 100:20:40 kg ha^{-1}had the highest yield of essential oil in aerial parts. Gharib et al. (2008) reported that the weight of essential oil in oregano plant increased with the increase in total nitrogen concentration. Arango et al. (2012) showed using phosphorus chemical fertilizer and mycorrhiza, the most effective fertilizer combination was phosphorus chemical fertilizer and mycorrhiza. Essential oils are terpenoid compounds whose building blocks (isonoids) such as isopentenyl pyrophosphate and dimethylallyl pyrophosphate have an urgent need for ATP and NADPH, and considering the fact that the presence of elements such as nitrogen and phosphorus is necessary for the formation of the latter compounds, finally the improvement of essential oil yield was observed following the increase of nutrients in plants (Rezvani Mogadam et al., 2013; Aslani et al., 2014). Nutrients play a key role in the primary and secondary processes of plants, and nutrients increase the fresh and dry weight of plants by increasing the rate of photosynthesis; And considering that the essential oil content of plants is affected by the dry weight of plants, as a result, nutrients increase the essential oil content of plants by increasing the dry weight of plants treated with chemical fertilizers.

Oil composition

The components of the essential oil of leaves and inflorescences of oregano plants harvested from the best treatment during the years 2017 and 2018 are shown in Table 3. In the table, the essential oil components that were higher than 1% are given. Among the essential oil compounds, Carvacrol with 58.36 to 82.32% had the highest concentration in oregano plant organs. After that, p-Cymene with 2.03% to 7.56%, γ-Terpinene with 1.65% to 4.52% and Carvacrylacetate with 0.68% to 1.46%. The amount of Carvacrol, β-Pinene, 1-Octen-3-ol, Myrcene, α-Terpinen and Carvacrylacetate was higher in inflorescences than in leaves. Also, α-Thujene, β-Pinene, Camphene, Sabinene, p-Cymene, γ-Terpinen, cis-Sabinene hydrate, Borneol, α-Terpineol, Geraniol, Thymol, β-Caryophyllen, β-Bisabolene, Spathulenol and α-Cadinol in the leaves were higher than the oregano inflorescences. The main components of essential oil mainly depend on genetic factors, plant root morphology, nutritional status and different parts of the plant (stem, leaf and inflorescences) (Rattanachaikunsopon and Phumkhachorn, 2010).

Table 3. The constituents of the oregano (*Origanum vulgare* L.) essential oil (%, v/v) extracted from leaves and inflorescences on harvest during 2017 and 2018. The plants harvested from the application of NPK fertilizers in order of 100:30:40 kg ha^{-1}

Compounds	Inflorescences		Leaves	
	2017	2018	2017	2018
α-Thujene	0.10	0.32	0.08	0.45*
β-Pinene	0.20	0.54	0.41	0.38
Camphene	0.10	0.09	0.13	0.15
Sabinene	0.41	0.52*	1.02	1.95*
β-Pinene	0.41	0.39	0.02	0.03
1-Octen-3-ol	0.19	0.23	0.31	0.49*
3-Octanone	0.12	0.13	-	-
Myrcene	0.73	1.23*	0.42	0.38
α-Phellandrene	0.08	0.12	-	-
δ-3-Carene	0.02	0.03	-	-
α-Terpinen	0.98	1.08*	0.52*	0.43
p-Cymene	2.03	3.12*	6.12	7.56*
Limonene	0.29	0.34	0.41*	0.30
β-Phellandrene	0.16	0.15	0.21	0.19
(Z)-β-Ocimene	0.06	0.04	-	-
(E)-β-Ocimene	0.01	0.03	-	-
γ-Terpinen	1.65	2.36	4.52*	2.98
cis-Sabinene hydrate	0.52	0.65	2.03	1.98
Terpinolen	0.09	0.12	-	-
trans-Sabinene hydrate	-	0.06	0.09	0.07
Borneol	0.19	0.21	0.36	0.56*
α-Terpineol	0.08	0.09	0.12	0.23*
Geraniol	0.06	-	1.03*	0.05
trans-para-mentha-2-one	-	0.09	0.12	0.34*
trans-Dihydrocarvone	0.03	0.1	0.23*	0.05
Carvacrol methylether	0.09	0.1	0.12	0.23*
Thymoquinon	0.10	0.13	0.21	0.16
Thymol	0.23	0.36*	0.65	0.97*
Carvacrol	82.32*	73.06	66.03*	58.36
Carvacrylacetate	0.68	1.26*	0.93	1.46*
β-Caryophyllen	0.03	0.32*	0.25	1.46*
α-Humulene	-	0.19	1.10	0.13
Allo-Aromadendrene	0.54*	0.36	1.03*	0.09
α-Muurolol	0.58	-	1.02*	0.03
β-Bisabolene	0.32*	0.09	2.06*	0.65
γ-Cadinene	1.05*	0.09	0.24	2.21*
δ-Cadinene	0.09	0.68*	0.06	0.98*
Spathulenol	1.32*	0.65	3.31*	1.81
Globulol	0.04	0.01	0.34*	0.06
Veridiflorol	1.03*	0.32	0.95*	0.09
Humulene epoxide Π	0.03	0.1	0.02	0.05
Caryophyllene oxide	0.03	0.40*	0.5	0.65*
epi-α-Cadinol	0.03	0.5*	0.4	0.9
epi-α-Muurolol	-	-	0.6	1.32*
α-Cadinol	0.17	1.03*	1.14	2.01*
Total	97.19	91.69	99.11	92.49

* The year exhibiting significantly higher percentage ($p < 0.05$) of each constituent.

The effect of year had a significant effect on some components of oregano essential oil. The content of compounds Sabinene, α-Terpinen, p-Cymene, Thymol, Carvacrylacetate, β-Caryophyllen, Caryophyllene oxide and α-Cadinol in 2018 was higher than in 2017. An increase in rainfall and a decrease in temperature lead to an increase in the percentage of oregano essential oil (Karamanos and Sotiropoulou, 2013). In this study, the percentage of oregano essential oil was higher in the second year than in the first year. One of the reasons can be attributed to the higher rainfall and lower temperature in the second year of the experiment compared to the first year. Carvacrol, which is the major constituent of oregano essential oil, increases its amount in the plant in conditions of low humidity and

rainfall and high temperature (Panagopoulos, 2012), and in this study, the amount of Carvacrol in the first year of the experiment was higher because The temperature during the growth period was higher than the second year of the experiment. Similar results were reported by Karamanos and Sotiropoulou (2013).

CONCLUSION

The application of chemical fertilizer has increased the dry weight of leaves, inflorescences and the entire shoot of oregano plant in both years of testing. The increase in the dry weight of the aerial parts was higher in the second year than in the first year of the experiment. With the application of maximum NPK fertilizer, the highest dry weight of aerial parts was obtained.

The concentration of essential oil in the leaves, inflorescences and the entire shoot of the plant was also affected by the use of chemical fertilizers. With the application of chemical fertilizer, the concentration of essential oil in the plant increased. The highest concentration of essential oil in aerial parts under the conditions of NPK application was 100:20:40 kg ha^{-1}respectively. Also, the results showed that the lack of application of chemical fertilizer has reduced the yield of essential oil in the plant. Thus, the lowest yield of essential oil was observed in the condition of not using NPK. The components of oregano essential oil varied in different years. Among the compounds, Carvacrol, p-Cymene, γ-Terpinen and Carvacrylacetate had the highest percentage of essential oil.

Conflict of Interest: The authors declare that they have no conflict of interest.

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EFFECT OF DIFFERENT PLANT DENSITY ON THE FORAGE YIELD AND SOME FORAGE QUALITY CHARACTERISTICS OF MORINGA (*Moringa oleifera* Lam.)

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ABSTRACT

In recent years, sustainable animal husbandry has increasingly emphasized the use of highly adaptable shrub and tree species as alternative forage crops. Among these, *Moringa oleifera* Lam., commonly known as Moringa, has emerged as a promising feed source due to its exceptional nutritional value. This study aimed to evaluate the potential of Moringa as a forage crop suitable for the Mediterranean climate. The research was conducted during the 2020 and 2021 growing seasons in the experimental fields of the Department of Field Crops, Faculty of Agriculture, Ege University, Türkiye. The study investigated the effects of four different plant densities (20x60 cm, 30x60 cm, 40x60 cm, and 60x60 cm) on various forage quality traits. The Moringa cultivar "PKM-1" served as the plant material, and parameters such as plant height, stem diameter, biomass yield, dry matter, crude ash, crude protein, crude fat, neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose were determined across two consecutive vegetation periods. Results indicated that, under Mediterranean ecological conditions, Moringa exhibited average plant heights ranging from 159.2 to 170.3 cm, with total biomass yields between 33.10 and 69.70 t ha^{-1}. The crude protein content varied from 17.12% to 18.15%, while ADF and NDF ratios ranged from 35.31% to 37.85% and 45.66% to 49.71%, respectively. Higher planting densities led to increased biomass yield, with the highest values observed at a 20x60 cm planting density. This density also demonstrated favorable results for crude protein, NDF, and ADF, suggesting its suitability for optimizing forage quality in Moringa cultivation.

Keywords: *Moringa oleifera*, plant density, forage quality, Mediterranean climate, sustainability

INTRODUCTION

The use of roughage is crucial in both physiological and economic aspects of ruminant animal nutrition. A wide variety of plant species can be utilized as roughage (Amad and Zentek, 2023). In Türkiye, meadows, pastures, forage crops, and straw are the primary roughage sources (Hanoglu Oral and Gokkus, 2021). However, other developed countries employ alternative sources. In the pursuit of sustainable animal nutrition, the evaluation of drought-resistant shrubs and tree species as alternative forage sources has gained prominence in recent years (Alavilli et al., 2022). Many of these alternative plant species possess the potential to thrive under Türkiye's climatic conditions. Unlike conventional forage crops, there is an increasing demand for alternative plants that can provide high-quality roughage and adapt to diverse climatic conditions (Ambadi and Basmacioglu-Malayoglu, 2022; Budakli Carpici et al., 2023).

One such plant is the Drumstick tree (*Moringa oleifera* Lam.), renowned for its highly nutritious and beneficial leaves, making it one of the most promising food sources globally (Patil et al., 2022). *Moringa oleifera* belongs to the Moringaceae family, which includes 13 known species worldwide, with *Moringa oleifera* being the most valuable. Native to South Asia, *Moringa oleifera* is cultivated in numerous countries. The plant is widely used for human nutrition, fodder, medicinal purposes, and water purification (Amaglo et al., 2006). Given its adaptability, nutritional content, and agricultural value, *Moringa oleifera* is considered a suitable species to help mitigate the effects of climate change in vulnerable regions (Trigo et al., 2021). Its resilience to arid conditions enables sustained productivity even during periods of food scarcity, underscoring its significance as a vital resource (Fahey, 2005). Moreover, *Moringa oleifera* has been shown to enhance the health status, feed efficiency, and growth performance of various animal species (Amad and Zentek, 2023). These attributes contribute to its recognition as a high-quality and valuable feed plant. Often referred to as the “miracle tree” *Moringa oleifera* is celebrated for its rich nutrient profile (Patil et al., 2022). Research consistently highlights that the nutritional content and value of *Moringa oleifera* far exceed those of other plants (Koul and Chase,

2015; Gopalakrishnan et al., 2016). For instance, Yameogo et al. (2011) found that Moringa contains 31.65% crude protein, 34.80% crude fat, and 6.53% crude ash. Notably, nearly every part of the plant, from seeds to leaves and roots to essential oil, is valuable. Moringa contains significantly higher levels of vitamins and minerals than most other plants. For example, Moringa has four times more calcium than milk, seven times more potassium than oranges, and three times more vitamin C than bananas (Islam et al., 2021).

In Moringa, various cultural practices, such as cutting and planting density under different agroecological conditions, have been identified as critical management practices affecting biomass yield and leaf quality (Sánchez et al., 2006). Mabapa et al. (2017) emphasized that plant density is crucial, with higher densities leading to increased yields in Moringa plants. Basra et al. (2015) reported optimal row spacings for Moringa at 15x30 cm (narrow) and 15x60 cm (wide) with mowing frequencies of 15, 20, and 30 days. They concluded that narrow spacing (15x30 cm) and an optimum mowing frequency of 30 days maximize nutrient composition and biomass production. Similarly, other studies suggest that dry matter yield increases with higher planting densities, recommending high-density planting for enhanced leaf production (Adu-Dapaah et al., 2017).

Effective plant management is particularly vital for the sustainability of perennial forage crops. The yield and quality of forage crops are directly influenced by factors such as cutting time, frequency, and height (Atis et al., 2019; Ileri et al., 2020). In this study, the effects of different planting densities of *Moringa oleifera* Lam., a novel and unique plant in Türkiye's agriculture and animal husbandry sectors, on yield and specific feed quality characteristics were investigated under Mediterranean climate conditions.

MATERIAL AND METHODS

The research was conducted in 2020 and 2021 in the experimental fields of Department of Field Crops, Faculty of Agriculture, Ege University, Türkiye (27°13'E, 38°27'N and altitude 26 m) which has a Mediterranean climate zone.

Table 1. Soil characteristics of the experimental area

Physical and Chemical Traits	Value	Unit	Physical and Chemical Traits	Value	Unit
pH	7.3		Phosphorus (P)	1.6	mg kg^{-1}
EC	0.5	mS cm^{-1}	Potassium (K)	398	mg kg^{-1}
Clay	19.6	%	Calcium (Ca)	377	mg kg^{-1}
Sand	61.5	%	Magnesium (Mg)	450	mg kg^{-1}
Loam	18.9	%	Sodium (Na)	19	mg kg^{-1}
$CaCO_3$	2.86	%	Iron (Fe)	14	mg kg^{-1}
Soil texture	Silty-clay loam		Copper (Cu)	9	mg kg^{-1}
Organic matter	1.06	%	Zinc (Zn)	5	mg kg^{-1}
Nitrogen (N)	0.081	%	Manganese (Mn)	59	mg kg^{-1}

The soil of the test area has the characteristics of sandy-loamy texture (Table 1). The pH value of 7.3 in the test area shows that the soil of the test area reacts close to neutral (Kacar and Inal, 2008). Organic matter content is quite low and lime content is at medium level. Nitrogen, phosphorus and calcium contents are low and iron, copper, zinc and manganese contents, which are microelements, are high (Gunes et al., 2000). Table 2 shows the average temperatures and total amounts of precipitation for the trial years and over the long term. When the data of the trial location and years were evaluated, it was observed that Moringa plant could be grown and there were no problems due to the lack of extreme differences.

Table 2. Climate data 2020-2021 and long-term (30 years)

Years	Months												
	1	2	3	4	5	6	7	8	9	10	11	12	X - Σ
Average Temperature (°C)													
2020	8.3	10.8	13.5	16.4	21.6	25.1	29.7	29.3	26.9	20.9	14.3	12.4	19.1
2021	10.6	11.1	11.1	16.7	22.9	25.4	30.6	29.9	24.9	18.7	-	-	20.2
Long term	8.8	9.5	11.7	15.8	20.8	25.6	28.0	27.6	23.6	18.8	14.1	10.5	17.9
Total Precipitation (mm)													
2020	37.5	76.6	83.0	56.1	55.2	24.9	1.4	0.4	0.5	53.6	2.2	126.0	517.4
2021	213.5	138.0	98.0	25.4	0.6	31.4	1.3	0.0	0.3	27.9	-	-	536.4
Long term	121.0	101.9	74.3	47.0	29.3	8.3	2.0	2.2	15.7	44.3	95.0	144.1	685.1

X: Mean, **Σ**: Total

The experiment was established with 3 replications according to the randomized complete block design experimental design (Acikgoz et al., 2004). In the experiment, plot sizes were arranged as 3.6 m x 2.4 m = 8.64 m^2 and 2 m path was left between the plots. The factor was different plant densities (20x60 cm, 30x60 cm, 40x60 cm and 60x60 cm). PKM-1 variety of Moringa (*Moringa oleifera* Lam.) plant originating from India was used as plant material. Seeds were germination tested before sowing. Sowing was done on 16.05.2020 by hand. Each plot consisted of 6 rows. After planting, the seeds were covered with 1-2 cm soil and irrigation was carried out after planting. Irrigation was done regularly to maintain the soil moisture at field capacity in the summer. The first hoeing was done when the plants were about 15-20 cm tall and hoeing was repeated when needed according to weed status.

Before sowing, compound fertilizer (15-15-15) was applied to all plots with 50 kg ha^{-1} N, 50 kg ha^{-1} P_2O_5 and 50 kg ha^{-1} K_2O as basic fertilizer and 50 kg ha^{-1} N fertilizer was applied when the plant height reached 50-60 cm. After harvest, fertilization treatments were repeated in both years. There was no need for pest and disease spraying etc. in the experiment. The first harvest (H-I) in the *Moringa oleifera* plots was carried out on August 17, 2020, after the plants were given a growth period of approximately 90 days (Gadzirayi et al., 2019). After this date, the plants in the plots were given a growth period of 60 days and the second harvest (H-II) was carried out on October 17, 2020. In the second year, the first harvest (H-I) was made on August 16, 2021, similar to the first year. The second harvest (H-II) was made on November 9, 2021, depending on the climate conditions and the growing status of the plants.

In this study, the distance from the soil surface to the tip of the plant was measured with a ruler and the plant height (cm) was calculated. For this purpose, 10 randomly selected plants were used. Stem diameter of 10 plants was measured by a digital caliper in each plots. During the harvesting process, in plots with 6 rows of plants, the rows on the edges were separated as a border effect and the middle 4 rows of plants were harvested with the help of a hand sickle, leaving a stubble height of 30 cm above the soil level (Basra et al., 2015). Samples were dried at 65°C for 48 h, weighed and dry matter % was estimated. The dry weight samples were then ground in a grinding mill and prepared for chemical analysis. Nitrogen was determined using Kjeldahl method, and nitrogen content was multiplied by a coefficient of 6.25 to calculate crude protein content. Crude ash and crude fat content were determined as described by AOAC (1997). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose were carried out (Van Soest et al., 1991).

ANOVA analysis was performed on the data obtained from the study (Table 3). Biomass yield trait was arranged as the sum of harvests and other traits were arranged as the averages of harvests. In addition, the study years were also considered as a factor to determine the year effect. Differences were determined using the LSD test and 5% probability levels were both used to determine the separate groups (Acikgoz et al., 2004).

RESULTS AND DISCUSSION

The statistical analysis of plant height indicated that only plant density had a statistically significant effect ($p < 0.05$), whereas the effects of year and the interaction between plant density and year were non-significant (Table 4). Among the different plant densities, the highest mean plant height was observed at the 60x60 cm density, with a value of 170.3 cm, while the lowest mean plant height was recorded at the 20x60 cm density, with a value of 159.2 cm. The overall two-year average plant height under Mediterranean climate conditions was determined to be 165.1 cm. Plant height is predominantly influenced by genetic factors. Mih et al. (2008) reported that plant height increased with rising plant density. These differences are due to climatic conditions and other agronomic treatment differences.

Table 3. Results of variance analysis of the examined characteristics

Sources of Variance	df	PH	SD	BY	DM	CA	CP	CF	NDF	ADF	HEM
D	3	134.92**	0.08**	1474.68**	0.69**	0.28**	1.19**	0.02**	18.60**	7.15**	2.81**
Y	1	25.42^{ns}	0.05**	1343.26**	39.89**	0.20**	4.91**	0.01^{ns}	6.25**	4.57**	0.13^{ns}
DxY	3	6.39^{ns}	0.01**	51.28**	0.02^{ns}	0.02^{ns}	0.02^{ns}	0.01^{ns}	0.05^{ns}	0.27**	0.24**

*ns: not significant, D: plant density, Y: year, *: significant at 0.05 level, **: significant at 0.01 level*
PH: plant height, SD: stem diameter, BY: biomass yield, DM: dry matter, CA: crude ash, CP: crude protein, CF: crude fat, NDF: neutral detergent fiber, ADF: acid detergent fiber, HEM: hemicellulose

Table 4. Effects of different plant densities on plant height and stem diameter of *Moringa oleifera*

Plant Height (cm)									
Plant	**2020**			**2021**			**Means of 2 Years**		
Density (cm)	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**
20 x 60	149.3	168.8	159.1	170.2	148.7	159.4	159.8	158.7	159.2
30 x 60	159.0	172.9	166.0	171.9	151.0	161.4	165.4	162.0	163.7
40 x 60	159.2	176.8	168.0	176.4	156.7	166.5	167.8	166.7	167.3
60 x 60	159.2	183.9	171.6	178.2	159.7	168.9	168.7	171.8	170.3
Mean	156.7	175.6	166.1	174.2	154.0	164.1	165.4	164.8	165.1
LSD (0.05)				D: 4.0	Y: ns	DxY: ns			
Stem Diameter (cm)									
Plant	**2020**			**2021**			**Means of 2 Years**		
Density (cm)	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**
20 x 60	1.90	2.06	1.98	2.07	2.20	2.13	1.98	2.13	2.06
30 x 60	2.08	2.26	2.17	2.14	2.33	2.24	2.11	2.29	2.20
40 x 60	2.13	2.31	2.22	2.17	2.42	2.30	2.15	2.37	2.26
60 x 60	2.17	2.40	2.29	2.24	2.46	2.35	2.21	2.43	2.32
Mean	2.07	2.26	2.16	2.16	2.35	2.25	2.11	2.31	2.21
LSD (0.05)				D: 0.02	Y: 0.02	DxY: 0.03			

ns: not significant D: plant density Y: year

The analysis results for stem diameter were statistically significant ($p < 0.05$) for plant density, year, and the interaction between plant density and year (Table 4). The highest two-year mean stem diameter, recorded at the 60x60 cm plant density, was 2.32 cm. Comparing the yearly averages, the stem diameter in the second year (2.25 cm) was higher than in the first year (2.16 cm), which aligns with expected growth patterns. The interaction between plant density and year revealed that the highest average stem diameter was 2.35 cm at the 60x60 cm density in the second year, whereas the lowest average stem diameter was 1.98 cm at the 20x60 cm density in the first year. Stem diameter is recognized as a trait significantly influenced by a plant´s genetic composition. In plants, particularly woody species, the stem functions as the trunk, playing a critical role in maintaining an upright posture and supporting other organs (Roddick and Hanson, 2007). It is generally anticipated that the stem diameter of *Moringa oleifera* and similar species will increase with each growing year a finding supported by our study. As a perennial and rapidly growing plant, *Moringa oleifera* exhibited an increase in stem diameter over the years, with the highest values observed in plots with lower planting densities. The stem diameter measurements obtained in this study are consistent with those reported by other researchers (Goss, 2012; Pradhan et al., 2023).

Table 5. Effects of different plant densities on biomass yield of *Moringa oleifera*

Biomass Yield (t ha^{-1})									
Plant	**2020**			**2021**			**Means of 2 Years**		
Density (cm)	**H-I**	**H-II**	**Total**	**H-I**	**H-II**	**Total**	**H-I**	**H-II**	**Total**
20 x 60	36.90	22.10	59.00	52.00	28.40	80.40	44.45	25.25	69.70
30 x 60	31.05	19.85	50.90	46.15	23.15	69.30	38.60	21.50	60.10
40 x 60	26.20	18.05	44.25	34.85	19.95	54.80	30.53	19.00	49.53
60 x 60	14.60	13.75	28.35	21.75	16.10	37.85	18.18	14.93	33.10
Mean	21.79	18.44	45.63	38.69	21.90	60.59	32.94	20.17	53.11
LSD (0.05)				D: 1.54	Y: 1.09	DxY: 2.18			

ns: not significant D: plant density Y: year

The statistical analysis of biomass yield data revealed significant effects ($p < 0.05$) of plant density, year, and the interaction between plant density and year (Table 5). When evaluating the results by plant density, the highest biomass yield was observed at a plant density of 20x60 cm (69.70 kg ha^{-1}). Conversely, the lowest biomass yield was recorded at a plant density of 60x60 cm (33.10 kg ha^{-1}). There were also notable variations between the years, with biomass yield increasing from 45.63 kg ha^{-1} in the first year to 60.59 kg ha^{-1} in the second year. Furthermore, the interaction between plant density and year showed that the highest biomass production occurred at a density of 20x60 cm, yielding 80.40 kg ha^{-1} in the second year. In contrast, the lowest biomass production was recorded at 28.35 kg ha^{-1} at a plant density of 60x60 cm in the first year.

These findings align with the report by Nouman et al. (2013), who noted that plant growth significantly impacts the biomass yield of *Moringa oleifera*. Additionally, higher planting densities are positively correlated with increased forage production (Kumalasari et al., 2017). Our study supports these conclusions, demonstrating that higher plant densities result in increased biomass yield. These results are consistent with the findings of Amaglo et al. (2006) and Mabapa et al. (2017).

Table 6. Effects of different plant densities on dry matter, crude ash, crude protein and crude fat ratios of *Moringa oleifera*

Dry Matter Ratio (%)									
Plant	**2020**			**2021**			**Means of 2 Years**		
Density (cm)	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**
20 x 60	15.91	17.93	16.92	18.58	20.58	19.58	17.25	19.26	18.25
30 x 60	16.34	18.03	17.19	18.81	20.67	19.74	17.58	19.35	18.46
40 x 60	16.57	18.40	17.49	19.04	20.85	19.95	17.81	19.63	18.72
60 x 60	16.58	18.86	17.72	19.38	21.33	20.36	17.98	20.10	19.04
Mean	16.35	18.31	17.33	18.95	20.86	19.91	17.65	19.58	18.62
LSD (0.05)				D: 0.15	Y: 0.11	DxY: ns			
Crude Ash Ratio (%)									
Plant	**2020**			**2021**			**Means of 2 Years**		
Density (cm)	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**
20 x 60	9.82	9.77	9.80	10.05	9.87	9.96	9.93	9.82	9.88
30 x 60	9.71	9.55	9.63	9.90	9.68	9.79	9.81	9.61	9.71
40 x 60	9.66	9.48	9.57	9.73	9.56	9.64	9.69	9.52	9.61
60 x 60	9.25	9.15	9.20	9.59	9.47	9.53	9.42	9.31	9.36
Mean	9.61	9.49	9.55	9.82	9.64	9.73	9.71	9.56	9.64
LSD (0.05)				D: 0.10	Y: 0.07	DxY: ns			
Crude Protein Ratio (%)									
Plant	**2020**			**2021**			**Means of 2 Years**		
Density (cm)	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**
20 x 60	18.92	18.18	18.55	18.06	17.45	17.76	18.49	17.82	18.15
30 x 60	18.87	17.96	18.41	17.86	17.02	17.44	18.36	17.49	17.93
40 x 60	18.50	17.64	18.07	17.61	16.84	17.22	18.06	17.24	17.65
60 x 60	18.09	17.15	17.62	17.11	16.12	16.62	17.60	16.64	17.12
Mean	18.59	17.73	18.16	17.66	16.86	17.26	18.13	17.29	17.71
LSD (0.05)				D: 0.11	Y: 0.08	DxY: ns			
Crude Fat Ratio (%)									
Plant	**2020**			**2021**			**Means of 2 Years**		
Density (cm)	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**
20 x 60	2.14	1.81	1.97	2.03	1.92	1.98	2.08	1.87	1.97
30 x 60	2.16	1.85	2.01	2.10	1.92	2.01	2.13	1.88	2.01
40 x 60	2.08	1.82	1.95	2.06	1.90	1.98	2.07	1.86	1.97
60 x 60	2.03	1.75	1.89	1.98	1.78	1.88	2.01	1.77	1.89
Mean	2.11	1.81	1.96	2.04	1.88	1.96	2.07	1.84	1.96
LSD (0.05)				D: 0.03	Y: ns	DxY: ns			

ns: not significant D: plant density Y: year

The statistical analysis of dry matter ratio revealed significant effects ($p < 0.05$) of plant density and year factors. However, the interaction between plant density and year was not significant (Table 6). The highest average dry matter ratio was recorded at a plant density of 60x60 cm, at 19.04%, while the lowest was observed at a 20x60 cm plant density, at 18.25%. Notably, the dry matter ratio significantly increased in the second year, rising from 17.33% in the first year to 19.91% in the second year. This increase suggests that the potential for dry matter production may continue to improve in subsequent years. Our findings are consistent with the results reported by Sánchez et al. (2006) and Arif et al. (2020).

The study also found significant effects ($p < 0.05$) on the crude ash ratio due to plant density and year. However, the interaction between plant density and year was not significant (Table 6). The highest average crude ash ratio was recorded at a 20x60 cm plant density, at 9.88%, while the lowest value was observed at a 60x60 cm plant density, at 9.36%. Significant differences were also noted between the years, with the lowest average crude ash value recorded in the first year (9.55%) and a slight increase to 9.73% in the second year.

Crude ash, which is crucial for the formation of nucleoproteins and the facilitation of oxygen transport, is defined as the residual value remaining after the dry matter in plants is incinerated at high temperatures. Determining crude ash content is vital in roughage, as maintaining a certain ash ratio in feed is preferred for optimal animal nutrition. Researchers such as Al-Masri (2003), Sánchez et al. (2006), and have reported that Moringa possesses a high ash concentration, despite wide chemical variability. The crude ash values obtained in our study ranged from 9.15% to 10.05%, aligning with the values reported by Sánchez-Machado et al. (2010) and Valdivié-Navarro et al. (2020), which ranged from 7.62% to 14.60%. However, our results are lower than the 12.41% reported by Alarape et al. (2023). These differences may be attributed to variations in climatic conditions and cultural practices.

The study observed that plant density and year significantly ($p < 0.05$) affected crude protein ratios. However, no interaction was detected between year and plant density (Table 6). The highest crude protein ratio was recorded at a 20x60 cm plant density, averaging 18.15%, while the lowest was found at a 60x60 cm plant density, at 17.12%. Significant differences were also noted between the years, with the highest crude protein ratio recorded in the first year (18.16%) and a decrease to 17.26% in the second year. Crude protein content is a crucial indicator of a plant's nutritional quality and is essential for their use as feed or food resources (Khanal et al., 2020). In our findings, *Moringa oleifera* exhibited an average crude protein ratio of 18.16% in the planting year, which decreased to 17.26% in the second year. The two-year average was determined to be 17.71%. Researchers have noted that crude protein ratios in Moringa vary depending on the plant parts and can range from 11.4% to 40% (Nouman et al., 2013; Mendieta-Araica et al., 2013). Our findings are highly consistent with the crude protein ratios reported by Chodur et al. (2018), Hassanein (2018). Various researchers have attributed such differences to genetic and climatic factors, as well as cultural practices like irrigation and fertilization (Sarwar et al., 2020). The variations observed in our study are likely due to differences in ecological and agricultural conditions, as well as variations in plant varieties and harvest times.

The analysis of crude fat ratios from this study, conducted in a Mediterranean ecology, showed that plant density had significant effects. However, the effects of year and the interaction between plant density and year were not significant (Table 6). The highest value over the two-year average was recorded at 2.01% at a 30x60 cm plant density, while the lowest value was observed at 1.89% at a 60x60 cm plant density. The fat content in various plant parts (root, stem, leaf, flower, seed), which protects these organs, varies in proportions (Eris, 2007; Yurtvermez and Gidik, 2021). These proportions can also vary depending on species and varieties (Mahajan et al., 2020). As with other characteristics, the fat ratio changes according to the developmental stages of plants (Pallardy, 2008). Studies on different plants have indicated that crude fat ratios are lower in the early stages of growth and increase in the later stages (Singh and Todaria, 2012). Our results are consistent with the crude fat ratios reported by Sánchez-Machado et al. (2010), which ranged from 1.28% to 4.96%.

Table 7. Effects of different plant densities on NDF, ADF and hemicellulose of *Moringa oleifera*

NDF (%)									
Plant	**2020**			**2021**			**Means of 2 Years**		
Density (cm)	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**
20 x 60	44.61	45.68	45.15	45.77	46.59	46.18	45.19	46.14	45.66
30 x 60	46.14	47.72	46.93	47.73	48.21	47.97	46.93	47.97	47.45
40 x 60	47.43	48.93	48.18	48.90	49.92	49.41	48.17	49.43	48.80
60 x 60	48.68	49.96	49.32	49.27	50.94	50.10	48.97	50.45	49.71
Mean	46.72	48.08	47.40	47.92	48.92	48.42	47.32	48.50	47.91
LSD (0.05)				D: 0.18	Y: 0.13	DxY: ns			
ADF (%)									
Plant	**2020**			**2021**			**Means of 2 Years**		
Density (cm)	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**
20 x 60	34.49	34.80	34.65	35.47	36.47	35.97	34.98	35.64	35.31
30 x 60	35.57	36.62	36.10	36.68	37.76	37.22	36.13	37.19	36.66
40 x 60	36.62	37.28	36.95	36.97	38.23	37.60	36.79	37.76	37.27
60 x 60	37.25	38.06	37.65	37.26	38.84	38.05	37.25	38.45	37.85
Mean	35.98	36.69	36.34	36.59	37.82	37.21	36.29	37.26	36.77
LSD (0.05)				D: 0.15	Y: 0.11	DxY: 0.21			
Hemicellulose (%)									
Plant	**2020**			**2021**			**Means of 2 Years**		
Density (cm)	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**	**H-I**	**H-II**	**Mean**
20 x 60	10.12	10.88	10.50	10.30	10.12	10.21	10.21	10.50	10.36
30 x 60	10.57	11.10	10.84	11.05	10.46	10.75	10.81	10.78	10.79
40 x 60	10.82	11.65	11.23	11.93	11.70	11.81	11.37	11.67	11.52
60 x 60	11.43	11.90	11.67	12.01	12.10	12.05	11.72	12.00	11.86
Mean	10.74	11.38	11.06	11.32	11.09	11.21	11.03	11.24	11.13
LSD (0.05)				D: 0.24	Y: ns	DxY: 0.34			

ns: not significant D: plant density Y: year

The analysis of neutral detergent fiber (NDF) data revealed significant ($p < 0.05$) effects of plant density and year. However, the interaction between plant density and year was not significant (Table 7). The NDF values from our study indicated that the highest average value, 49.71%, was observed at a plant density of 60x60 cm, whereas the lowest value, 45.66%, was recorded at a plant density of

20x60 cm. Additionally, the NDF value increased from 47.40% in the first year to 48.42% in the second year.

NDF is a crucial criterion for evaluating the quality and digestibility of roughage, making it important in animal feding (Sarikaya et al., 2023). It is well-established that NDF values increase with the plant's developmental stage (Acar et al., 2021). As the crude cellulose content—the structural component of plant cell walls—increases with development, the NDF value also rises. Our results align with studies investigating different genotypes and ecological conditions of Moringa plants (Bashar et al., 2020; Valdivié-Navarro et al., 2020).

The analysis of acid detergent fiber (ADF) data revealed significant ($p < 0.05$) effects of plant density, year, and the interactions between plant density and year (Table 7). When analyzing ADF values by plant density, the highest average ADF value of 37.85% was recorded at a plant density of 60x60 cm over two years, while the lowest average ADF value of 35.31% was observed at a plant density of 20x60 cm. The lowest average ADF value, 36.34%, was recorded in the first year, while an ADF value of 37.21% was recorded in the second year. Considering the plant density × year interaction, the highest ADF value of 38.05% was recorded in the second year at a plant density of 60x60 cm, while the lowest ADF value of 34.65% was observed in the first year at a plant density of 20x60 cm.

ADF represents the portion remaining after subtracting the hemicellulose from the NDF value. This criterion is particularly informative regarding digestibility and the animal's energy intake (Van Soest, 1991). Our findings are consistent with studies conducted on *Moringa oleifera* under various genotypes, ecological conditions, and agronomic treatments (Bashar et al., 2020; Valdivié-Navarro et al., 2020).

The analysis of hemicellulose data revealed significant ($p < 0.05$) effects of plant density and plant density × year interaction (Table 7). The highest two-year average hemicellulose was recorded at 11.86% at a plant density of 60x60 cm, while the lowest value was 10.36% at a 20x60 cm plant density. Additionally, the interaction between plant density and year revealed that the highest hemicellulose was achieved at 12.05% at a plant density of 60x60 cm in the second year, whereas the lowest hemicellulose was 10.21% at a plant density of 20x60 cm in the second year.

The proportions of cellulose and hemicellulose are higher in young and fresh plants at the beginning of growth, gradually increasing as the vegetation matures. Since these components are difficult to digest, lower proportions in feed are preferred (Oktem et al., 2021). Quintanilla-Medina et al. (2018) reported that the hemicellulose in Moringa ranges from 4.01% to 6.98%. The current findings are higher than these reported values. These differences may be attributed to variations in climatic conditions and cultural practices.

CONCLUSION

The research conducted under Mediterranean ecological conditions yielded promising results regarding biomass yield and forage quality characteristics of *Moringa oleifera*. The data analysis indicated that a plant density of 20x60 cm (69.70 t ha^{-1}) provided optimal conditions, resulting in the highest biomass yield and superior forage quality characteristics. This plant density is therefore recommended for optimizing *Moringa oleifera* cultivation in similar ecological settings.

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IMPACT OF NITROGEN FERTILIZATION ON BREAD WHEAT: SCREENING FOR CHANGES IN QUALITY, ANTIOXIDANT AND ESSENTIAL AMINO ACID CONTENT

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ABSTRACT

Wheat grain has a unique nutritional value and contains health-promoting and essential components in the daily human diet. Increasing consumer awareness of health and association of whole grains with several health benefits has led to a greater focus on sustainable and healthy wheat production. The aim of this study was to determine the effect of nitrogen on yield and protein characteristics as well as antioxidant capacity and essential amino acid profile of bread wheat genotypes adapted to different ecological conditions. Different nitrogen doses (0, 60, 120 and 180 kg ha^{-1}) were applied to 15 genotypes (3 lines, 1 hybrid and cultivars) with different growth habit to determine yield, quality, antioxidant and amino acid composition parameters. As a result of this study, total phenol content, antioxidant activity and gluten index of wheat decreased although grain yield potential increased in genotypes. In the results where the genetic factor is the primary focus, it was established that the responses to nitrogen fertilizer doses exhibited variability across different years particularly the case during the dry season. With regard to the YearxNitrogenxGenotype interaction, a notable increase was observed in total phenol content and antioxidant activity, while a decline was evident in yield, protein, and wet gluten parameters, particularly in the nitrogen dose applied during the dry season. The increase in protein content contributed significantly and positively to the essential amino acid composition. However, increasing the amount of some amino acids negatively affects others. The objective of this study is to identify and contribute insights into the impact of nitrogen factor on product quality, health and nutrition issues, grain yield potential of genotypes, plant breeding and agronomic studies.

Keywords: ***Triticum aestivum*** **(L.), nitrogen, protein, gluten, antioxidant, lysine, methionine**

INTRODUCTION

Wheat is a staple food and cultivated globally as supplying >20% of human calories and protein meeting the daily nutrient requirement of global population (Zhang et al., 2024). It has played an indispensable role in influencing society's culture and lifestyle and has constituted an indispensable food source for humanity throughout history. Considering the global climate change, scientists are engaged in formulating novel strategies to develop tolerated wheat varieties to sustain the crop's yield potential. The rising temperatures and irregular rainfall cause an adverse impact on crop productivity, not only leading to a decline in the potential yield of wheat but also quality (Tatar et al., 2020). The quality of the wheat grain has become a primary objective for breeders and farmers, as it is a crucial factor in achieving premium prices and meeting market demands for high-quality wheat products (Rossini et al., 2018). Aside from genetic and environmental factors, nitrogen fertilization exerts a considerable influence and serves as critical agronomic determinant in the formation of both yield and grain quality (Meng et al., 2024). Nitrogen (N) is a significant factor and essential nutrient for cereals, playing a crucial role in crop growth and yield. However, despite its importance, excessive nitrogen application does not substantially improve yields and may have detrimental effects on the environment (Kong et al., 2017; Koppensteiner et al., 2022). Therefore, the timing and application amount of nitrogen fertilization are important factors in achieving optimum yield and quality. N is not only the most important nutrient in terms of yield formation, but it also plays a crucial role in determining and involved in the formation of storage proteins in grains. The application of effective nitrogen fertilization (dosage and timing) allows for the regulation of grain N accumulation in wheat, resulting in an elevated grain protein concentration in response to an increased nitrogen fertilizer input (Massoudifar et al., 2014; Zhang et al., 2017; Zorb et al., 2018). Sohail et al. (2018) demonstrated that the application of nitrogen fertilizer during the early growth stages enhanced vegetative growth

and tillering potential. Conversely, the application of nitrogen fertilizer during the stem elongation and grain filling periods led to an increase in grain protein and gluten content, underscoring the pivotal role of nitrogen in determining quality. It is also known that nitrogen (N) requirement for protein synthesis in the developing wheat grain is determined by the mobilization of pre-assimilated N in vegetative tissues (in the range of 50-70%), as well as the direct uptake and assimilation of N during grain filling. However, the mobilization and recycling ability depend on genotype and is also influenced by maturity time (Götz and Erekul, 2023). Moreover, nitrogen fertilization is vital for biochemical components that play a role in human health and nutrition as well as contributing to the agronomic and quality characteristics of wheat. Wheat-based food ingredients and food products rich in natural antioxidants, especially those produced from whole grain products, as a beneficial dietary choice. In recent years, there has been a notable increase in consumer interest in whole grains and their products, largely due to their perceived health-promoting properties (Yu, 2008).

Antioxidants and phenolic compounds are believed to have beneficial effects on human health and also attributed to have protective affect against chronic diseases suggested in epidemiological evidence. They are thought to react directly with free radicals (ROS), thereby reducing peroxides and stimulating antioxidant defense enzyme systems. Free radicals occur naturally within the body however, increasing the level of these free radicals, leading to an imbalance with the existing antioxidants, this can result from certain stress factors (Narwal et al., 2014; Sonntag et al., 2020).

The impact of nitrogen fertilization on grain antioxidant and phenol content has been the subject of several studies, with findings that vary across research. N fertilization caused inverse effects in different fractions of phenolics in wheat. This result was linked to most phenolic compounds are predominantly situated within the external layers of the grain so it is expected that increasing proportion of bran may cause higher phenolic compounds (Stumpf et al., 2015). In general, bran was confirmed as rich in phenolics and antioxidants so it is understood that a lack of nitrogen may result in an increase in the bran layer within the grain, which can be attributed to the fact that the grains are unable to form adequately due to the absence of nitrogen (Mazzoncini et al., 2015).

Wheat grain is a significant source of protein in human nutrition, in addition to its role in maintaining health. In addition to protein quality, amino acids are regarded as nutrients that provide essential amino acids for a healthy diet. Wheat proteins are characterized by a relatively low content of essential amino acids, including lysine, tryptophan and threonine, which are crucial for human nutrition. These limited amino acids in question serve to determine the quality of the protein, which in turn affects the amino acid composition of the grain (Zhang et al., 2017; Siddiqi et al., 2020). Anjum et al. (2005) provide considerable insight into newly released wheat varieties, exhibiting superior nutritional characteristics particularly in terms of essential amino acids, with notable enhancements observed in lysine levels. This may be attributed to the higher nitrogen requirement of the new varieties. Amino acid composition basically depends on the genotype and environmental factors, nitrogen application time and concentration in the soil, availability of water budget and temperature in grain filling periods (Qabaha, 2010).

Amino acids represent the primary forms in which nitrogen is remobilized from leaves to the grain during the maturity period. Therefore, to improve the nutritional value of wheat, it is essential to ensure an optimal nitrogen supply. Zhang et al. (2017) observed a notable elevation in essential amino acid concentrations in response to augmented nitrogen doses. Both essential and non-essential amino acid profiles demonstrated a linear correlation with protein content, exhibiting a concurrent increase. With in the light of this knowledge, our objective was to investigate the nitrogen and genotype-related changes in healthy compounds and nourishment properties of wheat grain, in addition to yield and quality responses. Furthermore, we focused on evaluating and gain insight into the evaluated characteristics of varieties with different growth habits and newly released lines.

MATERIALS AND METHODS

A field experiment was conducted in Aydın (37°45′22′′N 27°45′36′′E) ecological conditions during 2017 and 2018 growing seasons. The material set consist of 15 wheat genotypes with different genotypic and agronomic characteristics: 3 advanced lines, 11 cultivars and 1 hybrid wheat shown in Table 1. The genotypes were grown in five nitrogen doses (0, 60, 120 and 180 kg ha^{-1} hereafter referred to as N_0, N_{60}, N_{120} and N_{180}). The experimental soil type (sampled in the topsoil to a depth of 0.30 m) is loamy sand with 69.9% sand, 21.3% silt, and 8.65% clay with typical alkaline soil pH level (8.0). The experiment was laid out in randomized split plot design with three replications where N doses were applied as the main plot effect. Plot size and sowing distance between rows were 1,2 x 6 m and 20 cm, respectively. Experiment area consisted of 180 sub-plots, in total carried out on 3360 m^2.

Nitrogen mineral fertilization was split as follows; 30 kg ha^{-1} as 20.20.20 form applied prior to the sowing for all doses without N_0. For treatments N_{60}, N_{120}, N_{180} when first tiller visible (BBCH 21 growth period) 30 kg ha^{-1} applied for N_{60}, 45 kg ha^{-1} applied for $N_{120,}$ and 75 kg ha^{-1} applied for N_{180}. At stem elongation stage (BBCH 31) 45 kg ha^{-1} applied for N_{120} and 75 kg ha^{-1} applied for N_{180} in the form of urea (46% N).

Weeds and diseases were controlled by using chemical control throughout the growing seasons. In tillering stage weed control was provided by herbicides: Arrat® (25% triosulfuron and 50% Dicamba) and Topcup® 240 EC (240 g/l Clodinafop-propargyl) and in the beginning of heading Sonfix® 5 EC (50 g/l Diniconazole) fungucide applied for wheat leaf rust diseases (*Puccinia* spp.) during 2017 and 2018 seasons.

Table 1. General characteristics, origin and description of genotypes

Genotype	Growth habit	Spike type	Institute/company
Golia	Facultative	White, awned	Italian origin, Tigem
Kate A	Facultative	White, awnedness	Bulgarian origin, Alfa seed
Selimiye	Winter	Red, awnedness	Trakya Agricultural Research Institute
Ceyhan 99	Facultative	White, awned	Eastern Mediterranean Agricultural Research Institute
Tosunbey	Facultative	White, awned	Field Crops Central Agricultural Research Institute
İkizce-96	Winter	White, awned	Field Crops Central Agricultural Research Institute
Müfitbey	Winter	White, awned	Transitional Zone Agricultural Research Institute
Line 1	Winter	Red	IWWIP Programme, Bahri Dağdaş International Agricultural Research Institute. CHAM//1D13.1/MLT/4/C126-6/C190-12//AU/3/TZPP/BEZ
Line 2	Winter	Red	IWWIP Programme, Bahri Dağdaş International Agricultural Research Institute. ZANDER-17/3/KAUZ*2/YACO//KAUZ
Line 3	Winter	Red	IWWIP Programme, Bahri Dağdaş International Agricultural Research Institute. PLK70/LIRA"S"/5/C126-15…/4/KRC/7/NECOMP1/5/BEZ//TOB/8156/4/ON/3/TH*6/ KF//LEE*6/K/6/TAST/SPRW..
Eraybey	Winter	White, awned	Bahri Dağdaş International Agricultural Research Institute
Bozkır	Winter	White, awned	Bahri Dağdaş International Agricultural Research Institute
Euclide	Winter	White,awned	Syngenta Seed, Quality group: A (High)
Julius	Winter	White, awnedness	KWS Seed, Quality group: A (High)
Hybery*	Winter	White, awnedness	Saaten-Union GmbH, Quality group: B (good), Hybrid

Hybery: Hybrid wheat cultivar seeds obtained for sowing in both experimental years.

Table 2. Monthly and long term (1941-2018) meteorological conditions during the growing seasons

Months	Mean Temperature (°C)			Total Precipitation (mm)		
	2017	2018	1941/2018	2017	2018	1941/2018
November	12.7	11.6	13.4	51.4	85.0	83.3
December	5.3	10.3	9.5	11.9	98.9	121.7
January	5.9	8.8	8.1	221.5	119.2	116.5
February	9.2	12.5	9.4	21.7	112.9	93.8
March	12.2	15.2	11.8	112.5	90.4	71.1
April	15.2	20.9	15.9	46.4	8.2	48.2
May	20.1	24.6	20.9	45.0	71.0	35.7
June	25.3	27.0	25.8	16.0	28.5	13.9
Mean T°C/mm*	13.2	16.3	14.3	526.4	501.2	584.2

*: Mean temperature and precipitation values during long term and growing seasons, Turkish State Meteorological Service (Mevbis). Mean annual temperature for long term: 17.7°C, total annual precipitation amounts for long term: 647.0 mm

Annual temperature, precipitation and long-term climate values are shown in Table 2. In 2017 the seedlings suffered from the lower (12.7, 5.3 and 5.9°C) field temperature (especially facultative growth habit cultivars) compared to long term values. In the first year of experiment, deficit rainfall amount observed in November (51.4 mm) and December (11.9 mm) but in January excess rainfall observed and caused short time flooding stress in wheat seedlings during field emergence period. In generally the weather conditions were favorable (compared to long term conditions) in 2017 during springtime and generative growth period. In terms of mean temperature, favorable values observed in vegetative development periods in 2018 compared to long term values, but it is noteworthy that March, April and May had high temperature (and also April had lowest precipitation value) values compared to long term and first year of experiment. This situation caused adverse conditions during flowering period and caused shortening of grain filling period. Furthermore, the unfavorable climatic conditions observed in 2018 resulted in a significant acceleration in the average number of days to ear emergence and the average number of days to flowering, with both occurring 12 and 11 days earlier, respectively, compared to the first year of the study.

Border lines in each plot were removed at both harvesting times (7th of June 2017 and 24th of May 2018) and the remaining area was harvested to calculate the yield. The harvested seeds were then threshed, cleaned and stored at 4°C until chemical analysis. Grain protein content (%) was determined by Velp® Dumas Nitrogen Analyzer in Aydın Adnan Menderes University Agricultural Biotechnology and Food Safety Laboratory (ADU-TARBIYOMER) according to AOAC 997.09 method. Bread making quality traits were measured following the Standard Method of the International Association of Cereal Chemistry (ICC). Wet gluten and gluten index were determined with the Bastak® 6000 (ICC Standard No. 137/1) and Bastak® Index 2002 (ICC Standard No. 155) devices with using whole grain flour (milled with Perten Laboratory Mill to 0.8 mm). For the extractions used to determine total phenolic content (and total antioxidant activity, 1 g of ground whole wheat flour sample was mixed in acidic methanol solution (HCl/methanol/water; 1:80:10, v/v) in a shaker (Gerhardt, Thermoshake) under nitrogen gas for 1 h, then centrifuged (Hettick) at 5000 rpm for 20 min. The resulting extracts were transferred to tubes and stored at +4 ºC until analysis (Beta et al., 2005; Ragaee et al., 2006; Ma et al., 2014). Total phenolic content of wheat samples was determined according to the Folin-Ciocalteu method described by Kaluza et al. (1980) and Ragaee et al. (2006) by using gallic acid as standard. The total antioxidant activity of wheat samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to the method proposed by Brand Williams et al. (1995). The absorbance measurements of total phenolic content (725 nm wavelength) and antioxidant activity (517 nm wavelength) extracts were determined by Thermo Scientific spectrophotometer. The amino acid analysis was performed by using high-performance liquid chromatography (Shimadzu Nexara, HPLC System) at the Research and Application Center of Drug development and Pharmacokinetics, Ege University. For this purpose, seven essential amino acids were identified based on the procedure chosen depends on oxidation (for methionine) and hydrolysis (threonine, valine, isoleucine, leucine, phenylalanine and lysine) analysis of samples. The experimental data were subjected to analysis of variance for each parameter and all data were analyzed by using ANOVA and LSD test techniques of Tarist statistical analysis software (Acikgoz et al. 2004). In addition, the correlogram was performed in R studio using the "metan" package (Olivoto and Lúcio, 2020).

RESULTS AND DISCUSSION

Impact of Varied Nitrogen Applications on Grain Yield and Quality traits

Results of statistical analysis for the effects of experimental factors [Year (Y), Nitrogen Applications (N) and Genotype (G)] on yield, protein and bread-making quality parameters of bread wheat with mean square values and significant levels (*: $p\leq0.05$, **: $p\leq0.01$) were given in Table 3. When the results of the analysis of variance of nitrogen applications and genotypes combined for two years were examined, grain yield and quality traits were affected by year (except wet gluten), nitrogen and genotypes. In addition, YxN, YxG, NxG, and YxNxG interactions were statistically significant at the $p\leq0.05$ and 0.01 levels (Table 3).

Table 3. ANOVA results (mean square values and significance levels) for grain yield and quality traits

Experimental factors	Grain Yield (kg ha^{-1})	Crude Protein Content (%)	Wet Gluten Content (%)	Gluten Index (%)
Year (Y)	2090756.5**	570.6**	0.4	4165.4*
Nitrogen (N)	1139854.7**	70.4**	1139.9**	989.3**
Genotype (G)	75057.9**	7.7**	237.8**	1498.5**
YxN	129171.9**	6.8**	85.3*	797.2**
YxG	132242.2**	9.5**	149.6**	541.2**
NxG	28282.8**	3.3**	64.6**	314.0**
YxNxG	19116.8**	3.0**	79.3**	377.0**

Significance levels: *: $p\leq0.05$, **: $p\leq0.01$

In 2017, favorable climatic conditions (lower mean temperature and sufficient precipitation in generative growth stages) were conducive to wheat development. However, in the 2018 season, the mean temperature values increased from February and were observed to be higher than the long-term average values by approximately 4°C during the grain filling period. In springtime, the precipitation levels in April were below the long-term average and the previous year's levels (8.2 mm). These conditions negatively affected wheat development (occurred at an earlier period: ear emergence and flowering) and grain filling, resulting in a decrease in grain yield (Tatar et al., 2020). The occurrence of post-flowering drought (decrease in spring rainfall) in wheat resulted in a detrimental impact on yield, leading to a reduction in grain yield (Aykut Tonk et al., 2011; Tatar, 2011). The first experimental yield values demonstrated an increase due to the lower mean temperature values and a good water supply (precipitation) condition and the application of elevated nitrogen fertilizer doses (N_{120} and N_{180}). The hybrid variety (Hybery) demonstrated a remarkably high yield value in the first year of the trial, under conditions of favorable climatic circumstances (a cooler winter season) and an N_{60} nitrogen dose. Furthermore, among the genotypes evaluated in the study, and those with a winter growth habit exhibited the highest yield values at the N_{120} nitrogen dose. In general, the cultivars exhibited a good response to increasing nitrogen doses (especially N_{120} and N_{180}) in terms of yield, reaching a high yield potential at these doses (except Julius and Hybery).

The newly released wheat lines (Line, 1,2 and 3) had also high yield potential compared to other genotypes. Additionally, they demonstrated a notable capacity for responding to nitrogen fertilization. The yield potential of the lines demonstrated positive response with the increasing nitrogen fertilizer dosages (Table 4).

The protein content demonstrated a positive response to both climatic conditions and increasing nitrogen doses. In conditions of elevated temperature and water scarcity, the supply of assimilates may be constrained, leading to a reduction in starch, protein and the nutritional value of the grain. The accumulation of starch and protein is determined during the early grain-filling period, with the final size of the cells influenced by water stress during this same period. Furthermore, exposure to elevated temperatures (>30°C) accelerates the grain-filling process, leading to a shortage during this period (Dupont and Altenbach, 2003). In 2017, the protein content of the grain increased under conditions of favorable climate (enough water supply and lower mean temperature conditions in grain filling) and with an increasing dose of nitrogen fertilizer. In contrast, in 2018, due to conditions of both unfavorable climate (higher mean temperature and lower precipitation values in grain filling) and unfavorable conditions, the protein content of the grain reached a sufficient level only at the highest nitrogen dose. It was demonstrated that the method of application of nitrogen fertilizer influences the grain nitrogen content. In particular, it was observed that the protein content increased in proportion to the amount of nitrogen fertilization. Furthermore, it was indicated that the grain nitrogen content was derived from the transport of nutrients from the stem and leaf parts. Additionally, it was observed that 50% of the nitrogen was absorbed from the soil following the flowering stage (Zorb et al., 2018). Accordingly, under dry season conditions subsequent to this period result in considerable reductions in protein content. In both experimental years, the hybrid cultivar (Hybery, B Quality Class) demonstrated a linear increase (from 10.5-11.7% to 14.1-14.5%) in protein content with increasing nitrogen doses. Similarly, other varieties (Selimiye, Ceyhan 99, Tosunbey, Line 2, Julius) exhibited a considerable increase at the same level. In previous studies, a negative relationship between grain protein ratio and grain yield was generally stated, but protein ratio increased with the increase in grain yield in the study due to nitrogen fertilizer applications (Aydogan et al., 2018; Cosentino et al., 2018). It is hypothesized that the primary cause of this phenomenon is the accumulation of protein in the milk stage, which is composed of protein previously accumulated in the stems and leaves. The reduction in protein content during the dry season (2018) can be attributed to the accumulation of less dry matter in the plant, which is a consequence of suboptimal developmental periods. (Naneli et al., 2015).

Wheat grain has a unique value for nutrition and production of bakery products. The protein content determines the ability of wheat flour to perform an important role in carbon dioxide retention, dough flexibility and development, and ultimately baking quality (Khalid et al., 2023). Basically, gluten content and its stability (index) are responsible for determining baking and bakery product quality. (Sharma et al., 2020). The significance of nitrogen regarding quality was underscored by the finding that nitrogen fertilizer applied at an early stage enhanced vegetative growth and tillering potential, whereas applied during the stem elongation and grain filling periods elevated grain protein and gluten content (Sohail et al., 2018). In both growing seasons, the wet gluten content (%) was observed to be almost generally lower in the initial three applications (N_0, N_{60} and N_{120}) of nitrogen doses, while a significant increase was noted with the highest nitrogen dose (N_{180}). This situation is more clearly understood, particularly during the dry season (2018) of the study. The application of nitrogen with increasing dosages was found to result in an increase in gluten content during the dry season. In general, a reduction in the quantity of wet gluten was observed in winter growth habit genotypes as the quantity of nitrogen applied was diminished. This phenomenon was most evident in the Line 2, Eraybey, Bozkır, Line 3 and Euclide genotypes. Moreover, these genotypes exhibited a positive response to the highest nitrogen dose (N_{180}) applied, demonstrating a notable increase in the quantity of wet gluten. In regard to the quality traits of the bread, the wet gluten ratio exhibited a range of 16.6-55.0%, with an observable increase in the gluten ratio corresponding to an increase in the nitrogen fertilizer doses. Gluten index values ranged between 41.4 and 98.9%, demonstrating an increase with the application of elevated nitrogen doses. However, the highest value was

observed at a nitrogen dose of N_{120} kg ha^{-1}, rather than the anticipated N_{180} kg ha^{-1} for both bread-making quality traits (Table 5). Consequently, the application of nitrogen had a considerable positive impact on the quality traits of the bread (Erekul et al., 2012a; Basyigit Koseoglu et al., 2024). The gluten index value, which is used to determine the strength of gluten, was observed to be generally higher during the dry growing season (2018) a finding that is consistent with the results reported by Barutcular et al. (2016). Furthermore, it was observed that there was an increase in gluten strength with a reduction in the quantity of wet gluten.

Table 4. Effects of nitrogen application on yield and protein content of evaluated genotypes in the period of 2017 and 2018.

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Grain Yield (kg ha^{-1})	Golia	2300	3013	6335	5967	1544	2708	4244	4490
	Kate A	3662	4736	7657	7163	1743	2721	4484	5245
	Selimiye	3783	4410	6539	5204	2624	4262	4007	4704
	Ceyhan 99	2369	2562	5767	5828	1621	1964	2346	2703
	Tosunbey	2370	2555	2491	3916	1356	2620	3605	3576
	İkizce-96	2350	2382	5802	5424	2020	2220	4521	4472
	Müfitbey	2339	2393	4360	4778	879	3263	3291	3838
	Line 1	3009	3168	4666	7524	2357	3356	3680	4603
	Line 2	2660	2634	4465	6429	1704	3864	3138	4961
	Eraybey	2780	2554	3982	4734	2139	5697	3671	4408
	Bozkır	1801	1217	5854	5765	2194	3029	3318	3513
	Line 3	3034	3703	8111	5590	1102	3592	3858	3876
	Euclide	2449	2760	7105	3162	955	2385	2313	2644
	Julius	4303	6314	5255	6767	1213	1096	2052	2028
	Hybery	6463	8349	7012	6103	465	2417	2490	1951
	Mean	3044	3516	5693	5623	1594	3012	3401	3800
	Lsd Y: 609; Lsd N: 228; Lsd YxN: 323; Lsd G: 442; Lsd YxG: 626; Lsd NxG: 885; Lsd YxNxG: 1252								
	N (kg ha^{-1}) /G	**2017**				**2018**			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Protein Content (%)	Golia	14.3	11.8	14.7	15.5	10.9	10.5	12.6	11.4
	Kate A	10.7	13.0	14.8	14.1	11.1	8.9	9.3	10.6
	Selimiye	11.3	12.7	13.7	13.7	9.3	8.3	11.0	11.3
	Ceyhan 99	12.0	12.8	14.0	14.7	9.0	8.1	11.4	12.7
	Tosunbey	11.4	14.1	14.9	14.2	9.4	10.1	10.0	12.9
	İkizce-96	13.2	14.0	15.4	14.8	11.5	9.8	11.7	12.7
	Müfitbey	13.6	14.1	14.5	14.6	9.8	8.5	10.6	11.9
	Line 1	15.0	12.1	13.9	14.4	10.2	9.5	10.3	11.3
	Line 2	12.5	12.6	12.9	13.3	9.0	8.3	10.7	14.4
	Eraybey	12.9	11.8	12.6	12.4	10.2	9.6	9.5	10.8
	Bozkır	14.1	15.7	12.3	13.0	8.8	9.2	9.9	10.3
	Line 3	12.3	11.2	11.9	13.5	9.6	8.5	9.4	11.5
	Euclide	10.4	9.0	11.5	13.1	12.6	9.4	10.8	11.4
	Julius	12.2	13.2	12.7	13.5	9.0	12.4	11.4	13.4
	Hybery	10.5	10.8	13.1	14.1	11.7	11.3	11.5	14.5
	Mean	12.4	12.6	13.5	13.9	10.1	9.5	10.7	12.1
	Lsd Y: 0.23; Lsd N: 0.40; Lsd YxN: 0.57; Lsd G: 0.79; Lsd YxG:1.12; Lsd NxG: 1.58; Lsd YxNxG: 2.24 color diagram: red-white-green. This sequence is characterized by an increase in value from left to right								

Table 5. Effects of nitrogen application on gluten content and gluten index of evaluated genotypes in the period of 2017 and 2018.

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Wet Gluten Content (%)	Golia	32.4	27.6	33.2	39.6	27.8	25.5	26.2	35.7
	Kate A	29.8	34.1	35.9	41.1	41.1	30.0	32.5	41.6
	Selimiye	23.6	28.0	33.3	35.3	30.9	22.6	32.8	36.2
	Ceyhan 99	25.4	32.0	27.1	33.5	27.0	18.4	30.7	37.9
	Tosunbey	37.4	42.2	38.8	30.6	23.8	31.1	27.4	31.9
	İkizce-96	35.1	37.9	25.4	41.2	36.5	29.6	32.1	36.9
	Müfitbey	27.8	28.6	33.1	46.0	30.9	19.8	31.7	38.3
	Line 1	32.9	33.8	34.1	36.4	28.1	24.6	30.2	40.7
	Line 2	28.4	22.7	55.0	29.6	27.9	20.1	28.6	40.1
	Eraybey	29.8	17.8	29.5	28.0	24.6	29.6	29.3	35.8
	Bozkır	30.1	19.6	34.2	38.3	26.9	30.4	25.8	35.8
	Line 3	28.6	26.1	32.5	35.2	30.3	41.7	36.9	34.8
	Euclide	19.5	16.6	22.0	25.0	28.6	25.6	31.9	36.8
	Julius	33.4	32.2	41.9	45.7	41.2	37.6	34.4	41.1
	Hybery	31.6	24.7	25.3	24.8	34.5	31.7	32.1	39.0
	Mean	29.7	28.3	33.4	35.3	30.7	27.9	30.8	37.5
	Lsd N: 1.4; Lsd YxN: 1.9; Lsd G: 2.7; Lsd YxG: 3.8; Lsd NxG: 5.4; Lsd YxNxG: 7.6								
	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Gluten Index (%)	Golia	67.1	81.5	67.7	75.4	86.6	84.7	92.6	81.7
	Kate A	41.4	49.7	61.2	41.9	52.2	73.2	67.8	53.3
	Selimiye	81.5	75.4	71.6	53.3	52.9	84.6	86.3	64.1
	Ceyhan 99	81.1	67.6	98.9	66.2	88.4	78.8	89.6	70.8
	Tosunbey	76.2	60.2	88.8	85.8	87.5	83.4	78.5	92.7
	İkizce-96	56.1	51.3	70.7	60.1	59.1	73.7	87.1	75.1
	Müfitbey	68.8	80.9	62.7	59.9	74.0	93.2	89.1	74.4
	Line 1	77.8	57.5	43.6	73.3	78.8	84.5	78.9	76.7
	Line 2	66.3	57.6	63.0	78.1	70.6	86.5	75.2	59.3
	Eraybey	77.4	88.1	75.1	66.2	85.7	70.8	94.4	63.1
	Bozkır	68.8	93.6	45.2	49.0	85.5	69.1	95.7	87.8
	Line 3	76.7	59.4	52.7	51.5	66.7	57.7	69.3	58.7
	Euclide	85.8	78.9	86.2	88.9	72.6	83.5	73.4	72.1
	Julius	70.9	70.3	42.2	45.4	60.3	61.3	79.0	63.4
	Hybery	89.0	55.3	87.6	76.0	65.2	65.6	73.1	48.1
	Mean	72.3	68.5	67.8	64.7	72.4	76.7	82.0	69.4
	Lsd Y: 2.9; Lsd N: 2.9; Lsd YxN: 4.2; Lsd G: 5.7; Lsd YxG: 8.1; Lsd NxG: 115; Lsd YxNxG: 16.3								
	color diagram: red-white-green. This sequence is characterized by an increase in value from left to right								

The gluten index value was observed to be generally lower at the highest nitrogen dose, and there was a concomitant decrease in gluten stability. Overall, nitrogen is found to be important factor affecting protein and gluten content and its quality largely influences dough and rheological properties of wheat (Hao et al. 2023).

Total phenolic content (µg GAE/g) and antioxidant activity (% inhibition) results

Wheat comprises a significant proportion of the human diet and has a valuable source of carbohydrates and protein and contains phytochemicals that confer significant health benefits (Punia and Sandhu, 2015). Phenolic compounds and antioxidants are the primary chemical components that directly interact with oxygen reactive species (ROS), which serve to protect cells from DNA damage. Whole grain products are well documented to be rich in phenolic compounds, which are primarily concentrated in the outer layers of grains, including aleurone, testa, and pericarp (Kerienė et al., 2015; Martini et al., 2015). The ANOVA results of total phenolic content and antioxidant activity with mean square values and significant levels (*: $p \leq 0.05$, **: $p \leq 0.01$) were given in Table 6. Total phenolic content and antioxidant activity of wheat grain were statistically significantly affected by Nitrogen Applications (N) and Genotype (G)] factors with $p \leq 0.01$ level while Year (Y) had $p \leq 0.05$ level. In addition, YxN, YxG, NxG, and YxNxG interactions were statistically significant at the $p \leq 0.01$ level.

Table 6. Total phenolic content and antioxidant activity ANOVA results (mean square values and significance levels)

Experimental factors	Total Phenolic Content (µg GAE/g)	Total Antioxidant Activity (% Inhibition)
Year (Y)	41763509.6*	2951.0*
Nitrogen (N)	5416567.0**	248.8**
Genotype (G)	1626642.0**	309.2**
YxN	1412684.2**	359.8**
YxG	1044935.6**	346.0**
NxG	949391.8**	354.4**
YxNxG	1018981.3**	340.9**
Significance levels: *: $p \leq 0.05$, **: $p \leq 0.01$		

Nitrogen is an essential nutrition that causes major changes in grain chemical composition and end-use quality (Kong et al., 2017). We observed significant changes on phenolic content and antioxidant activity caused by nitrogen application.

When the year factor was taken into account, it was evident that higher values were obtained in the dry season for both parameters. It was determined that the total phenolic content and antioxidant activity of wheat grain increased in the dry season (2018). In general, lower values were obtained under favorable climatic conditions. Phenolic acids are secondary metabolites that are synthesised as a defence system against stress conditions. As with many other antioxidants, they are mainly located in the outer layers of grain. With regard to stress conditions (water deficit and high temperature), an increase in antioxidant synthesis has been observed in cereals during the grain filling period (Zrcková et al., 2018). The primary cause of this result can be attributed to the observation of elevated values during the dry and hot season following the flowering period in the second year. This situation is hypothesized to be a consequence of the expansion of the bran layer and fiber component, which is accompanied by a reduction in grain weight (Žilić et al., 2012). The total phenol content of bread wheat genotypes at varying nitrogen doses over two years exhibited in a wide range of 771.9-5179.9 µg GAE/g, with the highest value observed in the Golia variety at N_0 kg ha^{-1} nitrogen dose during the 2018 season, and the lowest value noted in the İkizce variety at N_0 kg ha^{-1} nitrogen dose during the 2017 season (Table 7). Stumpf et al. (2015) suggested that the application of nitrogen fertilizer resulted in inverse effects on the concentration of phenolic compounds are predominantly located in the outer layers of the grain, suggesting that an increase in the proportion of bran fraction within the grain may potentially lead to an elevated concentration of phenolic compounds. The phenolic content of some genotypes (Line 2, Eraybey, Bozkır, Euclide and Hybery) exhibited a notable increase in general at the highest nitrogen dose during the dry season. However, for some of the genotypes (Golia, Line 3 and Julius), the lowest dose of nitrogen resulted in the highest values. The highest antioxidant activity values were obtained from Line 2 with N_{60} kg ha^{-1} and Bozkır variety with N_0 kg ha^{-1} nitrogen applications (Table 7).

Table 7. The mean values of total phenolic content (µg GAE/g) and total antioxidant acitivty (% inhibition)

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Total Phenolic Content (µg GAE/g)	Golia	2118.7	1731.0	2187.8	3564.3	5179.9	2530.6	2614.4	2842.6
	Kate A	1943.0	2189.0	1788.8	1722.1	3274.1	2319.2	2404.6	2386.2
	Selimiye	2158.6	1362.9	1948.7	1905.3	2411.8	2388.3	2406.5	2884.4
	Ceyhan 99	2472.2	2508.6	2747.7	2375.6	2244.0	2217.7	2037.2	2467.1
	Tosunbey	3892.3	1405.0	1555.3	2271.2	2591.2	2393.9	2538.2	2568.6
	İkizce-96	771.9	1980.3	1806.7	1799.8	3495.8	2615.8	1960.9	2461.9
	Müfitbey	1526.4	1606.1	2136.3	2167.6	2982.0	2406.6	2310.8	2853.0
	Line 1	1727.1	2066.4	1863.5	1963.1	2392.4	2283.4	2446.0	2534.9
	Line 2	1712.7	2746.8	2239.8	1718.7	2278.7	2768.2	2368.3	4143.1
	Eraybey	1750.2	2128.0	1578.6	2166.1	2775.0	3164.6	2538.4	3976.2
	Bozkır	4553.6	2282.2	2464.5	1842.8	3143.6	3181.0	2286.5	3406.3
	Line 3	3549.4	2310.7	2347.8	2170.6	5146.7	2137.8	2384.2	2902.4
	Euclide	2051.9	1708.5	1628.9	2298.0	2561.9	2887.9	2084.4	2813.0
	Julius	1778.4	2051.1	1845.0	1819.8	4350.6	2973.7	2747.2	3136.7
	Hybery	2165.3	2005.8	1914.0	2121.9	2832.3	2619.7	2475.3	3558.8
	Mean	2278.1	2005.4	2003.5	2127.1	3177.3	2592.5	2373.5	2995.6
	Lsd Y: 620.9; Lsd N: 121.4; Lsd YxN: 171.7; Lsd G: 235.1; Lsd YxG: 332.5; Lsd NxG: 470.2; Lsd YxNxG: 664.9								
	N (kg ha^{-1}) /G	**2017**				**2018**			
		N_0	**N_{60}**	**N_{120}**	**N_{180}**	**N_0**	**N_{60}**	**N_{120}**	**N_{180}**
Total Antioxidant Activity (%)	Golia	47.1	16.9	23.4	53.9	42.4	22.4	27.3	28.4
	Kate A	20.7	22.6	18.9	20.1	17.4	27.2	30.7	33.9
	Selimiye	22.7	6.8	28.7	21.4	28.5	32.1	26.8	30.4
	Ceyhan 99	28.3	26.5	39.9	37.9	30.7	29.5	28.5	29.3
	Tosunbey	46.1	12.5	14.7	22.6	15.3	27.5	30.6	48.6
	İkizce-96	15.9	30.2	18.6	31.3	50.4	26.4	29.7	30.0
	Müfitbey	19.6	23.0	32.4	25.4	26.4	25.2	32.2	30.5
	Line 1	22.9	32.5	40.7	22.3	14.1	33.8	36.4	39.2
	Line 2	10.0	64.2	24.3	16.7	28.2	25.7	27.6	36.7
	Eraybey	39.6	19.5	20.0	44.4	32.7	30.2	26.6	34.5
	Bozkır	61.4	12.6	30.4	9.9	52.2	35.8	35.6	35.4
	Line 3	51.3	23.6	25.0	33.9	12.5	31.8	34.7	38.1
	Euclide	15.9	13.3	13.7	15.7	14.0	29.0	41.9	27.7
	Julius	18.8	20.4	11.6	13.5	29.6	44.3	28.4	36.1
	Hybery	8.9	19.7	19.6	22.6	32.9	32.7	29.2	45.0
	Mean	28.6	23.0	24.1	26.1	28.5	30.2	31.1	34.9
	Lsd Y: 4.0; Lsd N: 1.1; Lsd YxN: 1.6; Lsd G: 2.2; Lsd YxG: 3.2; Lsd NxG: 4.5; Lsd YxNxG: 6.4								
	color diagram: red-white-green. This sequence is characterized by an increase in value from left to right								

Similar to the results obtained for the total phenolic content, an increase in antioxidant activity values was observed in some genotypes at the highest nitrogen dose in the dry season. The similar values and responses to N applications in terms of total phenolic content and antioxidant activity results are thought to be due to the high correlation between phenolic content and antioxidant activity as noted in previous studies (Mpofu et al., 2006; Verma et al., 2008; Žilić et al., 2012; Arshad vd., 2017; Boukid et al., 2019).

Essential amino acids profile (g 100 g^{-1})

Protein quality depends on the profile of essential amino acid present in foods. The amino acid composition of cereal grains is largely determined by the endosperm, which constitutes approximately 80% of the grain's weight. The aleurone and embryo tissues of grains exhibit a higher essential amino acid content compared to other grain components (Shewry, 2007). Lysine is the most limiting amino acid in wheat. The nutritional quality is determined by the higher protein and limiting amino acid content, especially lysine (Chaudhary et al., 2022). In addition to its significance in nutritional physiology, the amino acid lysine plays a crucial role in plant stress resistance. Lysine is employed to enhance plant resilience against abiotic and biotic stresses, for the synthesis of glutamate amino acid, and most notably, for the synthesis of glutamate, which plays a pivotal role in the establishment of plant defense systems (Sumer and Erten, 2023). Statistically significant different results ($p \leq 0.01$) were observed according to YxNxG interaction in all evaluated essential amino acid parameters (Table 8). In contrast, the essential amino acid content exhibited variability in response to nitrogen and genotype factors. However, no significant influence of the year factor was observed (Lysine, Phenylalanine, Valine and Methionine) for a portion of amino acids. No interaction between year and nitrogen was observed with respect to the most valuable essential amino acids, namely lysine and methionine.

Table 8. Statistical values (ANOVA) of essential amino acid composition (mean square values and significance levels) (g 100 g^{-1})

Experimental factors	LYS	THR	PHE	ISO	LEU	VAL	MET
Year (Y)	0.016	0.037**	0.025	0.073**	0.102**	0.010	0.001
Nitrogen (N)	0.031**	0.012**	0.017**	0.008**	0.003	0.006**	0.001**
Genotype (G)	0.010**	0.004**	0.011**	0.004**	0.011**	0.003**	0.002**
YxN	0.002	0.021**	0.010**	0.002*	0.007**	0.021**	0.000
YxG	0.012**	0.002**	0.009**	0.007**	0.007**	0.007**	0.002**
NxG	0.015**	0.004**	0.010**	0.004**	0.005**	0.007**	0.002**
YxNxG	0.019**	0.004**	0.008**	0.007**	0.007**	0.005**	0.002**

Significance levels: *: $p \leq 0.05$, **: $p \leq 0.01$
Lysine: LYS; Threonine: THR; Phenylalanine: PHE; Isoleucine: ISO; Leucine: LEU; Valine: VAL; Methionine: MET

Interestingly, for higher values of the lysine amino acid was generally found in many genotypes (Golia, Kate A, Selimiye, Ceyhan 99, Tosunbey, İkizce-96, Müfitbey, Line 1, Line 2 and (Eraybey) at the highest nitrogen dose during the dry season (2018). Kate A (2017, N60 kg ha^{-1}) variety had highest lysine content (1.610 g 100 g^{-1}) than other genotypes. With regard to the specific point of lysine, the results indicated that Tosunbey exhibited higher values in all evaluated nitrogen doses and in both growing seasons. Furthermore, the stability of the lysine amino acid revealed the significance of genetic potential of Tosunbey.

The impact of the year factor on the outcomes for the amino acids threonine, isoleucine and valine is notable. The concentration of the threonine amino acid was observed to be higher during the dry season (2018), whereas the levels of the isoleucine and leucine amino acids were found to be elevated during the first year of the study (Table 10 and 11). The concentration of the threonine amino acid reached elevated levels at both the lowest (N_0) and highest (N_{180}) nitrogen doses during the dry growing season, contingent on the genotypes. Furthermore, a notable elevation in threonine amino acid levels was observed across all genotypes (with the exception of Hybery) when the nitrogen dose applied during the dry season was augmented from 120 to 180 kg ha^{-1}(Table 9). The mean values of isoleucine amino acid were analyzed, and it was determined that some genotypes (Ceyhan 99, Tosunbey, İkizce-96, Müfitbey, Line 1 and 2, Bozkır, Euclide, Julius, Hybery) with high values in the first year exhibited significant losses in isoleucine amino acid content during the dry season. It is assumed that these genotypes, which are typically winter-growing genotypes, lack an adequate level of isoleucine amino acid content in grain during the dry season. This may be attributed to a deficiency in assimilate accumulation in the plants. The values of valine, another amino acid significantly affected by the year factor, exhibited a general tendency to reach high values at a nitrogen doses of 60 (N_{60}) and 120 (N_{120}) kg ha^{-1} during the dry season (Table 10).

Noberbekova et al. (2018) reached the conclusion that, despite the differing change in amino acid amounts between the varieties in response to nitrogen fertilizer doses, the application of two times nitrogen fertilizer applications and an increasing nitrogen amount resulted in a significant increase in essential amino acids, namely valine, leucine, isoleucine, and threonine. Additionally, this resulted in an increase in the biological value of total protein. The application of nitrogen fertilizer resulted in a positive response from the phenylalanine amino acid, with an observed increase in phenylalanine content in

correlation with the increase in nitrogen content. The lowest phenylalanine content was observed in genotypes that did not receive nitrogen application and exhibited low doses, while higher values were attained with increasing nitrogen doses. In the first experimental period, considerable elevations in phenylalanine concentration were discerned in Eraybey, Bozkır, Line 3, and Euclide genotypes, spanning the range of nitrogen doses from the lowest to the highest (Table 10). The leucine amino acid, which is the amino acid where the effect of the year factor is most evident, exhibited low values across all nitrogen doses (particularly for N_{120}) and genotypes, particularly during the dry season. Furthermore, a similar trend was observed in 2017, with genotypes demonstrating higher leucine amino acid values under generally suitable climatic conditions and a 60 kg ha^{-1} nitrogen dose (Table 11). Another the most limiting essential amino acid methionine takes specific and significant parts in many biochemical processes in plants. It plays a crucial role in protein synthesis and carbon metabolism, and its sulfur-bound methyl group. Methionine contributes plant physiology as cope with drought by boosting antioxidants (Maqbool et al., 2022). The methionine amino acid values exhibited a narrow spectrum distribution, with a range of 0.151-0.244 g 100 g^{-1}. The methionine content of the examined genotypes remained unaltered when subjected to different nitrogen doses, and no definitive conclusion could be drawn. In the first experimental year climatic conditions, the samples from Tosunbey and İkizce-96 exhibited elevated methionine levels across all nitrogen doses (Table 12).

Table 9. Observed lysine and threonine mean results of genotypes grown in different nitrogen applications in the period 2017 and 2018.

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Lysine Content (g 100 g^{-1})	Golia	1.139	1.208	1.286	1.283	1.219	1.289	1.229	1.265
	Kate A	1.128	1.610	1.180	1.148	1.141	1.124	1.168	1.276
	Selimiye	1.094	1.274	1.082	1.202	1.273	1.237	1.208	1.266
	Ceyhan 99	1.207	1.210	1.173	1.237	1.141	1.296	1.197	1.236
	Tosunbey	1.251	1.254	1.222	1.260	1.261	1.244	1.251	1.296
	İkizce-96	1.169	1.144	1.284	1.214	1.164	1.226	1.256	1.253
	Müfitbey	1.287	1.182	1.195	1.164	1.155	1.148	1.271	1.246
	Line 1	1.196	1.083	1.287	1.175	1.188	1.191	1.160	1.287
	Line 2	1.096	1.119	1.148	1.211	1.169	1.161	1.273	1.231
	Eraybey	1.098	1.116	1.257	1.324	1.157	1.233	1.176	1.294
	Bozkır	1.169	1.267	1.213	1.236	1.274	1.174	1.299	1.178
	Line 3	1.203	1.179	1.250	1.163	1.298	1.170	1.177	1.209
	Euclide	1.157	1.161	1.152	1.165	1.181	1.263	1.133	1.251
	Julius	1.302	1.268	1.309	1.093	1.133	1.226	1.219	1.119
	Hybery	1.090	1.182	1.107	1.308	1.132	1.270	1.297	1.122
	Mean	1.172	1.217	1.209	1.212	1.192	1.216	1.220	1.235
	Lsd N: 0.017; Lsd G: 0.033; Lsd YxG: 0.047; Lsd NxG: 0.066; Lsd YxNxG: 0.093								
	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Threonine Content (g 100 g^{-1})	Golia	0.532	0.567	0.567	0.571	0.634	0.549	0.557	0.641
	Kate A	0.547	0.543	0.553	0.568	0.590	0.627	0.509	0.611
	Selimiye	0.581	0.575	0.487	0.505	0.518	0.581	0.516	0.626
	Ceyhan 99	0.567	0.519	0.546	0.539	0.59	0.550	0.512	0.585
	Tosunbey	0.582	0.562	0.517	0.566	0.582	0.511	0.544	0.500
	İkizce-96	0.534	0.531	0.583	0.611	0.524	0.534	0.578	0.612
	Müfitbey	0.573	0.529	0.564	0.543	0.623	0.620	0.504	0.606
	Line 1	0.509	0.547	0.567	0.581	0.624	0.498	0.525	0.625
	Line 2	0.492	0.597	0.553	0.596	0.641	0.514	0.528	0.571
	Eraybey	0.564	0.575	0.541	0.518	0.552	0.640	0.599	0.606
	Bozkır	0.538	0.558	0.582	0.540	0.609	0.635	0.504	0.569
	Line 3	0.577	0.542	0.594	0.527	0.638	0.584	0.492	0.541
	Euclide	0.571	0.536	0.621	0.567	0.614	0.607	0.592	0.631
	Julius	0.491	0.528	0.541	0.526	0.592	0.498	0.557	0.626
	Hybery	0.562	0.593	0.564	0.543	0.604	0.586	0.549	0.538
	Mean	0.548	0.553	0.558	0.553	0.595	0.568	0.537	0.592
	Lsd Y: 0.006; Lsd N: 0.006; Lsd G: 0.012; Lsd YxN: 0.009; Lsd YxG: 0.017; Lsd NxG: 0.024; Lsd YxNxG: 0.035 color diagram: red-white-green. This sequence is characterized by an increase in value from left to right								

Table 10. Observed phenylalanine and isoleucine mean results of genotypes grown in different nitrogen applications in the period 2017 and 2018.

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Phenylalanine Content (g 100 g^{-1})	Golia	0.795	0.635	0.646	0.716	0.677	0.669	0.761	0.743
	Kate A	0.801	0.677	0.671	0.790	0.624	0.661	0.718	0.691
	Selimiye	0.698	0.671	0.638	0.652	0.698	0.805	0.699	0.633
	Ceyhan 99	0.642	0.646	0.717	0.724	0.624	0.686	0.771	0.801
	Tosunbey	0.728	0.697	0.738	0.721	0.730	0.718	0.733	0.820
	İkizce-96	0.673	0.646	0.686	0.677	0.711	0.767	0.807	0.734
	Müfitbey	0.733	0.724	0.798	0.786	0.657	0.815	0.753	0.744
	Line 1	0.726	0.781	0.732	0.760	0.640	0.787	0.644	0.822
	Line 2	0.734	0.813	0.676	0.664	0.759	0.816	0.74	0.703
	Eraybey	0.631	0.801	0.778	0.785	0.749	0.806	0.732	0.770
	Bozkır	0.668	0.702	0.644	0.809	0.640	0.645	0.771	0.654
	Line 3	0.666	0.639	0.718	0.781	0.633	0.675	0.724	0.815
	Euclide	0.640	0.719	0.693	0.768	0.797	0.743	0.756	0.681
	Julius	0.683	0.702	0.679	0.634	0.791	0.718	0.745	0.719
	Hybery	0.758	0.648	0.801	0.715	0.771	0.762	0.698	0.748
	Mean	0.705	0.700	0.707	0.732	0.700	0.738	0.736	0.738
	Lsd N: 0,007; Lsd G: 0,013; Lsd YxN: 0,010; Lsd YxG: 0,018; Lsd NxG: 0,026; Lsd YxNxG: 0,037								

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Isoleucine Content (g 100 g^{-1})	Golia	0.429	0.422	0.457	0.456	0.373	0.355	0.464	0.411
	Kate A	0.459	0.358	0.357	0.484	0.420	0.364	0.455	0.323
	Selimiye	0.390	0.358	0.385	0.375	0.409	0.456	0.420	0.466
	Ceyhan 99	0.406	0.419	0.379	0.397	0.420	0.329	0.351	0.385
	Tosunbey	0.389	0.442	0.466	0.440	0.409	0.324	0.443	0.373
	İkizce-96	0.481	0.481	0.462	0.415	0.358	0.386	0.322	0.462
	Müfitbey	0.454	0.476	0.427	0.441	0.367	0.399	0.377	0.417
	Line 1	0.433	0.407	0.363	0.390	0.449	0.396	0.334	0.448
	Line 2	0.353	0.358	0.444	0.362	0.461	0.375	0.332	0.338
	Eraybey	0.465	0.379	0.453	0.473	0.458	0.366	0.418	0.338
	Bozkır	0.362	0.428	0.396	0.455	0.398	0.454	0.327	0.434
	Line 3	0.471	0.459	0.375	0.453	0.404	0.382	0.433	0.440
	Euclide	0.432	0.404	0.399	0.405	0.329	0.421	0.375	0.458
	Julius	0.466	0.461	0.443	0.459	0.378	0.417	0.340	0.396
	Hybery	0.447	0.487	0.410	0.367	0.414	0.326	0.331	0.444
	Mean	0.429	0.422	0.414	0.424	0.403	0.383	0.381	0.408
	Lsd N: 0.007; Lsd G: 0.014; Lsd YxN: 0.010; Lsd YxG: 0.020; Lsd NxG: 0.029; Lsd YxNxG: 0.040								
	color diagram: red-white-green. This sequence is characterized by an increase in value from left to right								

Table 11. Observed leucine and valine mean results of genotypes grown in different nitrogen applications in the period 2017 and 2018.

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Leucine Content (g 100 g^{-1})	Golia	0.691	0.639	0.662	0.596	0.583	0.573	0.571	0.561
	Kate A	0.568	0.716	0.635	0.562	0.638	0.619	0.611	0.597
	Selimiye	0.638	0.565	0.642	0.670	0.549	0.613	0.573	0.610
	Ceyhan 99	0.690	0.632	0.667	0.663	0.638	0.616	0.648	0.659
	Tosunbey	0.618	0.675	0.582	0.684	0.659	0.560	0.666	0.689
	İkizce-96	0.714	0.577	0.736	0.690	0.669	0.619	0.555	0.678
	Müfitbey	0.719	0.684	0.736	0.733	0.593	0.574	0.663	0.591
	Line 1	0.681	0.687	0.624	0.710	0.682	0.633	0.669	0.658
	Line 2	0.621	0.715	0.723	0.564	0.654	0.65	0.555	0.667
	Eraybey	0.572	0.591	0.584	0.672	0.605	0.608	0.583	0.653
	Bozkır	0.567	0.737	0.700	0.654	0.671	0.608	0.577	0.576
	Line 3	0.727	0.692	0.623	0.677	0.542	0.676	0.567	0.627
	Euclide	0.597	0.699	0.650	0.634	0.591	0.595	0.653	0.647
	Julius	0.655	0.712	0.674	0.605	0.661	0.603	0.661	0.694
	Hybery	0.637	0.596	0.585	0.637	0.593	0.647	0.545	0.636
	Mean	0.646	0.661	0.654	0.650	0.621	0.612	0.606	0.636
	Lsd Y: 0,013; Lsd G: 0,023; Lsd YxN: 0,017; Lsd YxG: 0,033; Lsd NxG: 0,046; Lsd YxNxG: 0,066								

	N (kg ha^{-1}) /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Valine Content (g 100 g^{-1})	Golia	0.686	0.639	0.625	0.689	0.631	0.629	0.655	0.593
	Kate A	0.557	0.721	0.598	0.701	0.652	0.738	0.58	0.701
	Selimiye	0.710	0.667	0.639	0.708	0.662	0.689	0.592	0.707
	Ceyhan 99	0.662	0.624	0.682	0.644	0.652	0.676	0.699	0.605
	Tosunbey	0.699	0.654	0.634	0.614	0.617	0.681	0.685	0.670
	İkizce-96	0.641	0.656	0.614	0.628	0.601	0.633	0.656	0.599
	Müfitbey	0.674	0.641	0.584	0.669	0.705	0.715	0.727	0.592
	Line 1	0.624	0.710	0.562	0.623	0.702	0.725	0.623	0.703
	Line 2	0.642	0.628	0.668	0.607	0.595	0.682	0.713	0.713
	Eraybey	0.734	0.624	0.629	0.607	0.631	0.709	0.631	0.649
	Bozkır	0.707	0.636	0.731	0.666	0.698	0.675	0.608	0.594
	Line 3	0.711	0.657	0.663	0.651	0.587	0.722	0.677	0.58
	Euclide	0.580	0.568	0.626	0.676	0.650	0.744	0.706	0.631
	Julius	0.643	0.590	0.692	0.722	0.633	0.635	0.679	0.681
	Hybery	0.674	0.645	0.635	0.642	0.668	0.708	0.708	0.660
	Mean	0.662	0.644	0.638	0.656	0.645	0.690	0.662	0.645
	Lsd N: 0.009; Lsd G: 0.017; Lsd YxN: 0.012; Lsd YxG: 0.024; Lsd NxG: 0.034; Lsd YxNxG: 0.048								
	color diagram: red-white-green. This sequence is characterized by an increase in value from left to right								

Table 12. Observed methionine mean results of genotypes grown in different nitrogen applications in the period 2017 and 2018.

	N (kg ha$^{-1)}$ /G	2017				2018			
		N_0	N_{60}	N_{120}	N_{180}	N_0	N_{60}	N_{120}	N_{180}
Methionine Content (g 100 g^{-1})	Golia	0.224	0.178	0.173	0.170	0.176	0.162	0.231	0.224
	Kate A	0.206	0.212	0.243	0.238	0.192	0.242	0.184	0.181
	Selimiye	0.203	0.220	0.229	0.190	0.209	0.186	0.206	0.192
	Ceyhan 99	0.167	0.222	0.196	0.189	0.192	0.170	0.193	0.172
	Tosunbey	0.238	0.204	0.211	0.221	0.178	0.168	0.208	0.225
	İkizce-96	0.216	0.190	0.216	0.198	0.177	0.168	0.220	0.185
	Müfitbey	0.188	0.217	0.181	0.163	0.238	0.194	0.215	0.232
	Line 1	0.244	0.198	0.220	0.172	0.225	0.176	0.164	0.163
	Line 2	0.184	0.159	0.160	0.203	0.163	0.222	0.190	0.206
	Eraybey	0.174	0.244	0.163	0.173	0.177	0.202	0.163	0.174
	Bozkır	0.193	0.166	0.240	0.226	0.196	0.213	0.185	0.178
	Line 3	0.198	0.184	0.192	0.243	0.230	0.170	0.204	0.196
	Euclide	0.167	0.176	0.156	0.206	0.188	0.200	0.214	0.214
	Julius	0.223	0.151	0.155	0.185	0.166	0.170	0.196	0.180
	Hybery	0.196	0.163	0.228	0.179	0.244	0.216	0.200	0.168
	Mean	0.201	0.192	0.197	0.197	0.196	0.190	0.198	0.192
	Lsd N: 0.004; Lsd G: 0.008; Lsd YxG: 0.012; Lsd NxG: 0.016; Lsd YxNxG: 0.023 color diagram: red-white-green. This sequence is characterized by an increase in value from left to right								

Relationship Between Yield, Bread-Making Quality, Health and Nourishment Properties

Figure 1. shows the relationships between the evaluated parameters grown genotypes under different nitrogen applications for the period 2017 and 2018. According to the correlation coefficient results, grain yield showed a significant positive correlation with protein content (%) and wet gluten content (%) of r=0.290** and r=0.153**, respectively. This is the most attractive result that increasing yield values caused positive effect on bread-making quality and dilution effect between yield and protein was not observed contrary to previous studies (Erekul et al., 2012; Bagulho et al., 2015). A highly positive and significant relationship was found between protein content and wet gluten content (Mut et al., 2017; Siddiqi et al., 2020). However, increasing protein and wet gluten content resulted in an adverse effect on gluten quality (gluten and gluten index, r=-0.54***). Despite an observed increase in gluten amount and protein content, the resulting dough quality and gluten strength were found to be unsatisfactory.

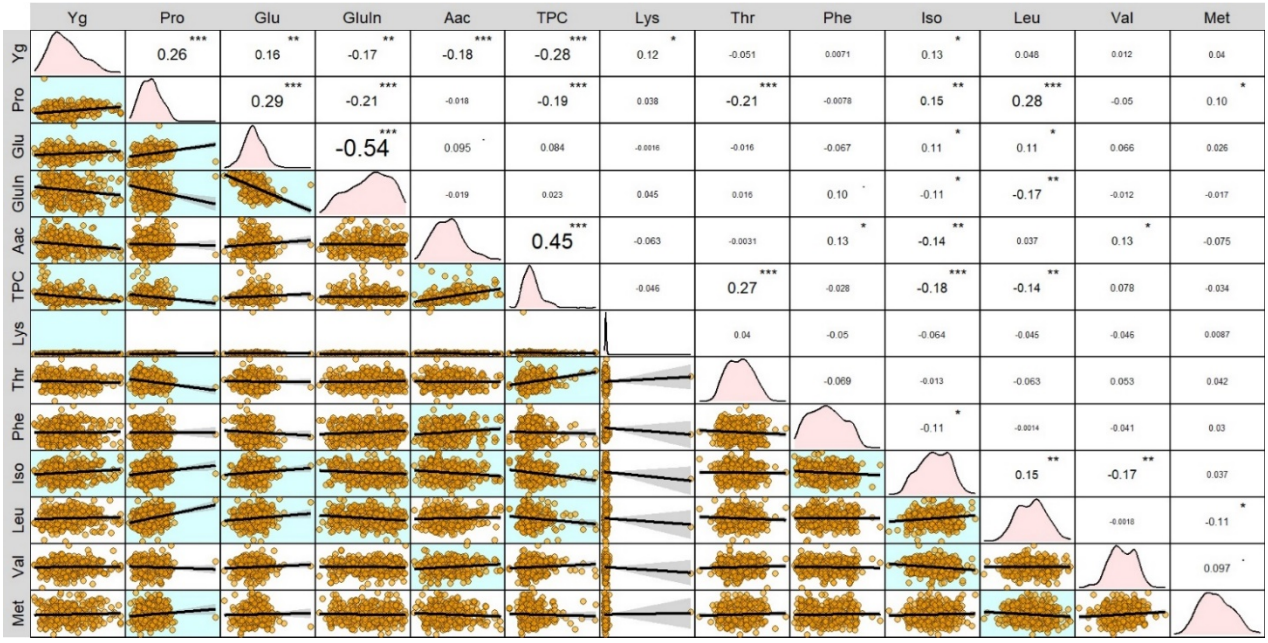

Figure 1. Correlation matrix showing the relationships between yield, bread-making quality, antioxidant and essential amino acids (Significance levels: ns: not significant, *: $p \leq 0.05$, **: $p \leq 0.01$, *** $p \leq 0.001$; Yg: Grain Yield; Pro: Protein Content; Glu: Wet Gluten Content; GluIn: Gluten Index; TPC: Total phenolic content; Aac; Total Antioxidant Activity; Lysine: Lys; Threonine: Thr; Phenylalanine: Phe; Isoleucine: Iso; Leucine: Leu; Valine: Val; Methionine: Met)

In contrast, an inverse and statistically significant correlation was observed between yield and health-related traits. The total phenol content and antioxidant activity properties demonstrated a decline with an increase in yield values.

Moreover, a notable and significant positive correlation (r=0.450**) was identified between total phenolic content and antioxidant activity, a finding that is consistent with the results reported by other researchers in the literature (Mpofu et al., 2006; Žilić et al., 2012; Zeibig et al., 2024). The increase in protein content contributed significantly and positively to the essential amino acid composition as also obtained same correlation results in our previous study (Yigit and Erekul, 2023). The amount of isoleucine (r=0.150**), leucine (0.284**) and methionine (r=0.104*) amino acids increased with the increase in protein content. Nevertheless, this is not the case for all essential amino acids (threonine; r=-0.210**), which results in a reduction in the quantity of certain amino acids, thereby indicating a complex relationship between protein and essential amino acid composition (Figure 1). Furthermore, when the interrelationships of essential amino acids are examined in general terms, it becomes evident that increases in the amount of some amino acids have negative effect on others. This illustrates that amino acid composition and physiology have complex structure.

CONCLUSION

Our study has clearly demonstrated the effects of nitrogen application on yield, bread-making, antioxidant and essential amino acid composition of bread wheat. In the research we conducted to determine the effect of nitrogen doses on genotypes with different genetic and growth habit properties, YxNxG interaction was significant in all traits due to the different climatic conditions observed in both growing seasons. The impact of climatic conditions on evaluated traits was notably identified, particularly during the second year of the study due to the prevalence of a dry growing season (2018). While a reduction was observed in yield and protein values, particularly during the dry season, an increase was noted in the gluten index, which is a key determinant of gluten strength and is therefore an important factor in bread quality, as well as in total phenolic content and antioxidant activity, which contribute to health. The total phenol content and antioxidant activity values exhibited a decline in certain genotypes because of the increased application of nitrogen fertilizers. However, an increase in health-relevant compounds in the grain was observed in some genotypes grown in the dry season (mostly winter-growth habit) with increasing nitrogen fertilizer doses. Due to the increasing sensitivities on climate change, environmental and human health and quality nutrition came to the forefront in recent years, important findings were determined by analyzing the yield, bread quality characteristics, antioxidant and amino acid composition characteristics of the results obtained within the scope of the research. Therefore, it was concluded that newly released and old genotypes have significant potential with respect to the nutritional and health traits. For this reason, these traits are important for the development of healthy products and varieties in breeding programs in the future.

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YIELD AND QUALITY RESPONSES OF SOYBEAN (*Glycine max.* L. Merr.) VARIETIES INOCULATED WITH *RHIZOBIA* STRAINS UNDER DROUGHT STRESS

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ABSTRACT

The aim of this study was to investigate the effects of nodule formation and nodulation performance of *Rhizobia* bacteria on yield and quality in soybean roots under limited water application conditions in soybean varieties depending on climate change. In this study, 4 different irrigation applications (limited vs irrigated: 25%, 50%, 75%, 100%), 2 soybean varieties (Cinsoy and Altinay) and 3 different *Rhizobia* inoculants (Control, AZOTEK-2, USDA-110) were applied for two years (2020-2021) in order to determine the yield and quality characteristics of soybean. The experiment was established according to randomized complete block split-split plots experimental design with 3 replications. In the experiment, the main factor was *Rhizobia* inoculant treatments, the first sub-factor was soybean varieties, and the second sub-factor was irrigation applications. Within the scope of the study, yield and quality parameters such as plant height (cm), first pod height (cm), number of pods plant^{-1}, number of seeds pod^{-1}, seed yield (kg ha^{-1}), 1000 seed weight (g), leaf area (cm^2 plant^{-1}), seed crude protein (%) and oil content (%) were examined. It was concluded that irrigation and inoculant applications and combinations of these factors had significant effects on yield parameters of soybean varieties. The study revealed that there was no discernible nodulation development observed in soybean roots under both irrigated and limited irrigation conditions. It was determined that under conditions of limited irrigation combined with high temperature conditions, the growth of the soybean was significantly impacted, resulting in a notable reduction in yield and leaf area but this was not observed in the quality characteristics.

Keywords: *Bradyrhizobium*, Drought, Irrigation, Nitrogen fixation, Soybean, Water stress

INTRODUCTION

Soybean (*Glycine max.* L. Merr.) is one of the most widely cultivated legumes in the world due to its valuable seed composition. Soybean is used in human and animal nutrition due to its oil (18-20%), protein (36-40%), carbohydrate (30%) and mineral substance (5%) content (Shea et al., 2024). Soybean is one of the most important oilseed crops worldwide and contains high amounts of protein for human food and animal feed (Wysokinski et al., 2024). Growing awareness of sustainable food systems has led to an increased demand for plant-based proteins, increasing the popularity of soybeans with their high protein quality (Zhang et al., 2021). Soybean is a legume plant that can form tubers, called nodules, on its roots with *Rhizobium* bacteria and bind free nitrogen from the air into the soil. *Bradyrhizobium japonicum* is the strain of bacteria that can form effective nodules in soybeans (Miransari, 2016).

Extreme weather events, such as droughts, reduce crop production, making it difficult to obtain and distribute crops worldwide. High temperatures occur during critical phenological stages, causing crop yield and quality losses (Yigit and Chmielewski, 2024). With climate change on the rise, long periods of high temperatures in semi-arid regions result in water scarcity for crop production (Parkes et al, 2022). Irrigation water is the most important input for increasing yield under arid and semi-arid conditions. Limited irrigation strategies are an important production alternative to reduce water demand in agricultural production (Miransari et al., 2022). Drought increases the stress levels in plants, shortens their development periods and decreases yield potential (Hatfield and Prueger, 2015). The most damaging stages of heat and water stress in soybean are during flowering and pod development. Water stress experienced during these stages leads to disruption of flower structure and pollen sterility. On the other hand, high temperature conditions affect assimilate accumulation in pods, leading to smaller seeds and therefore lower yields (Soba et al., 2022). Drought stress during the flowering and pod formation periods in soybean bean reduces the flowering rate, thus causing a decrease in the number of pods in the plant and leading to low yields (He et al., 2017). Restricted irrigation and drought conditions significantly

affect the protein and oil content in soybean seeds, and drought can increase the protein content and decrease the oil content (Hu and Wiatrak, 2012; Ravelambola, 2022). Poeta et al. (2016) also reported that the increase in protein content was positively and linearly related to the increase in water stress and negatively related to the oil content.

It is known that biological nitrogen fixation and photosynthesis are particularly sensitive in soybean under drought stress. Drought leads to inhibition of biological nitrogen fixation and limitation of nitrogen transport, resulting in reduced grain yield (Cerezini et al., 2020). Drought stress is an important factor affecting the symbiotic relationship. Nitrogen obtained by the soybean-*Rhizobia* association is of great agricultural importance. Under stress conditions, the number and development of *Bradyrhizobium japonicum* bacteria decrease (Akinrinlola et al., 2024). Nodulation in soybean occurs between 20-30 °C. At high temperatures, the number of roots in soybean decreases and capillary and lateral root development weakens. Therefore, nodulation initiation and nitrogen fixation may not occur under high temperature conditions (Miransari et al., 2022). Lumactud et al. (2023) reported that drought stress in the early vegetative stage significantly reduced nodule formation and nitrogen fixation in soybean. Under arid conditions, survival rates of *Rhizobia* present in the soil and introduced by seed/bacteria inoculation may be reduced (Thilakarathna et al., 2021). For this reason, it is necessary to select effective breeds with high nitrogen fixation power in sowing. Effective breeds are strain that can fix more nitrogen per unit of time and per plant (Sarimurat et al., 2022).

In this context, *Bradyrhizobium diazoefficiens* bacteria belonging to the USDA 110 strain was used in the inoculation of soybean seeds in the experiment, since it can grow under the temperature conditions in the soybean growing periods in Aydin province. The specific host plant of USDA 110 (*Bradyrhizobium diazoefficiens*) bacterial strain is soybean. This effective strain is a gram-negative (rod-shaped) bacterium that can develop a healthy symbiotic relationship and fix free nitrogen from the atmosphere. It is a bacterial strain that can fix nitrogen in the soil at higher rates than other species. It was first isolated from soybean nodules in the US state of Florida and is widely used in studies.

In this study, it was aimed to investigate the nodule formation performance of *Rhizobia* bacterial species in soybean, which is one of the most important plants for sustainable agriculture, and the effects of restricted irrigation conditions on both nodule formation and nitrogen fixation and plant growth stages in soybean varieties. In the study, it was aimed to provide an effective biological nitrogen fixation by working with USDA 110 *Bradyrhizobium diazoefficiens* bacteria strain, which has not been applied in Aydin ecological conditions before, and to develop this in sustainable agricultural systems. Depending on the treatments, yield and quality characteristics of soybean were also examined. Within the scope of the study, it was aimed to investigate the yield and quality parameters and nodulation performances of soybean varieties under high temperature and water scarcity conditions.

MATERIALS AND METHOD

Field experimental area and design

The field experiment was conducted during the 2020 and 2021 growing seasons in the experimental area, situated at 37°45'N 27°45'E on the Aydin Adnan Menderes University Faculty of Agriculture Research and Application Farm (Figure 1). In order to evaluate the yield and quality characteristics of soybean in the experiment, two mid-early soybean varieties (Cinsoy and Altinay) were provided as material from Aegean Agricultural Research Institute, Izmir. In the experiment, two *Rhizobia* bacterial materials were used for inoculation to soybean seeds. These materials were AZOTEK-2 (*Rhizobium spp.*) inoculant obtained from Ankara Soil, Fertilizer and Water Resources Central Research Institute and USDA 110 inoculant containing *Bradyrhizobium diazoefficiens* obtained from Humboldt-Universität zu Berlin, Germany.

Figure 1. Location and general view of field experiment

The experiment was carried out following the split-split plots over randomized complete block design. The main factor in the experiment was *Rhizobia* applications, the first sub-factor was soybean varieties, and the second sub-factor was irrigation (limited vs irrigated) applications. The experiment had 3 replications and consisted of 2 soybean varieties (Cinsoy and Altinay), 3 *Rhizobia inoculant* strain applications (Control, AZOTEK-2 and USDA-110) and 4 different irrigation (irrigated vs limited) treatments (25%, 50%, 75% and 100%). The experiment was established with a total of 72 plots. Each experiment plot was 5 x 2.80 m (4 row plot^{-1}) and had a total area 14 m^2 plot^{-1} at sowing.

The soil characteristics of the experimental site has a sandy loam soil structure with low organic matter (1.7%). The soil pH is alkaline (7.92), lime content was high (7.93%), sodium (89 ppm), potassium (224 ppm), calcium (2481 ppm) and phosphorus (11.53 ppm) levels were moderately useful.

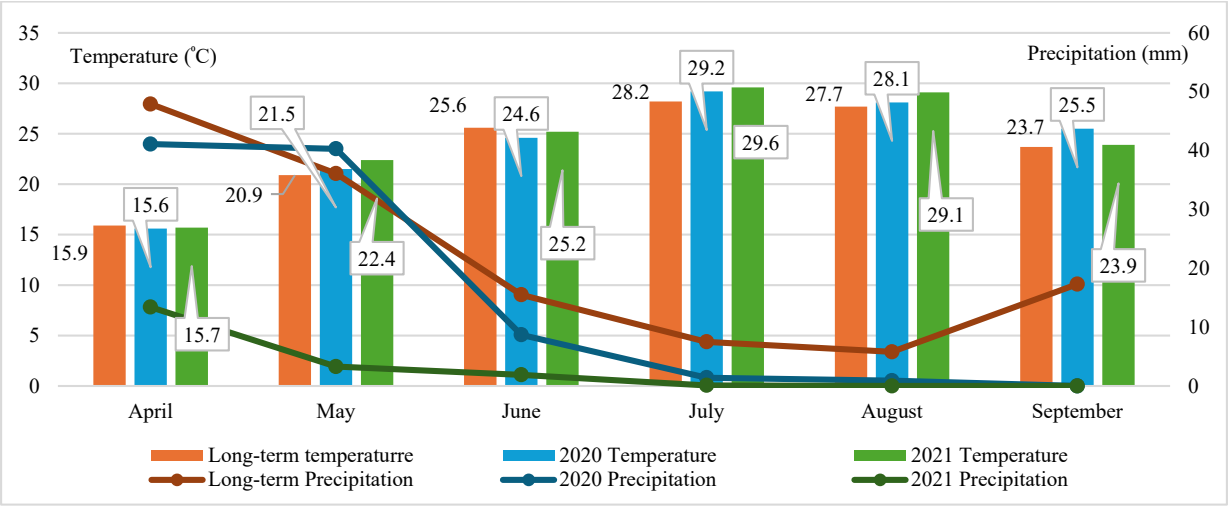

Figure 2. Monthly and long-term mean temperature and precipitation values (Turkish State Meteorological Service, Station: Kocarli/Aydin)

Figure 2 illustrates the comparison of temperature and precipitation values in Aydin province during the 2020 and 2021 soybean growing seasons, with data presented in relation to the long-term average. In both experimental years, it is observed that the total rainfall in the months of April through September is significantly below the long-term average rainfall. In general, the mean temperatures during the trial years were similar, with the exception of the July and August months for both years.

In the experiment, the irrigation water to be applied to the plots was calculated based on the cumulative evaporation amount (25%, 50%, 75% and 100%). The amount of irrigation water to be applied to the plots was calculated with different coefficients according to the evaporation amounts obtained from the Class A evaporation pan (US Weather Bureau Class A Pan) (Kanber, 1984), applications were made regularly once every 7 days using the drip irrigation system according to the equation below.

I=Kpc.Ep.P.A

[I: amount of irrigation to be applied to the plot, Kpc: evaporation container coefficient 100%, Ep: cumulative evaporation amount (mm), P: plant cover (%), A: plot area (m^2)]

In both years of the experiment, irrigation of plots started when the first flowers (BBCH 61) appeared and gradually terminated according to the maturity periods. The total amount of water applied to the plots; 858.77 mm irrigation water in 100%, 644.05 mm in 75%, 399.37 mm in 50% and 199.67 mm in 25% during the growth and development period in 2020. In the 2021 trial year, 816.98 mm irrigation water was given in 100%, 612.73 mm in 75%, 376.99 mm in 50% and 169.99 mm in 25% water application.

Sowing and maintenance

Sowing was carried out in the last week of April in both years of the experiment. Seeds were sown with a pneumatic seeder machine at a soil depth of 4-5 cm, taking soil moisture into consideration. In both years of the experiment, sowings were made one day apart due to different bacteria applications. In the sowing of the seeds, the varieties in the control blocks were sown without any *Rhizobia* inoculant application. In the sowing of AZOTEK-2 (*Rhizobium*) bacteria-treated plots, the varieties were inoculated in both experimental years so that the bacteria covered the entire surface of the seeds in a shaded environment. In USDA-110 bacteria application, soybean seeds were inoculated in both experimental years with USDA-110 bacteria in liquid form and sowing was completed. In both years, if nodulation was not at the desired level, fertilization was applied twice (at the beginning of flowering (BBCH 60) and at the beginning of pod setting (BBCH 70). During the development period, chemical control was carried out with the most common

pests (whitefly, green worm, thrips, Twospotted spider mite (*Tetranychus urticae* Koch), and *Spodoptera littoralis*).

Measurements and statistical analysis

In this study, agronomic, physiological and quality parameters such as plant height (cm), first pod height (cm), number of pods plant^{-1}, number of seeds pod^{-1}, 1000 seed weight (g), seed yield (kg ha^{-1}), leaf area (cm^2 plant^{-1}), crude protein content (%) and crude oil content (%) were investigated. No counts or statistical evaluations were made for nodules in the observations related to nodule formation in the experiment. Leaf area measurements were performed by sampling plants in each plot and the leaves were separated (all leaves plant^{-1}) and measured by using the LI-COR 3000 C (Lincoln, NE, USA) portable leaf area device in two times: the first at the flowering stage (BBCH 61-65, 10-50% flowering) and the second at the pod formation stage (BBCH 71-75, 10-50% pod formation). Grain protein content was determined in soybean flour (milled to 0.8 mm) samples according to the AOAC method 997.09 using Velp® Scientifica (Italy) NDA 702 DUMAS Nitrogen Analyzer at the Aydin Adnan Menderes University, Agricultural Biotechnology and Food Safety Application and Research Center (ADU-TARBIYOMER). Determination of crude oil content was made in the Solvent Extraction device in accordance with the Soxhlet method, based on the principle of extracting the oil using hexane solvent. Variance analyses of the data obtained in both years of the field trials were evaluated in the TARIST package program in accordance with the randomized complete block split-split plots trial design. The LSD test was used to compare the means (Acikgoz et al., 2004).

RESULTS AND DISCUSSION

The response of phenological development related to climate and observation of nodulation in roots

In both years of the experiment, observations were carried out on the plants rooted outside the edge rows and in the center of the plots in order to follow the nodule formations in the soybean roots. For this purpose, the soil in each plot was excavated to a depth of 0.50 m and 1 m and the root zones were examined under field conditions. In the first year of the experiment, although not in all plots, nodule formation on the roots was observed in AZOTEK-2 (*Rhizobium*) and USDA 110 (*Bradyrhizobium*) bacteria treatments. A better nodule formation was observed in both Cinsoy and Altinay varieties in USDA 110 inoculant application for 75% and 100% water treatments. However, the results of inoculation only observed in a few plants and upon examination of all plots, it was determined that stable and homogeneous nodule development was not evident. In the second year of the experiment, nodule formation in soybean roots in the plots were detected in much lower number as compared to the previous year where *Rhizobia* inoculants were applied. It is thought that the reason for this may be the high daily maximum temperature values experienced during the vegetative development periods in both years (Miransari et al., 2022). The impact of daily maximum temperature values on developmental periods is illustrated in Figure 3. In both experimental years, it was observed that daily maximum temperature values during the vegetative periods resulted in significant differences. Although the monthly temperature values during the phenological development periods were higher in the second year compared to the first year, the daily maximum temperature values did not exceed 35°C (except for a few days) in the second year in vegetative period. However, increasing daily maximum temperature values observed in flowering period of the second year. In the 2020 growing season, temperatures reached approximately 25°C at the time of sowing and remained at approximately 25-35°C range until the onset of flowering. In the 2021 experimental year, it was determined that the daily maximum temperature values exceeded 35°C between vegetation and end of flowering periods, commencing from the date of sowing. This period of elevated temperatures represents the most susceptible phase for the development of *Rhizobia* inoculation in soybean roots. It is hypothesized that this period of time coincides with the formation of signal compounds between bacteria and soybean roots (Soba et al. 2022). The daily maximum temperature values exerted a considerable influence on the flowering period (BBCH 60-69). In 2021, the daily temperature averages during the flowering period were below 35°C, indicating that the flowering period was more temperate than in 2020. In both years of the experiment, the pod setting (BBCH 70-79) periods exhibited minimal discrepancy. In the 2021 experimental year, higher daily maximum temperature values permitted the plants to reach maturity at a faster rate. Productivity under drought conditions varies depending on the intensity and duration of the stress occurred in which phenological developmental stages. The phenological development periods where drought is most effective are the period from sowing to emergence, flowering period and pod setting period (Lewandowska et al., 2020). The low yield of soybean is due to the stress conditions during the pod setting and grain filling periods rather than drought conditions during the flowering period (Staniak, et al., 2023). Drought stress at the onset of flowering results in a reduction in the number of pods. The stress experienced at the onset of flowering and at full flowering has been demonstrated to result in a reduction in the number of pods and grain size (Korte et al., 2023) Furthermore, the absence of elevated stress (heat) levels and earlier onset of the flowering period in the 2021 caused to reveal lower daily maximum temperature conditions (Figure 3) indicate that the yield was positively influenced (Table 2), aligning with the yield values obtained despite the elevated temperatures during the pod tying and grain maturity periods. Flower shedding is a consequence of drought stress during the flowering period. However, given that flowering occurs over a prolonged period (approximately 3-4 weeks) along the length of the stem, it is possible to offset the losses incurred through flower shedding. This suggests that the impact of drought stress during the pod setting period may be more pronounced than during the flowering phase (Mandić et al., 2020).

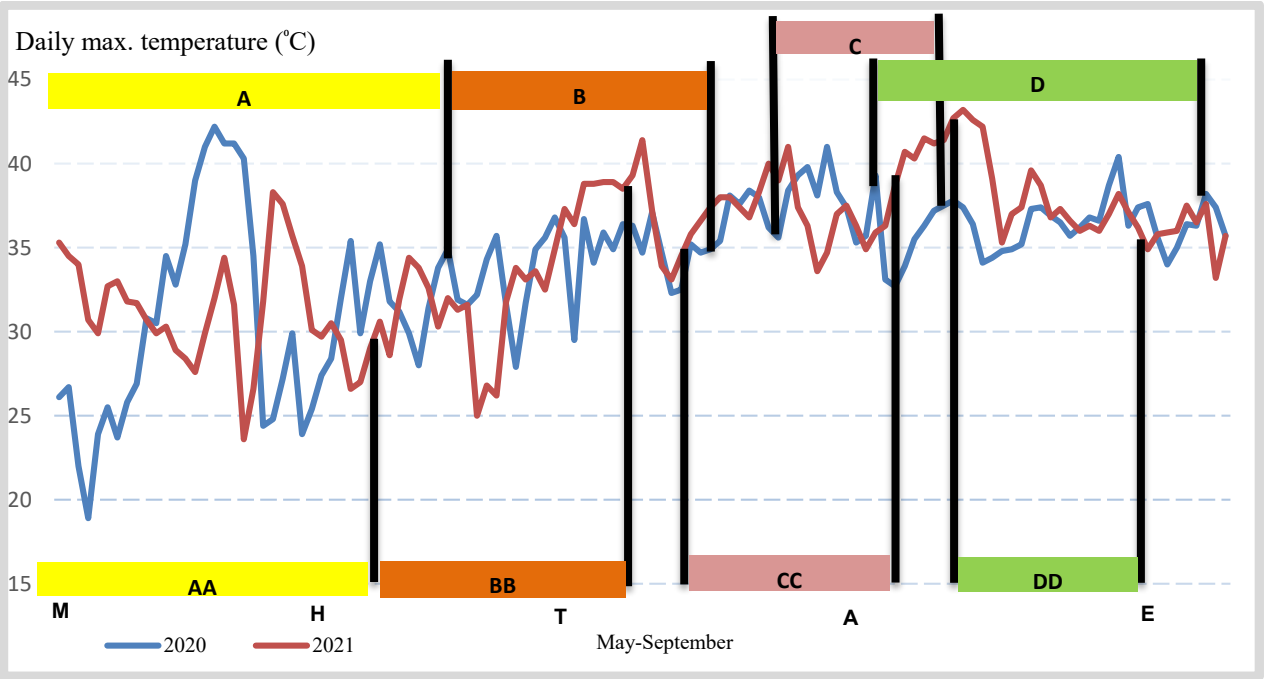

Figure 3. Daily maximum temperature values for the 2020 and 2021 soybean growing season (May-September)

A: Vegetative period 2020; AA: Vegetative period 2021; B: Flowering period 2020; BB: Flowering period 2021; C: Pod maturity period 2020; CC: Pod maturity period 2021; D: Grain maturity period 2020; DD: Grain maturity period 2021
M: May; H: June; T: July; A: August; E: September
BBCH Phenological growth stages of the soybean: 09: Cotyledons emerge, 11: First pair of true leaves unfolded, 60: Beginning of flowering, 69: End of flowering, 70: Beginning of pod development, 79: End of pod development, 80: Beginning of pod ripening, 85-89: End of pod ripening, 99: Harvest period (Munger et al., 1997)

The temperature values of July and August, when the pod-setting and grain-filling periods of soybeans coincide (BBCH 75-85), were observed to be higher in 2021 than in 2020. In the second year of the experiment, the phenological development periods in these months exhibited accelerated growth in correlation with the elevated temperatures, resulting in a reduction in the maturity periods compared to the first year of the experiment.

In this study, the effects of *Rhizobia* inoculation on some yield and quality characteristics of soybean varieties were investigated depending on different limited and irrigated water applications in order to ensure the continuity of soybean production in sustainable agriculture considering the importance of climate change. According to the variance analysis results we obtained, irrigation treatments (limited vs irrigated) were found to be statistically significant at the $p \leq 0.01$ level on yield parameters such as plant height, first pod height, number of pods plant^{-1}, number of seeds pod^{-1}, 1000 seed weight, seed yield and leaf area (Table 1). *Rhizobia* applications were found to be statistically significant in other parameters examined except protein content. In the experiment, bacteria, variety and water interaction were found to be statistically significant at the $p \leq 0.01$ in the number of pods per plant, the number of seeds per pod and seed yield, while it was found to be significant at the 0.05 level ($p \leq 0.05$) in 1000 seed weight. While water treatments were found to be statistically significant at 0.05 level ($p \leq 0.05$) on protein ratio, variety and bacteria treatments were found to be statistically significant at 0.01 level ($p \leq 0.01$) on crude oil ratio (Table 2).

Plant Height (cm)

The experiment on plant height revealed that the effects of years, *Rhizobia* applications, irrigation applications, and the year*inoculant*irrigation interaction on plant height were significant (Table 1). Upon examination of the mean values obtained, it was found that the mean plant height in the first year was 88.34 cm, while the mean plant height in the second year was 91.01 cm. It was observed that the mean plant height in the second year was greater than that observed in the first year. The data indicated a notable increase in plant height values with each incremental increase in irrigation application. The highest plant height value of 109.76 cm was obtained in the treatment group receiving 100% irrigation, as determined by the irrigation application averages. The lowest recorded plant height value was 63.28 cm, which was observed in the 25% irrigation treatment. The main reason for this is thought to be the decline in plant growth as the amount of irrigation decreases. The application of *Rhizobia* inoculant treatments did not result in a significant increase in plant height compared to control. A comparison of the plant height values obtained in the present study with those reported in previous studies revealed that Surgun Acar (2022) observed plant height values between 95.01 and 108.33 cm in soybean, while Ilker (2017) documented values between

63.10 and 94.90 cm in the first year and between 81.50 and 105.40 cm in the second year. In a study conducted in in 2021, the plant height of soybeans was found to range from 88.33 to 127.77 centimeters (Kars and Ekberli, 2021).

Table 1. Average values of yield and yield parameters in soybean for the combined trial years

Experimental Factor	Plant height (cm)	First pod height (cm)	Number of pods plant^{-1}	Number of seeds pod^{-1}	1000 seed weight (g)
Mean Year (A)					
2020	88.34b	20.68a	39.91b	2.66	147.35a
2021	91.01a	17.20b	76.73a	2.61	134.27b
Mean Bacteria (B)					
Control	93.30a	18.96b	54.41c	2.51b	136.17c
Rhizobium	88.34b	19.56a	57.88b	2.75a	141.09b
USDA 110	87.02b	18.73c	62.96a	2.65a	145.16a
Mean Variety (C)					
Cinsoy	89.09	19.41a	59.29b	2.60b	135.86b
Altinay	90.26	18.46b	60.35a	2.67a	145.76a
Mean Irrigation (D)					
%25	63.28d	16.83c	37.21d	2.56b	139.40b
%50	85.61c	20.97a	56.11c	2.68a	136.65b
%75	100.06b	19.22b	65.95b	2.72a	144.42a
%100	109.76a	18.73b	74.01a	2.59b	142.68a
LSD values					
A	4.07**	1.27**	3.86**	ns	5.65**
B	2.65**	0.46**	1.92**	0.12**	2.78**
C	ns	0.45**	2.40**	0.04**	2.19**
D	2.36**	0.64**	3.40**	0.06**	3.09**
A*B*C	4,.10**	ns	5.89**	ns	5.36**
A*B*D	5.80**	1.58**	8.34**	0.16**	7.58**
A*C*D	ns	1.29**	6.80**	0.13**	6.16*
B*C*D	ns	ns	8.34**	0.16**	7.58*
A*B*C*D	ns	ns	11.37**	ns	10.67**

ns: non-significant; *: significant at 0.05 level; **: significant at 0.01 level

Unal and Onder (2008) found plant height averages between 90.67-119.90 cm, Bateman et al. (2020) between 38.1- 142.2 cm, Cevheri and Yilmaz (2018) between 87.00-112.00 cm in a study conducted with soya bean lines. Boydak et al. (2018), in their study on the yield characteristics of 12 different soybean varieties, reported that plant height values were determined as 79.37-126.07 cm in the first year and 67.90-102.80 cm in the second year and found in agreement with our values.

First Pod Height (cm)

First pod height is an important parameter in soybean cultivation and in mechanized harvesting, it is important that the first pod is high in order to minimize harvest loss (Gizlenci et al., 2005). First pod height is positively correlated with plant height but negatively correlated with seed weight, number of seeds pod^{-1}, number of seeds plant^{-1} and number of pods plant^{-1} (Oz et al., 2009).

The effects of year, inoculant, variety, and irrigation on first pod height were found to be statistically significant ($p\leq0.01$). Upon analysis of the mean values presented in Table 1, it was determined that the first pod height was 20.68 cm in the initial year of the experiment. In the second year, a reduction in the initial pod height was observed, with a mean value of 17.20 cm. The positive effect of irrigation applications was observed in the 50% water application group (20.97 cm), and the results obtained from the 75% and 100% water application groups were statistically similar (Table 1). The results were obtained within the expected range for the first pod height values of the varieties. A comparison of the values obtained in the experiment with those from previous studies revealed similarities. The first pod height was determined to be between 9.67 and 20.33 cm by Unal and Onder (2008), between 12.4 and 22.17 cm by Yetkin and Arıoglu (2010), and between 12.86 and 19.37 cm by Ozer (2021) in soybean varieties. The values obtained in our experiment were found to be in agreement with those reported in previous studies.

Number of pods plant^{-1}

The effects of year, variety, inoculant, irrigation, and the interaction between year, inoculant, variety and irrigation on pod number per plant were found to be statistically significant ($p\leq0.01$). Significant differences were observed between the mean values of the experimental years. As illustrated in Table 1, the mean number of pods per plant was 76.73 in 2021, while the mean number of pods per plant in 2020 was 39.91.

This notable discrepancy is believed to be attributable to the deleterious impact of elevated temperatures experienced during the flowering phase in 2020 (Figure 2). It was observed that the application of irrigation resulted in a notable variation in the mean number of pods per plant.

The highest mean number of pods per plant was observed in the 100% water treatment (74.01 pods plant^{-1}), representing the average non-restricted water treatment. As the quantity of irrigation increased, the number of pods plant^{-1} also increased. The onset of water stress at the beginning of the flowering period has been observed to result in a reduction in the number of pods produced. Stress experienced subsequent to the onset of flowering and throughout the period of full bloom has been demonstrated to result in a reduction in both pod number and grain size. Limited irrigation has been demonstrated to result in a notable decline in grain yield and its constituent components (Korte et al., 2023). Additionally, statistical differences were observed in the number of pods produced under different *Rhizobia* inoculation applications. The application of the USDA-110 inoculant resulted in a higher mean number of pods plant^{-1} (62.96) than the control application (54.41). Gumus and Beyyavas (2020) reported that the number of pods in soybean varieties ranged from 81.50 to 133.90. Kumagai et al. (2022) reported a pod count of 57-101. Cevheri and Yilmaz (2018) reported a pod count of 103 in soybean under semi-arid climate conditions. The number of pods per plant was reported by Ilker et al. (2010) to be 50.92-111.11, by Boydak et al. (2018) to be 32.17-72.10, and by Surgun Acar (2022) to be 62.03-118.40. A comparison of the values in the literature with those obtained in our study reveals a high degree of consistency.

Number of seeds pod^{-1}

The impact of water stress on soybean yield is dependent on the developmental stage at which it occurs. While water stress can reduce yield at all developmental stages, the severity of this effect varies depending on the specific stage of development that is affected. Flowering is susceptible to drought stress, which can result in flower shedding. However, since flowering occurs over an extended period (approximately 3-4 weeks) along the length of the stem, it is possible to offset losses resulting from flower shedding. This evidence suggests that drought stress during the pod-setting period may be more severe than during the flowering period (Mandic et al., 2015).

The analysis revealed that the inoculant*variety*irrigation interaction was a statistically significant factor ($p \leq 0.01$) influencing the number of seeds per pod. In particular, the variety and irrigation factors were found to exert a significant influence on the number of seeds per pod. Table 1 illustrates that there is no statistically significant difference in the mean number of seeds per pod across the trial years. The application of the *Rhizobia* inoculant resulted in a significantly higher number of seeds pod^{-1}, with an average of 2.65 seeds pod^{-1}, compared to the control application, which yielded an average of 2.51 seeds pod^{-1}. It was observed that 50% limited irrigation (2.68 per plant) may be sufficient to achieve the highest value for the number of seeds pod^{-1}. The Altinay variety exhibited a higher seed number (2.67) than the Cinsoy variety (2.60). The primary discrepancies were noted during the final stages of pod formation and the initial phase of seed filling in both experimental years (BBCH 75-85). In 2021, the temperatures recorded at the conclusion of the pod setting period (August 10) and the onset of seed filling (August 22) resulted in notable discrepancies. During the specified period, daily maximum temperatures in 2021 exceeded 40°C, whereas in 2020, these values ranged between 35 and 37°C. The duration of phenological stages that coincided with these months was shortened due to the elevated temperatures, resulting in a reduction in maturity time in the initial year. Drought stress can be particularly impactful during the flowering, seed formation, and seed filling stages. The reduction in seed size is attributed to the shorter period of seed filling and the earlier onset of ripening (Ashley and Ethridge, 1978). Boydak et al. (2018) observed that the number of seeds pod^{-1} in soybeans was 2.78 in the first year and 2.83 in the second year of his two-year study. In contrast, Altinyuzuk (2017) reported values between 2.23 and 2.83, Ilker et al. (2010) between 2.83 and 3.00, Kobraee et al. (2011) between 2.3 and 3.5, and Shamima and Farid (2006) obtained values between 2.2 and 3.0. The number of seeds pod^{-1} obtained in our study is comparable to the values reported in other studies. Furthermore, Kumagai et al. (2022) reported that the number of seeds in soybean pods ranged from 1.62 to 2.07 and Surgun Acar (2022) from 1.99 to 2.54. These values were generally below those obtained in our study.

1000 seed weight (g)

Extreme environmental conditions, such as drought, accelerate the transition from the vegetative to the generative phase in plant development. This phenomenon is evidenced by a reduction in the duration of the generative and grain filling periods, which can be attributed to elevated temperatures and restricted irrigation. Consequently, there is a decline in photosynthesis and nutrient production, as well as a reduction in grain weight and, consequently, yield (Yigit and Chmielewski, 2024). The abiotic stress conditions experienced during the grain filling period has been demonstrated to result in a reduction in grain size, which in turn leads to a decline in grain weight and yield (Desclaux et al., 2000). The study revealed that during the experimental years, limited irrigation, variety, and inoculant treatments resulted in notable variations in the 1000-seed weight of soybean (Table 1). The highest 1000 seed weight was observed in 2020 (147.35 g), while the lowest 1000 seed weight was recorded in 2021 (134.27 g). It can be posited that the reason for this is that the elevated temperatures experienced during the pod-setting period in the second year of the experiment had an adverse impact on the filling period, resulting in a reduction in seed weight. A comparison of the varieties yielded mean values of 135.86 g for the Cinsoy variety and 145.76 g for the Altinay variety. The experiment revealed significant effects of average limited irrigation treatments (50 and 25%) on 1000-seed weight. Irrigation applications resulted in a range of 1000 seed weight values, with the highest observed in the 75% and 100%irrigation amount (144.42 and 142.68 g) and the lowest in the 25% and 50% limited irrigation amount (139.40 and 136.65 g), respectively.

In the context of the USDA-110 inoculant application, the highest value was observed among the inoculant applications, reaching a value of 145.16 g. Upon

examination of the studies conducted, it was found that the 1000-seed weight values of soybean ranged from 135.4 to 167.4 g (Yetkin and Arioglu, 2010), and from 140.5 to 180.4 g (Ilker et al., 2010). Boydak et al. (2018) obtained 1000 seed weight values of 117.10 to 156.96 g in the first year and 100.71 to 128.18 g in the second year in the soybean experiment. The data obtained in the present study are comparable to those of previous studies conducted in this area.

Seed yield (kg ha^{-1})

Restricted irrigation conditions result in a linear reduction in the average soybean yield. This is attributed to the shortening of the vegetative and generative periods caused by drought stress (Borowska and Prusiński, 2021). The period during which soybeans require the greatest quantity of water is that of flowering and pod maturation. Furthermore, this period is regarded as one of the most crucial due to the fact that insufficient water during the seed filling phase results in a reduction in the number of seeds and seed weight within the pod, ultimately leading to a decline in yield (Staniak et al., 2023).

The study revealed that the effects of year, inoculant, irrigation, and the interaction between inoculant, variety and irrigation were of particular significance ($p \leq 0.01$). The irrigation amounts resulted in notable alterations in seed yield. As illustrated in Table 2, the mean seed yield exhibited a notable increase from 441.5 kg ha^{-1} in limited irrigation (25%) to 3086.7 kg ha^{-1} in 100% full irrigation. Significant differences were observed in the mean yield values among the trial years. In 2020, the mean seed yield was 1700.7 kg ha^{-1}, while in 2021, it increased significantly to 2165.6 kg ha^{-1}.

It is hypothesized that increased irrigation amounts result in notable increases in seed yield, attributed to the elevated temperatures observed during the trial years, which frequently exceed the norm. Additionally, the impact of intermittent periods of exceptionally high daily temperatures is a contributing factor. In applications of *Rhizobia* inoculant, the highest grain yield was obtained with the USDA-110 inoculant (2083.1 kg ha^{-1}). In studies conducted with soybean, seed yields have been reported to vary considerably. For instance, Kresovic et al. (2017) reported yields between 1630 and 3210 kg ha^{-1}, while Kabraee et al. (2011) reported yields between 2266.9 and 3700.1 kg ha^{-1}. Ozer (2021) reported yields between 1434 and 3801 kg ha^{-1}, while Feng et al. (2022) reported yields between 1600 and 4100 kg ha^{-1}. Istemil (2015) reported that the grain yield of soybean exhibited considerable variation, with values ranging between 2709 and 3553 kg ha^{-1}. Kumagai et al. (2022) observed a similar trend, with soybean grain yield values ranging between 2570 and 3640 kg ha^{-1}. Similarly, Gojic et al. (2018) reported that the highest grain yield was achieved when 100% water was applied to soybean in four distinct irrigation treatments (control, 40%, 60%, and 100%). The highest yield was observed in the 100% water treatment, with a value of 3690 kg ha^{-1}.

Table 2. Average values of yield, leaf area and quality traits for the combined trial years

Factor	Seed Yield (kg ha^{-1})	Leaf Area $_{(FP)}$ (cm^2 plant^{-1}) (BBCH 65)	Leaf Area $_{(PD)}$ (cm^2 plant^{-1}) (BBCH 75)	Crude Protein (%)	Crude Oil (%)
Mean Year (A)					
2020	1700.7b	1061.64	1699.19	35.47a	20.85b
2021	2165.6a	1764.22	1691.01	32.59b	22.67a
Mean Bacteria (B)					
Control	1719.6b	1611.21a	1906.99a	32.65	22.36a
Rhizobium	1996.7a	1601.91a	1497.74b	34.48	21.55b
USDA 110	2083.1a	1025.67b	1680.57ab	34.59	21.38b
Mean Variety (C)					
Cinsoy	1942.1	1324.56	1661.63	34.21	21.10b
Altinay	1924.1	1501.30	1728.54	33.81	22.42a
Mean Irrigation (D)					
%25	441.5d	927.67c	1042.96c	35.00a	21.54
%50	1795.4c	1551.15ab	1467.31b	33.85ab	21.84
%75	2409.0b	1338.71b	2219.84a	34.47a	21.76
%100	3086.7a	1843.19a	2050.29a	32.72b	22.30
LSD values					
A	75.8**	ns	ns	0.97**	0.97*
B	158.7**	308.85**	287.94*	ns	0.41**
C	ns	ns	ns	ns	0.59**
D	118.6**	347.56**	365.69**	1.59*	ns
A*B*C	ns	ns	633.40**	ns	ns
A*B*D	290.6**	602.00**	895.77*	ns	ns
A*C*D	237.3*	ns	ns	ns	11.88*
B*C*D	290.6**	ns	ns	ns	ns
A*B*C*D	ns	ns	ns	ns	ns

ns: non-significant; *: significant at 0.05 level; **: significant at 0.01 level; Leaf Area $_{(FP)}$: Leaf Area amount in flowering period; Leaf Area $_{(PD)}$: Leaf Area amount in pod formation period

Leaf area (cm^2 $plant^{-1}$)

Leaf area is an important factor affecting light retention and biomass production in plants (Yao et al., 2017). Reducing the leaf area of the plant under drought and water stress conditions is considered as a strategy to reduce water loss in stress conditions. This situation leads to a decrease in biomass accumulation in soybeans in the following stages (Dong et al., 2019). It is known that drought conditions during the vegetative period reduce morphological characteristics such as leaf area, plant height, and first pod height (Staniak et al., 2023).

Leaf area amounts were quantified on two occasions during the course of the experiment, once during the flowering period (Leaf Area $_{FP}$) and once during the pod formation period (Leaf Area $_{PD}$). Table 2 illustrates the statistically significant impact of restricted irrigation and *Rhizobia* inoculant applications on leaf area amounts during the flowering period (BBCH 65). The observed effects were significant at the $p \leq 0.01$ level. As irrigation amounts increased, a linear increase in leaf area was observed in soybean plants. The greatest leaf area was observed in the treatment group that received 100% irrigation, with a value of 1843.19 cm^2 $plant^{-1}$. In the case of *Rhizobia* inoculant applications, the highest leaf area was observed in the control application, while the AZOTEK-2 bacteria application was found to be statistically similar to the control group (1611.21 cm^2 $plant^{-1}$). The effects of irrigation and *Rhizobia* inoculant applications on leaf area measurements taken during the pod formation period (BBCH 75) were found to be statistically significant (Table 2). The greatest quantity of leaf area (2219.84 cm^2 $plant^{-1}$) was observed in the irrigated condition (75% irrigation). Drought conditions restrict stomatal conductance, which in turn impairs photosynthetic activity. Furthermore, the leaf areas of plants subjected to water stress are markedly inferior to those of unstressed plants (Mangena, 2018).

In a study investigating the effects of inoculants and different doses of nitrogen fertilizer applications on yield and growth in soybeans, the combination of *Rhizobia* application and 50% nitrogen fertilizer application yielded the most optimal results. In the study, the leaf area per plant was determined to be 138.75 cm^2 (Herliana et al., 2019).

Protein Content (%)

Restricted irrigation conditions result in a deficiency of water in the tissues, which in turn impedes a number of physiological processes, including photosynthesis, transpiration, and stomatal conductance. This situation has an impact on plant growth and development, as well as seed yield and grain composition (Staniak et al., 2023). In arid conditions, there is a decrease in the ratio of crude oil and an increase in the protein content of soy seeds. These ratios change linearly with an increase in stress (Sobko et al., 2020). Ravelombola (2022) posited that drought can impact protein structure and protein synthesis. Additionally, the decomposed proteins are hindered from transferring amino acids to the leaf due to the effects of hydration.

As demonstrated in Table 2, the factors of year ($p \leq 0.01$) and irrigation ($p \leq 0.05$) were found to have a statistically significant impact on protein values. The mean protein values were found to be 35.47% in 2020 and 32.59% in 2021 among the trial years. The highest protein value among the irrigation amounts was observed in the 25% limited irrigation treatment (35.00%). As reported by Poeta et al. (2016), an increase in protein content was observed to be positively and linearly related to water stress and negatively related to oil content. While Unal and Onder (2008) reported a grain protein ratio of 34.40-38.61%, and Devi et al. (2013) reported a ratio of 34.40-36.71%, Cevheri and Yilmaz (2018) reported a protein ratio of 39.31-41.74%. Alsajri et al. (2020) reported that the oil and protein content of the seed is closely related to environmental factors, particularly with increasing temperature during the growth period, which has been observed to result in increased oil content and decreased protein content. The results of our study indicate that the highest protein content was achieved with a 25% limited irrigation amount, while the highest crude oil content was observed with a 100% full irrigated condition. These findings are largely consistent with those of other significant studies in the field.

Crude oil content (%)

High daily temperatures reduce oil content and increase protein content in soybean seeds (Gibson and Mullen,1996; Dornbos, 2020). It is established that water limitation influences the nutrient content of soybean seeds and accumulation to seeds during the flowering and seed development stages. Borowski and Michalek (2014) reported a decrease in crude oil content (13.8%) and an increase in grain protein content (6.2%) in soybean varieties with limited irrigation under drought conditions. The statistical analysis revealed that the applications of year ($p \leq 0.05$), variety and inoculant ($p \leq 0.01$) had a statistically significant impact on crude oil ratios (Table 2). Additionally, significant discrepancies were observed between the trial years. The mean crude oil content was 20.85% in 2020 and 22.67% in 2021. The differences in crude oil content between the two experimental years was approximately 2%. The effects of the *Rhizobia* inoculants on the crude oil ratio in the seed exhibited statistical differences, with the highest crude oil ratio observed in the control plot (22.36%). The applications had a notable impact on the variety averages. The mean crude oil ratio for the varieties was determined to be 21.10% for the Cinsoy variety and 22.42% for the Altinay variety.

The impact of restricted irrigation on the crude oil value of soybean was found to be relatively similar. The average crude oil contents of the irrigation treatments varied very little and exhibited similar results. Upon examination of previous studies on crude oil ratios, it was found that the crude oil ratio value was reported to be between 19.15 and 19.89% by Temory (2014), between 16.66 and 19.30% by Ay (2012), 18.65% by Kılınc and Arioglu (2018), and between 7.20 and 18.60% by Gaweda et al. (2017). In comparison to previous studies, our study yielded higher oil

content values (20.85-22.36%), and our findings indicate that oil content is not affected by irrigation treatments.

CONCLUSION

The objective of this study was to investigate the nodule formation performance of *Rhizobia* strains in soybean, a crop of paramount importance to sustainable agriculture, under conditions of limited and irrigated, as well as to evaluate the performance of soybean varieties in terms of yield and quality under conditions of Mediterranean climate. The study revealed significant insights into the interactions between the yield and quality potentials of different applications and related high temperatures in soybean varieties grown in the Aydin province. The experimental results demonstrated that the nodule formation performance of the *Rhizobia* inoculant was markedly influenced by irrigation practices and high temperatures. In the first year of the experiment, only USDA 110 *Bradyrhizobium* inoculant was applied, resulting in enhanced nodule formation in both the Cinsoy and Altinay varieties when 75% to 100% irrigation amount applied. However, almost no nodulation history was observed in any of the plants evaluated. This phenomenon was observed in a limited number of sampled areas and only in a subset of plant roots. It was observed that daily high temperatures were experienced in both trial years. However, the 2021 trial year exhibited longer and higher daily maximum temperatures in comparison to the 2020 trial year. It is hypothesized that during certain periods of plant development when elevated temperatures are present, *Rhizobia* inoculants can infect soybean roots, thereby affecting their capacity to form nodules. It has been observed that the average values obtained are significantly affected by limited water supply and varieties. The statistical significance ($p \leq 0.01$) of variety and irrigation in many parameters examined depending on the applications revealed that yield and quality traits were not influenced by a single factor. The highest values obtained in the examined parameters indicated that 100% irrigation amount was optimal, with the Altinay variety demonstrating superior performance. Furthermore, the global average yield for soybeans was achieved with a 75% irrigation amount in our experiment (2409.0 kg ha^{-1}).

The results demonstrated that a sufficient average yield can be achieved with 75% irrigation application (612.73-644.05 mm) under the ecological conditions of the limited water supply regions. Upon examination of both experimental years, it was observed that the period of highest water consumption (combined with high temperature) in soybeans was the developmental phase between the pod formation period and the seed filling period. The study revealed important results, especially regarding the significant effects of high temperatures and limited water applications on *Rhizobia* inoculation and nodulation performance during the vegetative period and the end of pod formation and grain filling periods.

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EFFECT OF WATER STRESS ON YIELD AND YIELD COMPONENTS OF PEANUT CULTIVARS

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ABSTRACT

To evaluate the effect of irrigation regimes on yield and water productivity, a split plot experiment was conducted with three replications in Iran in 2017 and 2018. The main treatment consisted of 40%, 60%, 80% and 100% water requirements, respectively, and the sub-treatment consisted of four peanut cultivars which are types of peanuts that are cultivated in Iran market (Guil, Gorgani, Jonobi and Mesri). Each 100 grams of these introduced peanuts contains 25.5 grams of protein and 48.4 grams of fat. Seed yield in 2017 with an average of 1316 kg ha^{-1} was higher than seed yield in 2018 with an average of 1022 kg ha^{-1} Due to irrigation, seed yield in the treatments of 40% and 60% of water requirement with the average of 1345kg ha^{-1} and 1379 kg ha^{-1}, respectively, had the highest value. Due to the year of irrigation, the maximum seed yield in 2017 and in treatments of 40% and 60% of water requirement were with an average of 1494 kg ha^{-1} and 1593 kg ha^{-1}, respectively. In peanut cultivars, Jonobi cultivar with an average of 1273 kg ha^{-1}, had the highest value compared to other cultivars. Due to irrigation×cultivars, 40% water requirement treatment and Jonobi cultivar with an average of 1732 kg ha^{-1}, and also 60% water requirement treatment and Guil cultivar with an average of 1667 kg ha^{-1} had the highest value. The maximum seed yield due in year ×irrigation ×cultivar was in 2017, and in the treatment of 40% of water requirement and in the Jonobi cultivar with an average of 1856 kg ha^{-1}. Water productivity on biological yield (4.32 kg m^{-3}) and pod yield (1.96 kg m^{-3}) in Mesri cultivar and Water productivity on seed yield in Gorgani cultivar were 0.54 kg m^{-3}.

Keywords: Cultivar, Irrigation, Peanut, Stress, Water Productivity.

INTRODUCTION

Peanut is one the oil crops in the world that is cultivated on a large scale for producing oil and nut consumption in many countries of the world, such as Iran. Peanut is the third major oilseed crop in the world next to soybean and cotton (Nayak et al., 2019). Peanuts are native plants of Brazil and have found their way from Brazil to other parts of the world (Abdzad Gohari, 2018). Peanut seeds contain about 25% to 30% protein which has the amino acids of tryptophan, lysine, methionine and cysteine (Amiri et al., 2015; Hammons et al., 2016; Virk et al., 2019). Produced peanut butter is available in many small shops, and small packets of roasted peanuts are commonly sold by street vendors (Fulmer et al., 2020). However, drought which is the single most devastating environmental stress, not only decreases crop productivity (Lambers et al., 2008). It has become increasingly apparent that crop maturity in seed peanut should be a priority for the peanut industry (Pierre et al., 2019). One of the most important economic sectors in Iran is the agricultural sector, and in the meantime water is the most important factor limiting agricultural production. In this regard, deficit irrigation is an optimal strategy to cultivate crops under water scarcity conditions which is accompanied by reduced crop yield. Peanuts exhibit drought resistance primarily due to their ability to maintain a functional root system under water stress. Water stress encourages peanut roots to grow deeper into the soil, allowing them to extract water from depths of at least 180 cm in fine sandy soils (Lenka and Mishra, 1973; Allen et al., 1976; Narasimham et al., 1977). When peanut cultivars experience soil moisture stress during the early vegetative stage, individual seed weight increases. However, water stress during the pod initiation and development stages reduces the suitability of seeds for planting (Nautiyal et al., 1991). Imposing water deficits during the vegetative phase results in higher final yields, improved water use efficiency, and increased dry matter production, including economic yield (Ong, 1984; Nautiyal et al., 2000). The

main objective of deficit irrigation is to increase water productivity by reducing the amount of irrigation water at each turn or elimination of irrigation methods which have the least efficiency. Deficit irrigation can be also utilized for expanding the cultivation level and maximizing or stabilizing the production of a region's crops. Some of the advantages of deficit irrigation include reducing production costs, reducing irrigation water costs and increasing application efficiency of the irrigation water (Giret and Rize, 2009). Adjusting the timing and amount of deficit irrigation throughout the growing season at various growth stages can potentially increase peanut yield by altering the distribution of dry matter between vegetative and reproductive organs (Ong,1984). peanuts require adequate water throughout all stages of physiological development to achieve optimal productivity (Rao et al., 1988; Meisner and Karnok, 1992), certain stages, particularly flowering and pod filling, are more sensitive to soil moisture levels compared to early vegetative or late maturity stages (Doorenbos and Kassam, 1979; Howell et al., 1980). Insufficient water during these critical stages can significantly reduce kernel yield and prevent the efficient use of available water (Pallas et al., 1979; Ike, 1986; Boote and Ketring, 1990).Water deficit significantly reduces yield potential of peanut worldwide. Availability of drought tolerant cultivars is essential, but their selection is difficult, in particular in environments where rainfall is unpredictable (Balota., 2020). Soil moisture retention is important for peanut production as well as water conservation in irrigated and non-irrigated fields (Hawkins et al., 2016). The results of Abdzad Gohari and Amiri (2018) study on peanuts indicated that deficit irrigation is good for the plant due to water conservation and increased water use efficiency, however, while extreme deficit irrigation results in a lot of water saving, it also decreases yield. One of the basic management methods of agriculture is providing conditions to maximize the crop production relative to the consumed water (Shinde and Laware, 2010). Water efficiency and productivity are indices of optimizing the consumption of water (Kijene et al., 2003). This concept is widely used in order to recognize and provide the yield ratio as biological yield or economic yield per unit volume of water consumed. Obtaining the optimum size of production function and its implications will be very beneficial for crop production and the future economic activity of farmers and also for maintaining and strengthening the status of agricultural products in the domestic and international consumption markets. The production function is a mathematical equation between product yield and consumption inputs in the production process. The general form of the production function can be written as equation (1):

$$Y = F(X_1,X_2,\ldots,X_n) \qquad \text{Equation (1)}$$

This equation shows that the product value (Y) can be calculated in different ways through multiple values of the production factors (X_i). Estimation of production function also makes it possible to separately identify the role and importance of each of the production inputs (Geerts and Raes, 2009). One of the applications of production functions is determining the optimal irrigation value (Bontang et al., 2010). Kijne et al., (2003) achieved a quadratic production function. In a study, Abdzad Gohari et al. (2018) examined the amount of water consumed and the amount of nitrogen on peanuts and they obtained quadratic nonlinear production functions. The purpose of the present study was to evaluate the performance and efficiency of water use on peanut cultivars with deficit irrigation and full irrigation conditions.

MATERIALS AND METHODS

For the examination of yield and water productivity in peanut cultivars, Randomized Complete Block Design (RCBD) arranged in Split Plots in replications in Guilan province (in Iran) with latitude of 37°16', and a longitude of 49°56', which is averagely -5 meters above sea level. This test was carried out in 2017 and 2018. The main treatments included irrigation with 40%, 60%, 80% and 100% water requirements for irrigation management, and the sub-treatment included four peanut cultivars (Guil, Gorgani, Jonobi and Mesri). The meteorological parameters are presented in Table 1. In order to determine physicochemical properties of soil, the farm soil was randomly sampled at different depths of 0-30 cm and 30-60 cm, prior to preparing the soil and applying the fertilizer. The soil type of the area was loam. The other properties of the soil in the regions are presented in Table 2. The field was plowed in april, and it was turned flat and soft by using a rotary. After that the seeds were sown in soil. Peanut seed emergence occurs between 6 to 11 d after planting, depending upon soil and air temperatures (Canavar and Kaynak, 2010). As a general rule, peanut germination is considered optimum in the soil temperature range of 20℃ to 35℃ at a 10 cm soil depth for three consecutive d and air temperatures between 27℃ and 32℃ are considered optimum for peanut growth and yield (Kvien et al., 2019; Virk et al., 2019). The amount of water that would be consumed during the growth period was provided through irrigation water and rainfall. In order to determine irrigation treatment, soil moisture depletion method was used and the amount of required water of the plant was considered as 100% irrigation treatment. Other irrigation treatments were considered as a percentage of this amount. To achieve the treatment of 100% irrigation, soil moisture at the root of the plant was calculated by Equation (2) in way to that the soil moisture up to the height of the root can reach the capacity value of the farm. The duration of irrigation depends on the time that the moisture reaches the root of the plant after starting the irrigation (Abdzad Gohari et al., 2018).

$$d_n = (\theta_{Fc} - \theta_i).\rho_b.D_r \qquad \text{Equation (2)}$$

$Ú_{Fc}$: water content weight percentage at field capacity. $Ú_i$: water content weight percentage in soil. ρ_b: Soil bulk density (Grams per cubic centimeter). D_r: Effective root height (cm). To measure biological function of seed and pods in each plot, 12 plants were randomly selected after removal of two rows of cultivars from each side. Then the pods, leaves and stems were separated from the plant and placed in the oven at 70°C for 48 hours. The samples were

weighed by scales (one-hundredth of a gram precision) after they were dried, and then the seeds were separated from the pods and converted to kg ha^{-1} unit. The amount of water productivity was obtained by dividing the yield (kg ha^{-1}) by the total amount of water used (Cubic meters per hectare). The amount of irrigation water and the water used can be seen in Table 3. For data analysis, MSTATC software was used to analyze the variance and mean comparison (Duncan at 5% probability level). Excel software was used for drawing the charts and the value of production functions was determined by using STATISTICA V5.5A model.

Table 1. Meteorological average of the study area in 2017 and 2018.

	Jun	Jul	August	September
Precipitation (mm)	27.4	41.3	65.6	21.8
Average Relative Humidity (%)	58.9	62	57.2	64.5
Wind speed (ms^{-1})	2.6	3.3	2.7	2.5
Maximum temperature (℃)	28.1	29	30.8	28.6
Minimum Temperature (℃)	19.3	21	22	19.4

Table 2. Characteristics of soil in the study area

	Particle size distribution %							
Soil depths (cm)	Sand	Silt	Clay	Organic Carbon	EC (%)	Total Nitrogen (%)	Total Phosphor (mg kg^{-1})	Total Potassium (mg kg^{-1})
0-30	47	32	21	0.65	0.646	0.03	3.17	181
30-60	49	31	20	0.66	0.632	0.03	2.33	150

Table 3. The amount of water use and the amount of irrigation in 2017 and 2018.

Irrigation management	Year	Amount of irrigation (mm)	Water use (mm)
40% water requirement	2017	150.9	349.6
	2018	118.4	232.8
60%water requirement	2017	154.1	352.8
	2018	158.8	272.9
80%water requirement	2017	271.7	470.4
	2018	249.4	363.8
100% water requirement	2017	389.2	587.9
	2018	340.4	454.8

RESULTS AND DISCUSSION

Biological yield

Effect of cultivars, effect of year × cultivars, effect of irrigation×cultivars and effect of "year × irrigation × cultivars" were significant at ($p<0.01$) n biological yield (Table 4). Jonobi cultivar with the mean of 9396 kg ha^{-1} had highest biological yield among all other cultivars (Figure 3). The highest biological yield was obtained for the mutual effect of year×cultivar for Jonobi cultivar in 2017 with a mean of 10075 kg ha^{-1} (Figure 4). Regarding the mutual effect between irrigation and cultivars, the maximum biological yield was obtained in 80% water requirement in Guil cultivar with a mean of 10880 kg ha^{-1} (Figure 5). Maximum biological yield was seen for the mutual effect of year×irrigation×cultivars in 2017 in the treatment with 100% water requirement and in the Jonobi cultivar with a mean of 12170 kg ha^{-1} (Table 11). By increasing vegetative growth duration and the effective life of the canopy, active photosynthetic absorption increases and it leads to increased dry weight of aerial organs (Anjam et al., 2011). Drought causes a reduction in the absorption of minerals and nutrients and this can also reduce the growth of aerial organs of the plant. Dry weight of aerial organs is greatly reduced under drought stress and eventually biological yield will also decrease. Because water stress eventually reduces carbon dioxide intake through closing the pores, and in this way, it results in a significant decrease in dry matter production by affecting metabolic activities (Anjam et al., 2011).

Pod yield

Effect of cultivars, effect of "year × cultivars", effect of irrigation×cultivars and effect of "year × irrigation × cultivars" were significant at ($p<0.01$) level on pod yield (Table 4). For the mutual effect of irrigation×cultivars, maximum pod yield was obtained in the treatment with 100% water requirement and in the Jonobi cultivar with a mean of 4513 kg ha^{-1}, and also in the treatment with 80% water requirement it was obtained in Guil cultivar with a mean of 4502 kg ha^{-1} (Figure 5). Maximum pod yield was seen for the mutual effect of year×irrigation×cultivars in 2017 in the treatment with 100% water requirement and in the Jonobi cultivar with a mean of 5230 kg ha^{-1} (Table 11).

In early stages of growth of pods, severe drought stress reduces their growth rate and leads to a significant reduction in the total number of pods (Liu et al., 2003; Anjam et al., 2011). In a study by Abdzad Gohari et al. (2011), It has been demonstrated that moisture is a crucial factor in the development of peanuts, and a lack of moisture during the pod growth phase can ultimately lead to a reduced pod yield. Shinde and Laware (2010) in their study showed that moisture deficiency at flowering time reduces peanut yield.

Table 4. Mean squares form the combined ANOVA for Agronomic Traits.

Source	df	Biomass yield	Pod yield	Seed yield	100-seed weight	Number of pods per plant
Year	1	22046458.59^{ns}	31711747.01^{ns}	2073288.17^{ns}	2734.93^{ns}	840.17^{ns}
Replication	4	9215010.25^{ns}	1329035. 63^{ns}	517149.79**	1027.27**	137.42^{ns}
Irrigation	3	17829265.45^{ns}	2397237. 53^{ns}	1210092. 40**	4024.67**	138.47^{ns}
Year×Irrigation	3	9118835.45^{ns}	188883.69^{ns}	77851.31**	510.13^{ns}	185.36^{ns}
Error	12	7741850.74	1605332.43	83893.71	169.32	84.92
Cultivar	3	5560716.04**	1005525.47**	196347. 07**	175.22**	88.36**
Year×Cultivar	3	3740454.04**	698157. 81**	64550.19^{ns}	103.46**	69.69**
Irrigation×Cltivar	9	6514854.16**	1027251. 33**	433553.99**	779.6**	43.15**
Year×Cultivar×Irr	9	4161357.38**	875999.32**	218508.85**	367.04**	42.93**
Error	48	252144.79	42778. 78	8424.70	15.22	4.514
CV (%)		5.75	5.48	7.85	6.07	7.47

ns = non-significant; * and ** = Significant at 5% and 1% probability level, respectively.

Seed Yield

Effect of year, effect of irrigation, effect of year×irrigation, effect of cultivars, effect of year×cultivars, effect of irrigation×cultivars and effect of year×irrigation×cultivars were significant at ($p<0.01$) on seed yield (Table 4). In irrigation management, seed yield with the treatment with 100% and 80% water requirements, had the highest value with the means of 1345 kg ha^{-1} and 1379 kg ha^{-1} respectively (Figure 1). In the effect of year×irrigation, maximum seed yield was in 2017, with the treatment with 100% and 80% water requirements with the means of 1494 kg ha^{-1} and 1593 kg ha^{-1}, respectively (Figure 2). In peanut cultivars, Jonobi cultivar had the highest value with a mean of 1273 kg ha^{-1} (Figure 3). For the effect of irrigation×cultivars, the highest values were obtained in the treatment with 100% water requirement in Jonobi cultivar with a mean of f 1732 kg ha^{-1}, and also in the treatment with 60% water requirement in Guil cultivar with a mean of 1667 kg ha^{-1} (Figure 5). The maximum seed yield for effect of year×irrigation×cultivars was observed in 2017 in the treatment with 100% water requirement in the Jonobi cultivar with a mean of 1856 kg ha^{-1} (Table 11). Water stress causes an extreme reduction in the yield (Bunari et al., 1992; Bontang et al., 2010). Amiri et al., (2010) in their study reported the maximum seed yield for peanuts with a condition of 100% water requirement. Abdzad Gohari et al., (2018) examined different peanut cultivars under water stress conditions and non-stress conditions. They concluded that seed yield was higher in all cultivars in non-stress conditions compared to conditions with water stress. Bontang et al (2010) examined the effect of intervals irrigation with periods of daily, two days and three days. They concluded that the maximum seed yield was achieved with daily irrigation conditions.

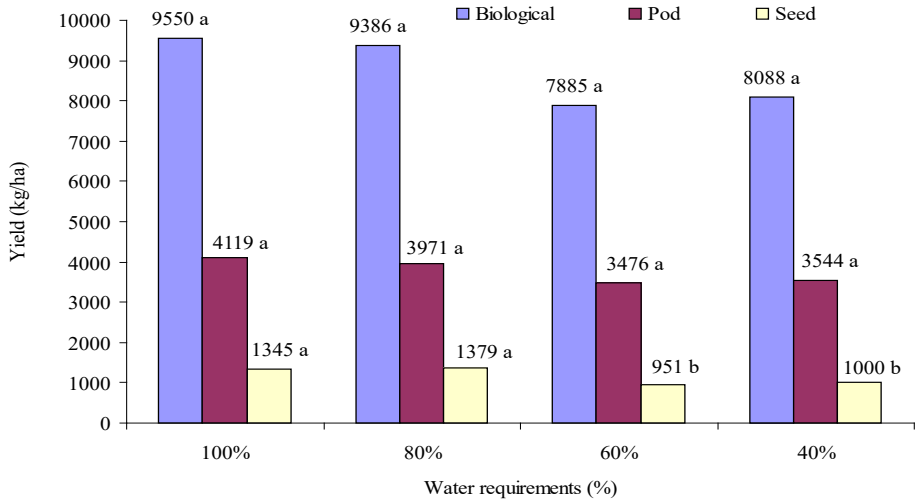


Figure 1. Effect of irrigation management on biological, pod and seed yield in peanut.

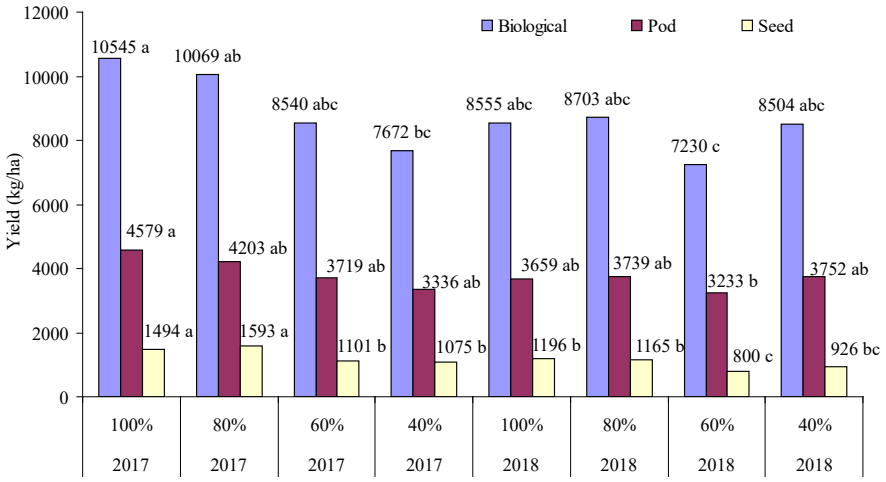


Figure 2. Effect of irrigation×year on biological, pod and seed yield in peanut.

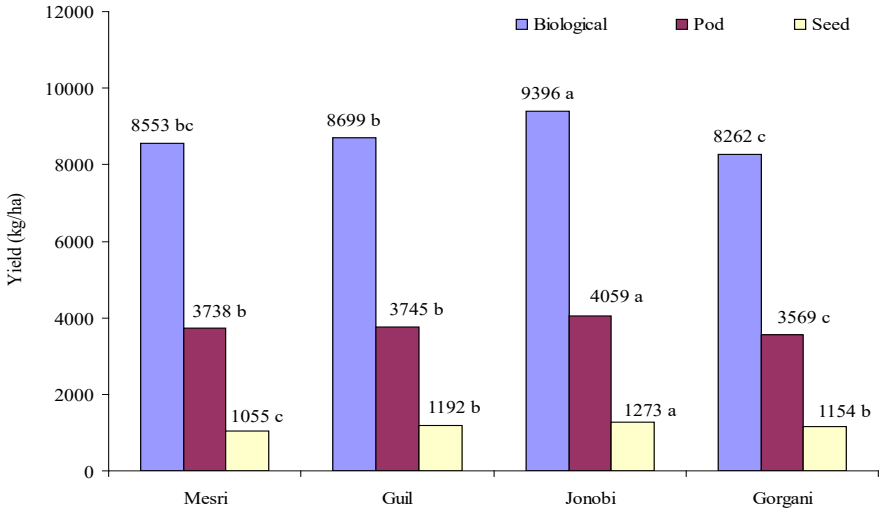


Figure 3. Biological, pod and seed yield in different peanut cultivars.

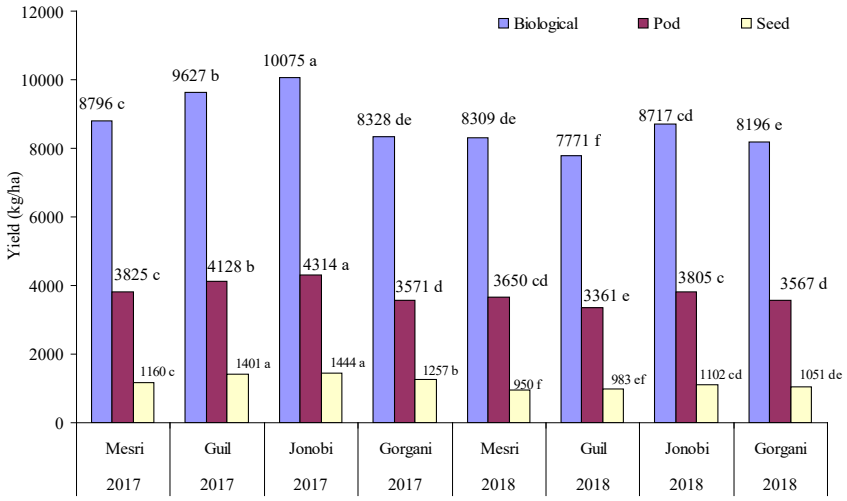


Figure 4. Effect of year×cultivars on biological, pod and seed yield in peanut.

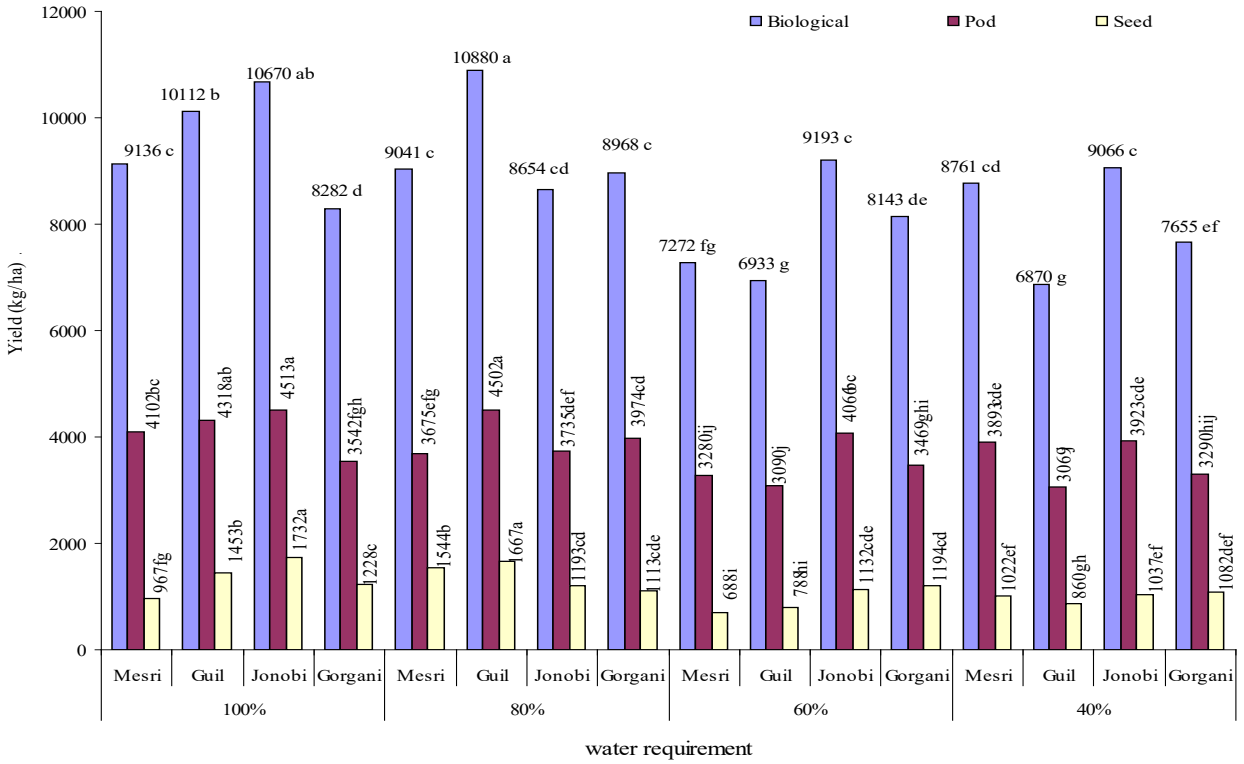


Figure 5. Effect of irrigation×cultivars on biological, pod and seed yield in peanut.

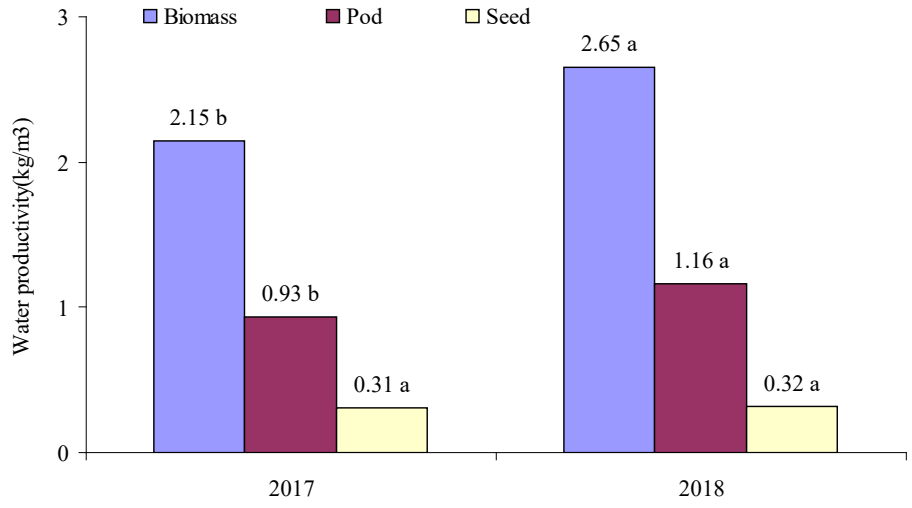


Figure 6. Water productivity in biomass, pod and seed yields in the studied years.

100-seed weight

The effects of year, irrigation, cultivars, irrigation × cultivars and year × irrigation × cultivars on 100-seeds weight were significant at ($p<0.01$) (Table 4). The 100-seeds weight was higher in the first and the second years with 69.6 g and 58.9 g, respectively (Table 6). Higher average of 100-seeds weight was related to 100% and 80% of the water requirements; i.e. 72.8 g and 77.9 g, respectively (Table 7). Compared to other cultivars, the 100-seeds weight in the Jonobi cultivar with an average of 66.9 g and the Gorgani cultivar with an average of 66.1 g had the highest value (Table 8). The highest effect of "year × cultivar" as found in Jonobi , Gorgani and Mesri cultivars (70.4 g , 71.8 g and 72.5 g respectively) in the first year, compared to the second year (Table 9). The highest effect of irrigation×cultivars was related to the 80% of water requirement and Mesri and Guil cultivars with an average of 90.4 g and 89 g, respectively (Table 10). For the effect of year×irrigation×cultivars the maximum amount of 100-seeds weight was observed in the 60% of water requirement and in Mesri cultivar with an average of 96 g in the first year (Table 11). Kabadagi and Setty (2010) evaluated of peanut cultivars and observed that the 100-seeds weight under stress conditions was lower than stress-free conditions. Shinde and Laware (2010) showed that water stress reduces 100-seeds weight. At the seed filling

stage, drought stress affects 100-seeds weight either by reducing the movement of storage materials toward the seed due to water restriction, or by reducing the share of photosynthesis in leaves. For this reason, decreasing the movement of storage materials and water restriction because of drought led to the limited translocation of nutrients in the plant and reduction in 100-seeds weight.

Number of pods per plant

The effects of the peanut cultivars, year×cultivar, irrigation×cultivars and year×irrigation× cultivars on the number of pods per plants were significant ($p<0.01$) (Table 4). The highest number of pods per plant was in Jonobi cultivar with an average of 31 pods (Table6). For the effect of year×cultivar, maximum number of pods per plants was observed in Jonobi cultivar with an average of 34 pods in the first year (Table 9). About the effect of irrigation×cultivars, maximum number of pods per plants was obtained in 100% of water requirement and in Jonobi cultivar with an average of 35 pods (Table 10). For the effect of year×irrigation×cultivars, maximum number of pods per plants was observed in the first year for 100% of water requirement and in Guil and Jonobi cultivars with an average of 40 and 43 pods, respectively (Table 11). As the amount of irrigation water increases, the period of pods growth and maturation become longer and the speed of leaves aging becomes slower; then, the number of seeds per plant will increase. On the other hand, reducing the amount of water irrigation and also increasing the temperature, lead to premature aging of the plant.

Pod length

The effects of irrigation, cultivars, year×cultivar, irrigation×cultivars and year×irrigation×cultivars on pods length were significant ($p<0.01$) (Table 5). For the irrigation effect, the maximum pods lengths were observed in the 100% and 80% water requirements with an average of 4.4 cm and 4.9 cm, respectively (Table 7). Among the peanut cultivars, the maximum amount of pods length was related to Mesri cultivar with an average of 4.3 cm (Table 8). About the interaction effect of year×cultivar, the maximum pods length was observed for Mesri cultivar with an average of 4.6 cm in the first year (Table 9). For the interaction effect of irrigation×cultivars, the maximum length was found in 80% of the water requirements and in Mesri cultivar with an average of 5.3 cm (Table 10). For the effect of year×irrigation×cultivars the maximum pods length was observed in 60% of the water requirement and in Mesri cultivar with an average of 5.5 cm in the first year (Table 11). To achieve the best growth and maximum pods length and yield, the amount of water should not lead to drought stress nor to the accumulation of large amounts of water around the plant's roots; by deviating from this amount of water, it is necessary to determine the exact amount of yield decrease.

Table 5. Mean squares form the combined ANOVA for Agronomic Traits.

Source	df	Pod Length	Number of seeds per plant	Water productivity based on		
				Biomass	Pod	Seed
Year	1	0.375^{ns}	2330.51^{*}	5.861^{*}	1.290^{*}	0.005^{ns}
Replication	4	1.458^{ns}	538.23^{ns}	0.740^{**}	0.106^{ns}	0.039^{ns}
Irrigation	3	10.92^{**}	1240.96^{*}	4.889^{**}	1.044^{**}	0.039^{*}
Year×Irrigation	3	0.247^{ns}	291.54^{ns}	2.515^{*}	0.499^{*}	0.017^{*}
Error	12	1.073	280.174	0.700	0.142	0.009
Cultivar	3	0.792^{**}	462.43^{**}	0.470^{**}	0.092^{**}	0.013^{**}
Year×cultivar	3	1.664^{**}	191.51^{**}	0.432^{ns}	0.081^{**}	0.012^{**}
Irrigation×cultivar	9	0.448^{**}	180.62^{**}	0.676^{**}	0.112^{**}	0.033^{**}
Year×cultivar×Irr	9	0.437^{**}	97.98^{**}	0.558^{**}	0.114^{**}	0.024^{**}
Error	48	0.65	5.771	0.920	0.004	0.001
CV (%)		6.22	4.46	5.93	5.68	8.10

ns = non-significant; * and ** = Significant at 5% and 1% probability level, respectively.

Table 6. Effect of year on agronomic traits

Year	100-seed weight	Number of pods per plant	Pod Length	Number of seeds per plant
2017	69.6 a	31 a	4.0 a	59 a
2018	58.9 b	26 b	4.1 a	49 b

Table 7. Effect of water requirement on agronomic traits

WR	100-seed weight	Number of pods per plant	Pod Length	Number of seeds per plant
100%	72.8 a	31	4.4 a	60 a
80%	77.9 a	30	4.9 a	60 a
60%	53.1 b	27	3.6 b	47 b
40%	53.3 b	26	3.4 b	48 b

Table 8. Traits measured in different cultivars

Cultivar	100-seed weight	Number of pods per plant	Pod Length	Number of seeds per plant
Guil	61.2 b	27 b	4.3 a	52 b
Gorgani	66.9 a	27 b	4.0 b	52 b
Jonobi	62.9 b	31 a	3.9 c	60 a
Mesri	66.1 a	28 a	4.1 b	51 b

Number of seeds per plant

The effects of year, irrigation, peanut cultivars, year × cultivars, irrigation × cultivars and year×irrigation×cultivars on the number of seeds per plant were significant ($p<0.01$) (Table 4). For irrigation effect, the highest number of seeds per plant (average of 60 seeds) was related to 100% and 80% of water requirements (Table 7). Among the peanut cultivars, the highest number of seeds per plant was observed in the Jonobi cultivar with an average of 60 seeds (Table 7) for the interaction of year×cultivar, (Table 7). the maximum number of seeds per plant was found in the Jonobi cultivar in the first year (Table 9). For the interaction effect of irrigation×cultivars, the maximum number of seeds per plant was obtained in 100% of water requirements and in Jonobi cultivar with an average of 71 seeds (Table 10). About the effect of year × irrigation×cultivars the maximum number of seeds per plant was observed in 40% of the water requirement and in Jonobi cultivar with an average of 77 seeds in the first year (Table 11). At the beginning of flowering, the plant grows rapidly; by providing the available moisture, the length of the reproductive period and also the rate of photosynthesis increase. In this situation, more flowers are formed in the plant, which in turn affects the formation of fertile pods and seed production. The reason for decreasing the number of seeds under drought stress is the decline in pods number in the main and secondary brunches. During the flowering stage, supplemental irrigation increases the number of seeds per plant. Plants that were exposed to drought stress during pod formation and growth produced the least number of pods, seeds, and dry matter, compared to plants that experienced drought stress during other growth stages.

Table 9. The effect of different years and cultivars on the measured traits

Year	Cultivar	100-seed weight	Number of pods per plant	Pod Length	Number of seeds per plant
2017	Guil	63.8 b	30 c	4.6 a	57 c
	Gorgani	71.8 a	32 b	3.8 de	60 b
	Jonobi	70.4 a	34 a	3.6 e	64 a
	Mesri	72.5 a	28 d	4.1 bc	53 d
2018	Guil	58.5 de	25 e	4.0 cd	46 f
	Gorgani	62.1 bc	22 f	4.2 b	43 g
	Jonobi	55.5 e	28 d	4.1 bc	57 c
	Mesri	59.6 cd	27 d	4.0 bc	49 e

Table 10. Interaction of water requirements and different cultivars in measured traits

Year	Cultivar	100-seed weight	Number of pods per plant	Pod Length	Number of seeds per plant
100%	Guil	55.4 ef	30 bcd	4.8 b	58 d
	Gorgani	77.0 b	33 ab	4.0 d	61 c
	Jonobi	80.3 b	35 a	4.1 d	71 a
	Mesri	78.6 b	27 efg	4.6 bc	50 f
80%	Guil	90.4 a	28 def	5.3 a	58 d
	Gorgani	89.0 a	31 bcd	4.9 b	64 b
	Jonobi	69.3 c	29 cde	4.8 b	59 b
	Mesri	62.9 d	32 bc	4.4 c	60 cd
60%	Guil	44.7 h	25 fg	3.7 e	42 g
	Gorgani	50.0 g	25 fg	3.5 efg	41 g
	Jonobi	52.1 fg	32 ab	3.4 fg	57 d
	Mesri	65.7 cd	26 fg	3.8 de	48 f
40%	Guil	54.2 efg	27 efg	3.3 fg	48 f
	Gorgani	51.9 fg	22 h	3.6 ef	41 g
	Jonobi	50.1 g	29 de	3.2 g	54 e
	Mesri	57.2 e	26 fg	3.5 efg	48 f

Table 11. Effect of year× water requirements×cultivars on agronomic traits measured in 2017 and 2018

Year	water requirements	Cultivars	Yield (kg ha^{-1})			100-seed weight	Number of pods per plant	Pod Length	Number of seeds per plant
			Biomass	Pod	Seed				
2017	100%	Mesri	9696 def	4405 cd	977 klmn	48 jkl	35 b	5.1 b	68 bcd
		Guil	11310 b	4904 ab	1592 bcde	74.7 ef	40 a	3.6 g-m	70 b
		Jonobi	12170 a	5230 a	1856 a	80.0 de	43 a	3.4 k-o	77 a
		Gorgani	9004 efg	3779 gh	1551 cde	88.6 bc	31 c-f	4.7 bcd	58 fgh
	80%	Mesri	9679 def	3939 efg	1645 bc	96.0 a	31 c-f	5.5 a	62 ef
		Guil	10650 bc	4317 de	1755 ab	93.0 ab	32 b-e	4.8 bcd	69 bc
		Jonobi	9986 cd	4266 def	1488 cde	86.2 cd	34 bcd	4.8 bcd	63 de
		Gorgani	9961 cd	4292 def	1483 cde	81.4 de	35 b	4.5 de	65 cde
	60%	Mesri	8348 g-j	3738 gh	806 no	43.5 lm	30 def	4.0 fgh	54 hi
		Guil	8554 gh	3757 gh	1081 ijk	57.5 hi	30 def	3.0 op	52 ij
		Jonobi	9761 cde	4215 def	1422 efg	61.8 gh	34 bc	3.1 nop	64 de
		Gorgani	7498 jkl	3165 jkl	1096 ijk	67.9 g	24 ijk	4.1 efg	45 lm
	40%	Mesri	7461 jkl	3218 jk	1214 hi	67.9 g	25 hij	3.9 ghij	47 kl
		Guil	7997 h-k	3534 g-j	1175 hij	61.8 gh	27 f-i	3.7 g-l	50 ijk
		Jonobi	8380 g-j	3544 g-j	1010 j-m	53.5 ij	26 g-j	3.2 l-p	50 ijk
		Gorgani	6850 lm	3046 kl	900l mno	52.2 ijk	24 ijk	3.2 m-p	45 lm
2018	100%	Mesri	8576 gh	3799 gh	957 klmn	62.7 gh	24 ijk	4.6 cde	48 jkl
		Guil	8918 e-h	3733 gh	1314 fgh	79.2 de	25 hij	4.4 de	52 ij
		Jonobi	9166 d-g	3797 gh	1607 bcd	80.5 de	26 g-j	4.7 bcd	65 de
		Gorgani	7561 i-l	3306ijk	906lmno	68.5fg	23jkl	4.4de	42m
	80%	Mesri	8402 g-j	3411 hijk	1443 def	84.9 cd	26 g-j	5.1 b	54 hi
		Guil	11110 b	4686 bc	1578 cde	85.0 cd	29 efg	5.0 bc	59 f
		Jonobi	7323 kl	3204 jk	898l mno	52.4 ijk	25 hij	4.8 bcd	54 ghi
		Gorgani	7975 hijk	3656 ghi	743 o	44.4 lm	29 e-h	4.4 def	54 hi
	60%	Mesri	6197 mn	2823 lm	571 p	45.8 klm	21 kl	3.5 i-o	31 n
		Guil	531 1o	2422 n	495 p	42.5 lm	19 lm	3.9 f-i	30 n
		Jonobi	8626 gh	3916 fg	841 mno	42.5 lm	30 def	3.6 h-n	51 ijk
		Gorgani	8787 fgh	3772 gh	1292 fgh	63.5 gh	28 f-i	3.4 j-o	51 ijk
	40%	Mesri	10060 cd	4567 bcd	830 no	40.5 m	29 fgh	2.8 p	50 ijk
		Guil	5743 no	2604 mn	546 p	41.9l m	16 m	3.6 h-n	33 n
		Jonobi	9752 cde	4303 de	1063 ijkl	46.6 j-m	32 b-e	3.3 l-o	58 fg
		Gorgani	8460 ghi	3535 ghij	1265 gh	62.2 gh	28 f-i	3.9 g-k	50 ijk

Water productivity based on yield

Effect of year, effect of irrigation, effect of year×irrigation, effect of cultivars, effect of year ×cultivars, effect of irrigation×cultivars and effect of year×irrigation×cultivars were significant at 1% level on water productivity of biological yield and pod yield (Table 5). The highest amount of water productivity was obtained in biological yield with the treatment 40% water requirements with the mean of 2.92 kg m^{-3} (Table 7). For effect of year×irrigation, the maximum water productivity was in biological yield in 2018 and in 40% of the water requirement treatment with the mean of 3.65 kg m^{-3} (Figure 8). In different peanut cultivars, Jonobi cultivar with the mean of 2.6 kg m^{-3} had the highest water productivity in biological yield compared to other cultivars (Figure 9). For the effect of irrigation×cultivars, the highest value belonged to non-irrigation treatment and Jonobi cultivar with the mean of 3.29 kg m^{-3} (Figure 11). Maximum water productivity in biological yield was observed in effect of year×irrigation×cultivar in 2018, with 40% water requirements treatment in Mesri cultivar with a mean of 4.32 kg m^{-3} (Table 12). For the effect of irrigation, highest value of water productivity was for pod yield with 40% water requirements treatment with the mean of 1.28 kg m^{-3} (Figure 7). For the effect of year×irrigation, highest value of water productivity was in pod yield in 2018 with 40% water requirements treatment with the mean of 1.61 kg m^{-3} meter (Figure 8). Guil cultivar with the mean of 1.13 kg m^{-3} had the highest amount of water productive in pod yield among all peanut cultivars (Figure 9). For the effect of year×cultivar, Jonobi cultivar in 2018 had the highest value with the mean of 1.25 kg m^{-3} (Figure 10). For the effect of irrigation×cultivar, the highest values belonged to Jonobi cultivar with 40% water requirements treatment and Mesri cultivar with 40% water requirements treatment with the means of 1.43 kg m^{-3} and 1.44 kg m^{-3}, respectively (Figure 11). For the effect of year × irrigation×cultivar, the maximum water productivity was in pod yield in year 2018 and in 40% of the water requirement treatment in Mesri cultivar with the mean of 1.96 kg m^{-3} (Table 12). For the effect of irrigation, the highest water productivity belonged to seed yield, with 40% water requirements treatment with a mean of 0.35 kg m^{-3} (Figure 7). For the effect of year×irrigation, maximum water productivity was obtained in seed yield in 2018, with 40% water requirements treatment with a mean of 0.40 kg m^{-3} (Figure 8). In different peanut cultivars, Jonobi cultivar with the mean of 0.34 kg m^{-3} had the highest water productivity in seed yield compared to other cultivars (Figure 9). For the effect of year×cultivar, year 2018 and Gorgani cultivar had the highest value with a mean of 0.36 kg m^{-3} (Figure 10). For the effect of irrigation×cultivar, the highest values belonged to the treatment with 80% water requirement and the Guil cultivar with the mean of 0.41 kg m^{-3} and also in the 40% water requirements treatment and Gorgani cultivar with the mean of 0.40 kg m^{-3} (Figure 11). Maximum water productivity was obtained for seed yield for the effect of

year×irrigation×cultivar in 2018 with the 40% water requirements treatment for Gorgani cultivar with the mean of 0.54 kg m^{-3} (Table 12). Puang Bat et al. (2010) examined eleven peanut cultivars in the irrigation conditions with and without stress. They concluded that water stress reduces seeds' water productivity from 1.69 kg m^{-3} under non-stress conditions to 0.98 kg m^{-3} under stress conditions. Water stress has a negative effect on many plant processes such as photosynthesis, evaporation, precursor accumulation and allocation (Ohashi et al., 2006), and leads to a substantial reduction in production (Reddy et al., 2004). Therefore, water stress is one of the methods to maximize water productivity and increase yield per unit of water consumed in deficit irrigation, in which the plant is put under stress conditions at a certain stage of growth or the whole duration of the growing season (Liu et al., 2008).

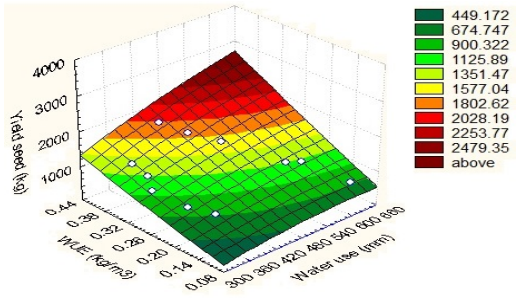
Table 12. Effect of year×water requirements×cultivars on agronomic traits measured in 2017 and 2018.

Year	water requirements	cultivars	Water productivity based on yield		
			Biomass	Pod	Seed
2017	100%	Mesri	1.67 d	0.76 mn	0.17 p
		Guil	1.94c d	0.84 lmn	0.27 jkl
		Jonobi	2.10 cd	0.90 jkl	0.32 g-j
		Gorgani	1.55 d	0.65 o	0.26 j-m
	80%	Mesri	2.70 cd	0.84l mn	0.35 e-i
		Guil	2.28 cd	0.92 ijkl	0.37 efg
		Jonobi	2.14 cd	0.91 ijkl	0.32 g-k
		Gorgani	2.13 cd	0.92 ijkl	0.32 g-k
	60%	Mesri	2.38 bcd	1.70 h	0.23 l-o
		Guil	2.46 bcd	1.80 h	0.31 g-k
		Jonobi	2.80 abcd	1.21 fg	0.41 cde
		Gorgani	2.15 cd	0.90 jkl	0.32 g-k
	40%	Mesri	2.13 cd	0.92 ijkl	0.35 f-i
		Guil	2.29 cd	1.10 hij	0.34 ghi
		Jonobi	2.4 bcd	1.20 hij	0.29 ijkl
		Gorgani	1.96 cd	0.87 lm	0.26 klmn
2018	100%	Mesri	1.89 cd	0.84 lmn	0.21 m-p
		Guil	1.97 cd	0.82 lmn	0.29 i-l
		Jonobi	2.20 cd	0.83 lmn	0.35 e-h
		Gorgani	1.67 d	0.73 no	0.20 nop
	80%	Mesri	2.32 cd	0.94 ijkl	0.40 def
		Guil	3.70 abcd	1.29 ef	0.44 bcd
		Jonobi	2.20 cd	0.88	0.25 lmn
		Gorgani	2.20 cd	1.10 hijk	0.21 m-p
	60%	Mesri	2.27 cd	1.30 hi	0.21 m-p
		Guil	1.94 cd	0.89 kl	0.18 op
		Jonobi	3.15 abcd	1.43 cd	0.31 h-k
		Gorgani	3.21 abcd	1.38 de	0.47 b
	40%	Mesri	4.32 a	1.96 a	0.36 efgh
		Guil	2.47 bcd	1.12 gh	0.23 l-o
		Jonobi	4.19 ab	1.85 b	0.46 bc
		Gorgani	3.63 abc	1.52 c	0.54 a

Evaluation of production function of peanut cultivars in conditions of complete irrigation and water stress

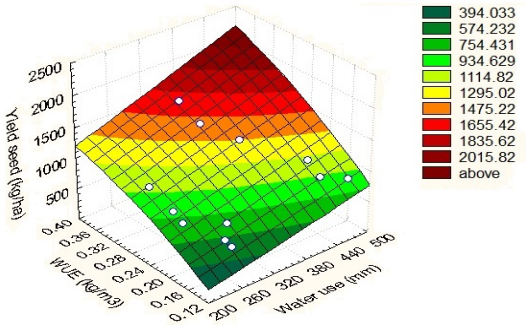
In this study all factors were assumed as fixed and crop yield was considered as a function for water requirement. Derivatives of the production function are the effects of two concepts of water use efficiency and amount of water consumed on the yield. The overall shape of the production function in this study is quadratic. Many researchers have examined the estimation of water demand function on crops, and they have suggested the quadratic function as the best production function (Abdzad Gohari et al., 2018; Amiri et al., 2015). Amount of water used and examination of the effect of deficit irrigation and water productivity on yield, represents the nonlinear quadratic equations in different peanut cultivars which can be seen in Figure 12.

Guil cultivar in 2017



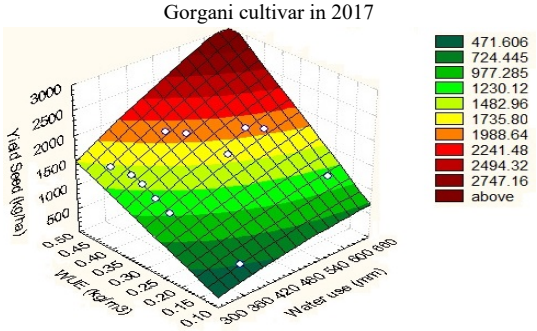
Y_{seed} = -665.276+3.204WU+47.657WUE-0.003WU^2+8.179 WU.WUE+1093.99WUE^2

Guil cultivar in 2018



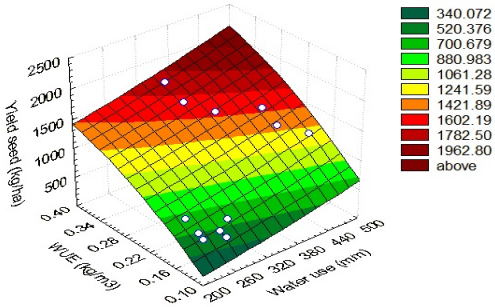
Y_{seed} = -722.707+1.027WU+5687.375 WUE+3.526e-4WU^2+5.121WU.WUE-6138.32WUE^2

Gorgani cultivar in 2017



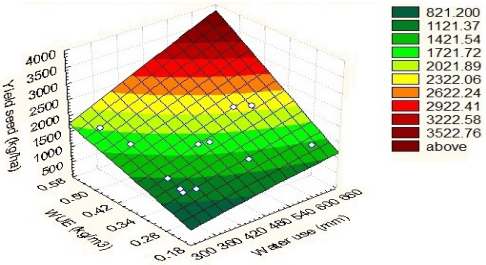
Y_{seed}= -682.716+2.562WU+818.54WUE-0.002WU^2+8.239WU.WUE-229.409WUE^2

Gorgani cultivar in 2018



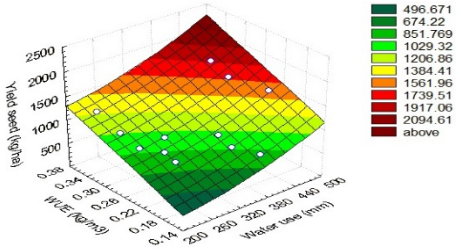
Y_{seed} = -86.175+0.458WU+7184.94WUE+0.001WU^2+ 2.544WU.WUE-7063.91WUE^2

Jonobi cultivar in 2017



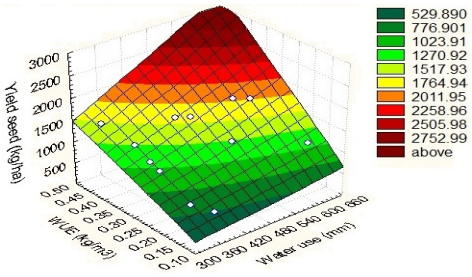
Y_{seed} = -137.098+0.492 WU+122.306 WUE-5.142e-4WU^2+9.919WU.WUE-26.005WUE^2

Jonobi cultivar in 2018



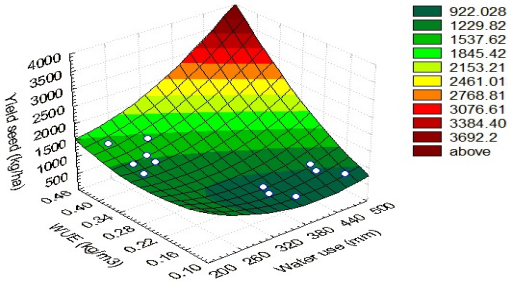
Y_{seed} = -275.88-1.115WU+ 4077.93WUE+0.005WU^2+1.867WU.WUE-759.608WUE^2

Mesri cultivar in 2017



Y_{seed} = 30.371-0.472WU+512.828WUE+6.084e-4WU^2+9.866WU.WUE-780.698WUE^2

Mesri cultivar in 2018



Y_{seed} =4968.76-18.936WU-12141.4WUE+0.02WU+29.633 WU.WUE +11463.35 WUE^2

Y: Yield (kg ha^{-1}), WU: Water use (mm) and WUE: Water use efficiency (kg m^{-3}).

Figure 7. Production Functions yield-Water productivity-Water use, in Peanut Cultivars in 2017 and 2018.

CONCLUSIONS

This study was conducted with the aim of optimization of water use in peanut cultivars under different irrigation managements. Increasing the amount of water to the optimum level of consumption is result in the increase in peanut yield. According to the analysis of the results, the amount of water consumed and the amount of water productivity is effective on the amount of yield. By increasing irrigation up to 80% of water requirement, in Guil cultivar, biological yield (10880 $kg.ha^{-1}$) and pod yield (4502 kg $.ha^{-1}$) and seed yield (1667 $kg.m^{-3}$) increased. Water productivity on biological yield (4.32 kg m^{-3}) and pod yield (1.96 $kg.m^{-3}$) in Mesri cultivar and Water productivity on seed yield in Gorgani cultivar were 0.54 $kg.m^{-3}$. The coefficients obtained from water production function of the consumed water on the yield in irrigation conditions, indicated that supplying the proper amount of water will maximize the yield production and supplying 100% of the required water will maximize the yield in peanut cultivars.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest or personal relationships.

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EFFECT OF MUNICIPAL SOLID WASTE COMPOST, GYPSUM AND MYCORRHIZA ON METALS CONTENT IN SOIL AND PEANUT GRAIN

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ABSTRACT

Municipal solid waste compost (MSWC) is widely used as an organic soil amendment and fertilizer on agricultural land. However, applying MSWC can cause adverse effects due to the heavy metals contained. This study aimed to determine the heavy metal content of MSWCs in the presence of mycorrhizae and gypsum and their effects on soil and peanut grain. The field experiment was conducted using a split factorial design based on a Randomized Complete Block Design (RCBD) with three replications in Iran during 2018 and 2019. The main factor includes two levels of gypsum (0 and 150 kg ha^{-1}) and the sub-factors include the presence and absence of Arbuscular mycorrhizal fungi (AMF) and different levels of MSWC at five levels (0, 2, 4, 6, and 8 t ha^{-1}). The findings showed that MSWC significantly increased the concentrations of manganese (Mn), lead (Pb), nickel (Ni), zinc (Zn), cobalt (Co), chromium (Cr), cadmium (Cd), and boron (B) in soil and grains. In addition, Co, Ni, and Zn concentrations in grain increased and Pb, Mn, Ni, and Zn concentrations in soil decreased with AMF application. Gypsum treatment also had no significant effect on metals in both grain and soil. According to the obtained results, the use of 4 t ha^{-1} of MSWC along with mycorrhiza in peanut cultivation is suggested in order to reduce the environment risks of soil and plants cause by the use of compost, and also use the benefits of urban waste compost.

Keywords: Gypsum, Heavy metals, Human health, Mycorrhiza, Peanut.

INTRODUCTION

Over the past few years, the use of MSWCs in agricultural fields has increased. MSWC provides the organic compounds required by the soil. A number of studies have shown that the addition of MSWC improves the physico-chemical properties of the soil due to the high amount of organic matter it contains (Batool et al., 2015; Yuksel, 2015; Tepecik et al. 2022). Using MSWCs improves soil microbial characteristics and crop production (Meena et al., 2016). Furthermore, they improve crop yields and improve soil nutrition, soil structure, and soil buffers. MSWCs are high in nitrogen, and humic compounds which include humic and fluvial acids (Srivastava et al., 2016). However, there are also negative impacts associated with the use of MSWC. One of the main concerns of its use is its heavy metals, which increase their accumulation in the soil (Yuksel, 2015; Tepecik et al. 2023). Ayari et al. (2010) showed that the using MSWC increased increased soil concentrations of Cd, Cr, copper (Cu), Ni, Pb, and Zn compared to control.

Heavy metals are the main contaminants causing environmental and human health problems because they do not biodegrade. Therefore, soil and crop exposure to these pollutants shortens life (Gu et al., 2019). They are essential for plant growth but toxic to animals, and are also toxic to plants if their concentrations exceed tolerance levels. Studies show that heavy metals accumulate in the soil as a result of the weathering of soil minerals and the use of treated wastewater, sewage sludge, and fertilisers (Nouri et al., 2016; Ongun et al. 2023). Besides polluting soil, heavy metals also affect food production, quality and safety. Heavy metals can affect plant metabolism and physical and biochemical processes, leading to reduced growth, reduced biomass production, and reduced metal accumulation (Edelstein and Ben-Hur 2018; Nouri et al., 2016). Plant exposure to heavy metals causes oxidative stress, cellular damage, and disrupts cellular ion homeostasis (Yadav, 2010).

To cope with high concentrations of heavy metals, plants use a variety of strategies to prevent them from entering the roots one such strategy (Rousta et al., 2023). Heavy metals can be immobilised by mycorrhizae, by sequestration of the metal and by complexation with organic compounds secreted by the roots of the plant (Antosiewicz et al., 2014). In addition to the provision of essential minerals to the plant, mycorrhizae also improve the physico-chemical properties of the soil (Nouri et al., 2020) and it also act as filters (Wu et al., 2019). They block

xenobiotics in their mycelium and immobilise heavy metals in the roots (Matinizadeh et al., 2022). The co-occurrence of mycorrhizal fungi with plant roots in areas of heavy metal contamination has already been reported (Riaz et al., 2021). The second strategy the tolerance mechanism for detoxification is activated if the plant cannot prevent heavy metals from entering the roots (Nouri et al., 2016). This mechanism includes the sequestration and compartmentalisation of metals in different intracellular compartments, the transport of metal ions, the binding of metals to the cell wall, the biosynthesis or accumulation of osmolytes and osmoprotectants (Emamverdian et al., 2015, Fryzova et al., 2018). Subsequent human intake can cause diseases including cancer, cardiovascular problems, depression, blood, gastrointestinal and renal failure, and osteoporosis (Edelstein and Ben-Hur, 2018).

Gypsum has been introduced as a soil remediation and fertilisation tool in many countries. Gypsum can meet soil sulphur requirements as a fertiliser. It provides a high level of exchangeable divalent cations and coagulates the colloids in the soil, particularly in temperate soils. It is also a source of calcium for clay flocculation in acidic and alkaline soils (Anikwe et al., 2016). Research shows that by altering water-holding capacity, soil structure, water infiltration rate and increasing water mobility in the soil, gypsum is involved in improving plant production, strengthening plant root systems, increasing nutrient availability and soil moisture (Batool et al., 2015). However, the extent of plant response and environmental improvement from gypsum application, influenced by different local conditions, remains unknown (Zoca and Penn, 2017). Symbiotic root endophytes can enhance plant growth. Arbuscular mycorrhizal fungi from the phylum Glomeromycota form symbiotic associations with 72% of plants, improving nutrient uptake from the soil. They also improve plant tolerance to biotic and abiotic stress (Tian et al., 2021). Mycorrhizal fungi increase plants' ability to absorb nutrients and water, and plants provide fungal carbohydrates (Wang et al., 2017; Cukurcalioglu et al., 2023).

This study was conducted in view of the nutritional benefits of peanuts, which are rich in protein and oil and are widely consumed in most countries of the world, particularly in Africa and Asia (Pandey et al., 2014). Peanut grain oil is a good source of monounsaturated fatty acids, including oleic acid, that help lower bad cholesterol in the blood. Peanut grains also contain vitamin E and niacin, which help to maintain the health of the brain (Mekdad et al., 2021). Therefore, the aim of the present study was to investigate the heavy metal concentration in peanut grains and soil after treatment with MSWC, gypsum and mycorrhiza over two growing years.

MATERIALS AND METHODS

Land preparation and planting operations

The field experiment was carried out in the split factorial design based on a completely randomized block in three replications in Astaneh-Ashrafiyeh, Iran (Long=49º 57'10.64", Lat=37º19' 21.96", 2 m a.s.l) in 2018 and 2019 cropping year. Rainfall was 1211 mm on average per year. Mean daily maximum and minimum temperatures during the peanut growing season ranged from 29.7 to 37.9 °C and from 14.6 to 26.6 °C, respectively. The mean daily relative humidity was between 45% and 91%. Table 1 presents temperature, precipitation, and sunny hours during the plant growth period and long term of Astaneh-Ashrafiyeh region.

Table 1. Mean temperature, total precipitation and sunny hours per month during plant growth (Astaneh-Ashrafiyeh Weather Station)

Year	Metrological parameters	May	Jun	Jul	Aug	Sep
2018	Temp.Mean (ºC)	19.4	23.1	28.1	27	25.1
	Precipitation (mm)	37.2	48.7	30.8	68.4	13.8
	Humidity (%)	74	75	73	77	74
	Sunny hours (h)	170.4	230.3	295.4	164.9	209.7
2019	Temp.Mean (ºC)	19.2	24.6	27.2	25.4	23
	Precipitation (mm)	64.4	9.9	58.3	25.3	156
	Humidity (%)	73	67	76	71	83
	Sunny hours (h)	222	306.4	253.3	226.1	123.5
Long term	Temp.Mean (ºC)	18.3	22.7	25.1	26.2	22.5
	Precipitation (mm)	84.5	62.2	39.2	41.5	44.6
	Humidity (%)	82.6	78.6	72.3	78.4	81.4
	Sunny hours (h)	158.1	219	232.4	176.3	193.2

Samples were taken from a depth of 30 cm to analyse the soil properties. The soil of the experimental field was sandy-loam in texture, with neutral pH (7.22), low in organic C (0.94%) and low EC (0.297 dS m^{-1}). The soil type is the Alfisol that is common in this area. The results of the soil analysis are presented in Table 2.

Table 2. Soil analysis at the start of cultivation.

Depth	pH	EC	OC	P	K	N	Ca	Sand	Silt	Clay
		(dS m^{-1})	(%)	(mg kg^{-1})		(%)	(mEq L^{-1})		(%)	
30 cm	7.22	0.297	0.94	9.15	256.8	0.08	6.4	31	63	6

Treatments and experimental design

The research was carried out as the split factorial design based on a Randomized Complete Block Design with three replications. The main factor includes two levels of gypsum (0 and 150 kg ha^{-1}) and the sub-factors include the presence and absence of Arbuscular mycorrhizal fungi (AMF), and different levels of MSWC at five levels (0, 2, 4, 6, and 8 t ha^{-1}).

Before sowing, the field was cultivated with a plough and a disc harrow. To plant the seeds of cv. 'NC2' (North Carolina variety), 6 cm deep furrows were made in the soil and the seeds were placed in the furrows and covered with soil. Before planting, 50 kg ha^{-1} of phosphorus (as triple superphosphate) and potassium (as potassium sulfate) fertilizers were added to the soil. The 60 kg ha^{-1} urea fertilizer (25.2 kg N ha^{-1}) was also added to the soil in two stages (before planting and before flowering). For the application of the treatments, 60 plots of 2.5 × 3 m^2 were prepared. A space was left between the plots to avoid interference with the treatments. Weeds were pulled by hand as soon as they appeared.

Gypsum (gypsum with the chemical formula $CaSO_4.2H_2O$ (with a sulphur content of 18% and a calcium content of 22%) was applied in one stage (V3 stage; 20 days after germination). MSWC was applied in the presence or absence of mycorrhizal fungi in the vegetative stage and before flowering at five rates of 0, 2, 4, 6, and 8 t ha^{-1} before sowing. Table 3 shows the characteristics of the MSWC. The mycorrhizal fungi were also obtained from Pishtaz Varian Knowledge Enterprise and for each kg of seeds, 10 g of arbuscular mycorrhizal fungi were mixed in 1 Liter of water and 400 g of sugar and treated for 1 h.

Table 3. MSWC characteristics used for the treatment

EC (dS m^{-1})	pH	OM (%)	OC (%)	Particle diameter (mm)	CEC (mEq $100g^{-1}$)	P (%)	N (%)	K (%)	C:N
10.93	7.56	36.77	21.32	1.18	8.11	0.001	1.66	0.31	12.84

Fe (%)	Ca	Na	Cd	Ni	Cr	Pb	Cu	Mn	Zn
	(mg kg^{-1})								
2.5	2.4	458.26	3>	7	100>	50	180	4	46

Irrigation and harvesting

Every two years, when rainfall was sufficient for peanut production in the area, the plants were irrigated by rain. Every two years, the harvest of the peanut crop was carried out at the same time as the physiological (grain care) arrival and 111 days after the planting, and by hand.

During harvest, plants in side rows and plants in beginning and end of plot were removed to eliminate marginal influence (Temel et al., 2024).

Heavy metal analysis

For this purpose, the prepared samples were crushed into particles smaller than 2 mm. Then 0.5 g of the sample with 6 mL of nitric acid was placed in a digestion vessel and stored for 1 h. Then 2 mL of hydrogen peroxide was added and the digestion vessel was placed in the microwave digester. After digestion, the tank was washed with water and the washing solution was filtered through a 0.22-µm filter. Finally, the heavy metal content was measured by inductively coupled plasma mass spectrometry (ICP-MS). A Perkin Elmer Elan 9000 ICP-MS with a quartz concentric nebuliser and an integrated quartz burner tube was used.

Statistical Analysis

Data analysis was performed with statistical analysis variance using SAS version 9.1 software and averages were compared using the Duncan's test at a 5% probability level, and charts were generated using Excel 2013.

RESULTS AND DISCUSSIONS

The results of the analysis of variance of this study showed that crop year had statistically significant effect on the concentration of studied metals in grain and soil (Table 4 and 5). Although applying different levels of MSWC had a significant effect on the concentration of studied metals in soil, it significantly affected B, Cd, Cr, Co and Pb in grain. Mycorrhizal treatment did not significantly affect the concentrations of the metals studied, except for Mn and Ni in grain. The gypsum treatment also did not have a significant effect on the metals in both the grain and the soil (Table 4 and 5). The results of the analysis of composite variance showed that the interaction effect of crop year × gypsum × mycorrhizal × MSWC had a significant effect on the concentration of the studied metals in both grain and soil. While the interaction effect of each of the three treatments did not have a significant effect on the concentration of studied metals in grain and soil (Table 4 and 5).

Overall, the maximum amount of B concentration in grain was 7.85 mg kg^{-1} obtained in the second year with 150 kg ha^{-1} gypsum and 8 t ha^{-1} MSWC in the presence of mycorrhizae, which was 9.94% higher than that in the first year. On the contrary, the highest value of boron in soil was observed in the absence of gypsum compared to values of boron in grain (Figure 1). Compared to the control and the MSWC rates of 2, 4 and 6 t ha^{-1}, the application of 8 t ha^{-1} MSWC increased the B concentration by 91.29, 47.51, 19.08 and 9.21% in the grain and by 71.04, 39.89, 19.38

and 8.05% in the soil (Figure 1). The lowest B concentrations in grain and soil were also observed in the plots not treated with MSWC. Moreover, the result of aplication of gypsum had positive effect on B concentraion in grain significantly. B is believed to play a crucial role in nitrogen metabolism by increasing nitrogenase and facilitating nitrate translocation and availability for reduction in leaves, stems, roots, and nodules (Ghazimahalleh et al., 2022).

Table 4. Combined variance analysis of the effect of year, gypsum, mycorrhiza and MSWC on the concentration of metals in grain of peanut

S.O.V	df	MS (Mean squares)							
		B	Cd	Cr	Co	Pb	Mn	Ni	Zn
Year (A)	1	26.3**	0.036	1.06**	20**	34.4**	15.4**	30.4**	2980**
Block (R)	4	1.05	0.0009	0.022	0.499	0.69	0.25	0.52	69.81
Gypsum (B)	1	94.8	0.164	4.43	76.1**	122	61.1	110.7	13369
B × A	1	13.6**	0.026**	0.71**	12.22	17.5**	9.72**	18.53**	2185**
Error 1	4	0.198	0.00007	0.00079	0.011	0.17	0.004	0.01	2.88
Mycorrhiza (C)	1	0.479	0.0038	0.036	0.686	0.67	0.48**	1.16**	133
MSWC (D)	4	30.8**	0.143**	1.46*	127**	55.4**	69.0	191	187
C × B	1	0.006	0.00075	0.002	0.032	0.008	0.01	0.01	20.2
C × A	1	1.2*	0.001*	0.078**	1.44**	1.56**	1.14**	2.31**	185**
C × B × A	1	1.15*	0.0005	0.07**	1.65**	1.59**	1.34**	2.18**	125**
B × D	4	2.63*	0.008	0.13*	7.06*	4.21*	3.94*	10.02	144
D × B × A	4	0.436	0.002**	0.019*	0.91**	0.62*	0.48**	1.42**	40.9*
D × C	4	2.14*	0.002	0.076*	1.34*	2.78 *	1.21 *	2.02 *	198
D × C × A	4	0.147	0.0002	0.011	0.162	0.19	0.14	0.18	33.9
D × C × B	4	0.933	0.001	0.038	0.536	1.16	0.5	0.71	119
D × C × B × A	4	0.46*	0.001*	0.026**	0.465**	0.59**	0.41**	0.68**	74.6**
Error 2	72	0.187	0.0002	0.006	13.8	0.2	0.06	0.12	15.29
CV (%)		9.2	7.3	7.6	8.6	8.54	6.84	7.28	6.87

*, ** Significant at 5% and 1% probability level, respectively and others are Non-significant

Table 5. Combined variance analysis of the effect of year, gypsum, mycorrhiza and MSWC on the concentration of metals in soil in peanut

S.O.V	df	MS (Mean squares)							
		B	Cd	Cr	Co	Pb	Mn	Ni	Zn
Year (A)	1	268.2**	0.183**	2345**	865**	17439**	2122**	2060**	24012**
Block (R)	4	10.2	0.0031	37.3	12.4	349	48.5	41.0	557
Gypsum (B)	1	1141.4	0.696	8646	3268	6424	8602	8229	91368
B × A	1	185**	0.111**	1468**	509**	10175**	1383**	1309**	14518**
Error 1	4	1.16	0.00002	7.31	0.094	20.66	6.47	0.57	14.26
Mycorrhiza (C)	1	9.08	0.005	75.9	43.08	563	75.0	73.9	797
MSWC (D)	4	273*	0.715**	8083**	4593**	149771**	5913**	7165**	148893**
C × B	1	0.641	0.00008	1.92	4.48	5.7	4.76	3.98	15.15
C × A	1	19.1**	0.014**	129**	52.7**	1319**	147**	145**	1777**
C × B × A	1	17.3**	0.014**	143**	60.6**	1702**	159**	158**	2157**
B × D	4	28.6*	0.043*	524*	277*	7484*	406*	460*	7945*
D × B × A	4	4.42	0.06**	64.3**	34.8**	920**	51.8**	57.8**	975**
D × C	4	19.0*	0.013*	155*	51.1*	1405*	150*	146*	1872*
D × C × A	4	2.83	0.0016	20.45	6.35	144.8	19.8	18.6	205
D × C × B	4	9.94	0.0056**	62.6**	18.14	491.08	68.0	63.3	707
D × C × B × A	4	9.95*	0.0046**	65.4**	19.3**	455**	53.1*	51.6**	627**
Error 2	72	2.43	0.00077	13.85	4.64	107.64	16.79	10.49	132
CV (%)		9.5	7.9	8.3	8.1	8.68	9.17	7.45	7.99

*, ** Significant at 5% and 1% probability level, respectively and others are Non-significant

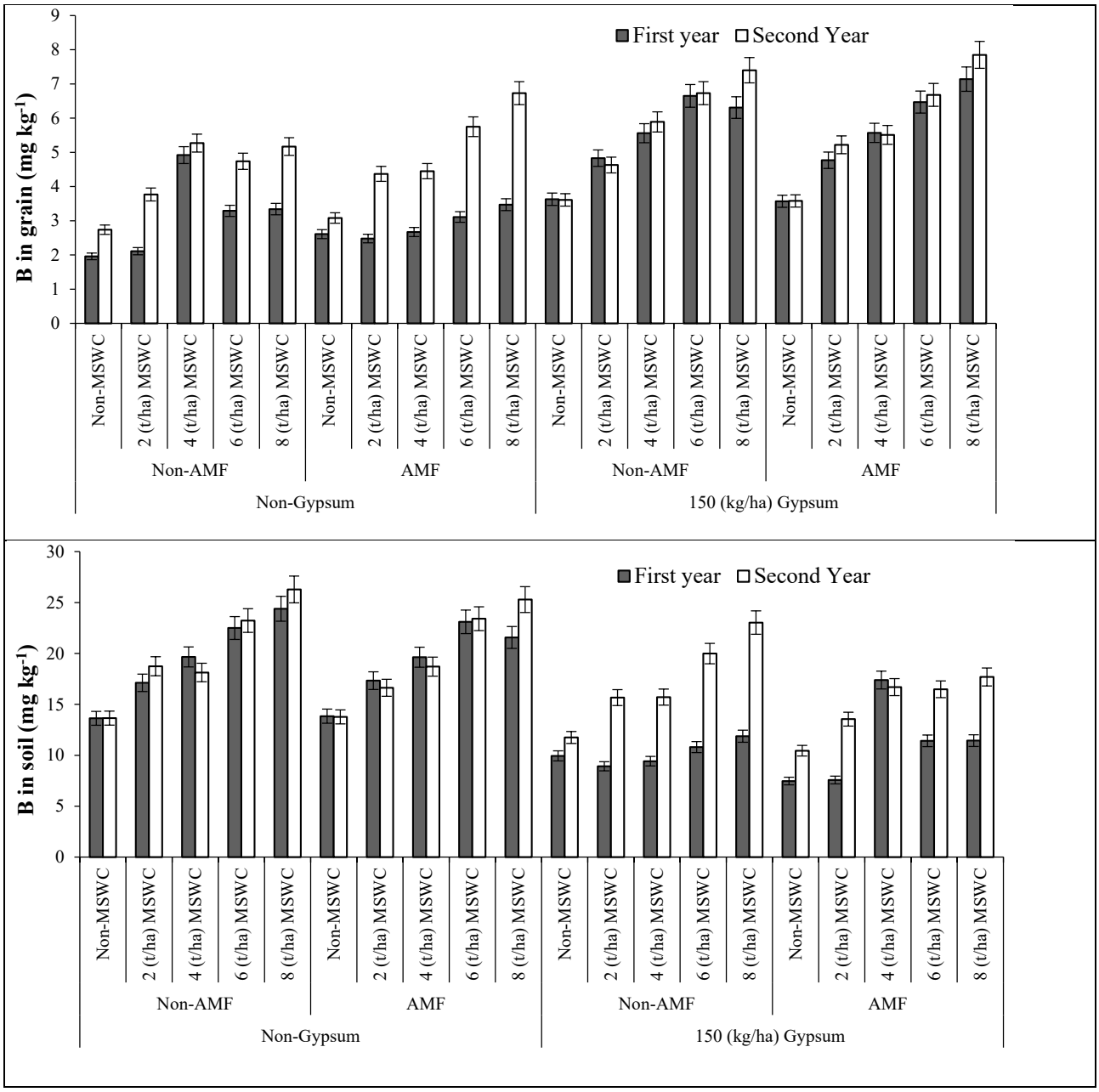

Figure 1. Effect of studied treatments on boron concentration in peanut grain and soil.

In both years, in both conditions of application and non-application of gypsum and mycorrhiza, the concentration of Cd in seeds and soil increased with the increase of MSWC application. But in the seed, the amount of this increase was lower under the application of mycorrhiza and gypsum, so that under the absence of application of mycorrhiza and gypsum, the level of 8 t ha^{-1} MSWC increased the concentration of Cd in the seed to 0.36 mg kg^{-1} in the first year and 0.39 mg kg^{-1} in the second year, while in mycorrhizal plants and under the application of gypsum, the concentration of Cd decreased to the same level as MSWC to 0.29 and 0.16 mg kg^{-1} in the first and second year, respectively. In other levels of MSWC application, the application of gypsum along with mycorrhiza, compared to the conditions of no application of gypsum and mycorrhiza, decreased the concentration of Cd in seeds from 6.6 to 25.6% in the first year and from 0.23% to 54.3% (Figure 2). Similarly, the results of the concentration of metals in peanut grown in fields contaminated with heavy metals were significantly higher than in those grown in a control plot, according to a study by Opaluwa and colleagues (2012). Similar findings have been reported in rice (Bakhat et al., 2017), wheat (Shrivastava et al., 2017) and veggies (Wang et al., 2006). There is evidence from studies that plants take up heavy and toxic metals through their roots and accumulate them in their tissues. Two main pathways, the apoplastic and symplastic pathways are often used for the entry of heavy metal ions into plant roots. A positive correlation between the amount of MSWC application and Cd uptake was reported, indicating that

increasing MSWC application leads to an increase in Cd phytoavailability (Wang et al., 2023). In addition, Cd may strongly bind to insoluble organic matter such as large molecules of humic acid and humin and increase the Cd absorption capacity of soil (Lee et al., 2022).

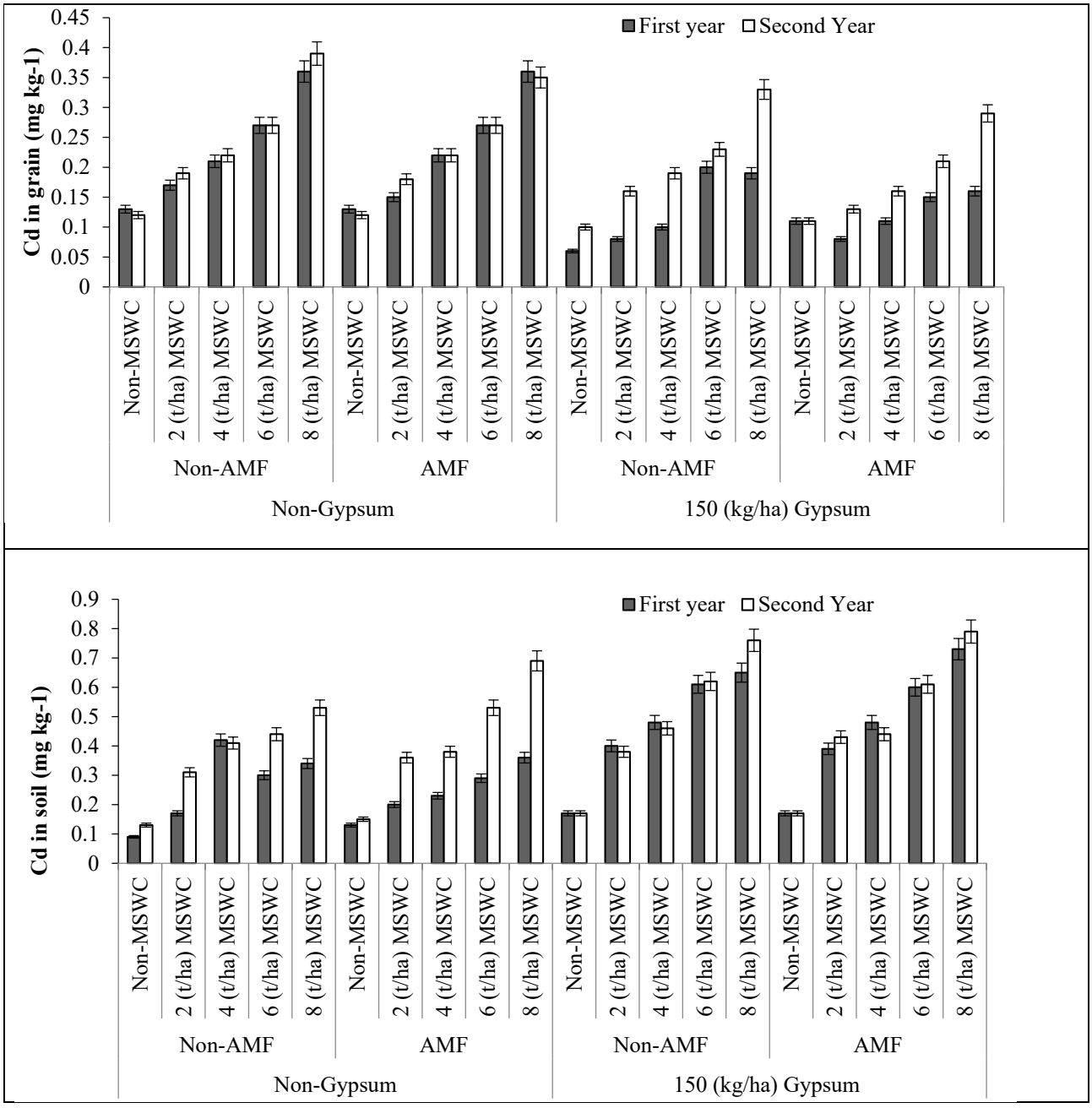

Figure 2. Effect of studied treatments on cadmium concentration in peanut grain and soil

In general, the maximum concentration of Cr in grain (1.57 and 1.69 mg kg^{-1} in the first and second year, respectively) was obtained from applying 8 t ha^{-1} MSWC without using gypsum in the presence of mycorrhiza. However, applying gypsum decreased the concentration of Cr in grain from 47.5 to 54.5% in the first year and from 12.1 to 26.3% in the second year compared to similar treatments under no application of gypsum. Also, the highest Cr concentration in soil (85.3 and 91.9 mg kg^{-1} in the first and second year, respectively) was obtained from the application of 8 t ha^{-1} MSWC along with the application of gypsum in the absence of mycorrhiza. In addition, at all levels of MSWC, the application of gypsum increased the concentration of soil Cr, whether in the presence or absence of mycorrhiza (Figure 3).

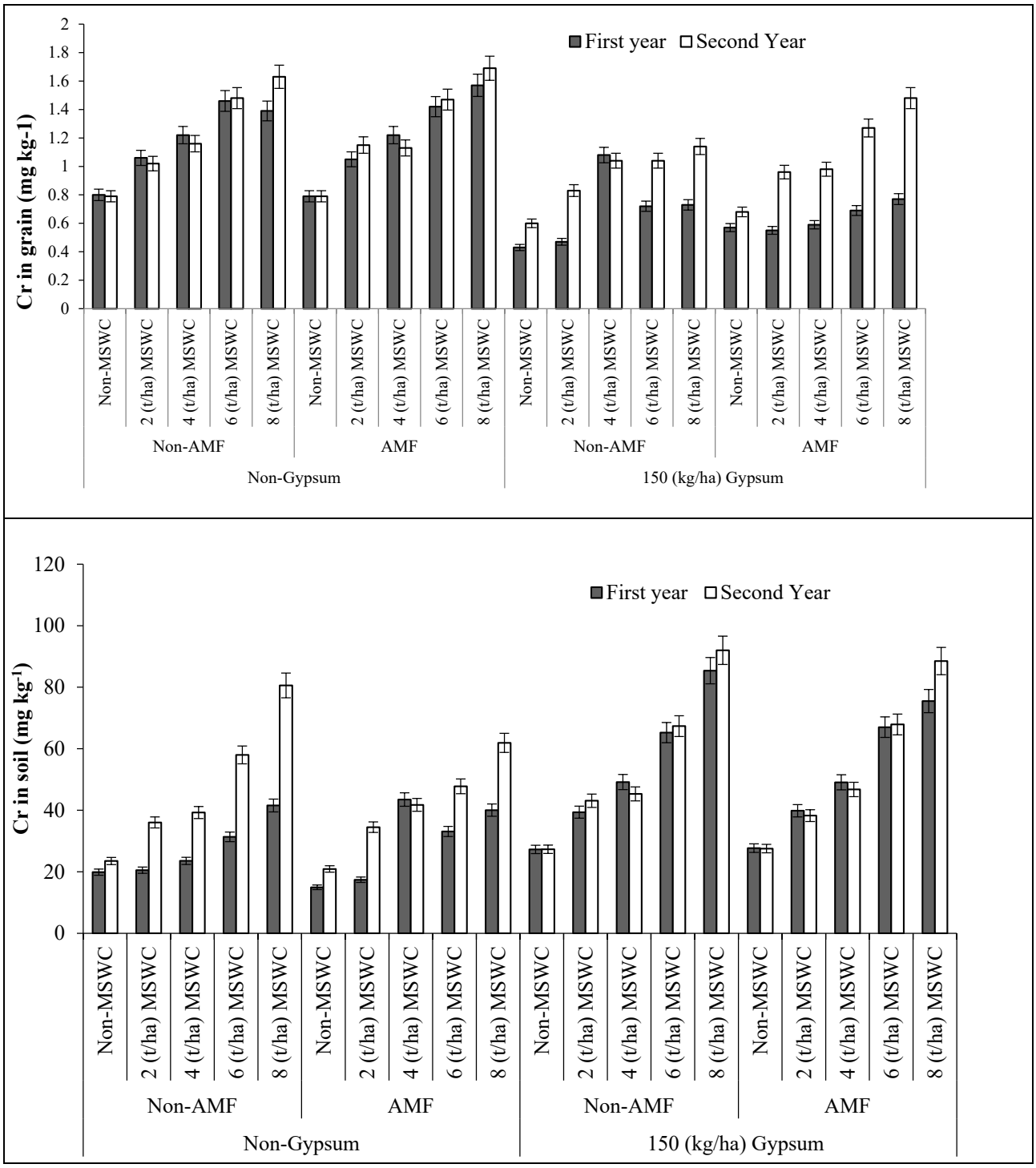

Figure 3. Effect of studied treatments on chromium concentration in peanut grain and soil.

Overall, the maximum amount of Co in the grain was 8.70 mg kg^{-1} obtained in the second year by application of 150 kg ha^{-1} gypsum and 8 t ha^{-1} MSWC under mycorrhizal conditions, and the highest Co concentration in the soil was 57.95 mg kg^{-1} observed in the second year without gypsum and mycorrhiza, and application of 8 t ha^{-1} MSWC (Figure 4). Significant concentrations of Co are highly toxic to crops. Co causes pale-coloured leaves, discoloured veins, leaf loss, and plant Fe deficiencies (Hu et al., 2021; Abugoufa et al., 2022). Peanut crops show hazardous effects when grown in soils with high Co concentrations (Abugoufa et al., 2022). Riaz et al. (2021) report that mycorrhiza might effectively immobilize Pb and Co in polluted soils. Mycorrhizal fungus has the ability to increase the absorption of less mobile nutrients such as P, Cu, Zn, Co, and Fe as a result of their interactions with soil cations such as Fe_3^+, Ca_2^+ and Al_3^+. This is possible because the fungus can create a wide network of hyphae with a very large surface area and with great potential to explore a larger volume of soil to extract nutrients (Udo et al., 2023).

Arbuscular mycorrhizae can provide both macronutrients and micronutrients. This is perhaps due to the key role of mycorrhizae in maintaining soil stability and soil ions and preventing their loss, and even though improve root characteristics (e.g., mass, root length, and root surface) (Lazcano et al., 2014).

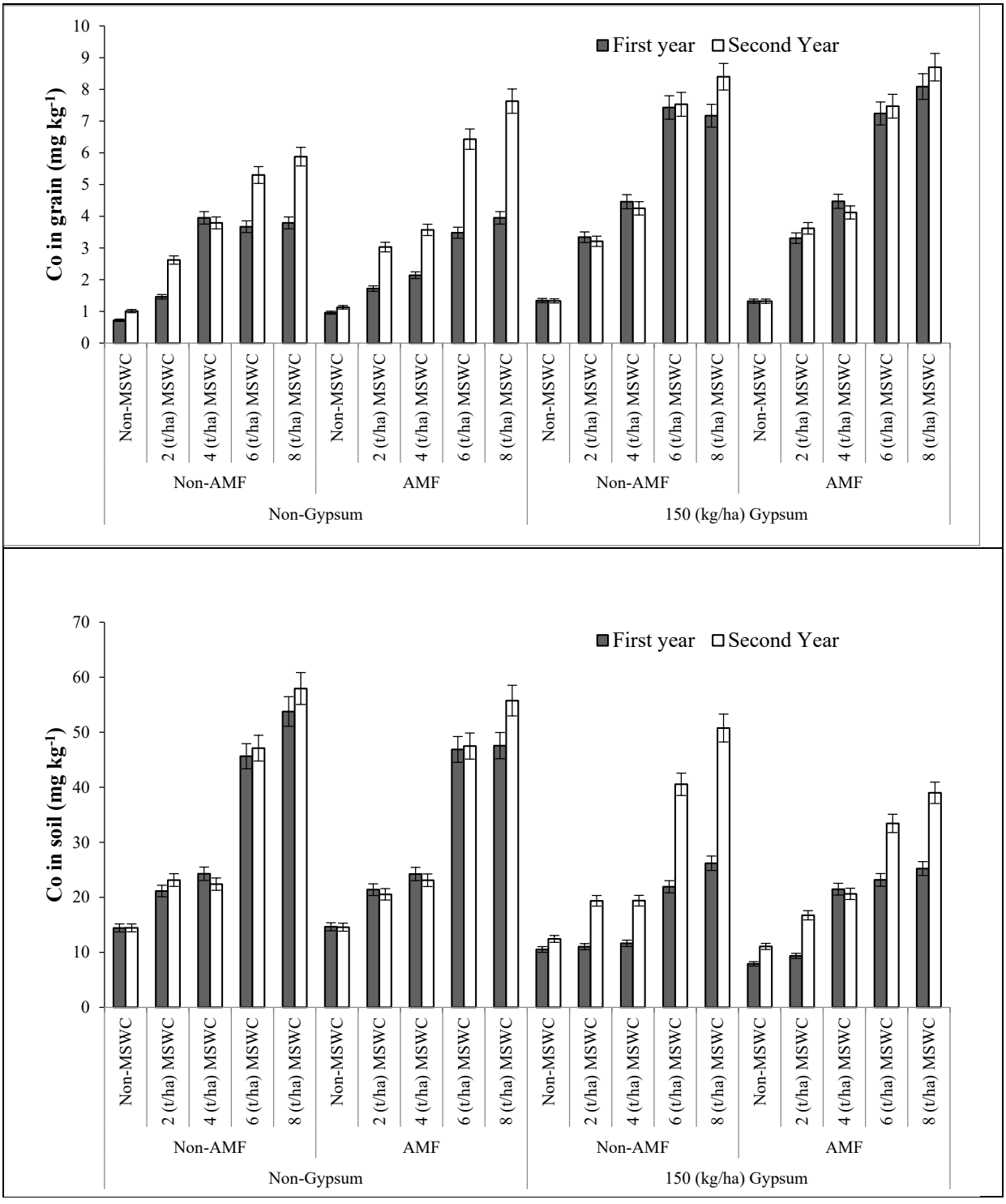

Figure 4. Effect of studied treatments on cobalt concentration in peanut grain and soil.

In general, the highest Pb concentrations in grain and soil were 9.42 and 285.55 mg kg^{-1}, respectively, observed in the second year at the gypsum level of 0 kg ha^{-1} in the presence of mycorrhizal fungi and the application of 8 t ha^{-1} MSWC, and in the second year treated with 150 kg ha^{-1} gypsum and 8 t ha^{-1} MSWC without mycorrhizal inoculation (Figure 5). As a result, the higher levels of all metals in the peanut soil are a cause of severe heavy metal

contamination in soil and grain of peanuts. Pb increased significantly in the soil with the application of MSWC, probably because Pb was the element with the highest concentration in the applied MSWC (Arrobas et al. 2022). Damera et al. (2014) found that soil heavy metals can significantly affect heavy metal concentrations in peanut roots, stems, leaves, and seeds. Soil heavy metal contamination is the main cause of heavy metal contamination in crops, and heavy metals can be transferred to crops through the root system (Damera et al., 2014). Research in the last decade has shown a significant positive correlation between soil heavy metal concentrations and crops (Ran et al., 2016). The negative effect of heavy metals on germination indicators was shown by the results of Didwania et al. (2019) and Jaouani et al. (2018).

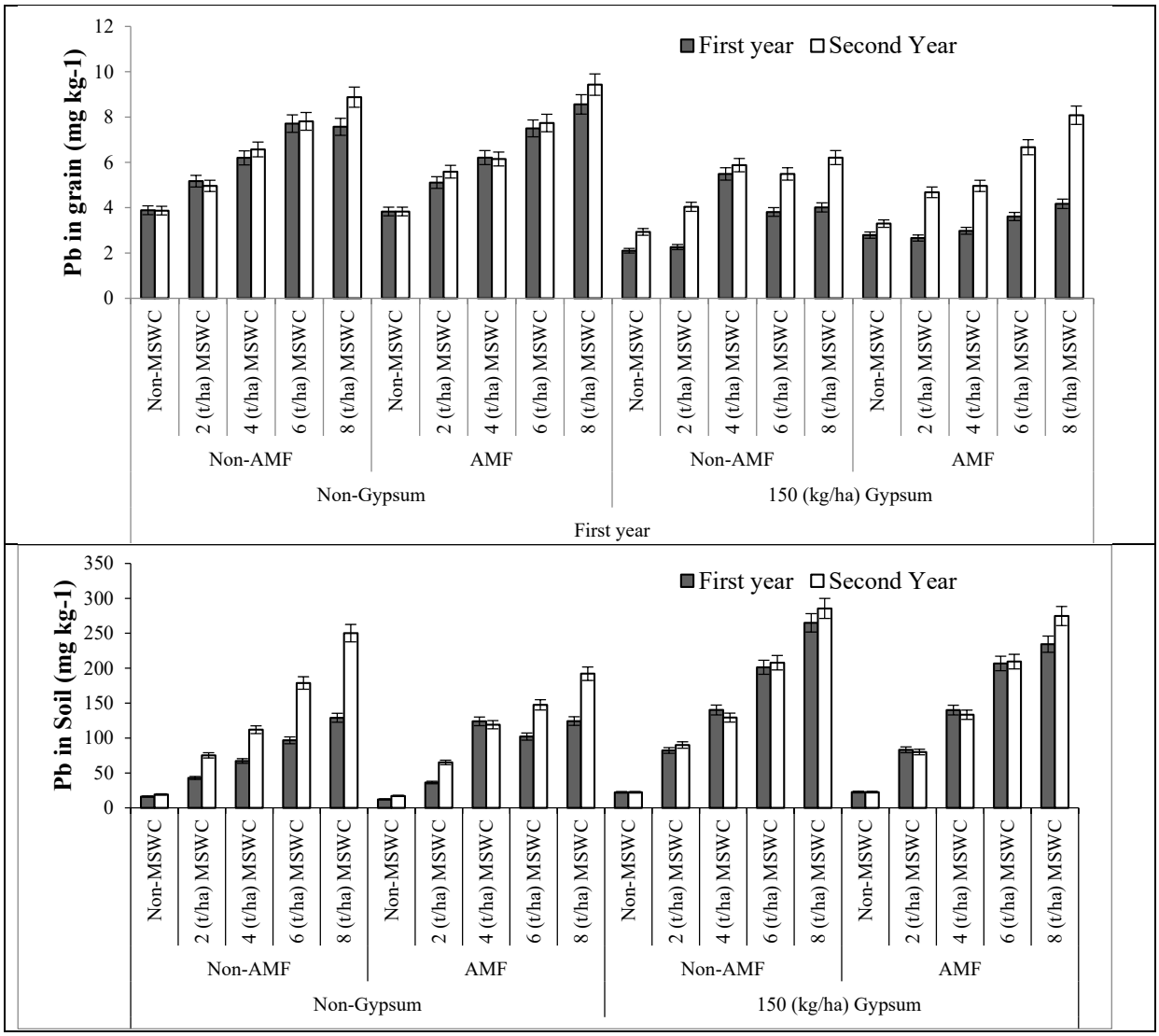

Figure 5. Effect of studied treatments on lead concentration in peanut grain and soil.

Heavy metals exert their inhibitory effect on germinating seeds in different ways. Some heavy metals reduce germination rate by inhibiting endosperm starch hydrolysis and preventing initial seed growth, others prevent seed germination by damaging embryo, and others prevent seed germination by damaging embryo. The highest increase in the amount of absorbable Pb in the soil was observed in the treatment of 50 tons of compost. Pb is one of the most important contaminants in the environment (Karak and Bhagat, 2010). Gu et al. (2019) revealed that the concentration of Pb in peanuts was 0.16 mg kg^{-1}. Blair and Lamb (2017) found that the Pb concentration of peanuts in the United States was 0.03 mg kg^{-1}. Zhao et al. (2010) analyzed the bioaccumulation of heavy metal in different parts of peanut and showed that peanut seed has the ability to bioaccumulate Cu, Zn and Cd.

Peanut shell had strong Pb and Cr bioaccumulation capability. Yang et al. (2020) reported the average concentration of heavy metals in peanut soil samples as 0.18, 47.49, 21.43, and 69.66 mg kg^{-1} for Cd, Cr, Pb, and Zn, respectively. Due to having higher amounts of Pb, urban waste compost has a greater effect on increasing this compound in the soil (Behaj et al., 2016; Marjavi and

Mashayikhi, 2018). Pb element is one of the most stable heavy metals in soil and it is stable in soil for about 150 to 5000 years (Kumar et al., 2017). The normal range of Pb concentration in plants is from 0.2 to 20 mg kg^{-1} and its critical limit is 30 to 300 mg kg^{-1} (Abbaspour et al., 2010). Pb prevents the division of meristem cells and the growth of root cells and reduces the function of plant roots. Also, this metal reduces the elasticity of the root cell wall and reduces the growth of plant roots (Kapata-Pendis, 2010). Mukai and Oyanagi (2021) showed that potential hazards could accompany the application of low-quality composts to the soil, the environment, and humans, caused by heavy metals and other pollutants. Consequently, the application of such composts could also Pb to low crop yields and economic returns to farmers, hence leading to its low use and adoption.

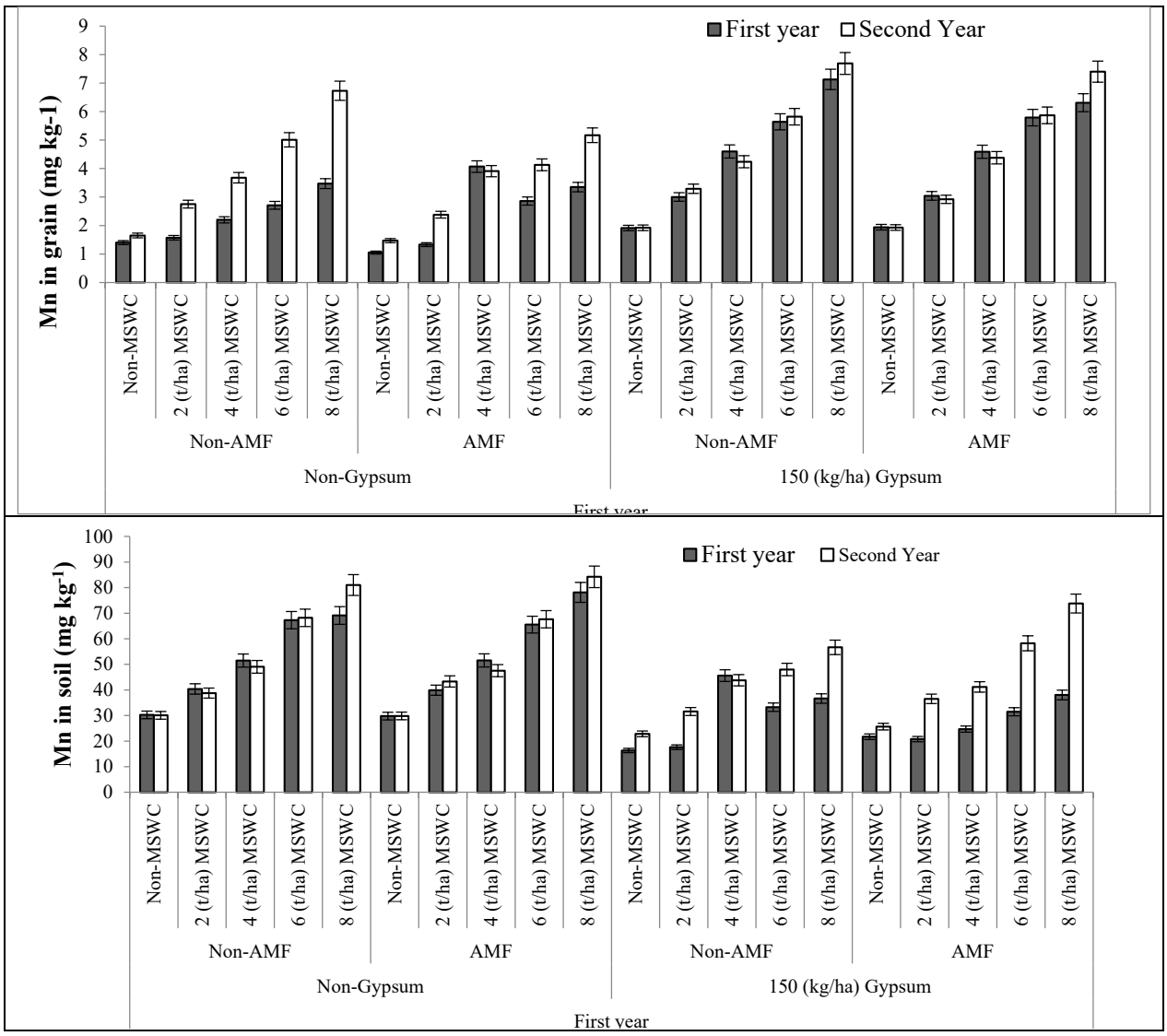

Figure 6. Effect of studied treatments on manganese concentration in peanut grain and soil.

The highest concentration of Mn in seeds (7.13 and 7.69 mg kg^{-1} in the first and second year, respectively) was obtained from the treatment of 150 kg of gypsum and 8 t ha^{-1} of MSWC without the application of mycorrhiza, while the highest amount of soil M (78.1 and 84.2 mg kg^{-1} in the first and second year, respectively) were observed in the treatment of 8 t ha^{-1} of MSWC in the presence of mycorrhiza (Figure 6). In addition, the presence of mycorrhizae in the soil reduced the concentration of Mn in the grain and increased its concentration in the soil. The application of 8 t ha^{-1} MSWC increased the Mn concentration in the grain by 256.02, 132.68, 42.24 and 24.95% compared to the control and the MSWC rates of 2, 4 and 6 t ha^{-1}, respectively. Similarly, application of 8 t ha^{-1} MSWC increased soil Mn concentrations by 150.72, 92.53, 45.87 and 17.74% compared to the control and MSWC rates of 2, 4 and 6 t ha^{-1}, respectively (Figure 6). Marjavi and Mashayikhi (2018) showed that organic matter treatments including municipal waste compost in the first stage of planting did not have a significant effect on the amount of plant-available Mn in the soil. However, in the second stage of onion planting, that is, after a period of five

years of using organic fertilizers in defined amounts, the consumption of different amounts of organic fertilizers studied increased the amount of plant-available Mn in the soil, and this amount increased only in the 50 tonnes per hectare treatment. Composting of municipal waste was significant. The amount of soil Mn in this treatment was 10.49 mg kg^{-1} soil. Moreover, Arrobas et al. (2022) depicted conversely that the concentrations of heavy metals in plant tissues tended to decrease with the application of MSWC, and the levels in the pulp remained below the maximum values established as safe for food. However, continuing to apply MSWC each year at such high rates and in neutral and alkaline pH soils does not appear to be sustainable, as there is a risk that increasing soil pH will lead to nutrient imbalances.

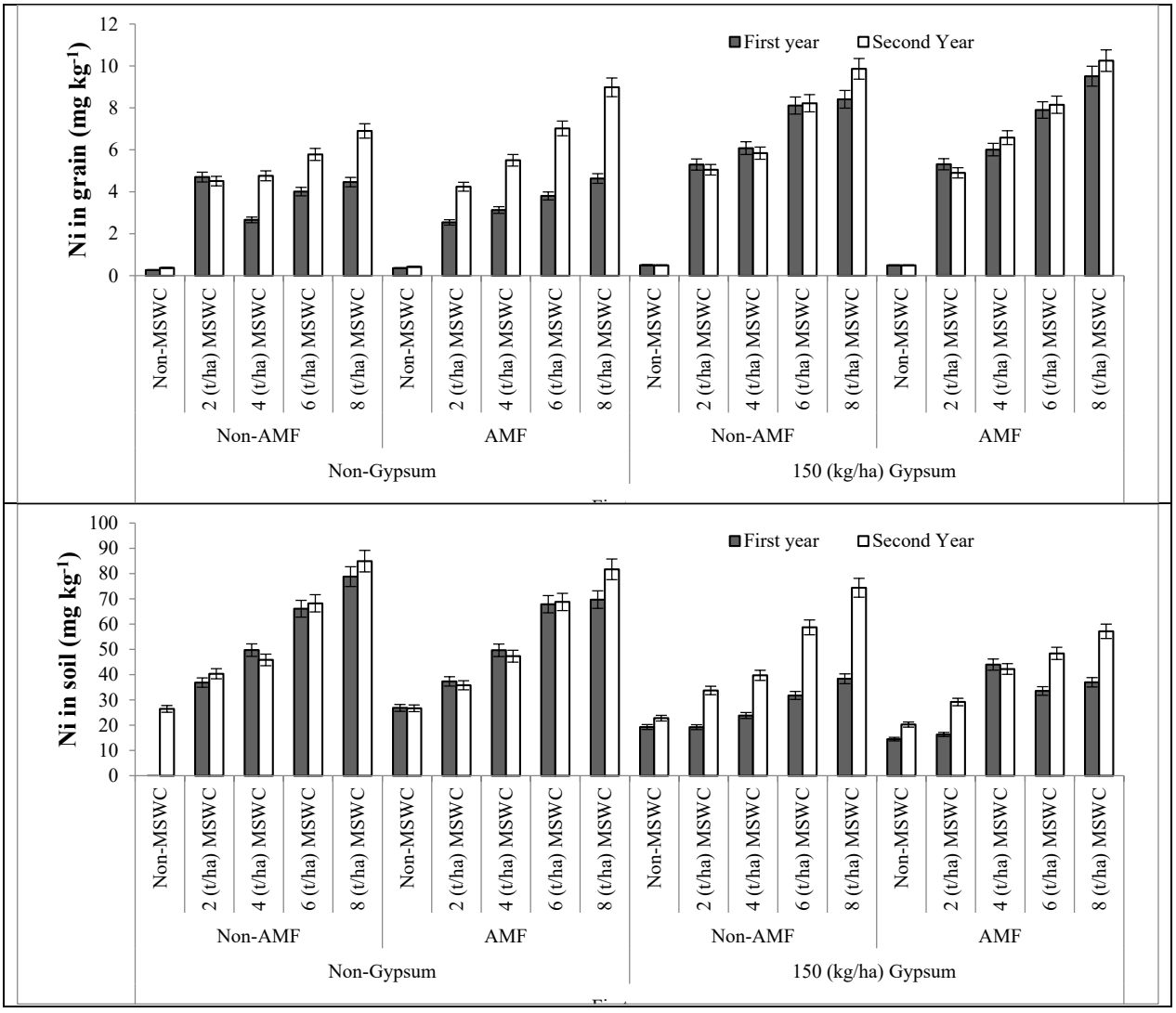

Figure 7. Effect of studied treatments on nickel concentration in peanut grain and soil.

In general, the maximum Ni concentration in the grain was 10.25 mg kg^{-1} in the second year by application of 150 kg ha^{-1} gypsum and 8 t ha^{-1} MSWC under mycorrhizal conditions, which was 7.78% higher than in the first year. Also, the maximum concentration of Ni in the soil was 84.95 mg kg^{-1} obtained in the second year by the application of 8 t ha^{-1} of MSWC, which was 7.77% higher than that of the first year (Figure 7). Therefore, compared to the control and the MSWC levels of 2, 4 and 6 t ha^{-1}, the application of 8 t ha^{-1} MSWC increased the Ni concentration in the grain by 1776.19, 72.43, 55.42 and 19.03%, respectively. Also, compared to the control and the MSWC levels of 2, 4 and 6 t ha^{-1}, the application of 8 t ha^{-1} increased the Ni concentration in the soil by 184.90, 109.74, 52.50 and 17.73%, respectively. However, in both years of the presence of mycorrhiza, especially under gypsum application, reduced soil Ni concentration at all levels of MSWC application (Figure 7). At all levels of MSWC and in both mycorrhiza application and non-application conditions, the application of gypsum increased the concentration of Ni in seeds, which according to García-Robles et al. (2022), this increase could be due to the effect of gypsum on soil pH. They reported that the effect of pH on the absorption of heavy metals from soil by

plants and their bioavailability is very important, so that in alkaline pH, the transfer of heavy metals from soil to plant tissues is very low, and the decrease in pH reduces the movement and dynamics of these metals in Soil increases. As calcium is one of the main antagonistic elements against some heavy metals sorption and metabolism, its presence in the soil solution enhances the selectivity in the uptake of metabolic important elements against unwanted ones (Madejón et al., 2018). Moreover, sulphur may have also enhanced the availability of some heavy metals such as Ni and Co (Madejón et al., 2018). These results are consistent with findings of Carbonell et al. (2011), who reported that the changes in the transfer factor for the Pb in compost and its consumption frequency are much lower than that of Ni, so that contrary to the constant trend of Pb increasing. Thereby the levels of municipal waste use also increase the transfer of Ni from the root to the aerial part.

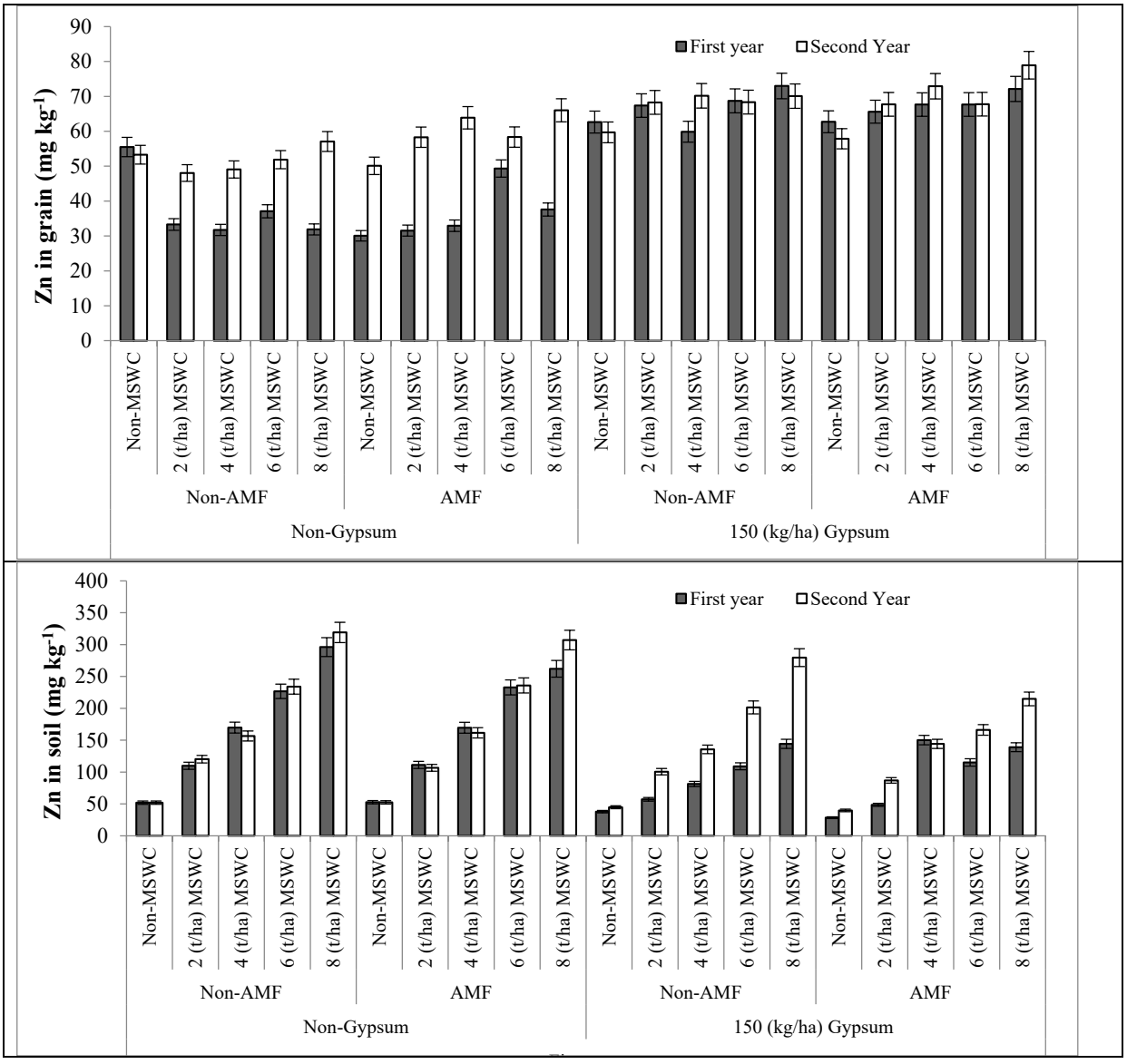

Figure 8. Effect of studied treatments on zinc concentration in peanut grain and soil.

The maximum amount of Zn in the soil was 319.14 mg kg^{-1} obtained in the second year using 8 t ha^{-1} MSWC in under non-use of gypsum and non-mycorrhizal conditions, which was 7.78% higher than that in the first year (Figure 8). The presence of mycorrhizal fungi in the soil, increased the Zn concentration in the grains and decreased its concentration in the soil (Figure 8). Zn, however, showed a slightly different behaviour in the grain, with an increase within the soil and a smaller reduction within tissues compared with other metals. High application rates of P fertilizers to soils low in available Zn can induce Zn deficiency (Broadley et al., 2012). An increase in the amount of compost in the soil resulted in a significant increase in the Zn concentration in the soil and an increase in the amount of MSWC of more than 4 ton/ha resulted in a significant increase in the Zn concentration in the grain. While the application of gypsum and mycorrhizae decreased the Zn concentration in the soil and increased the

Zn concentration in the grain, this concentration may be due to the interaction of calcium and Zn ions in the soil.Yang et al. (2020) reported that the average concentration of Zn in peanut grain samples was 31.42 mg kg^{-1}. Zhao et al. (2010) analyzed the bio-accumulation of heavy metals in different parts of peanuts and reported that the peanut grains had a strong bio-accumulation ability of Cu, Zn, and Cd. Peanut shells were strong bio-accumulators of Pb and Cr (Zhao et al., 2010). The highest amount of usable soil Zn in both stages was observed in the treatment of 30 tons of sewage sludge in the onion plant. In this treatment, the amount of plant usable Zn in the soil increased from 0.6 mg kg^{-1} soil in the control treatment to 4.46 mg kg^{-1} in the first stage and from 0.33 mg kg^{-1} soil to 82.5 mg kg^{-1} was reached in the second stage (Marjovi and Meshaikhi, 2012). The result depicted that the highest Cd, Cr, Pb, Mn contents were 0.3 and 0.6 mg kg^{-1}, 1.69 and 91.99 mg kg^{-1}, 9.42 and 285.55 mg kg^{-1}, 7.69 and 84.22 mg kg^{-1} in the grain and soil respectively. Also, the maximum Ni and Co concentration in the grain was 10.25 and 8.70 mg kg^{-1} in the grain and the maximum amount of Zn in the soil was 319.14 mg kg^{-1}. Other studies showed different results as: The mean concentrations of heavy metals in peanut soil samples were 0.18, 47.49, 21.43 and 69.66 mg kg^{-1} for Cd, Cr, Pb and Zn, respectively, reported by Yang et al. (2020). Lanre-Iyanda and Adekunle (2012), Blair and Lamb (2017), and Dai et al. (2016) found that peanuts from Nigeria and China contained 0.077 and 0.017 mg kg^{-1}, respectively. Among the heavy metals, Cd is of particular concern because of its potential to accumulate in plant organs and thus enter the food chain without showing signs of toxicity (Prince et al., 2002). The results of a study on sunflower showed that different concentrations of Cd in soils with roots inoculated with *P. indica* increased plant growth rate and photosynthetic pigment content in them. The results of the research showed that they had *P. indica.* In contrast to soil with uninoculated roots plant growth rate and the content of photosynthetic pigments and a reduction in the accumulation of Cd. It is also involved in improving the growth and performance of crops against the accumulation of ROS (Shahabivand et al., 2018). In addition, Yang et al. (2020) reported that the average Cr content in peanut kernels was 0.44 mg kg^{-1}, which was lower than that of our results.

The correlation between the studied traits in the first year showed a strong positive correlation among the Cr and Pb in grain with Cd in grain and Co, Zn , Ni, B and Mn in soil and also with each other, and smothly negative and positive correlation among with B, Mn, Ni and Co in grain and Cd and Pb in soil. Also the amount of Zn in grain had strong negative correlation with these parameters (Figure 9). While in the second year all metals in grain and soil except for Zn in grain had a strong positive correlation with each others. In most cases, metal ions are insoluble and immobile in the vascular system and are therefore immobilised in the apoplastic and symplastic compartments after forming carbonate, sulphate or phosphate precipitates. Heavy metal ions enter the xylem through the root symplasm and cross the plasma membrane. The electrochemical gradient across the plasma membrane, which has a high negative resting potential, facilitates the movement of metal ions. In the xylem, metals are transported by membrane transporter proteins. This is due to an active transport mechanism that requires energy (Edelstein and Ben-Hur 2018). Mounissamy et al. (2021) also concluded that after adding municipal waste compost to the soil, a significant increase in the amount of Pb, Mn and Cr was observed. Of course, the changes of heavy metals after adding compost depended on the amount of compost added and its characteristics. Aylaj et al. (2019) found that the application of municipal waste compost in an loamy sandy soil in a field study had no effect on the amounts of Cd, Cr, Ni and Pb, but the repeated application of compost increased the amount of total heavy metals in the surface layer of the soil. Studies show that the mobility and dynamics of elements change depending on the type of plant, the amount of organic matter, pH of the soil, metal concentration in the soil and the growth stage of the plant. The uptake and transport of elements in different plants is not the same. Usually, a plant species according to physiology, may act more specifically towards the transport of a specific metal, thereby increasing the amount of movement and transport of that metal. Many studies showed that the type of plant is one of the most important influencing factors in transfer of metals in soil and plant system (Mazumder et al., 2023; Ongun et al., 2023).

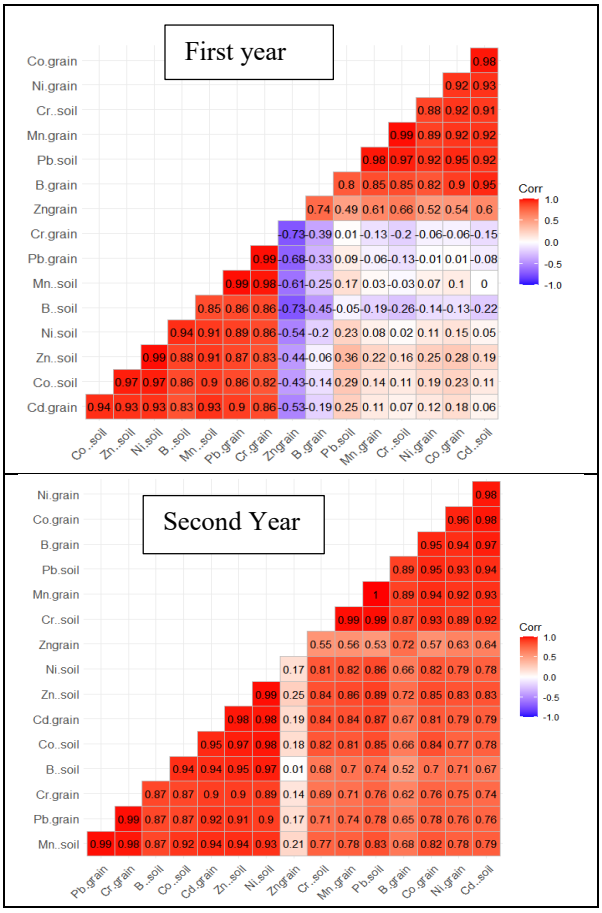

Figure 9. Correlation plot of traits of peanut under different treatments in two years.

CONCLUSION

MSWC can be a useful organic fertilizer for improving soil conditions and plant growth. However, the presence of heavy metals elements and toxic substances is one of the disadvantages of this fertilizer, which leads to soil and plant pollution, and our hypothesis was that the use of mycorrhiza and gypsum can reduce the negative effects caused by the use of MSWC in peanut cultivation. Our results showed that by increasing the application of MSWC from 2 to 8 t ha^{-1} compared to the absence of application, MSWC increased the concentration of Cd, Cr, Co, Ni, Mn and B in soil and grain. Alternatively, the application of 150 kg ha^{-1} of gypsum led to a significant increase in the concentration of Ni, Mn, Co, B, and Zn in the grain and Pb, Cd, and Cr in the soil. While the application of AMF led to an increase in the concentration of Ni, Co, and Zn in the grain and a decrease in the concentration of Ni, Mn, B and Zn in the soil. Therefore, according to the obtained results, the use of 4 t ha^{-1} of MSWC along with mycorrhiza in peanut cultivation is suggested in order to reduce the environment risks of soil and plants cause by the use of compost, and also use the benefits of urban waste compost.

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IMPACT OF INTEGRATED ORGANOMINERAL FERTILIZER APPLICATION ON GROWTH, YIELD, QUALITY, AND HEALTH-RELATED COMPOUNDS OF SWEET CORN

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ABSTRACT

In this study, the effectiveness of two organomineral formulations (OMF I and OMF II) on the growth, yield, kernel quality and health-related compounds of sweet corn were evaluated. Organomineral fertilizers were compared with chemically fertilized and unfertilized control to evaluate their effects as a basic fertilizer source. Field experiments were conducted using a randomized complete block design, with three replications over 2 years. Two cultivars (cv. 'Sentinel' and cv. 'Khan') were used as plant material. The results indicated that (1) compared to control, the application of organomineral and chemical fertilizers resulted in improvements in most growth, yield and quality traits of sweet corn in both cultivars; (2) use of organomineral fertilizers led to similar or significantly higher than chemical fertilizer in plant height, leaf number per plant, ear size, ear weight, ear yield (husked and de-husked) and total soluble solids. However, these effects showed responses that varied with type of organomineral fertilizer or cultivar; (3) use of OMF I treatment with cv. 'Khan' significantly improved ear size, ear weight, ear yield (husked and de-husked), colour traits, total phenolic content and antioxidant activity compared to other treatments.

Keywords: antioxidant activity; ear yield; plant height; total phenolic content; total soluble solid*; Zea mays* L. var. *saccharata*

INTRODUCTION

Despite recent innovations and modern approaches, most of the world's agriculture still relies on conventional practices and faces ongoing sustainability and soil fertility challenges. Chemical fertilizers (CFs) are among the most widely used, but their long-term and excessive use has numerous adverse effects, including soil fertility degradation, reduction in soil microbial populations and increased soil erosion and acidification (Shen et al., 2021). Additionally, the overuse of CFs can reduce food quality by leading to higher nitrate accumulation in crops and reduced synthesis of ascorbic acid and phenols (Ye et al., 2020). Using organic fertilizers (OFs) in agricultural production represents an alternative to using CF. OFs have been employed to enhance soil structure by increasing its organic matter content, which improves water retention, aeration, and root development (Toor et al., 2020). They also support the activity of beneficial microorganisms and facilitate the recycling of waste materials, thereby contributing to a more sustainable agricultural system (Rehman et al., 2020). However, OFs have limitations, such as lower nutrient concentrations, often requiring larger quantities to achieve the same fertility levels as CFs (Verma et al., 2020).

Integrated nutrient management has been widely recognised as a method for maximising agronomic efficiency and crop productivity while maintaining sustainable soil health and fertility (Selim, 2020). In this context, the development of organomineral fertilizers (OMFs), which combine organic materials with mineral nutrients, has gained attention as a sustainable approach to preserving soil fertility in agricultural production (Syed et al., 2021). Smith et al. (2020) highlighted that OMFs provide a balanced approach to plant nutrition by harnessing the complementary benefits of organic matter and synthetic nutrients. Research has shown that OMFs are a superior alternative to CFs, offering a high potential for nutrient provision to plants and contributing to the long-term maintenance of the physical, chemical and microbiological properties of the soil (Ayeni and Ezeh, 2017). Additionally, OMFs can reduce nutrient losses, such as potassium leaching, phosphorus fixation and nitrogen volatilisation, compared to OF combined with CF (Tejada et al., 2005). Abdulraheem et al. (2023) noted that OMFs also offer environmental benefits by reducing the amount

of organic waste that could otherwise pollute water, soil and air. Despite these advantages, the effectiveness of OMFs can vary significantly due to differences in their composition, which affects crop growth, yield and quality. Bouhia et al. (2022) attributed this variability to the diverse raw materials and mineral sources used in OMF formulations. Furthermore, Srinivasarao et al. (2024) emphasised that the successful use of OMFs depends on proper application practices and timing to ensure that plants receive a balanced and timely supply of nutrients for optimal growth. In line with the Sustainable Development goals, countries such as India and Türkiye have implemented government policies that include financial support schemes to promote the use of OMFs among farmers (Adanacioglu and Yag, 2023; Srinivasarao et al., 2024).

Sweet corn (*Zea mays* L. var. *saccharata*) is becoming increasingly popular in cuisines worldwide, valued for its dietary fibre, vitamins and antioxidants, which contribute to a healthy diet (Alan et al., 2013). With rising demand, greater emphasis is now placed on the cultivation of sweet corn, which is produced for three distinct markets: fresh, frozen and canned (Szymanek et al., 2015). To meet stringent market requirements, sweet corn crops must exhibit high quality and appearance standards. While characteristics such as marketable yield, plant height (PH) and ear height are important to growers, the processing sector prioritizes the appearance, dimensions of the ears and quality properties of the kernels. These traits can be influenced by genotype, environment and fertilization practices (Szymanek and Tanas, 2019).

Currently, limited data are available on the use of OMFs for sweet corn cultivation (Etukudo et al., 2015; Ajibola et al., 2020; Intansari and Subiksa, 2022). Although existing studies provide a foundation for OMF use in sweet corn, further research is needed to assess the effectiveness of OMFs under various application practices and environmental conditions. Considering these points, the aims of this study were as follows: (i) to compare the effects of two OMF compositions with CFs on growth, yield, kernel quality traits and health-related compounds in sweet corn in an open-field setting; (ii) to evaluate the fertilizer value of the two OMFs across two sweet corn cultivars; and (iii) to assess new fertilization regimes where OMFs can be used as a primary fertilizer source to for sustainable produce yields and quality characteristics comparable to those of CFs.

MATERIALS AND METHODS

Plant Material and Field Management

Field experiments were conducted over 2 years in the experimental fields of the Odemis Vocational School at Ege University, Izmir, Turkey (latitude 38°12'N, longitude 27°52′E and altitude 111 m a.s.l.). Two sweet corn cultivars were used as plant material: 'Sentinel F_1' and 'Khan F_1'). These cultivars, which carry the sh2 mutant gene (shrunken or super sweet), are widely grown for industry of sweet corn production in Türkiye.

The physical and chemical properties of the soil are provided in Table 1. The air temperature and mean total rainfall recorded during the cropping cycles (April–July) were 38.8°C–4.5°C and 17.0 mm in 2018 and 40.3°C–0.8°C and 30.1 mm in 2019, respectively (Figure 1).

Table 1. Physical and chemical properties of the soil

Properties	Values	Properties	Values
pH[a] (1:2.5)	7.74	Available P[g] (mg kg^{-1})	7.04
Total salt[b] (%)	0.067	Available K[h] (mg kg^{-1})	452
$CaCO_3$[c] (%)	1.11	Available Ca[h] (mg kg^{-1})	540
Sand (%)	76.92	Available Mg[h] (mg kg^{-1})	145
Clay (%)	6.78	Available Fe[ı] (mg kg^{-1})	4.72
Silt (%)	16.30	Available Zn[ı] (mg kg^{-1})	1.18
Texture[d]	Sandy loam	Available Mn[ı] (mg kg^{-1})	10.21
Organic matter[e] (%)	1.28	Available Cu[ı] (mg kg^{-1})	0.42
Total N[f] (%)	0.072		

a: 1:2.5 water extract, b: 1:2.5 Soil: conductimetric in water extract, c: calcimetric, d: Hydrometric, e: Walkley-Black method, f: Kjeldahl method, g: available olsen, h: available 1 N NH4OAc extract, ı: available DTPA extract

The experiment utilized two types of OMFs. OMF I, with an 8:8:8 N–P–K formula, contained 30% organic matter and 9% humic + fulvic extract. OMF II, with a 12:15:5 N–P–K formula, contained 10% SO_3, 20% organic matter and 7% humic + fulvic extract. For each treatment (CF, OMF I and OMF II), the fertilizers were evenly spread manually on the soil surface homogeneously. Then, all experiment plots were tilled at a depth of 0–15 cm with a rototiller. This was done 1 week before sowing the sweet corn seeds in early April of both experimental years. Seeds were sown into the field within the third week of April in both years, with 70 cm × 25 cm spacing. The experiment was conducted using a randomized complete block design with three replications. Each experimental plot covered an area of 21 m^2 (2.8 m × 7.5 m). Drip irrigation was applied as required, and weeds were controlled manually. No fungicides or insecticides were used during cultivation.

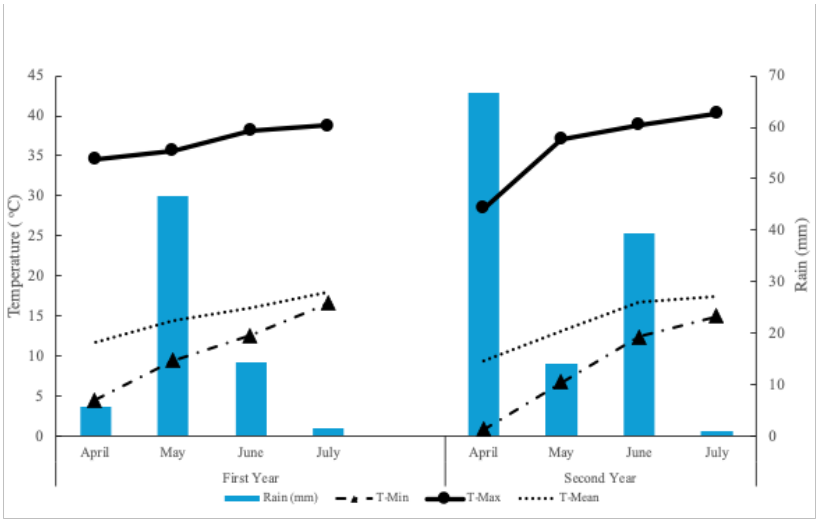

Figure 1. The air temperature and total rainfall recorded during the cropping cycles (April to July) in the 2-year experiment. (T-Min: minimum temperature, T-Max: maximum temperature, T-Mean: monthly mean temperature)

For one sweet corn crop cycle, 280 kg ha^{-1} of N, 110 kg ha^{-1} of P_2O_5 and 110 kg ha^{-1} of K_2O (Turgut, 2000) were applied based on the nutrient requirements of sweet corn plants. Field experiments were conducted under four treatment regimens: (1) control: unfertilized; (2) CF: (3) OMF I and (4) OMF II (Table 2).

Table 2. Fertilization and fertilizer dosage

Fertilization	**Fertilizer dosage (kg/ha^{-1})**					
	Composite fertilizer (15:15:15-NPK + Zn)	Urea (46% N)	Monoammonium phosphate (12-61-0)	Potassium sulphate (0-0-50)	OMF I (8:8:8 NPK)	OMF II (12:15:5 NPK)
Control	0	0	0	0	0	0
CF	700 (BF)	380 (SD)	0	0	0	0
OMF I	0	480 (SD)	93 (SD)	0	500 (BF)	0
OMF II	0	450 (SD)	50 (SD)	160 (SD)	0	500 (BF)

BF: before sowing, SD: side dressing

Growth and Yield Assessment

Days to tasseling (DT, i.e. the period from sowing to tassel appearance), days to silking (DS, i.e. the period from sowing to silk appearance), plant height (PH), leaf number per plant (LNP) and ear number per plant (ENP) were recorded on a whole plot basis. PH and LNP were measured after tasseling. Harvesting occurred when the sweet corn reached maturity, indicated by the juice consistency of the kernels, typically within the first 10 d of July each year. Twenty ears from the centre of each replication were randomly harvested by hand in the morning. The harvested plants were then taken to the processing lab, where the ears were divided into two categories for analysis: 10 ears for morphological measurements and 10 ears for quality measurements. ENP, ear diameter (ED), ear length (EL), number of rows per ear (NRE), number of kernels per row (NKR), husked ear weight (HEW) and de-husked ear weight (DEW) were recorded for morphological traits. Husked ear yield (HEY) was calculated from HEW, while de-husked ear yield (DEY) was calculated from DEW according to Alan et al. (2013).

Kernel Quality Assessment

For fresh kernel quality traits, kernels were cut from the ear 1 h after harvest, and the following measurements were recorded: kernel colour, dry matter (DM) content, total soluble solids (TSS) content, total phenolic content (TPC) and antioxidant activity (AA). Kernel colour was measured with a colorimeter at the CIE (Commission Internationale De L'eclairage) L* a* b*. Kernels were measured for each replicate, and the colour was characterised by lightness (L*), hue angle ($h^\circ = \tan^{-1}(b^*/a^*)$) and chroma ($C^* = \sqrt{a^{*2}+b^{*2}}$). Regarding DM, kernels were dried in an oven at 65°C and the weight loss between measurements was < 0.05 g. The percentage difference between the fresh and dry weights was used to calculate the dry matter content of the kernel. For TSS, kernels were cut from the centre section of ten ears from each plot, after a 2-inch section was removed from each end of each ear. Fifteen grams from each ear were placed on a double layer square of cheesecloth. Extract was collected and placed on the a digital refractometer (PR-1, Atago, Tokyo, Japan) for analysis (Alan et al., 2014).

TPC (mg GAE/100 g FW) and AA (µmol TE/g FW)

Five grams of fresh kernels were mixed with 25 mL of methanol and homogenised using an Ika Ultra-Turrax homogeniser. The homogenates were kept at 4°C in the dark for 14–16 h before being filtered through Whatman No. 4 filter paper. The supernatants were collected and stored at −20°C until analysis (Thaipong et al., 2006). The TPC of the phenolic extract from the fresh kernels was determined using a modified Folin–Ciocaltaeu method (Swain and Hillis, 1959), with an incubation period of 120 min for colour development. Absorbance was measured at 725 nm using a spectrophotometer (Carry 100 Bio; Varian, Australia), and the results were expressed in milligrams of gallic acid equivalent (GAE) per 100 g^{-1} based on a standard curve of gallic acid (0–0.1 mg mL^{-1}). The spectrophotometric method with the ferric reducing antioxidant power was applied to measure AA in fresh kernels (Benzie and Strain, 1996). The absorbance of the supernatant was recorded using a spectrophotometer at 593 nm. The final results were calculated in µmol Trolox equivalents (TEs) per gram using a Trolox standard curve (25–500 µmol).

Statistical Analysis

Statistical analysis of variance was conducted using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). Data from the cultivars were analyzed separately. The trait data generated from the fertilization treatments over 2 years were analyzed using a 2 × 4 factorial design (2 years × four fertilization treatments) arranged in a randomized complete block design with three replications. For quality attributes and health-related assays, 10 ears were analyzed for both varieties, with all assays performed in triplicate. Significant differences among groups were determined using Duncan's multiple range test at $p \leq 0.05$. Pearson's pairwise correlations were computed using the 'corrplot' package (Wei et al., 2017) in RStudio version 2022.12.0 (RStudio Team, 2020). Principal Component Analysis (PCA) was conducted with the JMP16 (SAS, USA) to explore interactions between fertilization treatments, cultivars and various traits. Additionally, data visualization included heatmap analysis using the 'Bioconductor' package in R (Gentleman et al., 2004).

RESULTS AND DISCUSSION

Effect of fertilization treatment on growth parameters

The DT, DS, PH and LNP of the treatments are presented in Table 3. The DT was not significantly influenced by the fertilization regimes for either cultivar, with mean values ranging from 53.8 d (OMF I) to 55.3 d (control) for cv. 'Sentinel' and from 52.2 d (OMF I and OMF II) to 53.0 d (CF) for cv. 'Khan'. Regarding DS, fertilizer treatments showed no significant differences for cv. 'Khan'; however, significant differences were observed for cv. 'Sentinel' ($p \leq 0.05$). Over the 2-year experiment, the control treatment increased DS compared with CF, OMF I, and OMF II (Table 3). Notably, DT and DS were significantly higher ($p \leq 0.01$) in the second year of the experiment for both cultivars. Similar findings were reported by Lahay *et al.* (2019), who compared inorganic and organo-bio fertilizers for sweet corn and found no differences in DT. They also noted that DS was influenced by fertilization treatments. They emphasised that soil fertility is among the factors affecting plant flowering. Phosphorous is crucial for assimilation and respiration processes and is necessary for the reproductive development of plants, which accelerates flowering. Moreover, Alan et al. (2011) noted that DT and DS can vary based on genotype or genotype–environment interactions.

Statistical analysis revealed significant differences in PH among fertilizer treatments for both cultivars ($p \leq 0.01$) (Table 3). Mean values indicated that the CF (cv. 'Sentinel' = 171.0 cm; cv. 'Khan' = 164.5 cm), OMF I (cv. 'Sentinel' = 171.7 cm; cv. 'Khan' = 169.3 cm) and OMF II (cv. 'Sentinel' = 171.2 m; cv. 'Khan' = 164.7 cm) fertilizer treatments exhibited similar and higher PH values compared to the control treatment for both cultivars. LNP was not significantly influenced by fertilizer treatments for cv. 'Khan' (Table 3). However, for cv. 'Sentinel', the fertilizer treatments showed a significant effect on LNP ($p \leq 0.05$). Among the treatments, OMF II (n = 10.98) and OMF I (n = 10.95) produced the highest values, followed by CF (n = 10.37). These findings align with Ajibola et al. (2020), who compared fertilizer types (urea, NPK, OMF and OF) for sweet corn. They demonstrated that OMFs improved PH and leaf area in sweet corn compared to other nutrient amendments. In the present study, both cultivars showed increases in PH and LNP (significant for cv. 'Khan' only) in the first year of the experiment. The observed differences between experimental years may be attributed to climatic conditions, especially during the first 2 months (Figure 1). In the second year, the minimum and maximum temperatures from April and May were 0.8°C and 37.1°C, respectively. Conversely, the first year had more favourable temperatures (4.5°C minimum and 35.7°C maximum) during the same period.

Effect of fertilization treatment on yield and yield-related parameters

The ENP was not significantly influenced by fertilizer treatments for either cultivar (Table 4). On average, ear number values ranged from 1.20 (control) to 1.45 (OMF I) for cv. 'Sentinel' and from 1.25 (control) to 1.42 (OMF I) for cv. 'Khan'. ENP was significantly higher in the second year of the experiment for cv. 'Sentinel', whereas cv. 'Khan' exhibited higher values during the first year (both $p \leq 0.01$).

Statistical analysis indicated that fertilization significantly affected ED for both cultivars (both $p \leq 0.01$). Additionally, the ED was significantly higher ($p \leq 0.01$) in the second year of the experiment for cv. 'Khan' only (Table 4). On average, the highest ED value was recorded in the OMF I treatment (49.8 mm for cv. 'Sentinel' and 45.2 mm for cv. 'Khan'), followed closely by the OMF II treatment (49.7 mm for cv. 'Sentinel' and 44.4 mm for cv. 'Khan'). The results for EL showed significant variations among fertilization treatments for both cultivars ($p \leq 0.01$ for cv. 'Sentinel' and $p \leq 0.05$ for cv. 'Khan'). The mean values indicated that the CF, OMF I and OMF II treatments

had similar and higher EL values compared to the control treatment for both cultivars (Table 4). Notably, EL was significantly higher (both $p \leq 0.01$) in the second year of the experiment for both cultivars.

Table 3. Changes in the plant growth parameters of cv. 'Sentinel' and cv. 'Khan' based on the different fertilizer treatments

cv. Sentinel				
Fertilization	DT	DS	PH (cm)	LNP
First year				
Control	51.0	59.3	172.0	10.37
CF	51.7	56.7	179.7	10.67
OMF I	50.0	57.3	183.3	11.10
OMF II	51.0	57.7	182.0	10.90
Second year				
Control	59.7	65.0	156.3	9.87
CF	58.3	64.3	162.3	10.07
OMF I	57.7	64.0	160.0	10.80
OMF II	59.3	64.0	160.3	11.07
First year	50.9 b	57.8 b	179.3 a	10.76
Second year	58.8 a	64.3 a	159.8 b	10.45
Mean of the years				
Control	55.3	62.2 a	164.2 b	10.12 b
CF	55.0	60.5 b	171.0 a	10.37 ab
OMF I	53.8	60.7 b	171.7 a	10.95 a
OMF II	55.2	60.8 b	171.2 a	10.98 a
(LSD 0.05)				
Year	1.128**	0.844**	2.735**	ns
Fertilizer	ns	1.193*	3.868**	0.674*
Year × fertilization	ns	ns	ns	ns
cv. Khan				
Fertilization	DT	DS	PH (cm)	LNP
First year				
Control	48.7	55.3	164.3	10.30
CF	50.0	54.3	171.3	10.40
OMF I	48.3	54.0	182.0	10.40
OMF II	49.0	55.0	168.0	10.10
Second year				
Control	56.3	63.3	153.3	9.80
CF	56.0	62.7	157.7	9.93
OMF I	56.0	62.3	156.7	10.27
OMF II	55.3	63.0	161.3	10.13
First year	49.2 b	54.7 b	171.4 a	10.29 a
Second year	55.9 a	62.8 a	157.3 b	10.03 b
Mean of the years				
Control	52.5	59.3	158.8 b	10.05
CF	53.0	58.5	164.5 a	10.17
OMF I	52.2	58.2	169.3 a	10.33
OMF II	52.2	59.0	164.7 a	10.10
(LSD 0.05)				
Year	1.315**	0.779**	3.471**	0.227*
Fertilizer	ns	ns	4.908**	ns
Year × fertilization	ns	ns	6.941**	ns

CF: chemical fertilizer, OMF: organomineral fertilizer, DT: days to tasseling, DS: days to silking, PH: plant height, LNP: leaf number per plant, *: $p \leq 0.05$, **: $p \leq 0.01$, ns: non-significant

Fertilizer treatments had no significant effect on NRE for either cultivar. Although the difference was not statistically significant, the control treatment for both cultivars showed the lowest NRE (Table 4). The effect of year on this trait was significant ($p \leq 0.05$) for cv. 'Sentinel' only, with higher NRE observed in the first year of the experiment. Fertilizer treatments had no significant effect on NKR for cv. 'Khan'. Although the difference was not statistically significant, the control treatment exhibited the lowest NKR. Conversely, significant differences in NKR were observed for cv. 'Sentinel' ($p \leq 0.01$). According to the mean results, the CF, OMF I and OMF II treatments exhibited similar values, increasing NKR by 5.2%, 8.6% and 5.7%, respectively, compared to the control treatment (Table 4).

Table 4. Changes in the plant yield and yield-related parameters of cv. 'Sentinel' and 'Khan' based on the different fertilizer treatments

cv. Sentinel									
Fertilization	ENP	ED (mm)	EL (cm)	NRE	NKR	HEW (g)	DEW (g)	HEY (ton ha^{-1})	DEY (ton ha^{-1})
First year									
Control	1.13	45.3	19.6	18.1	39.0	402.3	324.7	23.0	18.6
CF	1.20	48.7	20.9	18.2	40.7	444.3	350.0	25.4	20.0
OMF I	1.20	49.5	20.8	18.2	42.3	437.7	349.0	25.0	20.0
OMF II	1.30	49.7	21.3	18.4	40.0	452.0	357.0	25.8	20.4
Second year									
Control	1.27	46.0	21.2	16.8	38.0	382.0	275.3	21.8	15.7
CF	1.63	47.4	21.8	17.9	40.3	432.0	326.7	24.7	18.7
OMF I	1.70	50.0	21.9	18.0	41.3	438.7	330.0	25.1	18.9
OMF II	1.50	49.7	21.7	17.9	41.3	450.7	310.3	25.8	17.7
First year	1.21 b	48.3	20.7 b	18.2 a	40.5	434.1	345.2 a	24.8 a	19.7 a
Second year	1.53 a	48.3	21.7 a	17.6 b	40.3	425.8	310.6 b	24.3 b	17.8 b
Mean of the years									
Control	1.20	45.7 c	20.4 b	17.5	38.5 b	392.2 c	300.0 b	22.4 c	17.1 b
CF	1.42	48.1 b	21.4 a	18.1	40.5 a	438.2 b	338.3 a	25.0 b	19.3 a
OMF I	1.45	49.8 a	21.4 a	18.1	41.8 a	438.2 b	339.5 a	25.0 b	19.4 a
OMF II	1.40	49.7 a	21.5 a	18.1	40.7 a	451.3 a	333.7 a	25.8 a	19.1 a
(LSD 0.05)									
Year	0.160**	ns	0.267**	0.488*	ns	ns	19.151**	0.379*	0.433**
Fertilizer	ns	1.197**	0.378**	ns	1.520**	12.353**	27.084*	0.536**	0.613**
Year × fertilization	ns	ns	0.535*	ns	ns	ns	ns	ns	0.867*
cv. Khan									
Fertilization	ENP	ED (mm)	EL (cm)	NRE	NKR	HEW (g)	DEW (g)	HEY (ton ha^{-1})	DEY (ton ha^{-1})
First year									
Control	1.40	39.6	19.7	16.4	44.0	399.3	324.0	22.8	18.5
CF	1.43	40.6	20.4	16.2	45.7	417.3	331.3	23.9	18.9
OMF I	1.50	41.5	20.2	16.4	46.3	426.7	339.3	24.4	19.4
OMF II	1.43	41.7	20.8	16.3	47.3	422.7	329.0	24.2	18.8
Second year									
Control	1.10	44.6	22.2	15.2	46.7	425.7	297.3	24.3	17.0
CF	1.37	46.8	23.5	15.7	46.7	447.3	315.3	25.6	18.0
OMF I	1.33	48.8	23.8	16.4	47.0	474.7	341.3	27.1	19.5
OMF II	1.20	47.1	23.5	16.2	46.3	432.0	310.0	24.7	17.7
First year	1.44 a	40.9 b	20.3 b	16.3	45.8	416.5 b	330.9 a	23.8 b	18.9 a
Second year	1.25 b	46.8 a	23.2 a	15.9	46.7	444.9 a	316.0 b	25.4 a	18.1 b
Mean of the years									
Control	1.25	42.1 c	20.9 b	15.8	45.3	412.5 c	310.7 b	23.6 c	17.8 c
CF	1.40	43.7 b	21.9 a	16.0	46.2	432.3 b	323.3 b	24.7 b	18.5 b
OMF I	1.42	45.2 a	22.0 a	16.2	46.7	450.7 a	340.3 a	25.8 a	19.4 a
OMF II	1.32	44.4 ab	22.1 a	16.2	46.8	427.3 b	319.5 b	24.4 b	18.3 bc
(LSD 0.05)									
Year	0.137**	0.897**	0.552**	ns	ns	9.240**	10.499**	0.326**	0.389**
Fertilizer	ns	1.269**	0.780*	ns	ns	13.067**	14.847**	0.461**	0.550**
Year × fertilization	ns	ns	ns	ns	ns	18.479*	ns	0.652**	0.777*

CF: chemical fertilizer, OMF: organomineral fertilizer, ENP: ear number per plant, ED: ear diameter, EL: ear length, NRE: number of rows per ear, NKR: number of kernels per row, HEW: husked ear weight, DEW: de-husked ear weight, HEY: husked ear yield, DEY: de-husked ear yield, *: $p \leq 0.05$, **: $p \leq 0.01$, ns: non-significant

For HEW, fertilization treatments significantly affected both cultivars ($p \leq 0.01$) (Table 4). The highest HEW was obtained from the OMF II treatment (451.3 g) for cv. 'Sentinel', while cv. 'Khan' recorded its highest HEW value from the OMF I treatment (450.7 g). Additionally, HEW was significantly influenced by year only for cv.

'Khan' ($p \leq 0.01$), with higher values recorded in the second year of the experiment.

Statistical analysis showed that fertilization significantly affected DEW for both cultivars ($p \leq 0.05$ for cv. 'Sentinel' and $p \leq 0.01$ for cv. 'Khan'). The CF (338.3 g), OMF I (339.5 g) and OMF II (333.7 g) treatments had similar values, with the highest DEW for cv. 'Sentinel' (Table 4). For cv. 'Khan', the OMF I treatment produced the highest DEW value (340.3 g), which significantly differed from both unfertilized and fertilized treatments, resulting in increases of 9.5%, 5.3% and 6.5% compared to the control, CF and OMF II treatments, respectively. Furthermore, DEW was significantly affected by year for both cultivars ($p \leq 0.01$), with higher values observed in the first year of the experiment.

Fertilizer treatments significantly affected HEY for both cultivars ($p \leq 0.01$) (Table 4). The mean results showed that HEY was highest with the OMF II treatment (25.8 ton ha^{-1}) for cv. 'Sentinel', whereas the highest HEY for cv. 'Khan' was observed with the OMF I treatment (25.8 ton ha^{-1}). Additionally, HEY was significantly higher ($p \leq 0.05$ for cv. 'Sentinel' and $p \leq 0.01$ for cv. 'Khan') in the first year of the experiment for cv. 'Sentinel', while it was higher in the second year for cv. 'Khan'.

For DEY, fertilization treatments also had a significant effect on both cultivars ($p \leq 0.01$) (Table 4). The mean results indicated that the CF (19.3 ton ha^{-1}), OMF I (19.4 ton ha^{-1}) and OMF II (19.1 ton ha^{-1}) treatments yielded similar values, increasing DEY for cv. 'Sentinel' by 12.9%, 13.5% and 11.7%, respectively, compared to the control treatment. For cv. 'Khan', the OMF I treatment (19.4 ton ha^{-1}) produced the highest DEY, significantly differing from both unfertilized and other fertilized treatments. OMF I treatment increased DEY by 9.0%, 4.9% and 6.0% compared to the control, CF and OMF II treatments, respectively. Furthermore, DEY was significantly influenced by year ($p \leq 0.01$), with higher values observed in the first year of the experiment for both cultivars.

The influence of fertilizer types on the yield and yield-related parameters of sweet corn shows that both OMF I and OMF II treatments, maintained or improved the ED, EL, HEW, DEW, HEY and DEY parameters compared to the CF treatment for both cultivars. This finding aligns with the results reported by Lahay et al. (2019), Etukudo et al. (2015) and Intansari and Subiksa (2022), who also observed an increase in yield and yield-related parameters of sweet corn when OMFs were combined with CFs. However, Ajibola et al. (2020) reported that NPK treatment led to higher yield and yield parameters in sweet corn. Comparing studies on sweet corn treated with various OMFs is challenging due to differences in cultivars, environmental conditions and agronomic practices employed in each study. Variations in yield results can be attributed to differences in OMF formulations, as well as the timing and method of application, which significantly influence nutrient availability. Some fertilization systems can also reduce nutrient loss (Bouhia et al., 2022; Srinivasarao et al., 2024). Furthermore, this study found that the effect of year on most yield parameters varied according to the cultivars. Similar results found by Ilker (2011) and Kara (2011) support our findings. However, HEY and DEY were found to be higher in the first year of the experiment when climatic conditions were more favourable (Figure 1).

Effect of fertilization treatment on quality traits and health-related compounds

The colour traits of the treatments are presented in Table 5. The kernel L* value was not significantly affected by the fertilizer treatments for either cultivar, with mean values ranging from 68.8 (CF) to 70.0 (OMF II) for cv. 'Sentinel' and from 74.9 (control and OMF II) to 75.2 (OMF I) for cv. 'Khan'. Notably, the L* value was significantly higher ($p \leq 0.01$) in the second year of the experiment for both cultivars. For C*, the statistical analysis indicated a significant effect of fertilization for cv. 'Khan', while cv. 'Sentinel' showed no significant influence from the fertilizer treatments. The highest mean C* value (n = 57.3) was observed in the CF treatment, followed by OMF I (n = 56.7). C* was also significantly affected by year for both cultivars ($p \leq 0.01$ for cv. 'Sentinel' and $p \leq 0.05$ for cv. 'Khan'). For cv. 'Sentinel', the C* value was higher in the first year, whereas it increased in the second year for cv. 'Khan'. Regarding the h° value of the kernels, no significant differences were found for cv. 'Sentinel'; however, fertilizer treatments resulted in significant differences for cv. 'Khan' ($p \leq 0.01$). Both the control and OMF I treatments exhibited the highest h° value (n = 88.9) for cv. 'Khan'. Additionally, h° was significantly higher ($p \leq 0.01$) in the second year for both cultivars. Similar findings regarding kernel colour traits were reported by Alan et al. (2014), who noted that kernel colour parameters are influenced by genotype, genotype-environment interactions and in-field management. To the best of our knowledge, this is the first comparative report on the colour traits of fresh sweet corn kernels in relation to OMFs and CFs.

For DM content, statistical analysis revealed significant differences between fertilizer treatments for cv. 'Sentinel' only ($p \leq 0.01$). The CF (22.2%), OMF I (22.2%) and OMF II (22.6%) treatments reduced DM content compared to the control treatment (23.5%). Conversely, for cv. 'Khan', DM content did not differ among the fertilization treatments and was significantly affected by year ($p \leq 0.01$), with higher DM content observed in the first year of the experiment (Table 5). TSS content was significantly influenced by fertilizer treatment for cv. 'Khan' ($p \leq 0.05$), while no significant differences were found in TSS content for cv. 'Sentinel'. According to the mean values, OMF II (16.7%) had the highest TSS for cv. 'Khan', followed by CF (16.3%). The year also had a significant effect on TSS for cv. 'Sentinel' ($p \leq 0.01$), with the highest TSS recorded in the second year of the experiment (Table 5). These results align with previous studies indicating that the effects of fertilization treatments on kernel quality largely depend on the tested genotypes (Warman and Havard, 1998; Lazcano et al., 2011). Moreover, Kleinhenz (2003) noted that the

refractometer, used to measure TSS, has been an effective pre-harvest method for determining sweet corn sugar content. Previous findings indicate that combining organic and inorganic fertilizers increases TSS and total sugar content compared to inorganic fertilization alone (Akinrinde and Lawal, 2006; Bharatti et al., 2020). To our knowledge, this is the first comparative report on the kernel DM and TSS content of sweet corn regarding OMF and CF.

Table 5. Changes in kernel quality and health-related compounds of cv. 'Sentinel' and 'Khan' based on the different fertilizer treatments

cv. Sentinel							
Fertilization	L*	C*	h°	DM (%)	TSS (%)	TPC (mg GAE 100 g^{-1})	AA (µmol TE g^{-1})
First year							
Control	69.0	51.9	88.3	23.9	14.7	71.2	3.30
CF	67.5	51.7	88.4	22.3	14.2	70.0	2.83
OMF I	68.7	52.8	88.6	22.2	14.4	72.3	3.12
OMF II	68.8	52.1	88.0	22.7	14.6	69.9	3.12
Second year							
Control	70.3	48.7	89.6	23.0	16.3	82.2	3.92
CF	70.0	49.4	89.3	22.0	15.5	85.9	4.12
OMF I	70.3	50.3	88.8	22.3	15.1	79.0	4.37
OMF II	71.2	50.7	89.2	22.4	15.6	85.0	4.34
First year	68.5 b	52.1 a	88.3 b	22.8	14.5 b	70.9 b	3.09 b
Second year	70.5 a	49.8 b	89.2 a	22.4	15.6 a	83.0 a	4.19 a
Mean of the years							
Control	69.6	50.3	88.9	23.5 a	15.5	76.7	3.61
CF	68.8	50.5	88.8	22.2 b	14.9	78.0	3.48
OMF I	69.5	51.6	88.7	22.2 b	14.8	75.6	3.75
OMF II	70.0	51.4	88.6	22.6 b	15.1	77.4	3.73
(LSD 0.05)							
Year	0.698**	0.767**	0.306**	ns	0.436**	1.469**	0.250**
Fertilizer	ns	ns	ns	0.745**	ns	ns	ns
Year × fertilization	ns	ns	ns	ns	ns	2.938**	ns
cv. Khan							
Fertilization	L*	C*	h°	DM (%)	TSS (%)	TPC (mg GAE 100 g^{-1})	AA (µmol TE g^{-1})
First year							
Control	73.9	55.5	88.2	25.7	15.5	82.5	3.83
CF	74.4	56.0	87.9	25.8	16.5	83.3	3.98
OMF I	74.6	56.4	88.4	25.5	16.1	83.3	3.73
OMF II	74.3	56.3	87.9	25.3	16.8	82.4	3.87
Second year							
Control	75.8	55.6	89.5	23.5	16.5	76.2	2.91
CF	75.8	58.6	89.3	23.6	16.0	78.0	3.39
OMF I	75.8	57.1	89.4	23.0	15.5	79.3	3.64
OMF II	75.5	56.3	89.0	23.5	16.5	79.6	3.38
First year	74.3 b	56.0 b	88.1 b	25.6 a	16.2	82.8 a	3.85 a
Second year	75.7 a	56.9 a	89.3 a	23.4 b	16.1	78.3 b	3.33 b
Mean of the years							
Control	74.9	55.6 c	88.9 a	24.6	16.0 b	79.3	3.37
CF	75.1	57.3 a	88.6 b	24.7	16.3 ab	80.6	3.68
OMF I	75.2	56.7 ab	88.9 a	24.2	15.8 b	81.3	3.69
OMF II	74.9	56.3 bc	88.5 b	24.4	16.7 a	81.0	3.63
(LSD 0.05)							
Year	0.416**	0.661*	0.183**	0.682**	ns	1.530*	0.224**
Fertilizer	ns	0.935**	0.259**	ns	0.612*	ns	ns
Year × fertilization	ns	1.322*	ns	ns	ns	ns	ns

CF: chemical fertilizer, OMF: organomineral fertilizer, DM: dry matter, TSS: total soluble solid, TPC: total phenolic content, AA: antioxidant activity, *: $p \leq 0.05$, **: $p \leq 0.01$, ns: non-significant

Concerning health-related compounds, TPC was not significantly affected by the fertilization treatments for either cultivar. Mean values ranged from 75.6 mg GAE 100 g^{-1} (OMF I) to 78.0 mg GAE 100 g^{-1} (CF) for cv. 'Sentinel'

and from 79.3 mg GAE 100 g^{-1} (control) to 81.3 mg GAE 100 g^{-1} (OMF I) for cv. 'Khan'. TPC was significantly higher in the second year of the experiment for cv. 'Sentinel' ($p \leq 0.01$), whereas it was higher in the first year for cv. 'Khan' ($p \leq 0.05$, Table 5). Fertilizer treatments did not significantly affect AA in either cultivar (Table 5). Mean AA values ranged from 3.48 µmol TE g^{-1} (CF) to 3.75 µmol TE g^{-1} (OMF I) for cv. 'Sentinel' and from 3.37 µmol TE g^{-1} (control) to 3.69 µmol TE g^{-1} (OMF I) for cv. 'Khan'. Although the differences were not statistically significant, the CF, OMF I and OMF II treatments showed higher AA than the control treatment for cv. 'Khan'. The effect of year was significant for this trait in both cultivars ($p \leq 0.01$), with the highest AA content observed in the second year for cv. 'Sentinel' and in the first year for cv. 'Khan'. Contrary to our findings, previous studies on fertilizer treatments indicated that combining organic and inorganic fertilizers increases phenolic content in sweet corn (Bharatti et al., 2020). This variation in TPC and AA can be explained by the fact that TPC and AA are influenced by genotype and the eco-physiological factors such as temperature and global radiation (Ziets et al., 2010). To our knowledge, this is the first comparative report on the TPC and AA of sweet corn using OMFs and CFs.

Effect of fertilizer treatment on growth, yield and quality traits using principal component and correlation analyses

In this study, Principal Component Analysis (PCA) was applied to assess the scientific validity of the results and to identify variations across different fertilizer treatments. The PCA showed a variance ratio of 86.20% (PC1 + PC2), highlighting the impact of OMFs on growth, yield, and quality traits in sweet corn varieties. In the PCA plot, the 'Khan' variety was positioned in the first region and stood out particularly in terms of L, C, TSS, and NKR traits. In contrast, the Sentinel F1 variety was prominent in terms of NRE (Figure 2). Both OMFs and CFs were plotted in the same plane and exhibited significant differences compared to the control group. The results indicated that OMFs and CFs were more effective in enhancing the agromorphological characteristics of sweet corn than the control group.

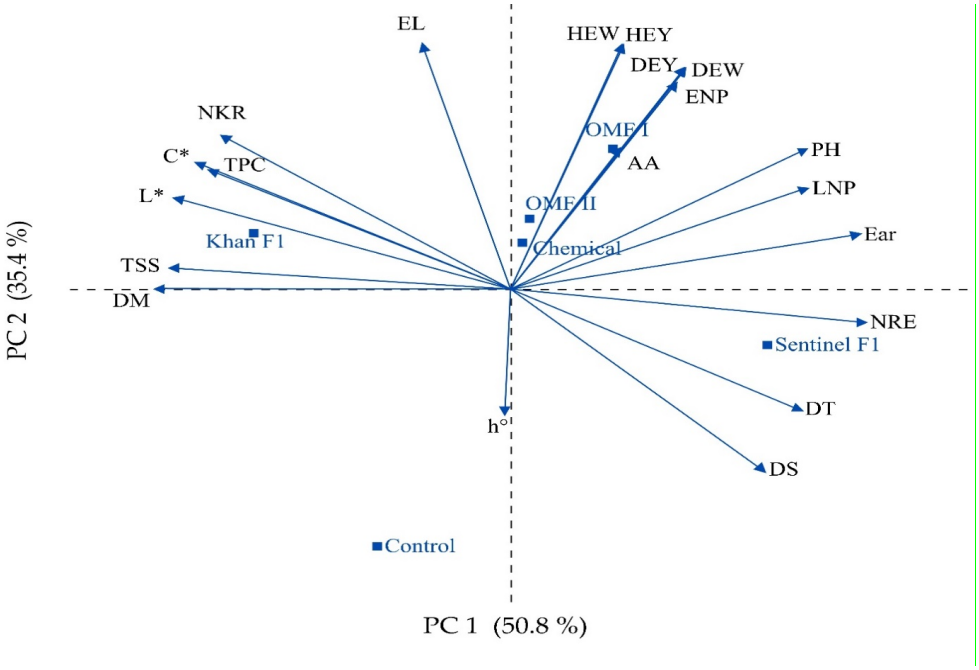

Figure 2. Correlation between sweet corn cultivars and fertilizer applications using PCA

According to the correlation analysis, a statistically positive correlation was found between DT and DS (r = 0.88, $p \leq 0.01$) and a negative correlation was observed between DT and NKR (r = -0.96, $p \leq 0.001$). The positive correlation rate between TSS and DM content of sweet corn was determined to be r=0.93 ($p \leq 0.001$). When comparing TPC with agromorphological characteristics, a positive correlation was identified between TPC and NKR (r = 0.88, $p \leq 0.01$), while a negative correlation (r = -0.89, $p \leq 0.01$) was observed with DS. In this study, positive correlations were determined between L* and C* color values and NKR, TPC, TSS, and DM (Figure 3).

The clustering analysis revealed the formation of two distinct groups among the treatments. The control treatment for cv. 'Sentinel' formed a separate group, while other treatments clustered together (Figure 4). Upon examining the grouping of agromorphological and biochemical characteristics based on the treatments, it was found that L*, C*, TPC, DM, TSS and h° values were grouped together, while other characteristics formed a different group. The heatmap analysis showed significant differences between cv. 'Khan' and cv. 'Sentinel' based on the treatments, resulting in their placement in different groups. Overall, in the OMF I treatment for cv. 'Khan', the values of HEW, HEY, DEW, DEY, EL, NKR, L*, C*, h°, TPC and AA were higher compared to the other treatments.

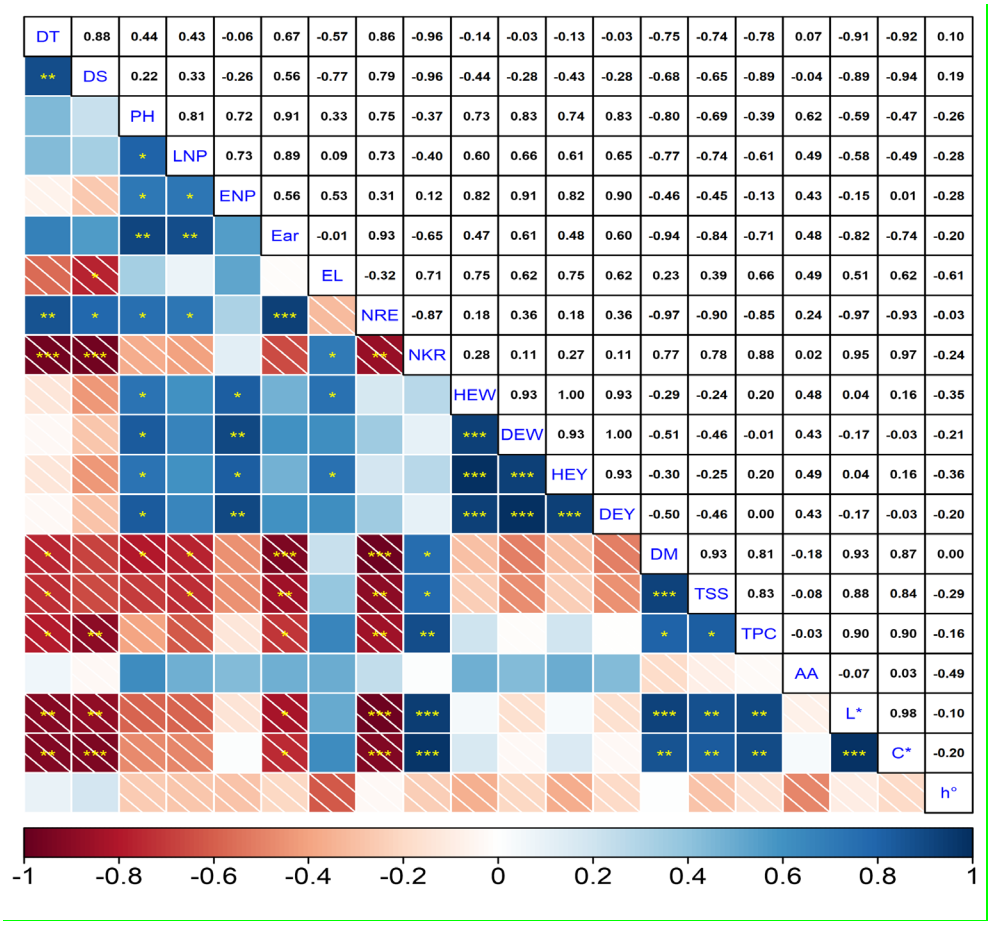

Figure 3. Correlation among physicochemical properties of. The color gradient ranging from red to blue represents correlation values between -1 and +1. *, **, and *** denote significance levels at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. DT: Days to tasseling, DS: Days to silking, PH: Plant height, LNP: Leaf number per plant, ENP: Ear number per plant, Ear: Ear diameter, EL: Ear length, NRE: Number of rows per ear, NKR: Number of kernels per row, HEW: Husked ear weight, DEW: Dehusked ear weight, HEY: Husked ear yield, DEY: Dehusked ear yield, DM: dry matter, TSS: total soluble solid, TPC: total phenolic content, AA: antioxidant activity.

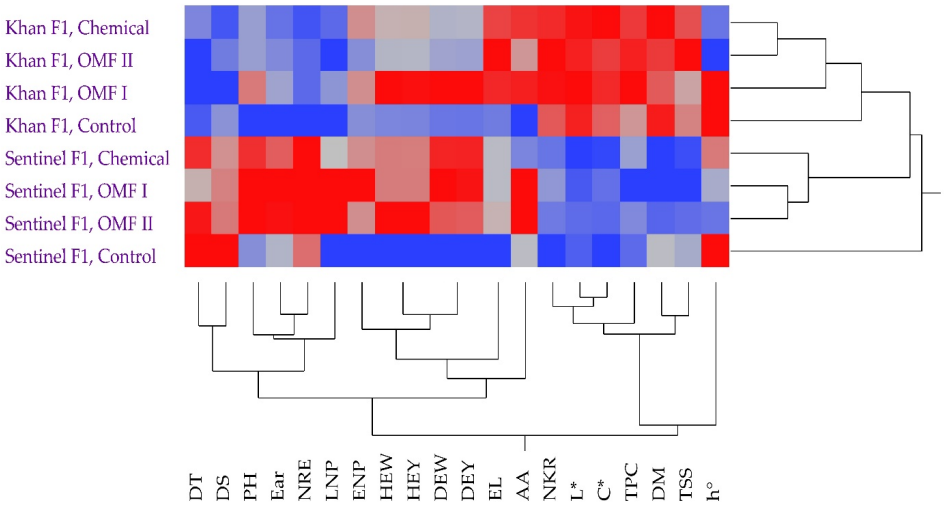

Figure 4. Clustering of cultivars based on physicochemical properties. The colour scale ranges from blue (indicating lower values) to red (indicating higher values). DT: Days to tasselling, DS: Days to silking, PH: Plant height, LNP: Leaf number per plant, ENP: Ear number per plant, Ear: Ear diameter, EL: Ear length, NRE: Number of rows per ear, NKR: Number of kernels per row, HEW: Husked ear weight, DEW: De-husked ear weight, HEY: Husked ear yield, DEY: De-husked ear yield, DM: dry matter, TSS: total soluble solids, TPC: total phenolic content, AA: antioxidant activity

CONCLUSIONS

This study demonstrated that sweet corn plants exhibited enhanced growth, yield and quality potential following the application of OMFs and CFs compared to the control group. Specifically, compared to CF, OMF I and OMF II either maintained or improved PH, LNP, ED, EL, NKR, HEW, DEW, HEY, DEY, C*, DM and TSS. However, the two OMF formulations elicited varying responses depending on the cultivar used. The application of OMF I with cv. 'Khan' resulted in increased ear size, ear weight (both husked and de-husked), ear yield (both husked and de-husked), colour traits, TPC and AA compared to the other treatments. These results suggest that OMFs are effective as a primary fertilizer source for speciality crops, such as sweet corn, in accordance with industry standards. Further research is needed to explore various OMF formulations and to assess different application timings and doses to achieve high yields without compromising the overall quality of the final product.

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THE EFFECT OF DIFFERENT STORAGE DURATION PERIODS AFTER HARVESTING IN A SEMI-ARID ENVIRONMENT ON THE QUALITY COMPONENTS IN SUGAR BEET

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ABSTRACT

The storage of the harvested sugar beet (*Beta vulgaris* L.) before the processing stage is crucial, particularly regarding quality and durability. To maintain the quality of the beet prior to processing, appropriate storage conditions must be provided. Although siloing is a standard method for beet storage, this process may have some negative effects. Given the limited information on the impact of siloing on yield and quality, it is essential to determine the optimal duration of siloing. This study investigated the effect of different siloing periods on the root and weight and quality criteria of sugar beet in 2020 and 2021. Four siloing periods (immediately after harvest, ten days after harvest, 20 days after harvest, and 30 days after harvest) were analyzed. The siloing periods significantly affected both the root weight and quality criteria in both years of research. It was determined that the examined characteristics were significantly influenced by the treatments, with weight loss, dry matter content, sugar content, and sugar yield of roots increasing with the length of the siloing period. The results highlight the importance of the waiting time during the siloing period for sugar beet stored in silos after harvest. To minimize losses in the examined characteristics, it is recommended that the processing occur immediately after harvest. If it must be delayed processing is recommended between 10-20 days post-harvest.

Keywords: *Beta vulgaris saccharifera* L., storage duration time, sugar beet, sugar content, weight loss.

INTRODUCTION

Storage practices for food products range from post-harvest storage in highly controlled environments to leaving the product in situ until the next stage of processing. The storage process is determined by a combination of the product's volume value and its tendency to deteriorate in the current environment (Wills et al., 2007). Sugar beet harvesting in our country varies from region to region for different reasons, such as climatic conditions, shipment, and enterprises' processing programs. The harvested products, which are directly related to the sugar beet processing capacities of the factories, are either kept in the fields where they are planted, in the weighbridge areas of the planting areas, or the collective silo areas determined by the enterprise or in the silo areas within the factory (Sarwar et al., 2008; Barna et al., 2011). This waiting period can be prolonged for various reasons, leading to significant losses in yield and quality. Sugar beetroots contain an average of 75% water, making siloed difficult and increasing losses. Therefore, the loss of tuber weight increases linearly as the storage period increases after harvest in tuber crops (Ozturk and Polat, 2016). For this reason, post-harvest losses can be as significant as harvest losses. Post-harvest yield and quality characteristics may vary according to siloing conditions, and even under suitable storage conditions, yield and quality may be adversely affected as the storage duration increases (Kenter and Hoffmann, 2009).

To reduce siloing losses, proper siloing techniques are essential (Barna et al., 2011). In general, as storage temperatures increase, respiration rate and cell membrane permeability increase and quality losses occur more rapidly (Kazaz et al., 2009). Relative humidity and temperature are the most critical environmental factors affecting the successful storage of sugar beet. The optimum temperature should be between 4-6 ^{0}C and relative humidity between 95-98% to minimize losses. Bacterial growth, fungal infestations, and germination in the roots can hinder the

formation of sucrose and raffinose in beets at 2 ^{0}C, 6% CO_2, and 5% O_2 (Demirel and Akinerdem, 2016).

Delaying the storage of beets after harvesting harvesting Results in a significant decrease in sugar yield; the highest sugar yield (12.37 tons ha^{-1}) was obtained in the control treatment. In the beets stored after leaf and head cutting and kept in the field for 48 hours, a sugar yield of 10.54 tons ha^{-1} was recorded, indicating a yield loss of 27.5%. The same study found no difference between sodium and amino nitrogen concentration and white sugar yield (Abdollahian-Noghabi and Zadeh, 2005). In another study, root yield, polar ratio, and sugar production of beet varieties stored both in sun and shade over 6 day periods and one-day intervals were monitored. It was found that root yield decreased, but polarity and sugar production increased in both storage conditions (Sarwar et al., 2008.).

There are differences between the outer surface and the inner part of the beet silo in terms of the rate of being affected by the external factors of the silo environment. It was noted that although the first 50 cm of the outer surface of the silo represents 17% of the whole silo, it accounts for 40-45% of the total losses, and sugar losses under covered and uncovered silo conditions differ. Kenter and Hoffmann (2006) stated that sugar yield decreases during storage, negatively impacting the processing quality of sugar beet roots. Consequently, sugar recovery becomes more expensive as the fabrication process extends.

In our country, sugar beet harvests vary by region due to climatic conditions, shipping and the processing programs of enterprises. The harvested sugar beet is kept in the harvested land, in weighbridge areas belonging to the cooperative, in the collective silo areas designated by the enterprise or in silo areas within the factory until it is processing (Ozgur, 2014). This waiting period can be prolonged for various reasons, resulting in serious losses in yield and quality.

The siloing of beet covers all the stages from the time it is harvested until it is processed (Ketizmen, 1987). Post-harvest losses are as critical as harvest losses due to the high moisture content in sugar beet. Yield and quality characteristics can vary according to siloing conditions, and even under suitable conditions, they may be adversely affected as storage duration increases (Yilmaz, 1987; Kenter and Hoffmann, 2009). To reduce siloing losses, it is essential to follow appropriate storage technique. Relative humidity and temperature are the most important environmental factors affecting successful storage. When roots are injured during harvesting or for other reasons, some parasitic fungi enter the root, increasing decay and losses (Lejealle and Cie, 1999).

The increase global demand brings nutritional challenges. Given that many countries face the threat of hunger and malnutrition, the importance of identifying crop losses in sugar beet becomes even more significant. This study aimed to determine the weight losses and changes in some quality characteristics that may occur in roots that cannot be processed immediately after harvest and must be kept in field conditions.

MATERIALS AND METHODS

Agronomic Practices

Field experiments were conducted (39° 47' 27" N and 40° 10' E; 1500 m above sea level) in Erzincan/Cayirli in 2020 and 2021. The soil of two experimental sites was a silty loam (fine, mixed, mesic assortments) with a pH 7.5, 2.21% organic matter, 140.6 kg ha^{-1} available P, and 2260 kg ha^{-1} available K in 2020 and pH 7.7, 2.30% organic matter, 154.0 kg ha^{-1} available P and 2280 kg ha^{-1} available K in 2021.

Temperature, rainfall, and relative humidity data during the crop-growing period are presented in Figure 1. Air temperatures during the two growing seasons were lower than the long-term mean. April to September temperatures, which averaged 18.7 ^{0}C, were slightly under normal in 2020 and 2021. There was considerable variability in rainfall amounts and distribution from year to year. The rainfall during the growing seasons of the Sentinel sugar beet variety was above the long-term average. The average rainfall for 2021 (18.67 mm) was lower than that observed (22.33 mm) in 2020.

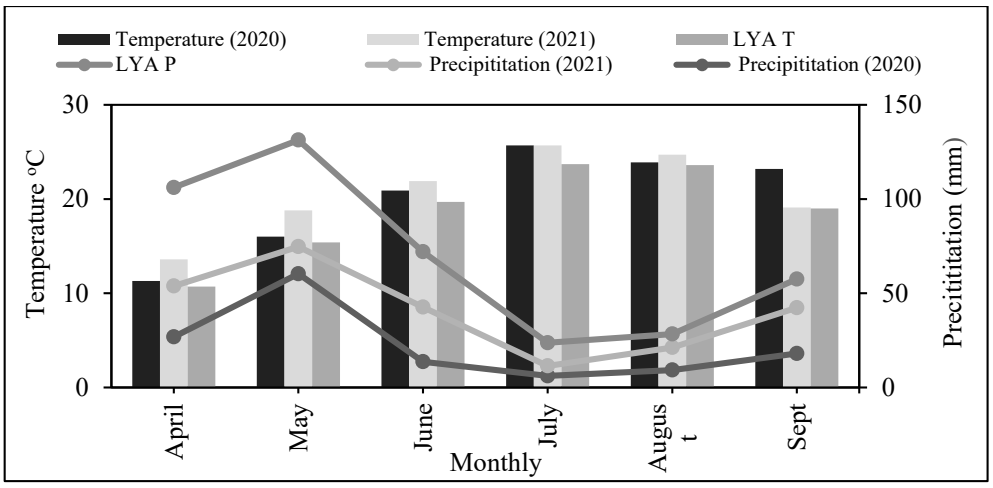

Figure 1. Some important climatic data of the experimental area for many years and 2020-2021(Erzincan/Cayirli)

The Sentinel sugar beet variety research with different siloing periods was conducted in two phases in 2020 and 2021. In the first phase of the study, three blocks measuring 20 m in lenght, 4.5 m in width, and an area of 90 m^2 (4.5 x 20 m) were prepared in a farmer's field in the productive village in Cayirli district, Erzincan province, for the production of plant material needed for the research. Each block consisted of four plots. This phase was carried out according to the "Randomize Complete Block Design" experimental design with three replications. Nitrogen containing fertilizers, including 15-15-15 and urea fertilizers containing 46% nitrogen were used at 150 kg per hectare. Fertilizers were applied by sprinkling and mixed into the soil. Sowing was conducted on April 14, 2020, and April 18, 2021, using a five-row precision beet seeder with a sowing depth of 5 cm, a distance of 45 cm between rows, and 8 cm above rows. Each plot consisted of 10 rows. After emergence, the row spacing was adjusted to 17 cm by thinning. On September 29, 2020, and September 31, 2021, when the plants reached a vegetation period of approximately 170 days, harvesting was carried out with a single-row harvester after separating one row from the edges and three plants from each head to account for the edge effect.

Second Phase: Application of trial factors.

Traits Measured

The plant material obtained after harvesting (first stage) was used in the second stage of this study. In the experiment, 100 kg of roots from each plot in three blocks (4 x 100 kg) were harvested separately. The sugar beet roots harvested from each plot have been siloed in open storage field in the form of piles, with an average height ranging from 2 to 2.5 meters and an average width not exceeding 3 to 5 meters. Firstly, the roots harvested from the production field were cleaned from soil residues and taken to the siloing field under open conditions in the Erzurum Sugar Factory experimental site on September 29-30, 2020-2021. Measurement, weighing, and analysis procedures for 10 kg beet samples taken from each silo at 10-day intervals from the beginning of siloing were conducted. Weight losses were calculated by weighing the 10 kg beet samples taken from each silo at 10 days intervals from the beginning of siloing. The climatic data of Erzurum Sugar Factory trial area determined at 10-day intervals are given in Table 1. Quality parameters were analyzed at the Sugar Factory Laboratory in Erzurum (Türkiye) according to Kavas and Leblebici (2004).

Table 1. Climatic factors in postharvest sugar beet storage duration (Erzurum)

YEAR	Storage periods			
	21-30 September	**01-10 October**	**11-20 October**	**21-30 October**
	Total rainfall for 10 days			
2020	1.67	0.03	0	0.62
2021	28.2	39	0	21.6
	Average temperature for 10 days			
2020	15.41	11.56	12.35	10.42
2021	10.54	9.13	10.07	2.86
	Relative humudity for 10 days			
2020	53.46	53.1	40.91	47.06
2021	64.42	69.88	57.82	63.78

Statistical analysis

All the data were analyzed using the SPSS package (SPSS, Version 20.0, SPSS Inc, Chicago, IL, USA). When the F-test indicated statistical significance at the P=0.05 level, the protected least significant difference (Protected DUNCAN) was used to separate the means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Statistically significant ($p<0.01$) differences were found between the years regarding the characters examined in the study (Table 2). Beet rootstocks ensiled in the first year exhibited greater weight loss and other quality parameters. The 2020 production season was drier and warmer than that of 2021 (Figure 1). The temperature values in 2021 negatively affected the quality criteria of sugar beet plants stored in the silo.

Weight Loss

In the study, the effects of year, storage duration, and year x storage duration interactions on weight loss were found to be significant ($p<0.01$) (Table 2). In the first year of the study, the weight loss of the roots in the silos was 1.6% higher than in the second year. It is thought that the higher air temperature in the first year of the research caused more weight loss in the roots due to rapid respiration and the resulting flushing. Indeed, Peterson et al. (1980) stated that the effect of temperature on siloing was significant. According to the storage period, the highest weight loss was observed at 30 days (38.29 kg), while the lowest was at ten days (18.5 kg). In the other storage period of 20 days, a weight loss of 32.6 kg occurred (Table 3, Figure 2). The change in weight loss values in all treatments was in the direction of increase compared to the values at harvest, which were accepted as the control. It was determined that the weight loss was 18.5% at the end of the

first 10-day storage period after harvest, 32.6% at the end of the 20th day, and 62.5% at the end of the 30 days.

The average weight loss during storage was 37.9% (Table 3; Figure 2).

Table 2. Analysis of variance results of yield and quality parameters of sugar beet varieties in different storage duration treatments

a)

Sources of Variation	df	Weight Loss		Sugar Content		Sugar Yield Loss		Dry Matter Content	
		F Values	Mean Square	F Values	Mean Square	F Values	Mean Square	F Values	Mean Square
Year (Y)	**1**	269.85**	583.12	189.80**	157.28	30.57**	16.375	541.00**	246.81
Storage Duration Periods (S)	**4**	1030.71**	1747.93	83.67**	62.71	9.19**	4.802	424.30**	117.09
Y x S	**4**	33.88**	73.23	5.31**	4.39	2.15	1.125	18.91**	8.62
Eror	**12**	23.41	1.93	9.09	0.79	6.27	0.52	4.56	0.37
CV		1.8		4.34		4.15		2.26	

b)

Sources of Variation	df	α- Amino Nitrogen		Ash Content		Polarization in Usare		Usare Purity	
		F Values	Mean Square	F Values	Mean Square	F Values	Mean Square	F Values	Mean Square
Year (Y)	**1**	1.01	0.00011	172.11**	5.57	24.61**	170.05	99.72**	47.77
Storage Duration Periods (S)	**4**	13.05**	0.00004	2.11^{ns}	0.08	65.89**	73.63	3.64^{ns}	6.90
Y x S	**4**	1.23	0.00013	6.40**	0.21	9.16**	6.39	5.93*	2.80
Eror	**12**	0.0002	0.0054	0.12	0.36	12.84	0.91	9.77	1.18
CV		9.77		4.86		1.01		4.81	

*. ** significant at the 0.05 and 0.01 levels, respectively. ns; nonsignificant.

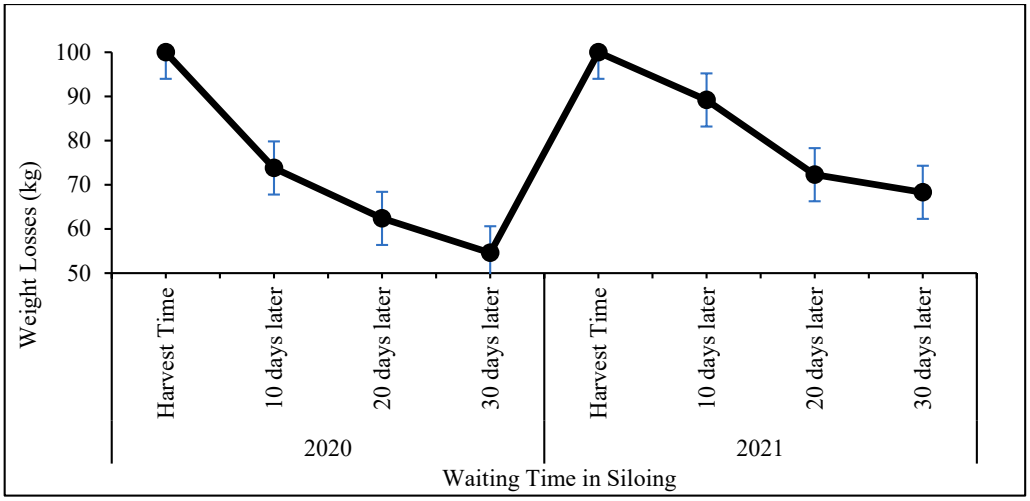

Figure 2: Effect of year × storage duration interaction on subsequent weight loss

As a result of the research, it was determined that the weight loss of roots kept after harvest increased in parallel with the number of days they were kept. Our study's average temperature during the thirty-day storage period was 11.42 ^{0}C. Sarwar et al. (2008) reported that 9.70%, 14.10%, 18.30%, 21.66%, and 25.11% weight loss was detected on average when sugar beets were stored in silos for 2, 3, 4, 5 and 6 days, respectively. In a similar study, Scalon et al. (2000) reported 55% weight loss in roots due to 12 days of storage when the siloing temperature was between 15-26 ^{0}C. Variety, climatic factors, physiological maturity of the beet, and silo size all effect post-harvest storage losses in sugar beet (Sefaoglu et al., 2016; Kocak et al., 2019). In addition, browning is observed depending on the size of the siloing, which causes the beet to lose weight and sugar (Haagenson et al., 2006).

Sugar Content

The effect of year, storage time in siloing, and year x storage time interactions on sugar content, which is one of the important factors in determining sugar yield, was significant ($p<0.01$). The sugar content of sugar beet averaged 22.63% and 14.49% in the 2020 and 2021 growing seasons, respectively. Ecological factors at the time of harvest play an important role in the sugar content of beet. The fact that respiratory activities continued at different levels in the siloing root under variable weather conditions led to changes in terms of sugar content.

Although the sugar content in the roots was the lowest at harvest (16.4%), it reached the highest value (23.7%) at the end of 30 days of storage. This was followed by a 20-day period with 21.22%; after 10 days of storage, the sugar content was 19.20% (Table 3, Figure 3). The storage duration significantly affects the amount of soil present and has a significant effect on availability; it has an increasing impact on sugar content compared to harvest time application. The sugar content, which increased by 19.70% in the 10-day application rose by 32.29% in the was 20 days application. The highest increase was 47.69% in the 30-day storage duration treatment.

Table 3. Effect of storage duration on sugar beet weight loss, sugar content, sugar yield loss, dry matter content.

Years	Harvest Time (Control)	10 Days Later	Change (%)	20 Days Later	Change (%)	30 Days Later	Change (%)
Weight Loss (kg)							
2020	100±0.00a	73.8±0.36b	-26.20	62.41±0.49c	-37.60	54.6±1.78d	-45.10
2021	100±0.00a	89.2±1.27b	-12.10	72.30±0.40c	-27.70	68.8±0.23d	-31.20
Mean	100±0.00a	81.5±3.49b	-18.50	67,36±2.22c	-32.64	61.7±3.26d	-37.95
Sugar Content (%)							
2020	17.4±0.06d	22.0±0.06c	26.90	24.2±0.05b	39.70	26.9±0.95a	54.8
2021	14.8±0.20c	16.4±0.98bc	10.80	18.2±0.42b	23.20	20.5±0.04a	38.7
Mean	16.0±0.59d	19.2±1.33c	19.70	21.2±1.35b	32.29	23.7±1.48a	47.7
Sugar Yield Loss (kg)							
2020	17.3±0.06a	16.2±0.03b	-6.4	15.1±0.10c	-12.7	14.6±0.14d	-15.6
2021	14.8±0.20	14.7±1.08	-0.7	13.2±0.38	-10.8	14.1±0.07	-4.7
Mean	16.1±0.59a	15.5±0.60a	-3,7	14.1±0,46b	-12.4	14.4±0,13b	-10.6
Dry Matter Content (%)							
2020	23.1±0.18d	29.1±0.40c	26.10	34.2±0.18b	48.00	35.4±0.82a	53.20
2021	19.9±0.07c	22.9±0.78b	14.90	25.9±0.11a	29.50	27.3±0.17a	36.70
Mean	21.5±0.70d	26.0±1.43c	20.90	30.0±1.86b	39.39	31.3±1.82a	45.56

For each main effect, values within columns followed by the same letter are not significantly.

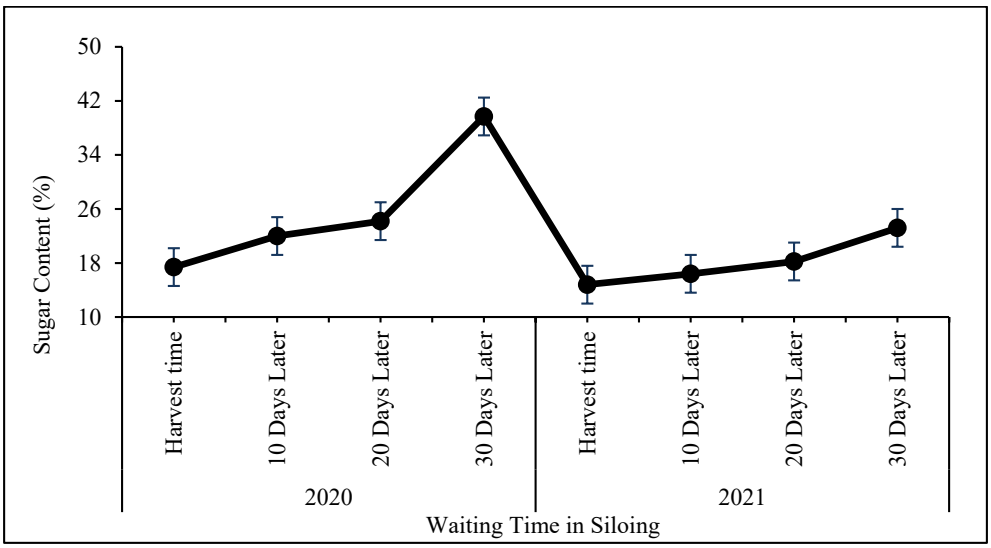

Figure 3: Effect of year × storage duration interaction on the sugar content losses

The impurities on the beet during storage (head, leaves, and soil amount) have a significant effect on the ensilability of the beet. Beets with neatly cut heads lose less sugar in silos. By siloing beets in this way, the silo temperature will be lower, and the losses that may occur due to respiration can be kept at lower levels. Steensen and Augustinussen (2002) reported that after 50 days of siloing, an average of 4.1% sugar loss occurred in silos made from undamaged beets, while 5.7% sugar loss occurred in silos consisting of damaged and bruised beets. Ada and Akinerdem (2006) reported that the highest sugar loss (19.53%) was observed after 90 days of storage. Similarly, Barna et al. (2011) and Kocak et al. (2019) reported that the sugar content increased with the extension of the storage period.

Sugar Yield Loss

While the effect of storage periods on sugar yield, based on stem yield and sugar content, was significant in the first production season, the impact of storage periods on sugar loss was insignificant in the second production year. On average, sugar loss in sugar beet was higher in 2020 than in 2021. This difference between the years was statistically significant at the $p<0.01$ probability level (Table 2). The higher sugar yield loss in the first year of the experiment, compared to the second year, is due to the greater average root yield loss and sugar content (Tables 2 and 3). When examining Table 3, which shows the sugar loss at the time of harvest and at 10, 20, and 30 days after harvest, it is observed that the response of sugar yield to storage periods is irregular. Beets kept for 30 days in the first research year and beets kept for 20 days in the second research year caused more sugar loss than beets kept for 10 days. In the first experimental year, 14.6 kg of sugar yield was obtained from beets kept for 30 days, while in the second year, 13.2 kg sugar yield was obtained from beets kept for 20 days. All treatments significantly decreased sugar yield compared to the harvest time (control). In the first year, the highest loss was 15.6% in roots stored for 30 days, followed by a 12.7% loss after 20 days of storage. In the 10-day storage period, the smallest loss was determined at 6.4% compared to the harvest time (Table 3). In the second experimental year, the highest loss was 10.8% in beets kept for 20 days, while losses 4.7% and 0.7% were observed in beets kept for 20 and 10 days of siloing, respectively. Harvested roots start to lose water rapidly through respiration immediately after harvesting, causing sugar losses (Ada and Akinerdem, 2006). Likewise, the longer the siloing period, the more rootstocks are exposed to frost damage. While 16.5% sugar is obtained from well-stored beet, this rate decreases to 12.5% in partially frost-damaged rootstocks (Batu, 2002).

Dry Matter Content

In the study conducted over two years under field conditions, the effect of years, storage duration, and the impact of year x storage duration interaction on dry matter content was significant ($p<0.01$). In the first experimental year, the dry matter content was higher than in the second year because the soils were rich in organic matter and potassium; the temperature was higher (17.1 ^{0}C), while precipitation (158.5 mm), and relative humidity (47.3%) were lower. It was determined that the storage duration treatments gradually increased the dry matter content. In both research years, the lowest dry matter content was obtained from sugar beet roots at harvest (23.1 and 19.97% g, respectively), and the highest dry matter content was obtained from the treatment kept for 30 days (35.4 and 27.29%, respectively). The results show that the dry matter content increased gradually with increasing storage duration. The amount of dry matter is directly related to the compounds that effect in root quality. Sohrabi and Heidari (2008) stated that sugar yield is a dry matter-related characteristic and that a high sugar yield can be obtained when a high proportion of dry matter is produced in the root. The findings of our research are supported by the results of Demirel and Akinerdem (2016), who reported a significant increase in weight loss due to the progression of storage duration and an increase in the dry matter ratio.

α- Amino Nitrogen

According to the results of variance analysis, the effect of storage periods on α- amino nitrogen ratio was insignificant in the first harvest year. In contrast, the impact of storage periods was significant in the second harvest year (Table 4). The α- amino nitrogen content was higher in the first harvest year than in the second crop year. In both research years, the highest α- amino nitrogen content was obtained in the 30-day storage duration, while the lowest α-amino nitrogen content was obtained in the control (at harvest) and 10-day storage duration (Table 4). These results showed that the α- amino nitrogen ratio increased with the storage duration compared to the harvest time. The highest increase of 14.29% was detected in roots kept for 30 days, while no change was detected in the 10-day storage period (Table 4). These results indicate that biological activity continues in beet after harvest. The absence of green parts in the plant during storage causes changes in the structure of nitrogenous compounds in the root. In addition, the increase in the dry matter content of root due to the prolonged storage period may also increase the amount of α amino nitrogen. Demirel and Akinerdem (2016) reported that the biological activity in the root of sugar beet continues after harvest, and the amount of amino nitrogen increases as this period extends.

Ash Rate

The amount of ash in sugar beet is a factor that reduces sugar yield and includes all the inorganic substances contained in beet fabrication products and white sugar (Kavas and Leblebici, 2004). Climate factors are critical in affecting the amount of ash in sugar beet. The highest amount of ash was obtained in the second harvest year when the climatic conditions were favorable. This difference in ash content between the years was statistically significant (Table 4). When examining Table 3, the ash percentage (13.82%) was highest in the first harvest year, when the storage duration in the heap was the longest (30 days). The value at 20 days was 3.14%. The lowest ash percentage was 1.59% at harvest time, and roots were kept for ten days. In the second crop year, the opposite was true; the highest value (2.93%) was obtained at 20 days of storage, while the ash percentage was lowest (2.26%) when the storage duration was the longest (30 days). In other words, the ash percentage increased by 5.50% in the 20-day storage period, where the highest ash percentage was obtained. The ash amount determined in the 30-day storage period decreased by 18.40% compared to the harvest time (Table 4). The 10-day storage period did not affect the ash percentage value.

Purity (Q) in Usare

A difference in purity was observed between 2020 and 2021 when the experiment was conducted. The purity obtained from roots was higher in the first and second experimental years (Table 4). The higher purity rate in 2020

compared to 2021 is thought to be due to the favorable effects of ecological factors in 2020. In both research years, beets kept for 30 days from different storage duration subject to the experiment showed higher purity than beets kept for 10 and 20 days at the time of harvest. In the first research year, the highest increase was 60.42% while in the second year, it was 28.00%. This increase was obtained with 30 days of roots in the pile, followed by increases of 40.35% and 19.80% with 20 days of waiting time. At 10 days of the siloing, the lowest increase was determined at 29.62% and 15.40%, respectively, compared to harvest time (Table 4). All treatments significantly increased purity compared to the harvest time (control). In cases where extremely low temperatures do not occur, the prolonged vegetation period leads to an increase in yield and quality and consequently to a rise in the purity of the juice (Cakmakcı and Tıngır, 2001). This difference in purity may be due to the gradual decrease in the ratio of soluble sugar to dry matter during siloing.

Polarization in Usare

The most important economic indicator in sugar beet production is white sugar content (Dadkhah, 2005). According to the results of variance analysis, the differences between years in terms of root refined sugar content were statistically significant ($p<0.001$) (Table 4). When examining Table 4, it was determined that root refined sugar content was 5.3% higher in the first research year. The higher polarity in the first year of the experiment compared to the second year may be due to the low rainfall, high temperature, and elevated lime and potassium levels in the soil during the growing period in this year. It was found that the polar sugar ratio of the harvested roots increased with the application of storage duration without processing. The polar sugar ratios of root in 2020 were 17.99%, 23.32%, 25.25%, and 28.86%, while in 2021 were 15.4%, 18.2%, 19.2%, and 21.4%, respectively (Table 4). According to the polar sugar content at harvest, the highest increase was observed at 30 days of waiting time, with increase of 60.42% and 28.00%, respectively, in both research years. The lowest increase rate was determined at 10 days of storage duration, with increase 29.62% and 19.2%, respectively. The study found that the polar sugar ratio increased with the prolongation of storage time of the harvested roots without processing. This may be due to the increase in the total sugar ratio resulting from the conversion of starch to sugar with the prolongation of the storage duration, which may have caused an increase in the polarization ratio in the juice. Demirel and Akinerdem (2016) reported that the rate of purified digestion increased with the extension of storage duration, and the rate of purified digestion increased in percentage with the increase in weight loss as the storage duration increased.

Table 4. Influence of storage duration on sugar beet α- amino nitrogen, ash rate, purity (q) in usare, polarization in usare.

Years	Harvest Time (Control)	10 Days Later	Change (%)	20 Days Later	Change (%)	30 Days Later	Change (%)
α- Amino nitrogen (g/100g)							
2020	0.043±0.0008	0.045±0.0002	4.7	0.047±0.0005	9.30	0.051±0.004	16.27
2021	0.034±0.0046b	0.039±0.0052ab	17.7	0.048±0.0003a	41.20	0.048±0.006a	41.20
Mean	0.042±0.0005b	0.042±0.0030b	-	0.045±0.0010a	7.14	0.048±0.004a	14.29
Ash Rate (%)							
2020	1.59±0.050b	1.59±0.020b	-	1.64±0.025b	3.14	1.80±0.021a	13.20
2021	2.77±0.098ab	2.54±0.230b	-9.10	2.93±0.063a	5.50	2.26±0.159c	-18.40
Mean	2.18±0.268	2.06±0.233	-5.83	2.29±0.289	5.05	2.03±0.123	-6.88
Polarization in Usare (g/100g)							
2020	17,99±0.490c	23.32±0.16b	29.62	25.25±0.10ab	40.35	28.86±1.14a	60.42
2021	15,40±0.050c	18.2±0.85b	15.40	19.2±0.23b	19.80	21.40±0.17a	28.00
Mean	16,71±0.627d	20.75±1.21c	24.18	22.20±1.371b	32.85	25.13±1.74a	50.39
Purity (Q) in Usare (g/100g)							
2020	88.36±0.030b	90.07±0.220ab	1.93	90.15±0.810ab	2.02	91.84±1.04a	3.93
2021	88.39±0.168a	87.01±1.045ab	1.60	85.86±0.499b	2.86	87.88±0.02a	0,57
Mean	89.27±0.539	89.43±1.267	0.18	87.11±0.602	2.48	88.98±0.500	0.32

For each main effect, values within columns followed by the same letter are not significantly.

CONCLUSIONS

Because roots maintain their vitality after harvesting, weight and quality losses during siloing constitute the most significant disadvantage of this plant. To reduce these losses, it is essential that siloing is carried out in suitable environments and for specific periods. Although the increase in the storage duration of roots after harvest generally causes weight loss, it can result in a partial increase in sugar content. While this leads to an increase in sugar availability, it also causes a significant decrease in sugar yield, which is the final product. When siloing is extended to 30 days, a 38.0% weight loss occurs, alongside a 47.7% increase in sugar availability, and a 10.6% loss in sugar yield.

The results highlight the importance of the waiting time during the siloing period for sugar beet stored in silos after harvest. To minimize losses in the examined characteristics, it is recommended that the processing occur immediately after harvest. If it must be delayed processing is recommended between 10-20 days post-harvest.

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IDENTIFICATION OF QTL CONFERRING ADULT PLANT RESISTANCE TO WHEAT STRIPE RUST IN BREAD WHEAT LANDRACE BWLR-2347

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ABSTRACT

Stripe rust is among the most destructive foliar diseases affecting wheat globally. The identification of novel adult plant resistance loci represents a critical strategy for mitigating the substantial yield losses attributed to stripe rust across diverse regions worldwide. Wheat landraces possess untapped genetic resources for abiotic and biotic stresses including diseases like stripe rust. This study was conducted to identify the genetic basis of adult plant resistance response in bread wheat landrace BWLR-2347 conserved at ICARDA gene bank. The mapping population of 178 F_2 individuals was genotyped with high quality genotype-by-sequencing derived SNPs. The phenotypic disease assessment was carried out in $F_{2:3}$ families derived from the cross between resistant bread wheat landrace BWLR-2347 and susceptible Avocet "S" in the field under artificial inoculation with a mixture of stripe rust races. Seven QTLs were identified for resistance to YR at the adult plant growth stage and mapped on five chromosomes. The QTLs were detected on the 1B, 2A, 2B, 2D, and 5A regions. The phenotypic variance explained by an individual QTL ranged from 2.01% to 5.59%. The study validated the six previously identified QTL and reported a novel QTL at chromosome 5A. The information obtained from the study will be helpful in wheat breeding programs towards the development of stripe rust resistant cultivars.

Keywords: DArT, QTL mapping, Stripe rust, Türkiye, Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) remains a crucial global food source, providing approximately 20% of human dietary calories and protein (Miner et al., 2022). Recent estimates suggest that wheat production must increase by 69 million ha by 2050 to meet growing food security demands (Bahar et al., 2020). This fundamental crop faces numerous climatic stresses, but rusts are the most damaging wheat disease worldwide. Wheat stripe rust, also known as yellow rust (Yr), is caused by the pathogen *Puccinia striiformis* f.sp. *tritici* (*Pst*) is the most devastating foliar disease for global wheat production (Wellings, 2011). Stripe rust mostly spread in cooler and moisture environments (0ºC to 23ºC) that cause global yield loss up to 1% (Savary et al., 2019) with a damage loss of more than 1 billion US dollars annually (Chen et al., 2021). Out of total wheat growing area of Turkey 25% mainly affect with stripe rust with 1-5% crop loss and epidemic occurs in 2 out of 5 year (Chen, 2020). This pathogen overcomes its resistance by producing new races through genetic variation and it also spreads over distance through air that cause the epidemic in relatively short periods. The most effective strategy to control this disease is to develop disease-resistant varieties, which is an environmentally friendly and economical approach as it limits the use of fungicides (Chen and Kang, 2017; Akcura et al., 2017).

Two major types of resistance prominently characterized to control the spread of *Yr*: all-stage resistance (race specific or overall resistance) and adult plant stage resistance (race non-specific or partial resistance) (Chen, 2005; Habib et al., 2020a). All-stage resistance is usually controlled by a single gene and can be detected at the seedling stage and is expressed at all developmental stages of plant, but it readily overcome due to emergence of new races and that cultivars become susceptible. Adult plant stage also known as high temperature adult plant (HTAP) resistance is durable as controlled by minor and effective multiple loci that expression depends upon growth stages of plant development and temperature (Chen, 2013; Zhou et al.,

2014). Therefore, the best approach is to combine the durable HTAP with the all-stage resistance through gene pyramiding that utilize the molecular marker to map the desired gene in the germplasm and its selection through marker assisted selection. Quantitative trait loci (QTL), genomic regions containing genes that influence quantitatively inherited traits, have become powerful tools in understanding complex disease resistance mechanisms in wheat. Modern genotyping approaches such as Diversity Arrays Technology (DArT), a microarray-based technique for DNA polymorphism analysis, and genotype-by-sequencing (GBS), a next-generation sequencing method for discovering genetic variants, have revolutionized our ability to identify and map these resistance loci. To date almost 80 stripe rust resistance genes and >300 QTLs have been reported in wheat (Li et al., 2018; Chen, 2020; Mourad et al., 2021). Recently, simple sequence repeats (SSR), DArT and single nucleotide polymorphism (SNP) markers have been widely used to estimate the genetic diversity and to map the QTLs in the wheat genome (Habib et al., 2020a; Hussain et al., 2022; Kocak et al., 2022). DArT markers have been widely used for QTL mapping in wheat against different traits due to its economical convenience and high genome coverage (Jighly et al., 2015; Ahmed et al., 2021).

For effective utilization of genetic resource in breeding strategy, the extent of variation and correlation is pre-requisite (Habib et al., 2020b) that can be depicts at genomic level using molecular markers. More genes need to be identified with major effect that show durability of resistance at HTAP (Zhou et al., 2014). The objective of this study is to characterize and map the QTLs/genes conferring the durable resistance to HTAP resistance to stripe rust in landrace BWLR 2347.

MATERIALS AND METHODS

Plant material

Seeds of the resistant bread wheat landrace parent BWLR 2347 (6571-landrace) were selected from the ICARDA gene bank based on its performance for adult plant resistance to stripe rust at Regional Cereal Rust Research Centre (RCRRC), in Izmir, Turkey. The landrace was crossed with stripe rust universal susceptible cultivar Avocet S. The mapping population was developed from cross of single plants of the susceptible 'Avocet S' (AvS) variety as the male parent and the resistant wheat landrace 'BWLR 2347' as the female parent. Individual F1 plants were selfed to produce the F_2 segregation population. The F_2 mapping population, consisting of 240 individual plants, was used for genetic map construction and phenotypic evaluation. F_2 derived F_3 ($F_{2:3}$) were used for adult plant phenotypic assessment of stripe rust.

DNA extraction and genotyping

Genomic DNA was extracted from fresh leaves collected from individual F_2 plants at 10-day old seedlings stage using a modified CTAB (cetyltrimethylammonium bromide) method (Hoisington et al., 1994). The seedling leaves were collected in labeled eppendorf tubes and stored in an Ultra freezer at -80°C for subsequent DNA extraction. Leaf samples were ground using a tissue lyser (Tissue Lyser II from QIAGEN) until a fine powder was obtained. 0.1g of the powdered leaf samples were used for DNA extraction using the CTAB method (Doyle and Doyle, 1990). The extracted DNA was dissolved in 100µl tris-EDTA (TE) buffer. The samples were analyzed on 1% agarose gel for the purity test and quantified with a spectrophotometer (NanoDrop ND1000). The DNA samples were stored at -80°C.

From the initial 240 F_2 plants, 178 lines were successfully genotyped and used for mapping. The reduction in population size was due to several factors: (i) DNA quality requirements for GBS analysis eliminated 35 samples, (ii) 15 plants did not survive to produce adequate tissue for DNA extraction, and (iii) 12 samples failed quality control during the genotyping process. The final population size of 178 individuals provides sufficient statistical power for preliminary QTL detection.

The extracted DNA samples of the F_2 individual plants and two parental lines were sent to Diversity Arrays Technology Pty Ltd. (Canberra, Australia, http://www.DiversityArrays.com/) for genotyping using the genotype by sequencing (GBS) method. The genotypic data obtained for 178 lines including the parents were filtered for markers with >10% missing data and with <0.1% minor allele frequency. The results obtained from 1115 polymorphic SNP markers were used to construct the genetic linkage map.

Phenotypic evaluation of adult plant resistance

The field experiments were carried out at the RCRRC during cropping season 2020. Fifty seeds from each $F_{2:3}$ accessions were planted in a 1-meter row with 30 cm spacing between the rows. To ensure sufficient inoculum production for disease infection, a mixture of the universally susceptible varieties 'Morocco', 'Seri 82', and 'Avocet S' along with the locally susceptible varieties 'Bolani', 'Basribey' (also derived from the CIMMYT cross 'Kauz'), and 'Cumhuriyet 75', 'Kunduru', 'Kasifbey', and 'Gonen' was planted as spreader after every 20 rows, as well as the spreader rows bordering the nurseries. The experiments were managed as per the standard local agronomic practices during the crop season.

PstS2 and *Warrior (PstS7)* pathotypes collected from previous years and preserved at RCRRC were multiplied using susceptible variety Avocet S. Freshly collected urediniospores were used for field inoculations. The $F_{2:3}$ accessions along with the spreader rows bordering the experiments were artificially sprayed with a mixture of the two races in talcum powder using a backpack sprayer at seedling, tillering, and booting stages. The field was irrigated through a mist irrigation system.

Field scoring started when disease severity reached 100% on the susceptible checks, 'Morocco' and 'Avocet S'. Because of conducive environmental conditions during January- February, the onset of the stripe rust (under artificial inoculation) usually starts at early February and reach full disease severity in susceptible genotypes by mid-

February, when the plants are generally at tillering stage. This is a unique condition for the evaluation of resistance in wheat germplasms at the regional rust phenotyping platform in Izmir. Adult-plant responses were recorded three times at 10-day intervals for the major infection types Resistant (R), Moderately resistant (MR), Moderately Susceptible (MS), and Susceptible (S) (Roelfs et al., 1992), and the disease severities (0-100%) following the Modified Cobb's Scale (Peterson et al., 1948). All three recordings were averaged and the Coefficients of Infection (CI) were calculated for infection types and disease severities following Saari and Wilcoxson (1974).

Map construction and QTL mapping

The QTL mapping strategy utilizing F_2 genotype and $F_{2:3}$ phenotype data follows established procedures in genetic mapping of disease resistance (Xu et al., 2017). This approach combines the advantage of precise genotyping at the F_2 generation with reliable phenotypic evaluation using $F_{2:3}$ families. The use of $F_{2:3}$ families allows multiple plants per line to be evaluated, reducing environmental variance and providing more accurate phenotypic data compared to single F_2 plants. The phenotypic means of $F_{2:3}$ families were used as trait values for their corresponding F_2 plants in QTL analysis. The composite interval mapping (CIM) method with the Kosambi mapping function was used for the detection of QTLs by Windows IciMapping v4.1. The threshold value for the logarithm of odds (LOD) score was determined through permutation testing (1000 iterations) at an experiment-wise error rate of 0.05, resulting in a population-specific threshold of 2.5. This empirically derived threshold accounts for multiple testing and provides more accurate control of false positives compared to arbitrary universal thresholds (Churchill and Doerge, 1994). Genetic maps with QTLs were drawn using MapChart v.2.32 software. For the markers with the same positions, only one single nucleotide polymorphism (SNP) maker was selected for the map.

RESULTS

Inheritance of YR resistance

Phenotypic data indicating the genetic variance to adult plant response in the BWLR 2347/AvS population varied from resistant to susceptible (CI= 0-9) in a field study at RCRRC-Izmir, Turkey. All the F_1 plants of BWLR 2347 were resistant to rust when tested at field conditions (CI=0) whereas all plants AvS were susceptible to stripe rust infection (CI=9). The 11 F1 plants from BWLR 2347/AvS were resistant producing mild uredinia (CI=3-4). That suggest the resistance gene in BWLR was partially dominant (Table 1). The 240 F_2 population was divided into 197 resistant (RR: Rr) plants and 43 susceptible (rr) which fit a segregation ratio of 3R:1S (χ^2= 6.4; P=0.011). The segregating 178 lines of F_2 derived F_3 population ($F_{2:3}$) the 70 plants were homozygous resistant (RR) (0-3) whereas 81 plants originating from F_2 plants were heterozygous representing the segregating alleles (Rr) (CI=4-6) and 27 plants were homozygous susceptible (rr) (CI=7-9). The segregating $F_{2:3}$ lines fitted the expected ratio of 1 resistant:2 segregating:1 susceptible ratio with chi-square value χ^2= 22.2, P=0.00001 (Table 1). The analysis entails the presence of 1 dominant stripe rust resistance genes in the BWLR 2347 landrace.

Table 1. Phenotypic segregation ratios and chi-square analysis of BWLR 2347 x AvS parental cross and $F_{2:3}$ hybrids for adult plant resistance to stripe rust.

Parents and Populations	Observed number of plants or lines [a]				Expected ratio[b]		
	Resistance	Segregation	Susceptible	Total	Res:Seg:Sus	χ^2 [c]	P-value
BWLR 2347	All		0				
Avocet S	0		All				
F_1	11			11			
F_2	197		43	240	3:1	6.4	0.011
$F_{2:3}$	70	81	27	178	1:2:1	22.2	0.00001

[a] The F_2 ratios are for resistant (CI= 0-4) and susceptible (CI=7-9) plants.
[b] The $F_{2:3}$ ratios are for homozygous Resistant, Segregating, and homozygous Susceptible lines.
[c] X^2 0.05=3.84

Identification of QTL for adult plant resistance to stripe rust in the $F_{2:3}$ population

Phenotypic data of stripe rust along with the genotypic data of 1115 SNP markers were subjected to one linkage group using software QTL IciMapping V4.1. One QTL *QYr.RCRRC.2D-1* was mapped on chromosome 2D by composite interval mapping (CIM) at 79 cM position flanked with SNP227-1318441 and SNP 220-1209547. This QTL explained 5.59% phenotypic variance with an LOD score of 7.98 (Table 2, Figure 1). A total of 7 QTLs were identified for resistance to YR at the adult plant growth stage using Interval mapping (IM) method. These seven QTLs were mapped on five chromosomes 1B, 2A, 2B, 2D, and 5A. One QTL *QYr.RCRRC.1B-1* is present at chromosome 1B flanked with SNP95-982151 and SNP93-306479 explaining the 2.67% phenotypic variance with LOD value of 3.57 (Table 2, Figure 1). Three QTLs were detected at chromosome 2A explaining the phenotypic variance as 3.61%, 4.75% and 5.03%, respectively (Table 2). At chromosome 2B, one QTL QYr.RCRRC.2B-1 present at 934 cM position flanked by SNP178-1316732 and SNP171-3948116. This QTL elucidate 4.17% phenotypic variance with 4.97 LOD score. Highest phenotypic variance explained by QTL *QYr.RCRRC.2D-1*

present at 2D chromosome i.e 5.59% the same QTL was identified through both mapping procedure IM and CIM. One QTL QYr.RCRRC.5A-1 mapped on chromosome 5A with LOD score 2.68 and phenotypic variance of 2.01%. The QTL was flanked with SNP611-11204643 and SNP610-1084102. The QTL that explained phenotypic variance of more than 10% is generally considered as major QTL therefore in the study all the QTLs detected were minor QTLs. The phenotypic variance explained by an individual QTL ranged from 2.01% to 5.59%. and the LOD scores of identified QTLs for *YR* were in the range of 2.68 to 7.98 (Figure 2).

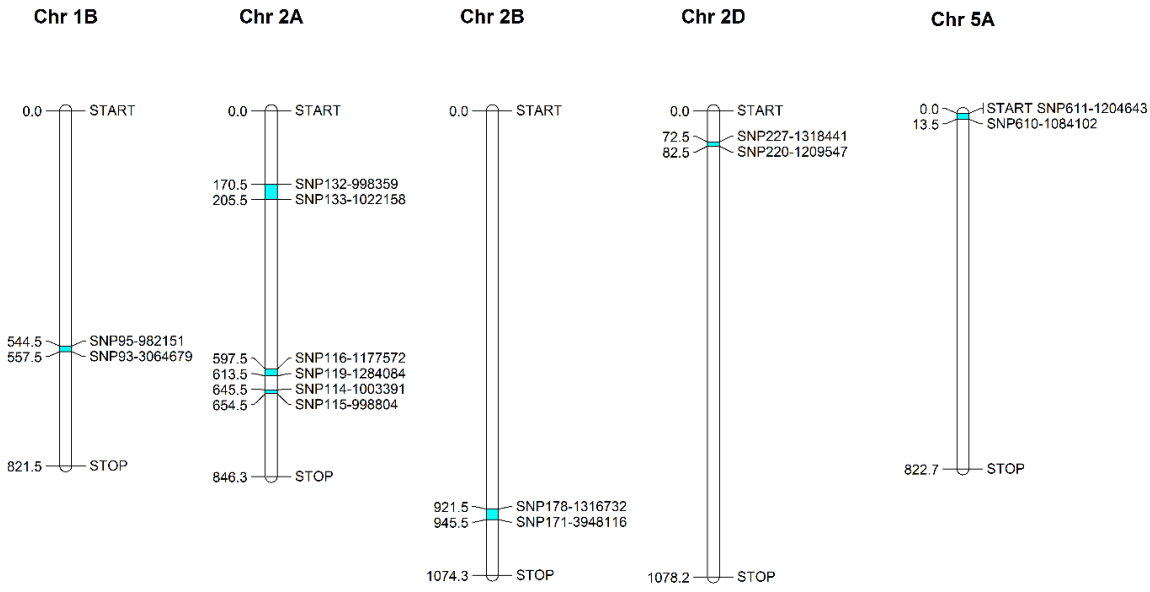

Figure 1. Genetic linkage map showing positions of QTLs conferring adult plant resistance to stripe rust in the $F_{2:3}$ population derived from BWLR 2347/AvS. Chromosome numbers are indicated at the top of each linkage group. Genetic distances (cM) are shown on the left side of each chromosome. The flanking markers are shown on the right side of each chromosome.

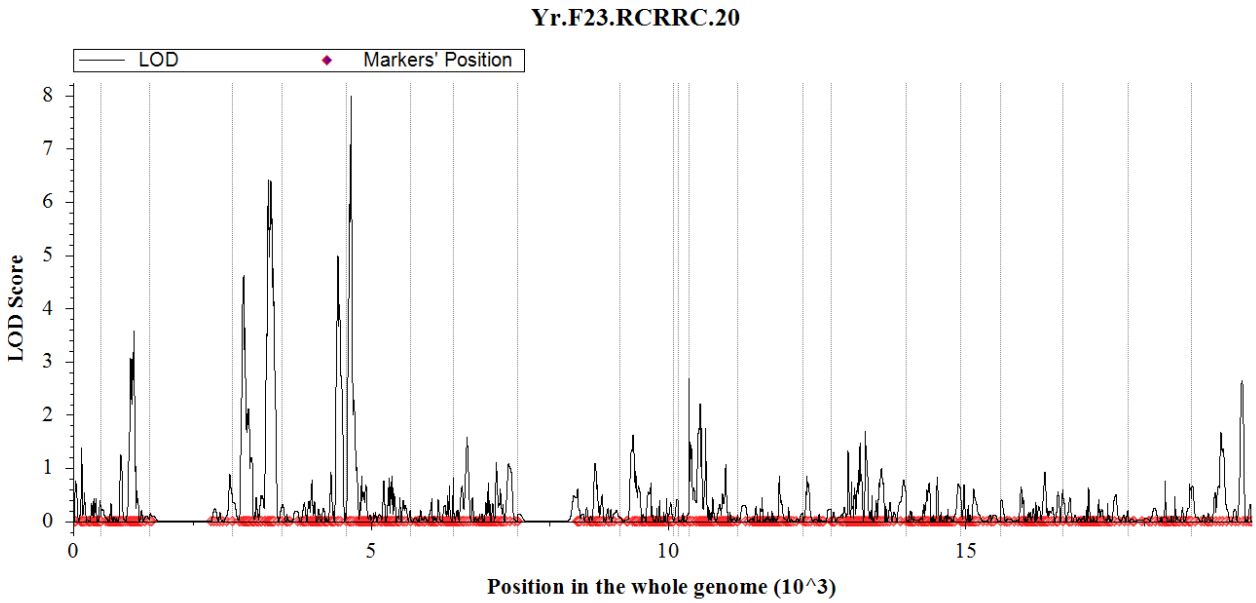

Figure 2. Genome-wide scan of $F_{2:3}$ population with LOD scores on y-axis and genome size on x-axis every single vertical line interval represents a chromosome.

Table 2. Quantitative trait loci for disease resistance to stripe rust at adult plant stage.

QTL Name	Chr	Position	Left Marker	Right Marker	LOD	PVE (%)	Add.	Left CI	Right CI	QTL/gene	Reference
QYr.RCRRC.1B-1	1B	549	SNP95-982151	SNP93-3064679	3.5755	2.671	-0.5912	544.5	557.5	Yr29/Lr46	William et al., (2003)
QYr.RCRRC.2A-1	2A	193	SNP132-998359	SNP133-1022158	4.6107	3.6132	-0.6915	170.5	205.5	Yr17	Helguera et al., (2003)
QYr.RCRRC.2A-2	2A	651	SNP114-1003391	SNP115-998804	6.4022	4.7497	-0.7975	645.5	654.5	Yr32	Eriksen et al., (2004)
QYr.RCRRC.2A-3	2A	606	SNP116-1177572	SNP119-1284084	6.4128	5.0361	-0.8122	597.5	613.5	QYr.ucw-2AS_PI610750	Lowe et al., (2011)
QYr.RCRRC.2B-1	2B	934	SNP178-1316732	SNP171-3948116	4.9792	4.19	-0.743	921.5	945.5	QYr-2B_Attila, QYrlu.cau-2BS1_Luke	Rosewarne et al., (2008); Guo et al., (2008)
QYr.RCRRC.2D-1	2D	79	SNP227-1318441	SNP220-1209547	7.9803	5.5949	-0.8562	72.5	82.5	QYr.caas-2DS_Libellula, QYr.wpg-2D.1 (IWA1939)	Lu et al., (2009); Naruoka et al., (2015)
QYr.RCRRC.2D-1	5A	0	SNP611-1204643	SNP610-1084102	2.6854	2.015	-0.5181	0	13.5	***Novel***	

CIM: Composite interval mapping; LOD: Logarithm of odds score; PVE: Percentage of phenotypic variance explained by individual QTL; Add: Additive effect of resistance allele; CI: Confidence interval

DISCUSSION

The frequent outbreaks of stripe rust epidemics in many countries seriously threaten wheat production and threaten food security. Growing disease resistance wheat cultivars, is the most feasible method to abate the stripe rust disease that developed from gene pyramiding and marker assisted selection (Mourad et al., 2021). In present study, we characterized the stripe rust resistance in wheat landrace BWLR 2347 as adult plant resistance source and mapped seven QTLs covering the five genomic regions. The integrated map constructed by Bulli et al., (2016) was used to compare the significant SNPs detected in the study with previously published *Yr* genes and QTL. From all the identified seven QTLs, the four were in the same genomic regions as previously described for Yr resistance genes. Whereas three identified QTLs were found in proximity of previously reported YR resistance QTLs.

Chromosome 1B

A minor QTL *QYr.RCRRC.1B-1* (SNP95-982151) was identified in association with adult plant resistance to stripe rust on chromosome 1B. The region overlapped the previously reported *Yr29* gene (William et al., 2003). This moderate adult plant resistance gene is linked with another leaf rust resistance gene Lr46 which is highly effective for LR resistance. The presence of *Yr29* gene in the same set of landraces has been reported previously by Tehseen et al. (2021). Therefore, it is likely that SNP95-982151 is tagging *Yr29* gene.

Chromosome 2A

Three QTLs *QYr.RCRRC.2A-1* (SNP132-998359), *QYr.RCRRC.2A-1* (SNP114-1003391), and *QYr.RCRRC.2A-3* (SNP116-1177572) were identified on chromosome 2A, which carries several *Yr* resistant genes. (Helguera et al., 2003; Eriksen et al., 2004; McIntosh et al., 2017). The stripe rust resistant gene *Yr17* lies within the confidence interval of SNP132-998359. *Yr17* resistance gene is linked with *Lr37* and *Sr38* a leaf rust and stem rust resistance respectively. Although rust pathogens with virulence to *Yr17* has been reported in many parts of the world, the combination of these resistance genes has proven effective against a wide range of cereal rust races (Kolmer et al., 2009). SNP114-1003391 and SNP116-1177572 were detected in close proximity with a *Yr* resistance gene *Yr32* and a QTL *QYr.ucw-2AS_PI610750*. The resistance gene Yr32 confers resistance to stripe rust at seedling and all stage resistance and is also within the proximity of several other stripe rust QTLs (Boukhatem et al., 2002; Eriksen et al., 2004; Mallard et al., 2005; Bansal et al., 2014). Since SNP114-1003391 lies in the same vicinity as *Yr32* and other QTL it is accepted that the resistance conferred by SNP114-1003391 is due to *Yr32* gene. A previously reported QTL *QYr.ucw-2AS_PI610750* and SNP116-1177572 overlap and, therefore are considered same both confer resistance to stripe rust at adult plant stage.

Chromosome 2B

The QTL region identified in the study on chromosome 2B was in the proximity of previously reported QTLs on the long arm of chromosome with moderate resistance and conferring slow rusting QTL (Guo et al., 2008; Rosewarne et al., 2008). The QTL *QYrlu.cau-2BS1_Luke* was identified to confer high temperature adult plant stage since our experiment did not undergo any temperature treatment therefore further studies are necessary to confirm the relationship however, based on genetic map distances and phenotypic evaluation the region detected in the study is the same as previously reported.

Chromosome 2D

The *QYr.RCRRC.2D-1* region on chromosome 2D was detected in both CIM and IM methods. This region overlaps with the previously reported QTL regions for adult plant resistance to stripe rust (Lu et al., 2009; Naruoka et al., 2015). The earlier reported QTLs were detected in an F_3 mapping population derived from Italian common wheat cultivars Libellula and Strampelli and a diversity panel of 402 winter wheat accessions. The QTL detected in the current study explained 7.98% of the phenotypic variance, similar to the previously reported QTLs. Furthermore, all these QTLs showed minor effect for resistance; therefore, it is suggested that this region on chromosome 2D exhibits adult plant resistance to stripe rust. When accompanied by other minor QTLs, it could prove to be a good source of horizontal resistance against stripe rust.

Chromosome 5A

An adult plant stripe rust resistance loci *IWA4767_APR* at the long arm of chromosome 5A has been reported Zegeye et al. (2014), however, the *IWA4767_APR* was identified at 113cm which is far away from the QTL detected in the current study at 0-13.5cm. Both QTL conferred stripe rust resistance at the adult plant stage and were detected in hexaploid wheat landraces however the distance between the two QTLs is more than 100cm. Therefore, both QTLs were suggested different. There was no other previously reported in this region thus making it a novel QTL for adult plant stripe rust resistance.

While segregation analysis in the $F_{2:3}$ population suggested a qualitative inheritance pattern controlled by a single dominant gene (Table 1), our QTL mapping revealed a more complex quantitative inheritance involving multiple minor-effect loci. This apparent discrepancy between inheritance patterns deserves careful consideration. The phenotypic segregation suggesting single-gene inheritance may reflect a complex genetic architecture where multiple linked QTLs segregate together, creating the appearance of simpler inheritance patterns (Periyannan et al., 2013). The resolution limitations of our mapping population may have prevented the separation of closely linked loci that could collectively behave as a single primary effect locus (Kertho et al., 2015; Ellis et al., 2014).

The nature of adult plant resistance itself may contribute to this complexity. Adult plant resistance typically shows intricate gene-by-environment interactions, where field conditions during phenotyping can influence the expression of resistance genes differently than controlled conditions (Boyd et al., 2013). This environmental interaction could

potentially mask or enhance certain QTL effects, leading to detecting multiple minor QTLs rather than a single major locus. Furthermore, the detected QTLs may participate in epistatic interactions that create threshold effects in resistance expression, resulting in more discrete phenotypic categories than typically expected for quantitative inheritance (Krattinger et al., 2016; Moore et al., 2015). Similar phenomena have been reported in other wheat disease resistance studies (Niks et al., 2015; Santra et al., 2008).

Our findings align with the growing understanding that durable adult plant resistance often results from the cumulative action of multiple partial resistance genes rather than single major effect loci (Figueroa et al., 2018; Klymiuk et al., 2018).

Identifying multiple minor-effect QTLs in BWLR-2347 has essential implications for wheat breeding programs. While individual QTLs explain relatively small proportions of phenotypic variance (2.01-5.59%), their combined effect could provide durable resistance through QTL pyramiding strategies (Rosewarne et al., 2013). These QTLs can be incorporated into elite breeding lines using marker-assisted selection, particularly utilizing the SNP markers flanking each QTL region identified in this study. The novel QTL on chromosome 5A (*QYr.RCRRC.5A-1*) represents a previously unreported source of resistance that could complement existing resistance genes. The co-location of several identified QTLs with previously reported *Yr* genes suggests the potential for developing multi-line resistance strategies.

CONCLUSIONS

This study used the wheat stripe rust resistance source BWLR 2347 to map the QTLs using GBS. Six QTLs were identified on 1B, 2A, 2B and 2D that are validated with previously identified QTLs and *Yr* genes. The SNP identified on the chromosome 5A was recognized as a novel QTL from the study and can be exploited in wheat breeding programs toward the development of stripe rust resistant cultivars. The newly identified and the previously validated QTLs will be a valuable source of resistance to adult plant stripe rust resistance that can be used for gene pyramiding to enhance the resistant source in the wheat cultivars.

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COMPARATIVE ANALYSIS OF EARLY ESTABLISHMENT PERFORMANCES OF PERENNIAL WHEAT GENOTYPES

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ABSTRACT

Perennial wheat (*Triticum aestivum* L. × *Thinopyrum* spp.) presents a promising alternative to conventional annual wheat for sustainable agriculture, offering advantages such as enhanced soil health and reduced environmental impact. This study evaluated the early establishment performances of 20 perennial wheat genotypes sourced from diverse donors alongside two commercial wheat varieties under rain-fed conditions in Bornova, Izmir, Türkiye. Two separate field trials were conducted over two growing seasons (2018/19 and 2020/21), assessed key yield components, including plant height (PH), spike number (SN), spike length (SL), thousand grain weight (TGW), and overall grain yield (GY). Results showed that perennial wheat genotypes exhibited higher plant height and spike length compared to common wheat but had lower grain numbers per spike and TGW. On average, perennial wheat achieved 40% of the grain yield of commercial wheat varieties, with significant variability among genotypes. Notably, the genotype Pw18 demonstrated satisfactory grain yield performance, achieving 5.21 tons ha^{-1}, close to common wheat yields evaluated in the study. These findings highlight the potential of specific perennial wheat genotypes for further development in sustainable cropping systems. However, further investigation is needed to assess the quality characteristics of these genotypes, which will be crucial for their potential use.

Keywords: perennial wheat, yield, annual wheat, comparison

INTRODUCTION

Perennial wheat, a hybrid derived from the crossbreeding of traditional wheat (*Triticum aestivum* L.) and wild perennial relatives such as *Thinopyrum spp*., represents a significant advancement in agricultural science aimed at addressing both food security and environmental sustainability challenges (Zhang et al., 2011; Jaikumar et al., 2012). In light of complex global challenges such as climate change, pandemics, and political conflicts impacting agricultural production worldwide, the sustainability of current food production systems is increasingly being challenged (Erenstein et al., 2022). The growing demand for food, driven by exponential population growth and shifting consumption patterns, is further strained by the limited availability of arable land (Zheng et al., 2021). Furthermore, contemporary agricultural practices have adversely affected the environment, including soil erosion, greenhouse gas emissions, and water contamination, despite their capacity to enhance crop yields (Monfreda et al. 2008, Liu et al., 2023). Under these circumstances, the efforts should be focused on ensuring food security while considering the environmental health and socio-economic situation (Chapman et al., 2022).

Perennial crops can be considered a promising option for the sustainability of agricultural production. Perennial crops don't need to be planted each year. Instead, they can regrow after the harvest for a couple of years, which would reduce the cost of production and field management (Soto-Gómez and Pérez-Rodríguez, 2022). The cultivation of perennial crops has the potential to enhance soil health by stimulating the activity density and soil richness of ground beetles (Burmeister, 2021), isolate the carbon and help to utilise the nutrients and water more effectively by their deep root systems (DeHaan et al., 2020). This speciality makes them more stable regarding yield properties during drought periods (Vico and Brunsell, 2018). Moreover, perennial crops have a superior by-product potential than their annual options, thanks to their higher aboveground biomass production (Soto-Gómez and Pérez-Rodríguez, 2022).

Perennial wheat (*Triticum aestivum* L. × *Thinopyrum* spp.) is a novel and promising hybrid species as a sustainable alternative to the annual wheat (Jaikumar et al., 2012). The attempts to develop perennial wheat has started by Soviet scientists in the 1930s (Jaikumar et al., 2012; Tsitsin, 1939; DeHaan and Ismail, 2017), today the efforts are ongoing to provide new perennial wheat cultivars that can meet the demands of wheat growers. In general, the key

properties in perennial wheat breeding are achieving a considerable grain yield and the plants staying long in the field (longevity) and meeting the essential quality characteristics (Hayes et al., 2018). Perennial plants generally allocate fewer resources to reproduction structures and prioritize their survival over multiple years (Bazzaz et al., 1987; Vico and Brunsell, 2018). The perennial wheat cultivars bred so far have 30% lower grain yield than commercial annual bread wheat (Baronti et al., 2022) and often reduce more after the first year of cultivation (Hayes et al., 2018), so the grain yield stands for as one of the most critical traits in perennial wheat development. In planning plant production activities, farmers tend to focus on the yield rather than other traits.

In perennial crops, the early establishment performance of a cultivar is crucial in terms of soil health and fertility. Perennial wheat is known for developing a deep and extensive root system that enhances soil structure and increases organic matter content (Kurmanbayeva et al., 2024a). This root development is complemented by the canopy's ability to protect the soil surface from erosion and nutrient leaching, which is particularly beneficial in regions prone to soil degradation (Bell et al., 2010). Therefore, the initial performance of perennial wheat lines becomes a significant factor in farmers' decision-making processes. Given that grain yield is a quantitative trait influenced by both genotype (G), environment (E), and their interaction (G × E) (Shewaye and Solomon, 2018; Mohamed, 2013), it is essential to assess the early establishment performance of perennial wheat germplasm across different environments. Although numerous studies have explored perennial wheat in terms of its future potential and agronomic evaluation (DeHaan et al., 2017; Glover et al., 2010; Hayes et al., 2018), there is limited research evaluating the early establishment performance of various perennial wheat lines. This study aims to evaluate the adaptation and early establishment performance of 20 perennial wheat genotypes, sourced from diverse perennial donors, in comparison with two commercial bread wheat cultivars to explore the potential of perennial wheat as a sustainable option for future agricultural systems

MATERIALS AND METHODS

Plant material and experimental site

A field experiment was conducted during the 2018/19 and 2020/21 wheat growing seasons, spanning the period from November to June. A total of 20 perennial wheat genotypes with different genotypic backgrounds (Table 1) provided by the International Maize and Wheat Improvement Center (CIMMYT) and 2 standard bread wheat (*Triticum aestivum* L.) varieties, *cv.*Basribey and *cv.*Masaccio were used as plant material. These varieties are known for their high yield potential and good adaptation to the Mediterranean climate conditions where the experiment was conducted. The experimental site was situated at an altitude of 6 meters (38°34'45" N, 27°1'22" E) in Bornova Plain, Izmir, Türkiye. The soil profile at depths of 0-20 cm and 20-40 cm was characterized as silt-clay with a pH of 8.2 and clay-loamy with a pH of 7.8, respectively. The climate data obtained from the Turkish State Meteorological Service for the 2018/19 and 2020/21 growing seasons in Izmir is shown in Table 2.

Table 1. The origin and identification of perennial wheat genotypes used in the study.

Accession Number	Genotype No	Name	Origin	Donor Wheatgrass
160018	Pw1	WHEAT-AGROPYRON PONTICUM PARTIAL AMPHIPLOID	OTHER	*Th.ponticum*
160020	Pw3	WHEAT-AGROPYRON PONTICUM PARTIAL AMPHIPLOID	USA	*Th.ponticum*
160008	Pw4	PI573182/BFC2-4//BFC2-N/3/PI440048/4/(TAM110/PI401201//JAG & 2137)/5/(PI636500/PI414667//PI414667/3/(PI573182/PI314190//BFC1-FF))	US-TLI	*Th.intermedium*
160012	Pw5	(KEQIANG/NANDA2419)/AG.INTERMEDIUM//WHEAT	CHINA	*Th.intermedium*
160009	Pw8	PI634318/PI414667	US-TLI	*Th.junceiforme*
160022	Pw9	WHEAT-AGROPYRON INTERMEDIUM PARTIAL AMPHIPLOID	RUSSIA	*Th.intermedium*
160019	Pw10	VILMORIN 27*2/AG.INTERMEDIUM	FRANCE	*Th.intermedium*
160014	Pw11	WHEAT-AGROPYRON INTERMEDIUM PARTIAL AMPHIPLOID	RUSSIA	*Th.intermedium*
160011	Pw12	(KEQIANG/NANDA2419)/AG.INTERMEDIUM//WHEAT	CHINA	*Th.intermedium*
160017	Pw13	WHEAT-AGROPYRON PONTICUM PARTIAL AMPHIPLOID	US-OSU	*Th.ponticum*
160006	Pw14	TAM110/PI401201//JAG & 2137	US-TLI	*Th.intermedium*
160021	Pw15	T.DURUM/AG.ELONGATUM	CIMMYT	*Th.elongatum*
160004	Pw16	MADSEN//CHINESE SPRING/PI531718	US-WSU	*Th.elongatum*
160007	Pw17	TAM110/PI401201//JAG & 2137/3/PI520054/4/PI401168/5/(TAM110/PI401201//JAG & 2137)	US-TLI	*Th.intermedium*
160013	Pw18	HEZUO#2/AG.INTERMEDIUM//WHEAT	CHINA	*Th.intermedium*
160015	Pw19	WHEAT-AGROPYRON PONTICUM PARTIAL AMPHIPLOID	RUSSIA	*Th.ponticum*
160017	Pw20	WHEAT-AGROPYRON PONTICUM PARTIAL AMPHIPLOID	US-OSU	*Th.ponticum*
160017	Pw21	WHEAT-AGROPYRON PONTICUM PARTIAL AMPHIPLOID	US-OSU	*Th.ponticum*
160017	Pw22	WHEAT-AGROPYRON PONTICUM PARTIAL AMPHIPLOID	US-OSU	*Th.ponticum*
160017	Pw23	WHEAT-AGROPYRON PONTICUM PARTIAL AMPHIPLOID	US-OSU	*Th.ponticum*

Table 2. Meteorological parameters (Monthly averages for temperature and humidity, monthly total rain amount) for İzmir in two experimental years (2018/19 and 2020/21).

		December	January	February	March	April	May	June
2018/19	Temperature (C°)	15.1	8.7	8.7	9.9	13.1	16.4	21.7
	Rain (mm)	38.8	97.4	119.6	50.0	58.6	16.9	-
	Humidity (%)	74.5	79.2	84.7	75.6	65.7	62.5	61.0
2020/21	Temperature (C°)	12.4	10.5	10.7	10.4	15.8	21.6	24.9
	Rain (mm)	172.8	164.0	62.6	129.6	33.2	0.2	16.8
	Humidity (%)	74.8	73.3	66.3	65.2	63.2	56.6	55.1

Experimental design

The experimental design utilized a randomized complete block design, with three replications and each replication consisting of one-meter-long row plots, and the distance between the rows was 50 cm. Sowing was conducted separately for each year following the method described by Hayes et al. (2018). Seeds of all wheat cultivars used in the study were hand-sowed at a rate of 25 seeds per row, ensuring a final seed density of 50 seeds per square meter. Due to the low regrowth rates of all perennial wheat genotypes after the first-year harvest in 2019, the experiment was repeated (replanting) in 2021 using the same genotypes. The same sowing pattern was applied for the perennial wheat genotypes and the commercial bread wheat cultivars.

Field management

The seeds were sown on December 1st, 2018, and November 20th, 2020 harvested on June 20th, 2019, and June 14th, 2021. Composite fertilizer (15.15.15), consisting of nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O), was applied to the experimental field at a rate of 0.33 ton ha^{-1} at the beginning of the experiment. Additionally, 0.14 ton ha^{-1} of ammonium sulfate fertilizer (21%) was applied during the initial stage of wheat's stem elongation at Z31 (Zadoks Stage = 31, Zadoks et al., 1974). The plants were grown under rain-fed conditions.

Data collection and analysis

Every single plant of each wheat genotype was sampled separately. Five representative plants per row were selected for the measurements, excluding the edges of the rows. Plant height (cm), spike length (cm), plant biomass (kg ha^{-1}), thousand grain weight (g), spike number (number m^{-2}), and plant yield (g $plant^{-1}$) were determined following the Wheat Special Report (No: 32) of CIMMYT (Bell and Ficher, 1994). After sampling, the border plants were removed, and the remaining plants were harvested for each plot. Then the wheat grains were threshed using a plot thresher, and grain yield (ton/ha) was calculated.

Statistical analysis

ANOVA was employed to assess the effects of the factors, and treatment means were compared using the least significant difference test (LSD) at a significance level of 0.05. All statistical analyses were conducted using the R software v.4.0.4 (R Core Team, 2021). The built-in function aov used to perform ANOVA, then the agricolae package (Mendiburu, 2023) was used for LSD test. Microsoft Excel (Microsoft Corporation, 2018) was used for visualization.

RESULTS AND DISCUSSION

The yield components of 20 perennial wheat genotypes were evaluated during the first year alongside two common wheat varieties (*Triticum aestivum* L.). Based on the analysis of variance, the differences between the genotypes were found statistically significant for all measured traits (Table 3). Besides, the years' effect was significant for four traits: spike length, spike number per square meter, grain number per spike, and grain yield. The genotype-by-year interaction effect was significant only for plant height and grain number per spike traits (Table 3).

Table 3. The mean squares observed from ANOVA for observed traits and variation sources

Variation Sources / Traits	Plant height (cm)	Upper Internode length (cm)	Spike length (cm)	Spike number m^{-2}	Grain Number $spike^{-1}$	Thousand Grain Weight (g)	Grain Yield (ton ha^{-1})
Genotype	1753.29**	338.09**	40.27**	12496**	574.09**	173.49**	162.27**
Year	86.81	1.34	180.24**	514360**	2012.57**	8.46	1775.41**
G x Y	164.18*	38.27	6.31	9152	271.12**	9.88	44.77
Replication	207.14	19.73	10.62*	16992*	374.24*	60.13**	206.10**

** : $p < 0.01$, *: $p < 0.05$

On average, the plant height of the perennial wheat genotypes was consistent at 94 cm. However, the plant height of the standard wheat varieties increased in 2021, reaching 70 cm compared to 61 cm in 2019 (Figure 1). Remarkably, the perennial genotype Pw15 exhibited significantly greater plant height (133 cm) than the other perennial genotypes, while Pw16, Pw17, and Pw18 displayed relatively shorter heights, ranging from 71 to 85 cm across both years. The greater plant height of perennial wheat compared to common wheat has also been reported

by Baronti et al. (2022). Similarly, in the present study, spike length in perennial wheat was approximately 5 cm longer than in common wheat in both years (Figure 1). The average spike length for perennial wheat was 14.8 cm, aligning with the findings of Pogna et al. (2013), who recorded a spike length of 14.3 cm, around 3 cm longer than common wheat. In addition to spike length, the upper internode length of perennial wheat genotypes averaged 37.5 cm, exceeding that of the common wheat varieties (29.6 cm) in both seasons (Figure 1). Kurmanbayeva et al. (2024b) reported that perennial wheat genotypes typically have four internodes, with five being rare. The greater plant height and relatively lower number of nodes (data not shown) observed in the perennial genotypes suggest that the plants have longer internodes in the upper part and throughout the stem.

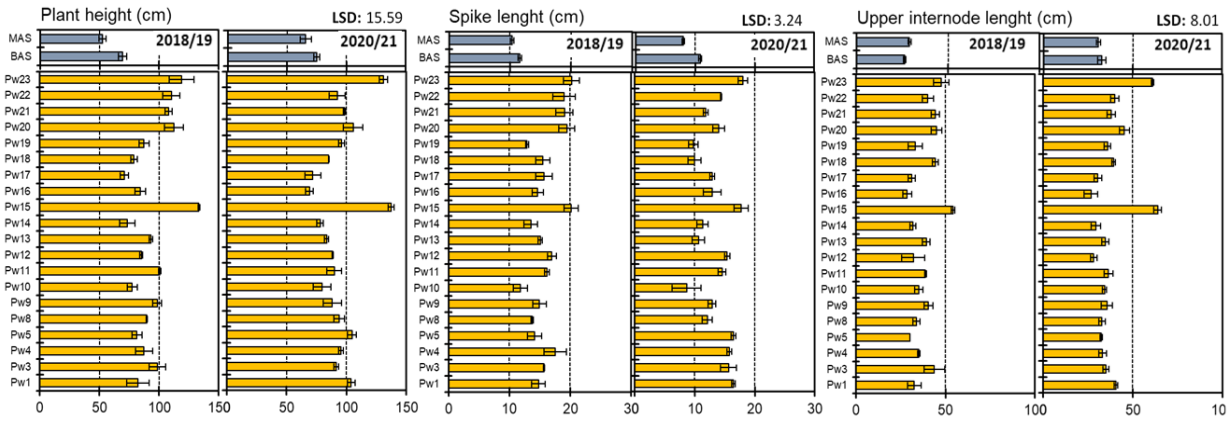

Figure 1. Comparison of plant heights (cm), spike length (cm) and upper internode length (cm) among 20 perennial wheat genotypes during the initial year of their growth cycle. The blue bars, presented above the figure, represent the plant heights of common wheat cultivars (*Triticum aestivum* L.), including Masaccio (MAS) and Basribey (BAS).

Grain yield is generally determined by the number of spike per unit area, the number of grains per spike, and the thousand-grain weight (TGW) (Xie et al., 2016; Tatar et al., 2020). In the current study, the average spike number, grain number per spike, and TGW for the common wheat varieties were 206 spikes m², 50 grains spike^{-1}, and 37.7 g, respectively (Figure 2). In comparison, the perennial wheat genotypes had lower values of 19% in spike number (167 spikes m²), 38% in grain number per spike (37 grains spike^{-1}), and 26% in TGW (28.1 g) compared to standard wheat genotypes. The lower grain number per spike in perennial wheat genotypes is likely due to the increased distance between spikelet, despite the longer spike length, as noted by Clark et al. (2019). Yan et al. (2022) also reported fewer spikelet per spike in perennial wheat lines, ranging from 17 to 24 spikelet. Despite the generally lower values of the grain yield components in perennial wheat, specific genotypes exhibited superior traits. Spike number is an important agronomic trait in yield formation and it depends on the productive tillering capacity of the plants (Fu et al., 2023) Pw14 had a notably higher spike number (246 spikes m^{-2}), while Pw12 and Pw17 showed higher grain numbers per spike (51 grains spike^{-1}), and Pw19 had the highest TGW (45.4 g) among the perennial wheat genotypes in the current study.

The average grain yield of perennial wheat genotypes was 2.5 tons ha^{-1} in the first year (2018/19) and 0.99 tons ha^{-1} in the second year (2020/21) (Table 4). In common wheat, the average grain yield was 4.82 tons ha^{-1} in the first year and 3.92 tons ha^{-1} in the second year. Among the perennial genotypes, Pw4 (6.20 tons ha^{-1}) and Pw18 (6.13 tons ha^{-1}) achieved the highest grain yields in the first year, while Pw18 continued to perform well in the second year with a yield of 4.29 tons ha^{-1}. For comparison, the common wheat variety BAS had the highest yields in both years, with 5.15 tons ha^{-1} and 4.55 tons ha^{-1}, respectively. This variability in yield values may be attributed to differences in climatic conditions between the two years. The relatively low precipitation in April and May, a critical period for yield formation, may have contributed to the lower yield observed in the second year (Table 2). These findings align with a previous study that reported a significant effect of drought during the grain-filling stage on wheat grain yield (Tatar et al., 2020).

Grain yield reductions in perennial wheat may result from resource trade-offs between reproductive growth, regrowth, and winter survival (Jaikumar et al., 2012). According to Bell et al. (2008), perennial wheat could become economically viable if it achieves 40% of the grain yield of annual wheat, especially when combined with forage production. In this study, the average grain yield of perennial wheat (1.75 tons ha^{-1}) was 40% of the grain yield of the common wheat varieties (4.37 tons ha^{-1}) (Table 4). However, there was substantial variability in the grain yield among perennial wheat genotypes, ranging from 0.16 to 5.21 tons ha^{-1}. Pw18 was particularly promising, with an average yield of 5.21 tons ha^{-1}, despite showing no standout performance in other yield components. Pw4 also demonstrated high performance, with a yield (4.06 tons ha^{-1}) approaching that of the common wheat varieties (Table 4).

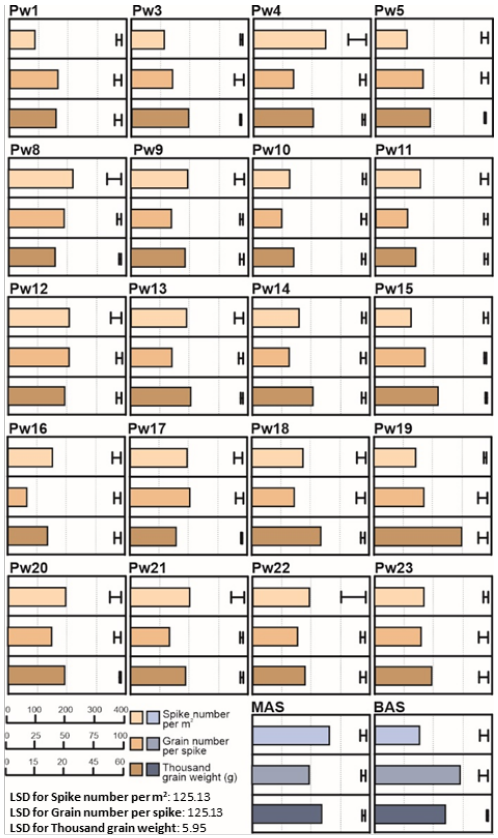

Figure 2. Spike number per m^2, grain number per spike, and thousand grain weight (g) among 20 perennial wheat genotypes during the initial year of their growth cycle. The blue bars, presented below the figure, represent the plant heights of common wheat cultivars (*Triticum aestivum* L.), including Masaccio (MAS) and Basribey (BAS).

Table 4. Comparison of grain yields (ton ha^{-1}) among 20 perennial wheat genotypes during the initial year of their growth cycle.

Genotypes		**Grain Yield (ton ha^{-1})**		
		2018/19	**2020/21**	**Mean**
Perennial Wheat	*Pw1*	0.82 ±0.30	0.79 ±0.17	0.80
	Pw3	1.42 ±0.28	0.84 ±0.31	1.13
	Pw4	6.20 ±0.02	1.93 ±0.26	4.06
	Pw5	0.74 ±0.22	1.33 ±0.20	1.04
	Pw8	3.02 ±1.55	1.20 ±0.24	2.11
	Pw9	2.89 ±1.07	0.77 ±0.22	1.83
	Pw10	1.15 ±0.64	0.26 ±0.12	0.71
	Pw11	1.44 ±0.77	0.39 ±0.10	0.92
	Pw12	0.65 ±0.20	0.66 ±0.02	0.66
	Pw13	3.65 ±0.49	0.41 ±0.033	2.03
	Pw14	2.46 ±0.74	1.14 ±0.41	1.80
	Pw15	2.51 ±0.44	1.04 ±0.15	1.78
	Pw16	0.18 ±0.08	0.14 ±0.02	0.16
	Pw17	3.32 ±0.74	1.12 ±0.10	2.22
	Pw18	6.13 ±0.98	4.29 ±0.43	5.21
	Pw19	2.18 ±0.47	0.89 ±0.22	1.53
	Pw20	3.15 ±0.54	0.47 ±0.06	1.81
	Pw21	3.04 ±0.93	0.40 ±0.09	1.72
	Pw22	2.63 ±1.17	0.68 ±0.22	1.65
	Pw23	2.50 ±0.09	1.01 ±0.08	1.76
	Mean	**2.50**	**0.99**	**1.75**
Common Wheat	*MAS*	4.49 ±0.76	3.29 ±0.19	3.89
	BAS	5.16 ±0.04	4.55 ±0.80	4.86
	Mean	**4.82**	**3.92**	**4.37**
	LSD	**0.86**		

In general, perennial wheat varieties have not surpassed 60–75% of common wheat yields, with significant yield declines after the first year (Pimentel et al., 2012). Jaikumar et al. (2012) reported that perennial wheat produced grain yields of 1.0 to 1.6 tons ha^{-1} - around 50% of North America's common wheat yield (2.7 tons ha^{-1}). Other studies have shown that perennial wheat genotypes can achieve between 18% and 64% of the yield of conventional common wheat varieties, though persistence varies widely (Scheinost et al., 2001; Bell et al., 2010).

In conclusion, perennial wheat genotypes exhibited higher plant height, spike number, and upper internode length than common wheat varieties. However, despite having more spikes per unit area, they showed lower grain numbers per spike and TGW. Significant variability was observed in the yield components of the perennial wheat genotypes. On average, perennial wheat yielded 40% of the grain yield of common wheat. Pw18 was particularly promising among the tested genotypes for its first-year grain yield performance relative to other genotypes. Further investigation is needed to assess the quality characteristics of these genotypes, which will be crucial for their potential use.

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EVALUATION OF MAIZE HYBRID IN MULTI-LOCATIONAL TRIAL USING GGE BIPLOT AND AMMI MODEL

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ABSTRACT

This study was executed to assess the twenty-one single cross maize hybrids with three local checks (BHM-12, BHM-13 and BHM-14) in five locations of Bangladesh for its adaptability and stability and also to study the genotypes and environment interaction. Yield data of twenty-four maize hybrids was analyzed through the additive main effects and multiplicative interaction (AMMI) and GGE biplot methods. Considering the grain yield, b_i~1 and S^2d_i~0 value, it was observed that G10 and G16 were the higher yielding and suitable across the environments. On the other hand, G20 and G14 were higher yielding but were responsive to favorable environments. Among the five locations, the environment of Hathazari was the poorest, whereas Barishal and Dinajpur were the most favorable environments for maize production. When hybrids were compared with ideal genotypes, it was observed that G14, G16, G10, and G20 were closed to the ideal genotypes so that they can be more desirable than other tested hybrids. The AMMI biplot indicated that G24, G16, G13, G17 and G14 were positioned adjacent to the biplot's origin which indicated their stability in performance across environments. Finally, stability analysis with the help of GGE and AMMI statistics identified two hybrids G14 and G16 that could be used as reference for future crop improvement program.

Keywords: AMMI Model, environment, GGE biplot, maize, stability

INTRODUCTION

Maize (*Zea mays* L.) is a crop of utmost importance for having its versatile uses along with wider adaptability and stands second position after rice in Bangladesh Maize is one of the prime staples crops for the nutrition of the world's population. Maize produces 1,170 million metric tons grain by covering more than 180 million hectares of land in all over the world (Ahmed et al., 2020). It is one of the leading productive C_4 plant with higher response of photosynthesis and it has the eminent potential for production of carbohydrate in unit area/day. Shiferaw et al. (2011) reported that animal feed industries utilized about 70% of total maize production and intensified growth of population of this sector will set off the need of meat and eggs as a protein source which eventually stimulated the production of maize. Maize is also exploited in the food industry as sweeteners and food additives, which is a

further significant end-user part of maize (Gulati et al., 2008). Maize plant's stem and foliage can be used as livestock feed. Husk, stalk and shelled cobs are usable as fuel (Ahmed et al., 2011). Maize has a potential nutritional value that contains about 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 Kcal/100 g (Hasan et al., 2018). At present, 4.7 million tons (BBS, 2021) have been produced in Bangladesh against around two million tons of annual demand (Islam et al., 2022). So there has been a crucial requirement to increase its yield, quality and production area to break the cycle of poverty during frequent climatic extreme conditions. Two factors influencing yield increase are modern management practices and plant breeding, which has a major impact on production.

Maize grows over a broad extent area with regard to the genotype by environment interaction (GEI) that hinders the pointing out of high-yielding and stable genotypes (Akcura et al., 2011). Genotype by environment interaction (GEI) deals with the various responses of genotypes across a broad environmental range. The prime focus of the new hybrid is to look for higher and stable yield in both favorable and unfavorable environmental conditions (Katsenios et al., 2021). The identification of maize varieties that are stable (hybrid's response across various environments) plays a major role in the enhancement of farmers' acceptability as well as the adoption of elite new varieties.

Genotype is supposed to be better adapted or stable if it has a higher mean yield and low fluctuation in yielding capability across diverged locations. The effect of genotype and environment interaction becomes more apparent during multi-location and multi-year trials for estimation and prediction of yield on the basis of based on defined experimental data. According to Lu'quez et al. (2022), higher yielding and better stable cultivar can be identified when cultivars are grown in various environments,

The GEI analysis has been conducted by various statistical methods such as stability analysis following AMMI model; principal component analysis (PCA) and linear regression analysis; ANOVA and GGE biplot analysis (Nzuve et al., 2013). GGE biplot analysis i.e. genotype main effect (G) and interaction of G × E (Hossain et al., 2018; Kizilgeci et al., 2019) is one of the convenient tools for geneticists and plant breeders that identifies superior genotypes (stable and high yielding) over multiple locations as well as by using graphical axes to detect the best suitable location for a particular genotype (Akcura et al., 2011).

The AMMI model is also a functional method that combines ANOVA and PCA, and the resulting output is a biplot that evaluates GE interactions graphically (Kaya et al., 2006). The results of AMMI analysis are regarded potential to evaluate the performance of yield of different genotypes under multi environment trials and to determine the suitable environments for all studied genotypes [Li et al., 2006; Agahi et al., 2020; Mafouasson et al., 2018 and Hongyu, et al, 2014]. Due to its high accuracy of results and contribution for understanding interaction between genotypes and environment, this model is extensively used by the researchers (Gauch, 2013). It also provides information for evaluation of improved cultivar, recommendations and selection of tested environment (Abay and Bjornstad, 2009).

Therefore, the aim of this research to find out the high yielding stable hybrids using AMMI and GGE biplot method which could have wide or specific adaptation in tested environment.

MATERIALS AND METHODS

Experimental sites

The study was executed by exploiting multi-location trials in five locations viz., Regional Agricultural Research Station (RARS), Rahmatpur, Barishal; RARS, Jashore; RARS, Jamalpur; RARS, Hathazari, Chittagong and Wheat Research Centre (WRC), Nashipur, Dinajpur during rabi season of 2018-19. The agro-climatic description of five experimental sites used in the study was given in Table 1. The environments had different soil texture (pH ranges from 5.2-7.5) and variable microclimate condition. Jashore location had a well-drained and clay loam structure of soil with pH 7.5 whereas Dinajpur has sandy clay loam soil and pH was 5.21.

Table 1. Agro-climatic description of five experimental sites used in the study

Particulars	E_1	E_2	E_3	E_4	E_5
Location	Rahmatpur Barishal	Jashore	Jamalpur	Hathazari, Chittagang	Nashipur, Dinajpur
Lattitude (decimal)	22.7875	23.1869	24.9354	22.5016	25.7524
Longitude(decimal)	90.2945	89.1874	89.9356	91.7936	88.6733
Soil type	Silty clay	Clay loam	Loamy	Silty loam	Sandy clay loam
pH	7.0	7.5	5.8	5.76	5.21
Climate					
Maximum temp. (^{O}C)	33.5	32.1	31.6	29.7	33.6
Minimum temp. (^{O}C)	25.2	22.9	22.4	23.4	23.4
Average rainfall (mm)	141.96	109.75	171.46	243.04	158.53

Experimental treatments and design

Twenty-one white maize hybrids (developed through the local cross) and three checks BARI hybrid maize (BHM-12, BHM-13 and BHM-14) were used in this study. Seven white kernelled maize inbred lines (CML-330, CML-332, CML-322, CML-311, CML-331, CML-518, CML-383) were used as a parental line then the lines were crossed in a half diallel fashion excluding reciprocals to produce 21 F_1's in the previous rabi reason 2017-2018. The experiment was laid out in a randomized complete block design with three replications in five locations.

Experimental procedure

Two seed per hill and each line were sown in two row plot with 4m long. The unit plot size was 4m×1.2m. In the field, 1.5 m distance from block to block, 60 cm from row to row, and 25 cm from plant to plant was kept. Fertilizers were applied in the form of urea-Triple Super Phosphate-Muriate of Potash-Gypsum-Zinc Sulphate- Borax @550-600-250-200-50-25kg ha−1respectively. Before sowing, one third of urea and the full doses of Triple Super Phosphate- Muriate of Potash, Gypsum, Zinc sulphate and borax were applied. The remaining two-thirds of urea was applied in two equal segments at the knee height stage and about one week prior to silking. The entire field management approaches were standard to assure favorable growing environments.

Data collection

Harvesting was started on 20th April 2019, depending upon the maturity of the plants, i.e., when plants showed visible signs of drying, the husk cover was entirely dried, and the grains were completely matured. Grain maturity was pointed out from the milk line of kernels or a black layer formation at grain and placenta's junction. To estimate grain yield (GY), ten middle plants from each row were harvested to avoid border effects and adjusted at 15 % moisture.

Statistical Analysis

Data collected from all tested locations were pooled and the presence of significant G×E were deal with analysis of variance. The stability parameters, regression coefficient (b_i) of the genotype over environmental indices and deviation from regression (S^2d_i) were calculated in accordance with Eberhart and Russel (1966). Using Cropstat 7.2 program, all collected data were processed and analyzed.

GGE Biplot and AMMI Model

The GGE biplot was constructed in view of the simplified model for two main components centered on the environment (PC1 and PC2) to evaluate stability and adaptability (Yan, 2007). The GGE biplot multiplicative model is close to the AMMI multiplicative model (Sousa et al., 2015). Principal components formed by putting environment-centered means to singular value decomposition (SVD). Then the first principal component (Axis 1) scores were laid out against their respective value/scores for the second principal component (Axis 2) to create biplot (Mohammadi et al., 2010; Hossain et al., 2018).

AMMI model analysis was done by using analysis of variance and principal component analysis for measuring stability and genotype environment interactions (Gauch, 1988; Yan et al., 2007).

The model equation is:

$$Y_{ij} = \mu + G_i + E_j + \sum_{k=1}^{n} \lambda k\ \alpha ik\ \gamma jk + e_{ij}$$

Where: Y_{ij} is the average yield of the ith genotype in the jth environment and is the overall mean yield; μ denotes the grand mean; G_i stands for the effect of genotype i; Ej is the effect of environment j; λ_k is the k^{th} singular value of the original matrix interaction; α_{ik} and γ_{jk} signify the genotype and environment principal component scores for axis k; n is the number of principal components kept in the model and e_{ij} stands for the average experimental error associated with observation, assumed to be independent.

RESULTS AND DISCUSSION

The full joint analysis of variance (Table 2) displayed considerable effects for genotypes, environment and the interaction between genotype and environment (GXE). Genotype's mean sum of squares were highly significant for yield that indicated the presence of genetic variation. Environment was the extensive initiator of variation for grain yield. The mean sum of squares for environments were highly significant suggesting that yield was significantly influenced by environments. Since the environment and G x E interactions was found significant which specify diverse environment and tested hybrids which implied differential responses of genotypes to different environments, it is necessary to find out the G×E interaction (Silveira et al., 2016; Ilker et al., 2018). The AMMI model is a powerful tool that can perfectly analyze genotype environment interaction. Different studies have been reported considerably different G×E interactions in maize grain yield (Carson et al., 2002; Makumbi, 2005; Menkir and Adepoju, 2005).

Table 2. Full joint analysis of variance for grain yield data inclusive of the partitioning of the G×E interaction of twenty-four maize hybrids

Source of variation	df	Mean sum of squares
Genotypes (G)	23	16.66**
Environment (E)	4	7.35**
Interaction (G x E)	92	2.01**
AMMI Component 1	26	3.38**
AMMI Component 2	24	1.62
AMMI Component 3	22	1.54
AMMI Component 4	20	1.23
Residuals	23	1.79

Assessment of phenotypic index and stability parameters

The grain yield in five locations, as well as overall mean yield coupled with phenotypic index (P_i), environmental index (I_j), regression coefficient (b_i), and deviation from

regression (S^2d_i) value, are presented in Table 3. The mean grain yield ranged from 6.91 t ha^{-1} to 11.75 t ha^{-1}. In case of phenotypic index, fourteen hybrids displayed positive phenotypic index while rest of the hybrids had negative index for yield. Thus, positive phenotypic index represented the higher yield and negative represented the lower yield. Again, the rich or favorable and poor or unfavorable environments are reflected by positive and negative environmental index (I_j) for this character, respectively. The range of environmental indices for grain yield was -0.37 to 0.51 which reflected the variation in performance from one location to another. Thus, the environment of Hathazari was the poorest, whereas Barishal and Dinajpur were the most favorable environmental for maize production. The environmental mean for grain yield ranged from 9.79 t ha^{-1} to 10.67 t ha^{-1}. The differences in b_i value ranges from 0.08 to 3.75 reflected the response of tested hybrid and indicated that these materials responded differently in different environment. The adaptability in performance across the location for all the genotypes was indicated by the non-significant regression coefficient value (b_i) different from unity. When b_i=1 and mean yield high, then the genotypes are well adapted to all environment; when b_i=1 and mean yield low, the genotypes are poorly adopted to all tested environments. A regression coefficient value significantly less than unity indicates, either, a lower than average response to high yielding environments or a better than average performance in low-yielding environments. Among the studied materials, G14 (11.75 t ha^{-1}), G16 (11.51 t ha^{-1}), G20 (11.43 t ha^{-1}) and G10 (11.28 t ha^{-1}) produced highest yield. Considering grain yield, b_i~1 and S^2d_i~0 indicated that G14 and G16 were the higher yielding and suitable across the environments. These results were in line with the reports of Kaundal and Sharma (2006). On the other hand, G20 & G10 were given higher yield but were responsive to favorable environments. Rahman et al. (2010) were also recorded significant differences of adaptability and yield stability in maize genotypes.

Table 3. Estimates of stability parameters for yield (t ha^{-1}) of twenty-four maize hybrids at five locations

Crosses	Yield (t ha^{-1})					Overall mean	P_i	b_i	S^2d_i
	BSL	JSR	DPUR	HAT	JML				
1. CML 330× CML 332(G1)	10.44	8.31	8.84	7.09	8.08	8.55	-1.60	3.75	0.48
2. CML 330× CML 322(G2)	10.50	8.86	11.17	9.61	10.97	10.22	0.07	1.25	0.46
3. CML 330× CML 311(G3)	10.68	10.99	10.21	7.58	9.93	9.88	-0.28	2.62	0.90
4. CML 330× CML 331(G4)	10.85	8.99	10.34	8.94	10.73	9.97	-0.18	2.26	0.10
5. CML 330× CML 518(G5)	11.79	8.92	11.33	10.83	9.84	10.54	0.39	1.90	0.73
6. CML 330× CML 383(G6)	9.31	10.29	10.74	9.70	9.47	9.90	-0.25	0.66	0.16
7. CML 332× CML 322(G7)	10.20	10.08	9.66	8.83	9.67	9.69	-0.47	1.26	0.41
8. CML 332× CML 311(G8)	11.33	10.58	10.17	9.81	9.50	10.28	0.12	1.69	0.29
9. CML 332×CML 331(G9)	6.84	6.67	7.00	8.89	5.15	6.91	-3.24	1.59	1.44
10. CML 332×CML 518(G10)	11.27	10.55	11.54	11.34	11.71	11.28	1.13	0.13	0.32
11. CML 332×CML 383(G11)	11.55	11.72	10.93	7.90	10.80	10.58	0.42	3.14	1.27
12. CML 322×CML 311(G12)	10.22	10.65	10.50	8.62	10.04	9.91	-0.25	1.25	0.06
13. CML 322×CML 331(G13)	10.92	9.30	9.61	9.37	10.26	9.89	-0.26	1.91	0.44
14. CML 322×CML 518(G14)	12.00	11.63	11.58	11.54	12.00	11.75	1.60	0.91	0.14
15. CML 322×CML 383(G15)	8.90	9.95	10.72	10.72	11.23	10.30	0.15	2.09	0.08
16. CML 311×CML 331(G16)	11.58	11.70	10.69	11.67	11.88	11.51	1.35	0.93	0.29
17. CML 311×CML 518(G17)	11.30	10.17	10.06	9.74	10.67	10.39	0.23	1.76	0.50
18. CML 311×CML 383(G18)	10.56	8.52	9.26	10.52	9.10	9.59	-0.56	0.80	0.43
19. CML 331×CML 518(G19)	10.24	10.20	10.48	9.52	10.78	10.24	0.09	0.58	0.34
20. CML 331×CML 383(G20)	11.86	11.04	11.11	11.83	11.30	11.43	1.27	0.08	0.44
21. CML 518×CML 383(G21)	11.41	10.60	9.54	10.18	10.86	10.52	0.36	1.36	0.17
22.BHM 12(G22)	11.74	11.19	10.02	11.79	10.23	10.99	0.84	0.26	0.34
23.BHM 13(G23)	11.81	10.84	9.70	10.93	10.69	10.79	0.64	1.16	0.00
24.BHM 14(G24)	8.86	8.55	9.39	7.97	7.74	8.50	-1.65	0.96	0.11
E.mean	10.67	10.01	10.18	9.79	10.11	**10.16**	-	-	-
E.index (I_j)	0.51	-0.15	0.02	-0.37	-0.05	-	-	-	-
LSD (0.05)	1.22	1.17	1.24	0.76	0.93	-	-	-	-

*BSL=Barishal; JSR=Jashore; DPUR=Dinajpur; HAT=Hathazari; JML=Jamalpur

For the identification of suitable or better performing hybrids in each location "Which-Won-Where" function of GGE biplot can be used. Data derived from the multivariate models of genotypes/varieties/hybrids and tested environments, GEI pattern can be identified effectively by plotting concurrently in one figure i.e GGE biplot polygon (Yan et al., 2001). Dehghani et al. (2009) also used GGE biplot method to pick out best maize genotypes for target sites. Fig. 1. displayed GGE biplot that showing the performance of twenty-four hybrids over five

environments. The principal component Axis 1 interpreted 69.73% genotype main effects, while second principal component Axis 2 elucidated 15.36 % G×E interaction and thus the GGE biplot demonstrated 85.09% of the total variation of grain yield. Fig. 1 showed a polygon that was formed by integrating different points, located apart from the centre, where on the vertexes some of the testing hybrids were positioned, while rests of the hybrids were inside the polygon. The genotypes placed in the vertex in their sector represent the high yielding genotypes in the area that fell inside the specific area (Yan et al., 2000; Makumbi et al., 2005). Those hybrids were considered to be the most responsive due to their position on the vertex because they had an extended distance from the origin of the biplot. Yan and Rajcan (2002) explained that responsive hybrids were either the best or the poorest at one or every environment. It is apparently set out which genotype won in which environments, thus supporting the documentation of mega-environment (Yan et al., 2000; Dimitrios et al., 2008). Other researchers (Sabaghnia et al., 2008; Choukan, 2011; Shiri, 2013) have also cited this method.

The vertex genotypes in this Fig. 1 were G1 (CML 330× CML 332), G9 (CML 332×CML 331), G11 (CML 332×CML 383), G14 (CML 322×CML 518) and G20 (CML 331×CML 383) that were the most responsive one can be visually determined. The biplot was divided into seven sectors by seven rays and for each sector the highest yielding genotypes were identified. Similar results were noticed by Bhartiya et al. (2017) and Ramos et al. (2017). They marked that the GGE biplot obtained for soybean genotypes for seed yield was divided into six or eight sectors. In this study, out of seven sectors tested environments fall in two of them. The vertex genotype for sector which encompassed environments Barishal, Jashore, Jamalpur and Dinajpur was G14 (CML 322×CML 518) and for sector content environment Hathazari was G20 (CML 331×CML 383), indicating that these genotypes were the winning genotypes for that environments. In this case GE can be exploited by recommending specific genotype to specific locations (Yan et al., 2007). Other corner genotypes, G9 (CML 332×CML 331) was the poorest yielding among the tested genotypes and the location of this genotypes reflecting the fact that as this was poor yielded at each location so this was located far away from all of tested locations. Ahmed et al. (2020) also found hybrids 'G4', 'G9' and 'G6'positioned at the pick of the GGE biplot polygon, but they had low GY in all the tested environments.

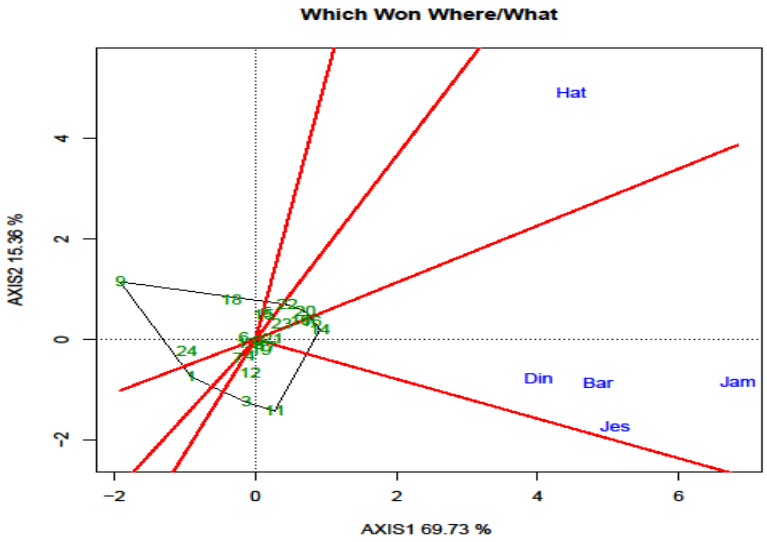

Figure 1. Polygon view of genotype + genotype × environment interaction biplot displaying performance of genotypes in each location.

In this GGE biplot method testing hybrids were compared with hypothetically determined ideal genotype (Fig. 2). The ideal genotype has maximum grain yield mean value and higher stability (PCA scores near zero) in that it will not exhibit any G×E interaction (Yan & Kang, 2003; Sharma et al., 2010; Akcura et al., 2011). According to Kaya et al. (2006), an ideal genotype is explained by having the maximum vector length of the high-yielding potential genotypes and with the lowest GEI that is presented by an arrow pointing to it. A genotype is considered best if it is located nearest to the center with an average higher grain yield and stability. Thus, keeping the ideal genotype as the center, a concentric circle has been drawn to assist in the visualization of the distance between all tested genotypes and the ideal/best genotype. In Fig. 2, G14 (CML 322×CML 518) was located at the center of concentric circles and was affirmed as an ideal genotype with maximum grain yield and higher yield stability. Whereas G16 (CML 311×CML 331), G10 (CML 332×CML 518), and G20 (CML 331×CML 383) were adjacent to the best genotype 'G14', also pointed out as better hybrids. However, CML 332×CML 331(G9) would not be regarded as a higher grain yielding and yield stable genotype due to having distance from genotype G14 (CML 322×CML 518). Similar findings related to our research were mentioned by Zhang et al. (2006) and Bhartiya et al. (2017). The genotype E14, E16, E10 and E20 could be utilized as a reference for evaluation of genotypes (Kaya et al., 2006) and these could be encompassed for further assessment in both selection of early and later stages (Mitrovic et al., 2012).

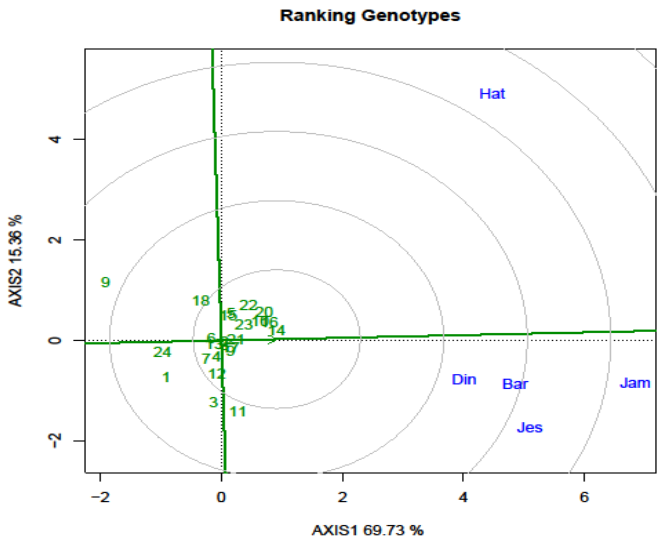

Figure 2. GGE-biplot based on genotype and environment focused scaling for comparison of the genotypes with the ideal genotype.

The indication of the stability of a genotype over locations/environments is interaction principal component analyses (IPCA) scores in the AMMI analysis. A Genotype or environment is regarded as stable genotype having IPCA1 scores nearly zero and has no interaction effect. Positive interaction of genotype or environment is reflected by the identical sign on the PCA axis, while negative interaction depicted by dissimilar sign. From the biplot, environments are distributed from low yielding genotypes and environment in quadrants I (top left) and IV (bottom left) to the High yielding genotypes (G) and favorable environment (E) in quadrants II (top right) and III (bottom right) (Fig. 3). In the present study it was observed that G14 (CML 322×CML 518), G16 (CML 311×CML 331), G10 (CML 332×CML 518), G20 (CML 331×CML 383), G22 (BHM 12) and G23 (BHM 13) were high yielding hybrids. G9 (CML 332×CML 331), G24 (BHM 14) and G1 (CML 330×CML 332) were low yielding and rest of hybrids are average yielding. Genotypes belonging to low-yielding environments are indicated at the lower left quadrant of the biplot. Among the entries, G14 (CML 322×CML 518), G2 (CML 330×CML 322), G24 (BHM 14) were more stable due to smaller IPCA1 score that was near to zero. G21 (CML 518×CML 383), G17 (CML 311×CML 518), G19 (CML 331×CML 518) and G8 (CML 332× CML 311) were average yielding and nearly stable but G11 (CML 332×CML 383) and G3 (CML 330×CML 311) were unstable. Ahmed et al. (2020) and Matin et al. (2017) also made similar findings using AMMI model.

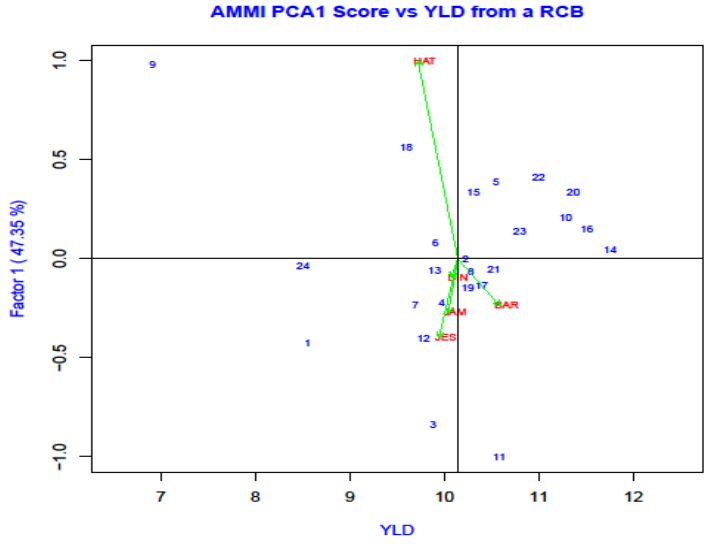

Figure 3. First AMMI interaction (IPCA1) biplot for yield (t/ha) of twenty-four maize hybrids and five environments using G × E scores

Since IPCA2 scores also have a notable part in describing the GEI; the IPCA1 scores were set against the IPCA2 scores for more exploration of adaptation. With the first two principal component scores the AMMI 2 biplot

generated a distinct relationship between genotypes and environments (Fig. 4). Interaction level is indicated by the length of sidelines. The longer sideline implies strong interaction, while the shorter sideline denotes weak interaction. The biplot conveyed that with the long spoke, the environments of Hathazari exert strong interaction. The AMMI 2 biplot also specified the relationship among the genotypes. According to Fig. 4 the hybrids G11 (CML 332×CML 383), G22 (BHM-12), G9 (CML 332×CML 331), G3 (CML 330×CML 311) and G15 (CML 322×CML 383) were unstable due to their dispersed position from the other hybrids in the biplot. G24 (BHM-14), G16 (CML 311×CML 331), G13 (CML 322×CML 331), G17 (CML 311×CML 518) and G14 (CML 322×CML 518) were located adjacent to the biplot's origin which indicated their stability in performance over environments when plotting the IPCA1 and IPCA2 scores**.** Ahmed et al. (2020) stated that genotypes near to the origin will have equivalent grain yield in all locations, while distant genotypes will response diversely in mean yield and over environments.

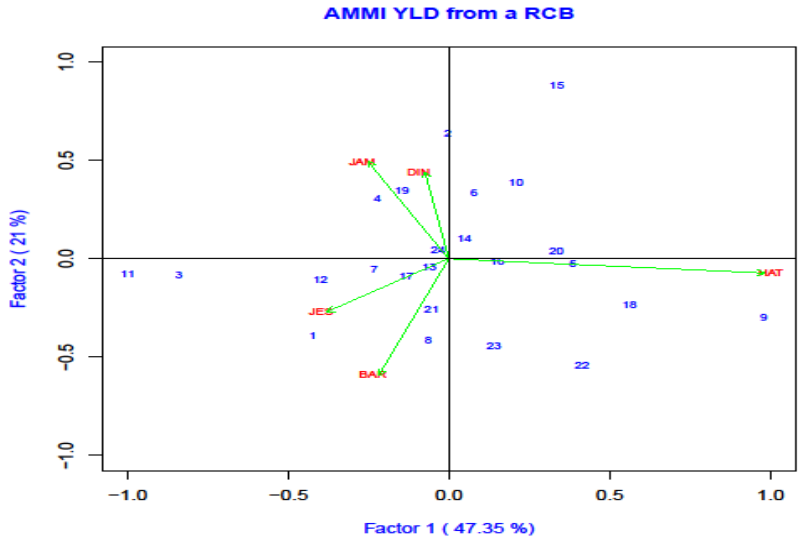

Figure 4. AMMI biplot 2 for grain yield (t/ha) displaying the relationship of IPCA against IPCA 1 scores of twenty-four maize hybrids grown in five environments

CONCLUSION

The present study revealed that the maize grain yield was greatly influenced by genotype × environment interaction that was followed by genotypic (G) and environment (E) effects, respectively. In the present investigations, phenotypic index, stability parameters, GGE, and AMMI were utilized to measure the stability of hybrids among the five tested locations in Bangladesh. Among the tested materials, G14 (CML 322×CML 518), G16 (CML 311×CML 331), G20 (CML 331×CML 383) and G10 (CML 332×CML 518) produced highest yield than check BHM-12 and BHM-14. Considering overall performance on yield, yield contributing characters and stability, G14 (CML 322×CML 518) and G16 (CML 311×CML 331) were found superior by providing information about GEI and stability as supportive addition.

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THE EFFECT OF DIFFERENT IRRIGATION LEVEL AND NITROGEN DOSES ON THE SILAGE YIELD AND QUALITY OF SORGHUM × SUDAN GRASS HYBRID (*Sorghum bicolor* L. × *Sorghum sudanese*)

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ABSTRACT

This study aimed to determine the effects of different irrigation levels (I30, I60, and I100) and nitrogen doses (N0, N50, N100, and N150 kg ha^{-1}) on the silage yield and quality of sorghum × Sudan grass hybrids (*Sorghum bicolor* L. × *Sorghum sudanese*). The experiment was conducted via a split-plot trial design with three replicates over two years, 2021 and 2022. Silage yield was evaluated over two years, and quality traits were evaluated over one year. Silage yield, irrigation water use efficiency (IWUE), water use efficiency (WUE), dry matter, pH, organic acids (lactic and acetic), relative feed value (RFV), crude protein, potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), total phenolic and total flavonoid content, DPPH free radical scavenging activity, and condensed tannin content were determined in the silage samples. The highest silage yield was observed in the I100×N1000 (64.3 t ha^{-1}) and I100×N150 (61.8 t ha^{-1}) treatments. The highest WUE was obtained from the I30×N150 interaction, whereas the highest IWUE value was obtained from the I30×N150 interaction. The lactic acid and crude protein contents of the silages ranged from 2.29-4.38% and from 6.51-9.70%, respectively. As a result, the silage yield decreased, whereas the silage quality was not affected by stress conditions. Accordingly, the I100xN1000 interaction, which results in the highest silage yield, is recommended.

Keywords: Drought stress, Fertigation, Silage yield, Silage quality

INTRODUCTION

Natural resources and agricultural areas are being depleted due to global climate change and population growth. However, the need for natural resources and agricultural land is growing daily, particularly owing to population expansion. This necessitates the efficient use of agricultural land and natural resources. In particular, maintaining the continuation of human existence depends heavily on the proper use of water, which is becoming a valuable resource.

Because of global warming and fast population growth, freshwater supplies are being depleted domestically and globally. However, as a result of rising daily water demands, which are necessary for all living things to survive, the supply–demand balance is starting to breakdown. Food shortages and water crises are predicted to result from the world's rapidly growing population. Consequently, either more agricultural land must be allocated to agriculture or agricultural methods that maximize output per unit area must be adopted to fulfill the demands of the growing population. Studies should concentrate on maximizing the unit's efficiency because expanding the agricultural area is not an option.

Water efficiency has become the most important criterion, especially for the agriculture industry, which uses approximately 70% of the water worldwide. Therefore, research on agricultural practices that may be used in dry and hot climates as well as plant species and types that can withstand these circumstances has become more important in recent years, and this field of study has seen an increase in activity (Khoshouei et al. 2024). Deficit irrigation and semiwetting irrigation are two methods that may be applied to use water efficiently. According to Geerts and Raes (2009), deficit irrigation is a significant and sustainable production technique utilized in areas with limited water supplies. Reducing the quantity of irrigation water or irrigation frequency is intended to increase plant water usage efficiency. A plant undergoing restricted watering is anticipated to conserve irrigation water without noticeably reducing production since it is subjected to varying degrees of water stress at any point throughout the growing season

or during development (Kırda, 2002; Erkovan and Afacan, 2024).

One of the most important inputs for enhancing agricultural output is fertilization, along with irrigation. The use of chemical fertilizers has increased significantly in recent years worldwide, including in our own nation. Nevertheless, a portion of the fertilizers are gassed off or washed out of the soil, or they are fixed in the soil and lose their useful forms, which decreases their efficacy. Because of this, it is crucial to apply the right quantity of fertilizer to the soil for the plant rather than using too much fertilizer.

It is crucial to select plants that are suited for the right circumstances in addition to the agricultural practices that will be used. While silage and grasses that have been cut and dried from field areas are frequently utilized to meet animal feed demands during the winter, meadows and pastures serve as sources of food for animals during warmer weather. When used as silage feed, one of the plants with the highest nutritional value is maize. However, in dry and semiarid climate zones, as well as in situations where irrigation is not carried out, the high water requirements of maize during its growth and development phase result in a decrease in productivity and a narrowing of the maize planting areas. Therefore, sorghum and its hybrids have become viable crops, especially in arid and semiarid areas, to replace maize (Tutar, 2024).

This study investigated the effects of different nitrogen doses and irrigation levels on the quality and yield of silage produced from a sorghum × Sudan grass hybrid.

MATERIALS AND METHODS

Experimental Site

Field experiments were conducted for two years, 2021 (03.06.2021) and 2022 (06.06.2022), at the University of Bilecik Seyh Edebali's application and Research Station (40° 6` N, 30°.0` E), in the province of Bilecik, Turkey. Bilecik is in a semiarid climate zone, and according to long-term climate data, the average temperature is 12°C, and the average annual rainfall is 459 mm. During the experimental plant growth period from 2021–2022, the total precipitation was 132.6 mm and 227.7 mm, respectively. The experimental area has a loamy soil texture suitable for agriculture, and some of its properties are given in Table 1.

Table 1. Some properties of the experimental area soil

Depth (cm)	Texture	Volume weight (g cm^{-3})	Field capacity PW (%)	pH	Organic Matter (%)	Phosphorus P_2O_5 (kg ha^{-1})	Potassium K_2O (kg ha^{-1})
0-30	CL	1.26	27.87	7.77	1.18	267.40	1162.80
30-60	L	1.21	24.57	7.81	1.24	274.50	915.90
60-90	L	1.27	26.67	7.71	2.07	210.20	964.20

CL: Clay-loam; L: Loamy.

Experimental Design and Treatments

A sorghum × sudan grass hybrid (*Sorghum bicolor* L. × *Sorghum sudanense* "SS hybrid") was used as the crop material for the experiment. The experiment was conducted via a split-plot trial design with three replicates over two years. The plot size was 6×2.8 m (16.8 m^2), the row spacing was 70 cm, the plant spacing was 5 cm, and each parcel had 4 rows. Four irrigation levels, 100% (I100), 60% (I60), and 30% (I30) 0% (I0) of the evaporation measured in Class A Pan, were placed in the main plots, and four nitrogen treatments (N0, N50, N100, and N150 kg ha^{-1}) were placed in the subplots. DAP base fertilizer was applied to all the plots at 8 kg P_2O_5 per decare during planting. After taking into account the amount of nitrogen we provided with the base fertilizer, we completed the missing amounts to 50, 100, and 150 kg per hectare and distributed them to the parcels. Irrigation was performed in 3 different critical growth periods for the plants as supplemented irrigation. Three irrigations were performed as follows: the first took place when the plants were 30 to 35 cm tall; the second was performed at the start of flowering; and the third was performed when the panicle appeared. The plants were irrigated with drip irrigation. A lateral line with a dripper spacing of 20 cm and a flow rate of 4 l h^{-1} was placed in each row. The amount of irrigation water to be applied before each irrigation event was calculated via Equation 1 according to the amount of cumulative evaporation occurring in the Class A Pan. (Kanber, 1984).

$$I = A \times kcp \times Ep \times P$$

where I is the irrigation amount (mm), A is the parcel area (m^2), kcp is the plant-pan coefficient (0, 0.30, 0.60, 1.00), Ep is the Class A Pan's total cumulative evaporation amount (mm), and P is the percentage of vegetation.

The soil water content was measured gravimetrically from 0.3 m depth to 1.2 m depth throughout the growing season. Crop evapotranspiration was calculated via a water balance equation (James, 1988).

$$ETa = I + P - Dp - Rf \pm \Delta S$$

where I is the irrigation amount (mm), P is the seasonal amount of precipitation (mm), Dp deep penetration is considered 0 (mm), Rf is the amount of surface runoff and ΔS is the change in soil moisture content between planting and harvest (mm). The P value in the equation is obtained from the Meteorological Station at Bilecik State. Deep penetration and surface runoff were considered insignificant because the irrigation water quantity was regulated.

The plants were harvested at the milk dough stage. Two cuttings were taken in both years. Therefore, silage yields are given as the sum of two harvests. In this study, silage

yield was evaluated over two years, and quality traits were evaluated over a single year.

Measurements and analysis

Silage yield, preparation, ensiling, and silo opening

Following the harvest and weighing of the plants, the yield of green forage was computed as t ha^{-1} on the basis of the fresh weight. The silage yield was calculated by reducing the green forage yield by 25%. The gathered plants were cut into 2 cm pieces and then packed into vacuum silage bags. The silages were stored under controlled conditions at 25 ± 2°C and opened after 45 days, and the necessary analyses were performed.

Water productivity

The most important indicators used to explain plant water yield relationships are water productivity and irrigation water productivity values. The irrigation water productivity shows the yield values obtained per unit of water applied to the plant, and the water productivity shows the yield values obtained against the seasonal plant water consumption. These values were calculated from the equations determined by Howell et al. (1990):

$$IWUE: \frac{Y}{I}$$

$$WUE: \frac{Y}{ETa}$$

where IWUE refers to the irrigation water use efficiency (kg m^{-3}), Y refers to the yield (kg da^{-1}), I refers to the volume of seasonal irrigation water applied (m^3 da^{-1}), WUE refers to the water use efficiency (kg m^{-3}), and ETa refers to the actual seasonal evapotranspiration (m^3 da^{-1}).

Dry matter and pH

The dry matter ratio (DM) (%) was computed after the silage samples were dried for 48 hours at 105°C in a hot-air oven. A digital pH meter was used to measure the pH of the silage samples.

Organic acid analyses

An electric blender was used to blend the 20-gram silage sample from the silage bags for 30 minutes, after which it was filtered. The mixture contained 100 mL of distilled water. Organic acid analyses (lactic acid, acetic acid and butyric acid) were performed via HPLC.

Crude protein

To determine the amount of crude protein, the nitrogen ratios of the samples were first determined via the Kjeldahl apparatus. The amount of crude protein was calculated by multiplying these determined nitrogen concentrations by the coefficient of 6.25 (FOSS 984.13).

Acid deterh-gent fiber (ADF), neutral detergent fiber (NDF) and mineral content analyses

The levels of ADF, NDF, and minerals (potassium, phosphorus, calcium, and magnesium) were measured via near-infrared reflectance spectroscopy (NIRS, 'Foss 6500').

Relative feed value (RFV)

The RFV was calculated via the following formulas from Rohweder et al. (1978).

Dry matter intake % (DMI) = 120/NDF

Digestibility of dry matter % (DDM) = 88.9 – (0.779 × ADF)

Relative feed value (RFV) = (DDM * DMI)/1.29

Total phenolic contents

The Folin–Ciocalteu technique (Singleton et al., 1999) was used to quantify the total phenolic content (TPC) in the SS hybrid extracts. Specifically, 200 µL of each chicory extract was combined with 0.2 mL of Folin-Ciocalteu solution and 9 mL of distilled water. Finally, the volume was adjusted to 10 mL by adding 0.6 mL of a 20% sodium carbonate solution. Absorbance readings at 760 nm were taken after the combinations were incubated at room temperature in the dark for two hours. The data are presented as mg of gallic acid equivalent (GAE) per gram of extract. Nine distinct concentrations of the gallic acid standard were used to generate the calibration curve (y = 0.004x − 0.0138; R2 = 0.9995).

Total flavonoid content

The technique used by Arvouet-Grand et al. (1994) was slightly modified to assess the total flavonoid content (TFC) of the extracts. One milliliter of potassium acetate (1 M) and one milliliter of aluminum nitrate (10%) were combined with each 200 µL of SS extract. Five milliliters, or 99% ethanol, was used to adjust the final volume. The absorbance was measured at 417 nm after the mixture was incubated for 40 minutes in the dark at room temperature. The quercetin standard's calibration curve (y = 0.0367x + 0.0003; R2 = 0.9900) was used to determine the extracts' total flavonoid content in µg of quercetin equivalent (QE).

DPPH free radical scavenging activity

Yaman et al. (2020) examined how SS hybrid silage extracts affect the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. A 3.2 mL solution of DPPH in 0.004% methanol was mixed with 200 µL of the sample. Absorbance measurements at 517 nm were taken after the combinations were allowed to stand at room temperature for 30 minutes in the dark.

Condensed tannins

The analysis of condensed tannins was performed in accordance with Bate-Smith (1975). A total of 0.01 g of the pulverized material was combined with 6 ml of the tannin mixture, placed in a tube, and vortexed. The samples were allowed to cool after the tubes were securely sealed and heated to 100°C for one hour. The samples were then measured with a spectrophotometer at 550 nm for absorbance. This formula was used to compute condensed tannins (CTs): dry weight (%)/absorbance (550 nm × 156.5 × dilution factor).

Statistical analysis

The yield and quality parameters were subjected to analysis of variance (ANOVA) via the Minitab 19 package program. An F test was performed to determine the significance of irrigation and nitrogen dose. In applications where the F test was significant, Tukey's (p<0.05) multiple comparison test was applied to irrigation, nitrogen dose and interactions.

RESULTS AND DISCUSSION

Variance analysis

The results of the variance analysis and significance levels of the effects of different irrigation levels and nitrogen doses on the quality traits of the sorghum–sudan grass hybrid are presented in Table 2. The effects of irrigation level on crude protein and nitrogen dose on total flavonoids were found to be statistically insignificant. However, the effects of irrigation level and nitrogen dose on other traits were found to be significant. Additionally, the interaction between irrigation level and nitrogen dose had a significant effect on all the examined traits.

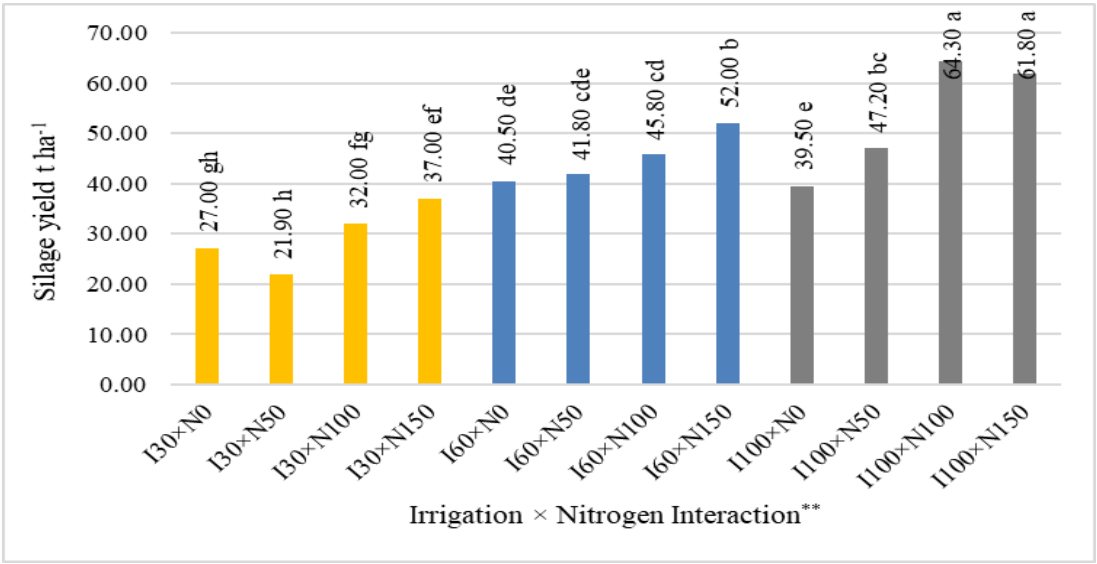
Table 2. Analysis of variance for SS hybrid silage under different irrigation levels and nitrogen doses

		Mean of squares													
	DF	DM	pH	LA	AA	CP	RFV	P	K	Mg	Ca	TP	TF	DPPH	CT
Rep	2	0.686	0.00016	0.00172	0.00316	0.0432	1.914	0.00004	0.0026	0.0048	0.00067	8.338	0.091	0.00026	0.00006
IL	2	8.515**	0.039**	7.271**	0.021*	4.624	339.81**	0.0068**	2.530**	0.0002*	0.0038**	817.747**	16.692**	2799.26**	0.0582**
Error (1)	4	0.209	0.0015	0.0035	0.0027	0.974	0.487	0.0003	0.00041	0.000024	0.000038	Eyl.67	0.061	17.997	0.0006
ND	3	4.103**	0.125**	0.465**	0.061**	2.986**	147.82**	0.0037**	0.047**	0.0063**	0.041**	418.723**	0.436	178.495**	0.043**
IL×ND	6	7.028**	0.033**	0.571**	0.034**	2.283**	95.438**	0.0016**	0.360**	0.0022**	0.0064**	204.625**	7.179**	71.113**	0.079**
Error (2)	18	2.168	0.031	0.061	0.021	5.008	31.812	0.0049	0.0567	0.0015	0.0099	135.065	2.534	132.663	0.02
CV	-	1.00%	1.00%	1.77%	7.04%	6.72%	1.38%	5.41%	2.89%	3.72%	7.97%	3.58%	5.59%	4.24%	4.56%

*: P≤0.05; **: P≤0.01; IL: Irrigation level; ND: Nitrogen dose; IL×ND: Irrigation level and nitrogen dose interaction; DF: Different degrees of freedom; DM: Dry matter; LA: Lactic acid; AA: Acetic acid; CP: Crude protein; RFV: Relative feed value; TP: Total phenolics; TF: Total flavonoids; CT: Condenced tannins

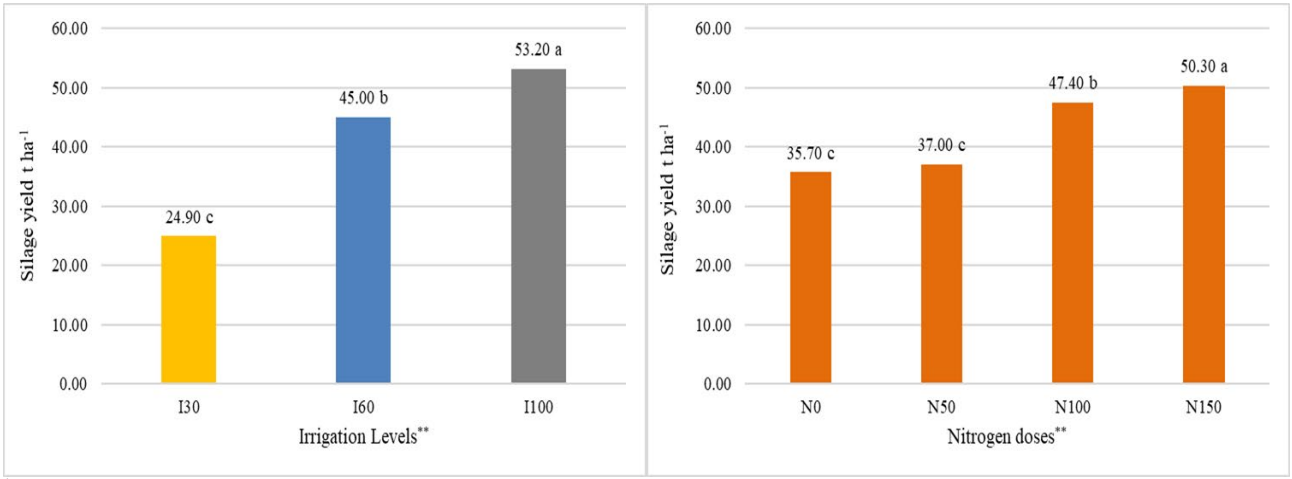
Silage yield

The irrigation level, nitrogen dose, interaction and year had significant (p<0.01) effects on the silage yield. According to the interactions, the silage yield of the SS hybrid ranged from 21.9 to 64.3 t ha^{-1}. The I100 × N100 and I100 × N150 treatments resulted in the highest silage yield, whereas the I30 × N50 treatment resulted in the lowest silage yield (Figure 1). This situation shows that the interaction effect between the irrigation level and nitrogen dose is strong. High irrigation levels eliminated the effectiveness of low nitrogen doses. Indeed, high irrigation levels and high nitrogen doses were more effective and increased the silage yield. Increasing the irrigation level and nitrogen dose alone increased the silage yield. However, although N100 and N150 resulted in significantly different groups, they presented similar values.



*: P≤0.05; **: P≤0.01

Figure 1. Silage yield of the SS hybrid according to the interaction



*P≤0.05; **: P≤0.01

Figure 2. Silage yield of the SS hybrid according to the irrigation level and nitrogen dose

Figure 2 shows that the silage yield increased with increasing nitrogen dose and irrigation water level. While the yield differences between nitrogen doses were relatively small, the differences were significantly greater across the various irrigation water levels. Research has shown that nitrogenous fertilizer typically increases plant production, although these improvements are not consistent (Subedi and Ma 2005; Islam et al. 2010). According to Kiziloglu et al. (2009), water deficiencies resulted in a lower output of green herbage. In a study that examined the effects of different irrigation levels and nitrogen doses on maize yield, Kaplan et al. (2016) reported that the herbage yield of maize ranged from 48.9 to 80.9 t ha^{-1}. Nematpour et al. (2021) reported that yield decreased significantly under water stress conditions.

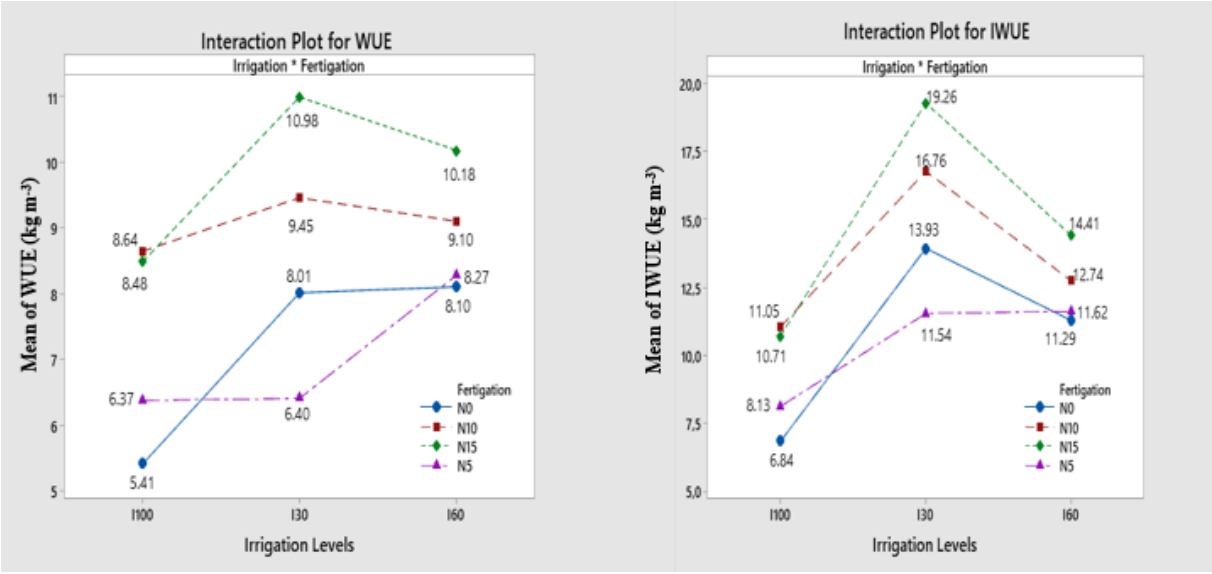


Figure 3. WUE and IWUE values of the SS hybrid silage yield

Water-producing SS hybrid silage

WUE and IWUE are crucial metrics for assessing irrigation practices. The WUE and IWUE values are given in Figure 3. The values varied according to the water levels used for irrigation. The lowest WUE values were obtained from the I100×N0 interaction, whereas the highest WUE values were obtained from the I30×N150 interaction. The lowest IWUE values were obtained from the I100×N0 interaction, whereas the highest values were obtained from the I30×N150 interaction. When we evaluated the averages of irrigation subjects separately, the highest IWUE value was obtained from the I30 irrigation subject, whereas the highest WUE value was obtained from the I60 irrigation subject. When we evaluated the fertilizer applications separately, the highest IWUE and WUE values were observed for the N150 fertilizer doses (Figure 3). The WUE and IWUE values increased as the irrigation level decreased, per the two-year mean results. This outcome demonstrates that under some irrigation application

restrictions, the WUE and IWUE values can increase. Deficit irrigation water may therefore be appropriate in areas where it is scarce. In other studies, researchers obtained similar results. They reported that the WUE and IWUE values increased with water shortage (Kaplan et al., 2019; Aydınsakir and Erdurmus, 2021; Farhadi et al., 2022; Khalaf et al., 2019; Akcay and Dagdelen, 2016).

Dry matter and pH of the SS hybrid silage

The pH and silage dry matter ratio of the SS hybrid were significantly (p<0.01) affected by irrigation level, nitrogen dosage, and their interaction (Tables 1 and 3). According to Ball et al. (1996), ripening duration, temperature, irrigation, fertilization, and ratios of leaves to stems may affect a cultivar's dry matter ratio. Regional climate, soil conditions, plant genetics, sowing time and cultural practices significantly influence dry matter yield (Cacan et al., 2018). Water stress can stop the buildup of dry matter in plants by reducing nutrient transport (Kruse et al., 2008; Setter and Parra, 2010). In our study, the dry matter content decreased with increasing irrigation level and nitrogen dosage. Additionally, the present dry matter ratios (32.02–37.13%) were between 25% and 40% of the desired ratios (Panyasak and Tumwasorn, 2013). According to Filya (2001), silage should have a pH of less than 5 to prevent Enterobacteria and Clostridial spores from growing and interfering with fermentation. The pH of the SS hybrid silage ranged from 4.34--4.77 and was below the critical level. Esen et al. (2022) reported that the average dry matter ratio and pH value of sorghum silage were 24.7% and 3.90, respectively.

Table 3. Dry matter ratio and pH values of the SS hybrid silage

Dry matter ratio (%)						pH					
	Nitrogen doses						Nitrogen doses				
IL	**N0**	**N50**	**N100**	**N150**	**Ave.**	**IL**	**N0**	**N50**	**N100**	**N150**	**Ave.**
I30	37.13a	32.95ef	35.59bc	35.28bc	35.23a	**I30**	4.73ab	4.50ef	4.40 fg	4.60cde	4.56b
I60	34.38cd	33.74de	34.81bcd	35.90ab	34.70b	**I60**	4.79a	4.57de	4.55de	4.77a	4.67a
I100	34.86bcd	34.88bcd	32.59ef	32.02f	33.58c	**I100**	4.70abc	4.34 g	4.70abc	4.63bcd	4.59b
Ave.	35.45a	33.85b	34.33b	34.40b		**Ave.**	4.74a	4.47d	4.55c	4.66b	

IL: Irrigation level

Organic acids of SS hybrid silage

The lactic and acetic acid contents of the SS hybrid were significantly (p<0.01) affected by irrigation level, nitrogen dosage, and their interactions (Tables 1 and 4). Butyric acid was not found in the present study. A lactic acid level greater than 2.0% is required for the development of high-quality silage. As a result, in comparison with the crucial value, the lactic acid contents (2.29--4.38%) of the silage samples used in this investigation were rather high. On the other hand, because acetic acid signals deterioration in silage, the percentage of acetic acid in the silage should not be greater than 0.8%. In the present study, the acetic acid content (0.30--0.67%) of the silages was below this value.

Table 4. Lactic and acetic acid contents of the SS hybrid silage

Lactic acid (%)						Acetic acid (%)					
	Nitrogen doses				IL	Nitrogen doses					
IL	**N0**	**N50**	**N100**	**N150**	**Ave.**	**IL**	**N0**	**N50**	**N100**	**N150**	**Ave.**
I30	3.87c	4.29ab	4.38a	4.14b	4.17a	**I30**	0.54bc	0.54bc	0.30e	0.40de	0.44b
I60	2.88e	2.55 fg	2.41gh	3.56d	2.85b	**I60**	0.60ab	0.39de	0.43cd	0.44cd	0.47b
I100	2.29 h	2.64f	3.41d	2.84e	2.79b	**I100**	0.67a	0.37de	0.63ab	0.43cd	0.52a
Ave.	3.01d	3.16c	3.40b	3.51a		**Ave.**	0.60a	0.45b	0.43b	0.43b	

IL: Irrigation level

Crude protein content and RFV of SS hybrid silage

The RFV of the SS hybrid was significantly (p<0.01) affected by the irrigation level, nitrogen dosage, and their interactions. The effects of nitrogen dose and its interaction with irrigation level on crude protein were found to be significant, whereas the effect of irrigation level alone was not significant. (Tables 1 and 5). The crude protein percentage of the silage samples ranged from 6.51% to 9.70% and increased as the nitrogen dose increased but decreased as the irrigation level increased. The RFV ratio is the same in this regard. The value of RFV is established via NDF and ADF. This means that RFV decreases as ADF and NDF increase. In addition, increases in the ratios of ADF and NDF make digestion more challenging, which ultimately lowers crude protein. The temperature, irrigation, fertilization, and ratio of leaves to stems may affect the crude protein content. Water stress can stop the buildup of dry matter in plants by reducing nutrient transport; therefore, the crude protein content may decrease (Ball et al., 1996; Kruse et al., 2008; Setter and Parra, 2010). In addition, in plants, nitrogen is essential for the production of enzymes and proteins (Islam et al., 2010). In this context, nitrogen increases the protein content of plants.

The most extensively used feed quality metric in the world, relative feed value (RFV), is based on estimations of feed intake from the NDF content and digestibility from the ADF content. The RFV value, which represents the forage quality, was therefore > 151 for the beginning quality standard, 151–125 for the first quality standard, 124–103

for the second quality standard, 102–87 for the third quality standard, 86–75 for the fourth quality standard, and < 75 for the fifth quality standard. The RFV values determined in the present study revealed that silages between the fourth and second quality classes were examined. Celik and Turk (2021) reported that the RFV of the Aneto SS hybrid was 94.55.

Table 5. Crude protein content and RFV of SS hybrid silage

Crude protein content (%)						Relative feed value (RFV)					
	Nitrogen doses						Nitrogen doses				
IL	**N0**	**N50**	**N100**	**N150**	**Ave.**	**IL**	**N0**	**N50**	**N100**	**N150**	**Ave.**
I30	7.33b-d	8.96ab	9.70a	8.22a-d	8.55	**I30**	95.46cd	107.99a	102.91b	102.32b	102.17a
I60	6.51d	7.51bcd	6.71d	8.88abc	7.40	**I60**	91.35ef	85.74 g	104.21b	101.56b	95.72b
I100	7.11cd	7.94a-d	8.19a-d	7.05d	7.57	**I100**	87.94 fg	90.59ef	91.86de	96.06c	91.61c
Ave.	6.98b	8.13a	8.20a	8.05a		**Ave.**	91.58c	94.77b	99.66a	99.98a	

IL: Irrigation level

The macronutrients of the SS hybrid were significantly *(p*<0.01) impacted by irrigation level, nitrogen dosage, and their interactions (Tables 1 and 6). K is crucial for maintaining an animal's water balance and osmotic pressure, whereas P is involved in every metabolic reaction and energy transfer inside the body. Calcium, the primary building block of bones and teeth, is the most common mineral in the body. In addition, the minerals that most affect a cow's ability to produce and reproduce. In the feed of dairy cows, magnesium is utilized to maintain proper blood magnesium levels, ideal ruminal pH values (6.2–6.5), and proper operation of the ruminal digestive processes. Consequently, at least 0.21% P, 0.8% K, 0.3% Ca, and 0.1% Mg are needed for roughage (Kidambi et al. 1993; Tekeli and Ates 2005). All the silages in the present study presented P, K, Mg and Ca contents within the acceptable range. In addition, as the irrigation level and nitrogen dose increased in the present study, the P and Mg contents of the silages increased, whereas the K and Mg contents decreased. (Table 6).

Table 6. Macronutrient contents of the SS hybrid silage

Phosphorus (%)						Potassium (%)					
	Nitrogen doses						Nitrogen doses				
IL	**N0**	**N50**	**N100**	**N150**	**Ave.**	**IL**	**N0**	**N50**	**N100**	**N150**	**Ave.**
I30	0.23de	0.26d	0.35b	0.35b	0.29b	**I30**	2.74a	2.44b	2.03ef	1.93 fg	2.29a
I60	0.24de	0.25de	0.34bc	0.28cd	0.27b	**I60**	2.10de	2.26c	1.83 g	2.22cd	2.10b
I100	0.19e	0.24de	0.33bc	0.44a	0.31a	**I100**	1.16ı	1.28hı	1.80 g	1.43 h	1.42c
Average	0.22c	0.25b	0.34a	0.36a		**Average**	2.00a	1.99a	1.89b	1.86b	
Magnesium (%)						**Calcium (%)**					
	Nitrogen doses						Nitrogen doses				
IL	**N0**	**N50**	**N100**	**N150**	**Ave.**	**IL**	**N0**	**N50**	**N100**	**N150**	**Ave.**
I30	0.26bc	0.24cde	0.21f	0.29ab	0.25a	**I30**	0.33a	0.34a	0.33ab	0.31abc	0.33a
I60	0.21f	0.22ef	0.27abc	0.28abc	0.24a	**I60**	0.32ab	0.31abc	0.28b-e	0.30abcd	0.30b
I100	0.20f	0.26bcd	0.23def	0.30a	0.24a	**I100**	0.34a	0.26de	0.27cde	0.24e	0.28c
Average	0.22c	0.24b	0.23bc	0.28a		**Average**	0.33a	0.30b	0.29bc	0.28c	

IL: Irrigation level

The secondary metabolites of the SS hybrid, with the exception of total flavonoids, were significantly *(p*<0.01) impacted by the irrigation level and nitrogen dosage, and the effects of their interactions with the nitrogen dose on the total flavonoid content were not significant (Tables 1 and 7). When plants are under stress, they produce secondary metabolites, which are crucial for their ability to adapt to shifting environmental conditions (Edreva et al., 2008). In the present study, the highest secondary metabolite contents were obtained from the least-irrigation-level (I30) treatments. Plants may have been stressed and produced secondary metabolites (Table 7).

Table 7. Secondary metabolite contents of SS hybrid silage

Total phenolic (%)						Total flavonoid (%)					
	Nitrogen doses						Nitrogen doses				
IL	**N0**	**N50**	**N100**	**N150**	**Ave.**	**IL**	**N0**	**N50**	**N100**	**N150**	**Ave.**
I30	86.11ab	91.10a	72.46cd	83.94ab	83.40a	**I30**	9.31a	8.74a	6.74bc	6.98b	7.94a
I60	79.21bc	81.11b	72.46cd	82.72b	78.87b	**I60**	6.83bc	6.95b	5.80c	6.79bc	6.59b
I100	54.35f	66.10de	62.19ef	86.92ab	67.39c	**I100**	3.59e	4.69d	6.92b	7.16b	5.59c
Ave.	73.22c	79.44b	69.03d	84.53a		**Ave.**	6.57	6.79	6.48	6.97	
DPPH Radical Scavenging Activity (%)						**Condensed tannin (%)**					
	Nitrogen doses						Nitrogen doses				
IL	**N0**	**N50**	**N100**	**N150**	**Ave.**	**IL**	**N0**	**N50**	**N100**	**N150**	**Ave.**
I30	77.58a	77.83a	74.79a	72.85a	75.76a	**I30**	0.56f	0.84bc	0.94a	0.90ab	0.81a
I60	74.17a	63.36bc	70.37ab	70.54ab	69.61b	**I60**	0.64ef	0.56f	0.93ab	0.59f	0.68b
I100	57.32c	35.39e	48.03d	46.37d	46.77c	**I100**	0.75cd	0.73de	0.57f	0.76cd	0.70b
Ave.	69.69a	63.25b	63.25b	58.86c		**Ave.**	0.65d	0.71c	0.81a	0.75b	

IL: Irrigation level

Research on the nutrition of ruminants has demonstrated the importance of secondary metabolites for the health of the rumen and the production of the animals. These substances have antibacterial and antioxidant properties and may significantly increase the quantity and quality of animals produced. Furthermore, several studies have shown the benefits of these compounds for animal production and health, rumen fermentation, and the management of nutritional stressors such as bloat and acidosis (O'Connell and Fox, 2001; Robbins, 2003; Rochfort et al., 2008; Santos Neto et al., 2009; Frozza et al., 2013; Seradj et al., 2014; Patra et al., 2006; Paula et al., 2016; Lee et al., 2017). On the other hand, condensed tannins lower greenhouse gas emissions by inhibiting some protozoans and methane-producing organisms that consume hydrogen directly in the rumen. In addition, condensed tannins also have anthelmintic effects, lessening internal parasites in animals and increasing animal output. Consequently, plants must have a condensed tannin concentration of no more than 2–3% (Kumar and Singh, 1984; Bary, 1987; Luscher et al., 2016). The condensed tannin concentration in the present study was below the essential threshold, ranging between 0.56% and 0.90% in the combined years (Table 6).

CONLUSION

While the silage yield increased with increasing irrigation level and nitrogen dose, the silage quality decreased. Accordingly, in terms of silage yield, the I30xN100 treatment resulted in the best results in terms of silage quality, and the I60xN150 treatment resulted in the best results in terms of IWUE and WUE. As a result, the I60xN100 treatment was determined to be suitable for improving the silage yield and quality of the SS hybrid.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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INFLUENCE OF THE TIMING OF THE APPLICATION OF SALICYLIC ACID ON THE QUANTITATIVE YIELD AND SOME BIOCHEMICAL CHARACTERISTICS OF BARLEY (*Hordeum vulgare* L.) UNDER DEFICIT IRRIGATION

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ABSTRACT

Using salicylic acid (SA) to feed drought-stressed plants plays a vital role in reducing the adverse effects of water stress and improving plant performance. This study explores the role of salicylic acid and different barley cultivars in mitigating the effects of drought stress on barley.The study examined three irrigation levels—one-time irrigation (severe stress), two-time irrigation (moderate stress), and four-time irrigation (control)—along with foliar and non-foliar applications of salicylic acid (SA) at three key stages of the Zadoks Growth Scale (ZGS): ZGS 29 (end of tillering), ZGS 34 (50% stem elongation), and ZGS 39 (completion of flag leaf emergence). These treatments were applied to three barley cultivars—Khatam, Reyhan, and Nosrat—which are considered semi-tolerant to drought stress. The findings showed that the interaction of reduced irrigation and SA increased chlora (8.8%) and b (7.12%) in the ZGS34 treatment under control conditions compared to the treatment without SA. The proline content increased with increasing drought stress, with the highest proline content obtained at the end of the tillering stage in the control condition. Compared to the control, which had no foliar spraying, the specific leaf area increased by 3.8, 1.8, and 0.4%, respectively. Relative water content in Khatam (35.6%), Reyhan (33.3%) and Nosrat (30.5%) decreased with increasing stress in the control treatment compared to the minimum stress. The most sensitive cultivar to lack of irrigation was Khatam. The rate of yield increase by SA compared to the control was (10.33%) among the barley cultivars cultivated, the cultivar Reyhan had a comparative advantage in more measures, mainly when applied at ZGS29. In conclusion, SA improved the drought tolerance of the barley and increased the yield by improving the biochemical characteristics.

Keywords: Antioxidant capacity, Barley, Cultivars, Drought stress, Phytohormone

INTRODUCTION

Barley grain remains found at archaeological sites in the Fertile Crescent indicate that it was domesticated there from its wild relative *Hordeum Vulgare* L. about 10,000 years ago (Badr et al., 2000). It is a perennial plant of the wheat family and one of the oldest cultivated plants with a wide range of distribution and climatic compatibility. This plant is usually grown for grain production and has many uses in human and animal nutrition (Langridge, 2018). Barley is a globally important crop with multiple uses (feed, food and beverage) (Rehman et al., 2021). It is the fourth most important crop in the world in terms of dry matter production after maize, wheat and rice, and in 2021 world barley production reached 145 million tones (FAO, 2022).

Rainfall and temperature are the most important abiotic factors affecting crop yield, especially in arid areas (Kheiri et al., 2021), and warming and climate change are expected to aggravate drought (Rousta et al., 2023). Drought stress is the most important factor limiting agricultural production (Meza et al., 2021). At any stage of plant growth, water deficit stress alters agronomic and physiochemical traits (Nouri et al., 2020; Matinizadeh et al., 2024). The lack of water reduces the uptake and production of substances in the leaf by closing the stomata and increasing respiration. This leads to a reduction in leaf weight. Finally, it reduces leaf area, increases tissue senescence, and a negative effect on assimilate metabolism (Rozentsvet et al., 2022). Changes in the physiological and morphological characteristics of leaves, such as changes in weight and leaf area, are responses of plants to drought (Moshki et al., 2024). These changes affect the rate of photosynthesis and ultimately the plant's yield. The change in leaf thickness and its degree of fleshiness is one of the leaf responses to drought

(Papkyadeh et al., 2023). It is assessed by measuring two indices of specific leaf area to estimate leaf thickness (Yan et al., 2019). Based on previous reports, there is a weak correlation between leaf thickness and yield (Khoshouei et al., 2024). Under drought stress, the leaf area decreases due to a reduction in cell size, which causes a reduction in its specific area. Based on researchers, the lower leaf area compared to its dry weight is one of the adaptive aspects of plants under dry conditions (Liu et al., 2022).

Drought tolerance is a major trait for increasing and stabilizing barley productivity in arid areas worldwide (Sallam et al., 2019). It appears that cultivars that produce the same yield under favorable and low irrigation conditions have a relatively higher tolerance to drought. Identifying and studying plant growth indicators can be very helpful in analyzing factors affecting yield and yield components. In addition, by measuring the dry matter produced during the growth period, we can better understand the distribution of photosynthetic substances in different organs and their accumulation (Ghanem and Al-Farouk, 2024). Maintaining optimal crop performance under drought stress conditions is an important goal shared by researchers and breeders working in semi-arid areas around the world (Williams et al., 2022).

It has been shown that salicylic acid (SA) to confer tolerance in plants to various abiotic stresses such as heat, salinity, heavy metal toxicity and drought (Singh and Usha, 2003). Plant responses to drought are modulated by plant growth regulators such as SA, auxins, gibberellins, cytokinins and abscisic acid (Singh, 2023). The use of chemicals such as SA is easier and cheaper than breeding methods that are time-consuming and costly (El-Tayeb, 2005). SA is a beta-hydroxyphenolic acid widely produced by prokaryotes and plants (Ding and Ding, 2020). In this regard, the role of SA as a growth regulator to induce tolerance to numerous biotic and abiotic stresses such as drought stress has been considered (Pirnajmedin et al., 2020; Kaur et al., 2022). In general, it appears that SA can improve nutrient uptake under low irrigation and salinity conditions and increase growth (external traits such as plant height, length and number of internodes) (Hafez and Farig, 2019; Pirnajmedin et al., 2020; Singh, 2023). SA is involved in germination, seedling establishment, cell growth, stomatal closure, senescence, increased enzymatic activity and photosynthesis under stress conditions (Shakirova et al., 2003; Kaur et al., 2002; Singh, 2002). The foliar application of SA on the leaves is a suitable method to mitigate the negative effects of drought stress (Safar-Noori et al., 2018). Studies have demonstrated the role of SA in improving plant biochemical characteristics such as soluble protein content, free proline, antioxidant activity, photosynthetic pigments and phytohormone levels, thereby increasing yield under stress conditions in many plants such as barley (El-Teyeb, 2005; Abdelaal et al., 2020; Kaur et al., 2022), wheat (Hafez and Farig, 2019; Shakirova et al., 2003) and rice (Asma et al., 2023).

Water is one of the most important inputs; however, water for irrigation is a scarce resource (Ledesma-Ramírez et al., 2023), while 73% of the land in Iran is under arid and semi-arid conditions (Maghsoudi et al., 2019). Water scarcity is the main constraint for barley production in the semi-arid Mediterranean, especially in Iran, and it is expected to become more severe under the current climate change. Therefore, a correct decision on irrigation regime should be based on a thorough understanding of the factors influencing plant growth, development, yield and quality. Indeed, a better understanding of the effects of irrigation levels on barley will promote better management of deficit irrigation. The present study evaluates the changes caused by foliar application of salicylic acid on some important agronomic and biochemical indices of barley cultivars during the growth period. In addition, the effect of deficit irrigation on these indices and on the trend of the total accumulation of dry matter will be evaluated. Therefore, the main objective of this study is to evaluate the positive effect of SA and cultivars in reducing the adverse effects of drought stress in barley.

MATERIALS AND METHODS

Experiment setup and treatment application

A factorial split-plot experiment based on a randomized complete block design with three replications was conducted in two cropping seasons (2020-2021 and 2021-2022) from December to May. The field experiment was conducted in Neyriz under the supervision of the College of Agriculture, Islamic Azad University in Fasa, Iran, at 29º 12' N, 54º 20' E and 1595 m above sea level. Figure 1 shows the monthly averages of minimum/maximum temperature and rainfall during the two barley cropping seasons in this area and Table 1 shows the different physicochemical properties of the soil collected from the study site.

Table 1. The physical and chemical properties of the soil of the experimental site (0-30 cm).

Year	EC	pH	N	P	K	Fe	Zn	Mn	Cu	OC	Sand	Silt	Clay
	dS m^{-1}					PPM						(%)	
2020	3.2	7.6	0.01	11.4	238	7.1	1.4	19.8	1.50	0.46	33	43.2	23.8
2021	3.5	7.1	0.02	11.9	222	7.1	1.5	18.8	1.55	0.45	35	42.2	22.8

The studied treatments consisted of irrigation treatments as the main factor, including 1-off irrigation from the tillering stage to the end of the growing season (severe stress), 2-off irrigation from the stem elongation stage to the end of the growing season (moderate stress), and 3-off irrigation after the milky stage (control). The second factor was foliar application of salicylic acid (SA) at a concentration of 1 mM applied at different growth

stages as none (control), sprayed at the end of tillering stage (Zadoks, 29), fifty percent of spike emergence (ZGS34) and flag leaf full emergence (ZGS39) and the third factor included three barley cultivars (Khatam, Reyhan and Nosrat). Table 2 shows the characteristics of the cultivars studied.

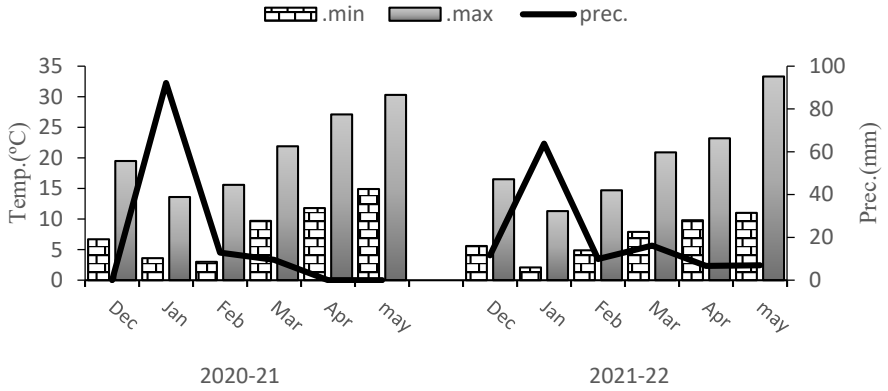

Figure 1. Average temperature (°C) and amount of precipitation (mm) in 2020, 2021 and 2022

Table 2. Some characteristics of studied barley cultivars

Cultivar name	Pedigree	Spike type	Maturity type	Growth type	Origin
Nosrat	Karoon/Kavir	six-rowed	Winter	Moderate maturity	Iran
Khatam	LB.Iran/Una 8271//Gloria"S"/Come"s"/3/Kavir	six-rowed	Spring	Moderate maturity	Iran
Reyhan	Rihane	six-rowed	Spring	Early maturity	ICARDA

ICARDA, International Center for Agricultural Research in the Dry Areas

The sowing date was the beginning of December and the harvest date was the beginning of June.

Rainfall during the growing season was 90 mm in the first year and 114 mm in the second year of the experiment.

Each plot was composed of six lines of 6m in length, sown at a distance of 20 cm between lines to achieve a density of 400 grains per m^2. Broadleaf weeds were controlled with 2,4-D herbicide at a rate of five litres per hectare prior to emergence. Grains were collected from the Agricultural Research Centre and Natural Resources of Fars Province. The SA was purchased from Merck Co. (Darmstadt, Germany).

Measurement of traits

Five plants were taken from the third and fourth rows by removing the two lateral edges and immediately taken to the laboratory to be measured. In this study, chlorophyll a and chlorophyll b contents (Lichtenthaler and Wellburn, 1983) and proline contents (Bates et al., 1973) were measured using a spectrophotometer (PG Instrument Ltd., UK). Total chlorophyll was measured using a SPAD-CCM-200 plus (Opti-Science). Samples were taken mainly from the apex of the plant, as these are the youngest fully developed leaves, to reduce variation due to leaf age.

Extract preparation for antioxidant enzyme assay, enzymatic antioxidants. Flag leaves of three barley cultivars (0.25 g) were extracted in 5 ml of potassium phosphate buffer (pH 7.8) at 4°C and centrifuged at 12,000 rpm for 15 minutes. The supernatant was used to determine the activities of enzymatic antioxidants.

The catalase activity of the extract was expressed as catalase activity units: min-1 mg-1 protein (Cakmak and Horst, 1991). The peroxidase activity was measured specifically with guaiacol. The increase in absorbance at 470 nm was recorded in a mixture of 0.1 ml of enzyme extract with 3 ml phosphate buffer (50 mM; pH=7) containing 0.05 ml guaiacol and 0.03 ml H2O2 (Nakano et al., 1980). Enzyme activity was expressed as units of enzyme activity per mg of protein.

To measure the relative water content (RWC) of the leaves, samples from the most recently developed leaf were collected from all experimental treatments. Fresh leaves were weighed, then submerged in distilled water for 24 hours, after which they were gently dried with tissue paper and reweighed to determine their turgid weight. Leaves were dried in a drying oven (at 70 °C for 48 h) to constant weight. The RWC was calculated using equation 1 (Ritchie and Nguyen, 1990):

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100 \quad (1)$$

In both years, agronomic yield was determined by measuring the plant height (PH; cm) of the main above-ground shoot at maturity in each plot after the plants had matured. Five spikelets were separated to determine the spike length (SL; cm) and weight (g) and the number of grains per spikelet (NG). Total aboveground biomass (i.e. straw and grain) was manually harvested on a surface of 1 m2 near the soil surface, sun-dried and then calculated as biological yield (BY; kg ha-1) weight, while grain yield (GY; kg ha-1) was determined after threshing the grain from the biomass. Specific leaf area (SLA) was calculated using equation 1 (Beadle 1993):

$$SLA = \frac{LA}{LDW} \quad (2)$$

In this formula, LA is the leaf area per unit area and LDW is the leaf dry weight.

Statistical analysis

After the physiological and biochemical evaluation, statistical analyses were carried out with the use of MSTAT-C and the SAS system software version 9.4 (Delwiche and Slaughter, 2019).

Data were analyzed using a combined analysis of variance and means compared with Duncan's multiple range tests. Finally, tables and calculations were carried out using Excel software. The results of the Bartlett's test to examine the homogeneity of the data in two crop years did not show a significant difference. They indicated the homogeneity of the data in the two years of the study. Two-year combined analysis of variance was performed on the data for this purpose.

RESULTS AND DISCUSSION

SLA and RWC

Analysis of variance showed that the mean squares due to drought stress (DS), cultivar (C), salicylic acid (SA) and their interactions were significant ($p \leq 0.05$ or $p \leq 0.01$) for SLA and RWC (Table 3). Year and often its interaction effects did not significantly affect SLA and RWC. Foliar application of salicylic acid increased SLA and RWC at all growth stages (Figure 2).

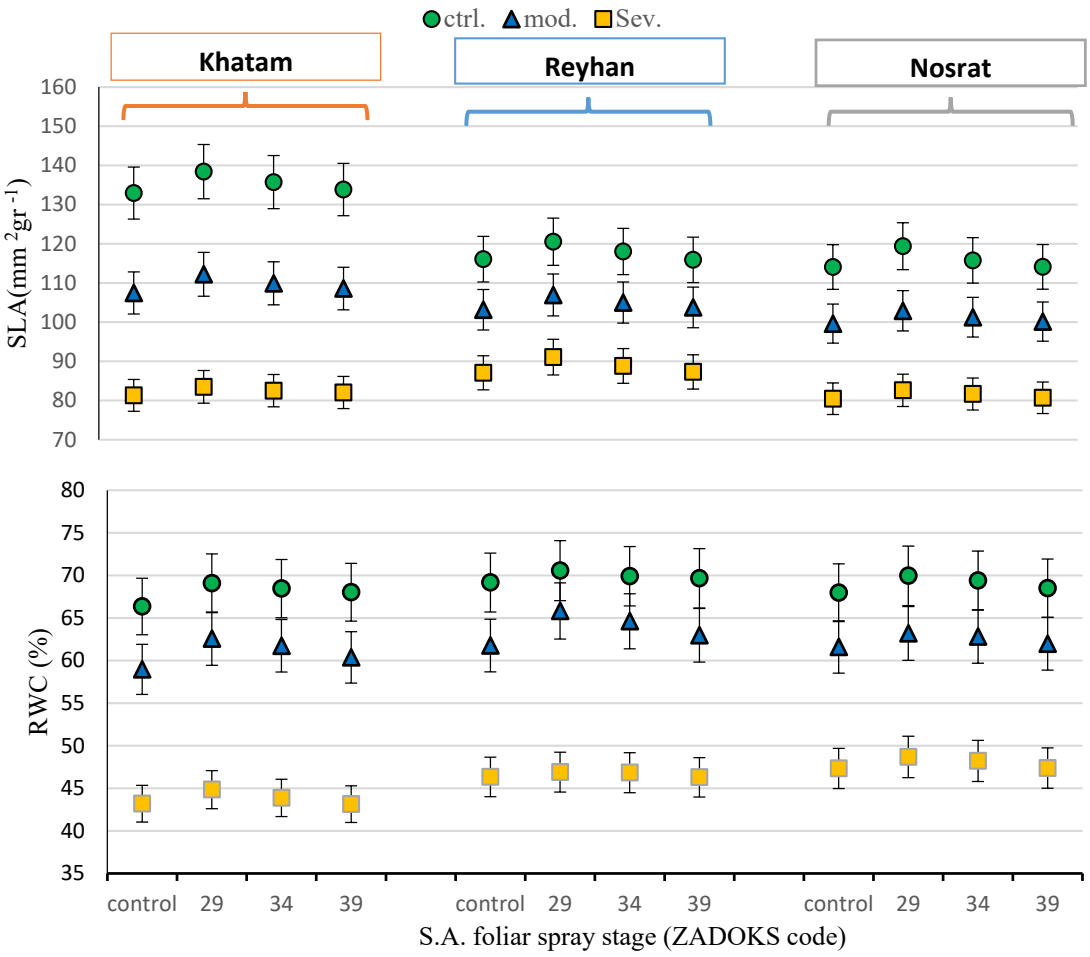

Figure 2. Triple interaction of investigated factors on the concentration of Specific leaf area (SLA), Relative water content (RWC), Columns with at least one letter in common do not have a statistically significant difference (Duncan 5%).

Based on the findings, the highest SLA of the flag leaf was with the SA leaf application at ZGS 29. A delay in foliar application reduced the plant's ability to use SA and its effect on SLA (Figure 2). The highest SLA was obtained from cultivar Reyhan in the high stress treatment (88.56 $mm^2.g^{-1}$). As the stress level decreased, Khatam cultivar's SLA increased compared with other cultivars. In the moderate and control treatments, the SLA of this cultivar was 109.54 and 135.23 mm2/g, respectively. In the severe stress treatment, the SLA of Khatam (39.1%), Reyhan (24.7%) and Nosrat (29.8%) cultivars decreased compared to the control, indicating the greater tolerance of Reyhan cultivar to the reduction in SLA due to low irrigation (Figure 2).

Table 3. Combined analysis of variance of the effect of levels irrigation, cultivar and time of salicylic acid foliar application on Specific leaf area (SLA), Relative water content (RWC), Plant Height (PH), Spike Length (SL), Number of Grains $Spike^{-1}$ (NG), Biological Yield (BY), Grain Yield (GY) during the two growing seasons

SOV	df	SLA	RWC	PH	SL	NG	BY	GY
Year (Y)	1	476.15^{ns}	1219.18^{ns}	2028.91^{ns}	1.0556^{ns}	91.00^{ns}	171760.56^{ns}	8957260.2^{ns}
Replications (R)	4	0.04	0.21	26.26	0.04861	0.21	45.16	197.3
Irrigation (I)	2	27180.97**	9960.59**	40830.72**	133.09**	3796.53**	10985868.29**	452554229**
Y × I	2	39.46^{ns}	468.01^{ns}	242.13^{ns}	0.05296^{ns}	9.57^{ns}	28797.23^{ns}	715622.3^{ns}
Yr × I	8	0.20	0.19	55.54	0.08627	0.21	43.64	699.4
Cultivar (C)	2	1684.14**	135.53**	4652.18**	17.1119**	161.28**	122386.79**	3180166.5**
Yr × C	2	30.70^{ns}	1.48^{ns}	106.70^{ns}	0.26601^{ns}	4.71^{ns}	2496.84^{ns}	401415.3^{ns}
I × C	4	942.80**	21.20*	517.32**	1.77359**	29.67**	30454.54**	1068303**
Yr × I × C	4	2.54^{ns}	2.69^{ns}	65.13^{ns}	0.27246*	6.25^{ns}	2097**	151209.3**
Salicylic acid (SA)	3	168.02**	38.64**	299.38**	5.33418**	13.24**	17474.17**	1239667.1**
Yr × SA	3	1.88**	0.39**	7.72^{ns}	0.09156^{ns}	0.81^{ns}	212.98^{ns}	7236.5^{ns}
I × SA	6	5.87**	3.91**	14.64**	0.33371**	0.42**	2726.29**	241932.8**
Yr × I × SA	6	1.16^{ns}	3.30^{ns}	17.69^{ns}	0.28531*	0.41^{ns}	267.59**	5536.1**
C× SA	6	0.81**	1.69**	2.58**	0.42666**	1.34**	168.16**	20706.3**
Yr × C × SA	6	0.86**	3.20**	9.29^{ns}	0.07411^{ns}	0.38^{ns}	314.91^{ns}	2463.6^{ns}
I × V× SA	12	1.19**	2.43**	3.42**	0.30924**	0.14**	138.25**	5224.2**
Yr× I × C × SA	12	1.30^{ns}	0.52^{ns}	4.80^{ns}	0.10736^{ns}	0.20^{ns}	354.49^{ns}	2635^{ns}
Error	132	0.11	0.09	6.38	0.10837	0.16	57.14	307.7
CV%		12.8	11.9	14.2	14.5	12.2	29.8	18.9

^{ns}, * and **: no significant, significant at the 5% and 1% probability levels, respectively

Under stress conditions, severe and moderate leaf RWC belonged to cultivar Khatam (63.06 and 68.57%). However, with increasing irrigation cycles under control conditions, the maximum RWC of flag leaves belonged to Reyhan and Nosrat cultivars. The RWC of each cultivar decreased in the severe stress treatment compared to the control. This reduction belonged to Khatam, Rehan and Nosrat cultivars at severe stress treatment with 7.7, 11.3 and 34.1%, respectively, compared to the control. Compared to the other cultivars studied; Khatam showed the lowest sensitivity to low irrigation (Figure 2). In the interaction of SA foliar application and stress treatments, it was observed that the RWC of the flag leaf with foliar application at ZGS29 was the highest among the foliar application treatments in each stress treatment. Flag leaf RWCs in this treatment were 2.6, 1.5 and 2% higher than control in heavy, medium and control treatments, respectively. Siosemardeh et al. (2004) stated that wheat tolerant cultivars have higher relative water content than drought-sensitive cultivars under stress conditions. Colem et al. (2003) considered the reason for the decrease in the relative moisture content of the leaf to be the decrease in water absorption from the roots in dry conditions. Colom and Vazzana (2003) considered the reason for the decrease in the relative moisture content of the leaf to be the decrease in water absorption from the roots in dry conditions.

Under drought stress, photosynthetic machinery can be damaged by stomatal and non-stomatal limitations (Reddy et al. 2004). Drought reduces the level of photosynthesis and the amount of photosynthesis, which reduces dry matter accumulation (Lawlor, 1981). Based on results from Ghotbi-Ravandi et al. (2014), stomatal conductance is the main factor limiting photosynthesis in Barley under mild drought stress. Low irrigation of wheat plants during the reproductive stage caused a reduction in most of the measured traits compared to the control. The greatest reduction was observed in the low irrigation treatment during the flowering stage. The decrease in photosynthesis and transfer of nutrients to the spike reduces the dry matter of each plant, which ultimately reduces yield (Barati et al., 2020).

Singh et al. (2023) have reported Moisture stress induced a reduction of relative water content. Moreover, a decrease in the RWC in plants under stresses such as drought and salinity may be due to the loss of turgor pressure, resulting from the cell's limited access to water (Soltys-Kalina et al., 2016). Cornic (2000) reported a decrease in the RWC has been known to induce stomata

closure and thus a parallel reduction in photosynthetic efficiency. Barley plants treated with salicylic acid have more stem length and weight than non-treated plants. These plants have higher sugar and proline content, indicating that they apply higher osmotic regulation to increase the RWC in the plant (Abdelaal et al., 2020). Singh et al. (2023) have shown that Application of SA and GB enhanced photosynthesis, improved stomatal conductance, and maintained higher RWC. Wheat treatment with salicylic acid increases the leaf's RWC (Hafez and Farig, 2019). In other words, the leaf's RWC is a key indicator of the degree of cell and tissue dehydration (Silva et al., 2007). Thus, measuring the RWC of the flag leaf is used as a significant index in determining the water status of the plant and identifying cultivars resistant to drought stress (El-Seidy et al., 2013).

Plant Height, Spike Length, Number of Grains Spike−1, Biological Yield and Grain Yield

Analysis of variance showed that the mean squares due to drought stress (DS), cultivar (C), salicylic acid (SA) and their interactions were significant ($p \leq 0.05$ or $p \leq 0.01$) for plant height, spike length, number of grains spike-1, biological yield and grain yield (Table 3).

The highest plant height in the control conditions belonged to Reyhan and Nosrat cultivars. As the stress level increased, the plant height and two cultivars' height decreased by 48.7% and 60.6%, respectively. The lowest plant height with an average of 28.25 cm belonged to cultivar Khatam under severe stress conditions (Table 4). SA applied on the leaves on ZGS29 had the highest plant height in all stress conditions. Foliar application at this growth stage increased plant height by 6.6, 5.7 and 21.5% under control, moderate and severe stress conditions, respectively, compared to the non-foliar treatment. Under control conditions, the maximum spike length was obtained from Reyhan and Nosrat with an average of 6.53 and 6.4 cm, respectively.

Table 4. Means comparison of interaction effects of levels irrigation, cultivar and time of salicylic acid foliar application on Plant Height (PH), Spike Length (SL), Number of Grains Spike^{-1} (NG), Biological Yield (BY), Grain Yield (GY)

Drought stress	Cultivar	PH (cm)	SL (cm)	NG	BY (kg ha^{-1})	GY (kg ha^{-1})
Control	Khatam	75.21 c	5.29 b	38.61 b	11460.67 c	5069.75 c
	Reyhan	84.29 a	6.53 a	39.64 a	13020.42 a	5934.17 a
	Nosrat	83.50 a	6.4 a	36.33 c	12230.58 b	5649.5 b
Moderate	Khatam	57.92 e	4.33 c	36.5 c	9900.96 f	4140.5 f
	Reyhan	81.75 b	5.49 b	37 c	10630.38 d	4339.54 e
	Nosrat	64.17 d	5.18 b	33.98 d	10500.58 e	4434.58 d
Severe	Khatam	28.25 h	3.17 d	24.58 f	4630.71 i	644.54 h
	Reyhan	43.25 f	3.52 d	26.34 e	4810.29 g	739 g
	Nosrat	32.92 g	3.43 d	22.08 g	4740.54 h	782.08 g
Drought stress	S.A. foliar spray stage (ZGS)					
Control	control	78.83 c	5.85 abc	37.49 c	11930.22 d	5269.06 d
	29	84.06 a	6.4 a	38.77 a	12590.83 a	5800.33 a
	34	81.72 b	6.26 a	38.57 ab	12320.78 b	5666.39 b
	39	79.39 c	5.95 ab	37.94 bc	12110.06 c	5468.78 c
Moderate	control	66.33 f	4.88 d	34.34 e	10150.39 h	4041.17 h
	29	70.11 d	5.46 a-d	35.57 d	10610 e	4502.67 e
	34	67.78 e	5.22 bcd	35.42 d	10410.39 f	4419.89 f
	39	67.56 e	4.45 cd	34.75 e	10220.11 g	4255.78 g
Severe	control	32.06 i	3.2 e	24 f	4690.89 k	702 j
	29	38.94 g	3.66 e	24.68 f	4800.61 i	746.11 i
	34	35.61 h	3.43 e	24.54 f	4720.39 j	730.33 ij
	39	32.61 i	3.2 e	24.11 f	4690.83 k	709.06 j
Cultivar	S.A. foliar spray stage (ZGS)					
Khatam	control	51.94 i	4.3 f	32.71 c	8506.1 k	3100.28 l
	29	56.61 g	4.53 de	33.5 b	8862.2 h	3394.33 i
	34	54.22 h	4.32 ef	33.41 b	8742.2 i	3363.44 j
	39	52.39 i	3.9 g	33.29 b	8573.9 j	3281.67 k
Reyhan	control	67.28 c	4.89 c	32.86 c	9325 d	3487.11 g
	29	73 a	5.7 a	34.23 a	9716.7 a	3859.83 a
	34	70.67 b	5.38 b	34.1 a	9531.7 b	3741.5 c
	39	68.11 c	4.75 cd	32.9 c	9387.8 c	3595.17 e
Nosrat	control	58 fg	4.75 cd	30.27 g	8953.9 g	3424.83 h
	29	63.5 d	5.28 b	31.28 d	9435.6 c	3794.94 b
	34	60.22 e	5.22 b	31.02 e	9191.7 e	3711.67 d
	39	59.06 ef	4.77 c	30.61 f	9068.3 f	3556.78 f

Averages with at least one common letter in each part of the column do not have a statistically significant difference (Duncan 5%)

Plant height and flag leaf are two important agronomic traits for crop yield (Cheng et al., 2023). For their part, Ledesma-Ramírez et al. (2023) evaluated three irrigation schedules, 2, 3 and 5 irrigations, where the phenological characters and especially the plant height were affected by the limited irrigation. These results are in agreement with the results of the present study. Drought stress causes a decrease in turgor pressure and thus a decrease in cell growth and development, especially in the spike and leaves. Drought-stressed plants had shorter duration of grain filling than well-watered plants. The reduction in cell enlargement and cell division reduces leaf area, photosynthesis rate, and ultimately plant height. The reduction in growth and development limits cell growth. In other words, a reduction in photosynthetic material due to a decrease in leaf area and a reduction in the transfer of photosynthetic products to the reproductive organs due to low irrigation leads to a decrease in yield (Lv et al., 2023). Samarah (2005) showed that drought stress treatments reduced grain yield of barley by reducing the number of tillers, spikes and grains per plant and individual grain weight. Salicylic acid will likely improve nutrient uptake under drought and salt stress conditions, increasing growth and plant height (Moharekar et al., 2003). Plant height is influenced by several factors that are closely related to yield and quality, broadly divided into endogenous hormones and the external environment (Cheng et al., 2023). Researchers reported a 13% increase in wheat yield under salinity stress conditions due to the application of salicylic acid (Arfan et al., 2006).

Under the severe stress conditions, the spike length of the investigated cultivars did not show any statistically significant difference. Spike length decreased by 40.1, 46.1 and 46.4% in Khatam, Reyhan and Nosrat, respectively, under severe stress compared to the control. SA foliar application caused a significant increase in spike length in control and moderate stress treatments. However, foliar application of SA under severe stress did not significantly affect spike length in any of the treatments studied. Each cultivar's highest spike length was associated with foliar application of SA at the end of tillering (ZGS29). The highest spike length was associated with cultivar Reyhan with a mean of 5.7 cm and foliar application at ZGS29 (Table 4).

Reyhan cultivar had the highest number of grains per spike in each stress level. 36.3, 33.6 and 39.2% in Khatam, Reyhan and Nosrat cultivars reduced the number of grains per spike in the severe stress conditions compared to the control treatment. In the control and SA, the highest number of grains per spike was found in stages 29 and 34 of Zadoks, without significant statistical difference. The foliar application of SA did not significantly affect the number of grains per spike under severe stress conditions. In each of the cultivars studied, foliar application of SA at the end of tillering had the most excellent effect on the number of grains per spike. Reyhan had the highest spike number in the experiment under the effect of leaf spray at the end of tillering stages 29 and 34 of Zadoks, with an increase of 4.2% and 3.8%, respectively, compared to the non-applied control (Table 4). The spike is the grain-bearing organ in cereals that is an essential proxy for grain yield and quality (Ling et al. 2023), and spike length is a trait that plays a key role in grain yield. Low irrigation can reduce spike length in wheat plants by shortening the growing season (Sallam et al., 2019) and increasing the growth rate. Drought can also reduce spike length in wheat plants through a direct negative effect on the terminal meristem that forms the spike (Gooding et al., 2003). Research on wheat plants reported a reduction in the number of grains per spike due to low irrigation (Cattivelli et al., 2008). The reduction in the number of grains per spike can be due to the role of drought in slowing down the spike formation or the division of meiosis in the gametes, and the fertility of the eggs and the earlier development of the grains. The present study results revealed that salicylic acid foliar application under drought stress significantly increased the number of grains per spike. It is in line with the results of a study conducted by Hafez and Farig (2019), who stated the Phytohormone Salicylic Acid positively affect the number of grains per wheat spike in low-irrigation conditions. The biological yield includes the dry weight of all the aerial parts of the plant, which is affected by genotype and growing environment conditions (El-Seidy et al., 2013).

The highest biological yield in each stress condition belonged to the Reyhan cultivar and the lowest in all stress conditions was obtained from the Khatam cultivar. After foliar application of SA in each growth stage, the biological yield increased compared to the control without foliar application (Table 4). In each stress condition, the biological yield increased after foliar application at ZGS29, which was 5.5%, 4.5% and 2.3% in minimum, moderate and severe stress conditions, respectively, compared to the control without foliar application. The foliar application in ZGS29 had the highest biological yield in each of the cultivars studied. The increase in yield due to foliar application at this growth stage compared to the control was 1.4%, 4.2% and 5.4% in Khatam, Reyhan and Nosrat, respectively.

Under control conditions, the highest grain yield belonged to the cultivar Reyhan with an average of 5934.17 kg per hectare (Table 4). The highest grain yield was obtained from Nosrat cultivar in moderate and severe stress conditions. Grain yield of Khatam cultivar was lower than other cultivars in all stress conditions. Applying SA in all stress conditions increased grain yield compared to the control without foliar application. This rate of increase due to foliar application in ZGS29 was greater than other foliar treatments. The foliar application of SA at this growth stage increased grain yield by 10.1%, 11.4% and 6.3%, respectively, in minimum, moderate and severe stress conditions compared to the control. The results showed that the highest increase in grain yield due to foliar application occurred under control conditions. In all the cultivars studied, the highest yield increase after foliar application was obtained in ZGS29. This increase in Khatam, Reyhan and Nosrat cultivars compared to the

control was 9.5%, 10.7 and 10.8%, respectively, indicating the greater effectiveness of SA foliar application on grain yield of Nosrat cultivar from Table 4. The delay in SA foliar application in the cultivars caused a reduction in grain yield (Table 4).

SPAD index Chlorophyll a and b

Analysis of variance showed that the mean squares due to drought stress (DS), cultivar (V), salicylic acid (SA) and their interactions were significant ($p \leq 0.05$ or $p \leq 0.01$) for the SPAD index chlorophyll a and b (Table 5). The highest level of this reduction in the severe stress treatment compared to the control belonged to cultivar Khatam (26.6%). The reduction in leaf chlorophyll index under severe stress compared to the control was 25% and 25.8% in Reyhan and Nosrat cultivars, respectively. Leaf chlorophyll index increased with increasing irrigation cycles in each SA foliar treatment (Figure 3). Despite the positive effect of SA foliar application, in the stress treatments, the control treatment (no foliar application) was not significantly different in terms of leaf chlorophyll index due to foliar application at the end of ZGS39 in each of the stress treatments. In each stress treatment, the foliar application of SA at ZGS29 had the highest leaf chlorophyll index. However, the foliar application of SA could not compensate for the reduction in leaf chlorophyll index caused by the decrease in the number of irrigations. The highest leaf chlorophyll index was obtained from the foliar application at ZGS29 with control irrigations (55.12 units).

Table 5. combined analysis of variance of the effect of irrigation, cultivar and time of salicylic acid foliar application on Leaf Chlorophyll Index (SPAD), Chlorophyll a (Chl a), Chlorophyll b (Chl b), peroxidase activity (POX), catalase activity (CAT), and Proline Concentration (PC) during the two growing seasons

SOV	DF	SPAD	Chl a	Chl b	POX	CAT	PC
Year (Y)	1	16.58^{ns}	0.130^{ns}	0.037^{ns}	544427.6^{ns}	35.46^{ns}	581.91^{ns}
Replications (R)	4	0.66	0.001	0.000	14413.75	23.33	4.41
Irrigation (I)	2	3445.23**	3.748**	1.855**	7148.26**	661.69**	397.07**
Y × I	2	2.49^{ns}	0.204^{ns}	0.009^{ns}	1518.15^{ns}	6.03 ns	15.88^{ns}
Yr × I	8	0.39	0.001	0.000	10345.12	46.69	10.65
Cultivar (C)	2	1103.20**	0.453**	0.023**	9708.38**	63.16**	50.75**
Yr × C	2	9.07^{ns}	0.034^{ns}	0.004^{ns}	5856.32	0.46	2.03^{ns}
I × C	4	11.43**	0.101**	0.110**	1506.18**	23.29**	17.74**
Yr × I × C	4	13.21^{ns}	0.015^{ns}	0.003^{ns}	730.81^{ns}	0.40^{ns}	0.71^{ns}
Salicylic acid (SA)	3	47.21**	0.018**	0.007**	5705.21**	12.62**	11.31**
Yr × SA	3	1.66**	0.005**	0.000**	19.57**	0.52**	0.45**
I × SA	6	0.39**	0.007*	0.001*	399.10**	4.02**	1.63**
Yr × I × SA	6	0.72^{ns}	0.001^{ns}	0.001^{ns}	335.21^{ns}	0.43 *	0.77^{ns}
C × SA	6	1.07*	0.003*	0.001**	1857.44*	27.53*	0.07**
Yr × C × SA	6	1.26**	0.004**	0.001**	468.04**	0.37**	0.03**
I × C× SA	12	1.28**	0.002**	0.001**	527.52*	4.77*	0.11**
Yr× I × C × SA	12	0.47^{ns}	0.002^{ns}	0.001^{ns}	428.21**	0.30**	0.00^{ns}
Error	132	0.36	0.001	0.000	575.37	5.36	0.01
CV%		12.1	9.8	11.3	22.5	13.80	12.27

^{ns}, * and **: no significant, significant at the 5% and 1% probability levels, respectively

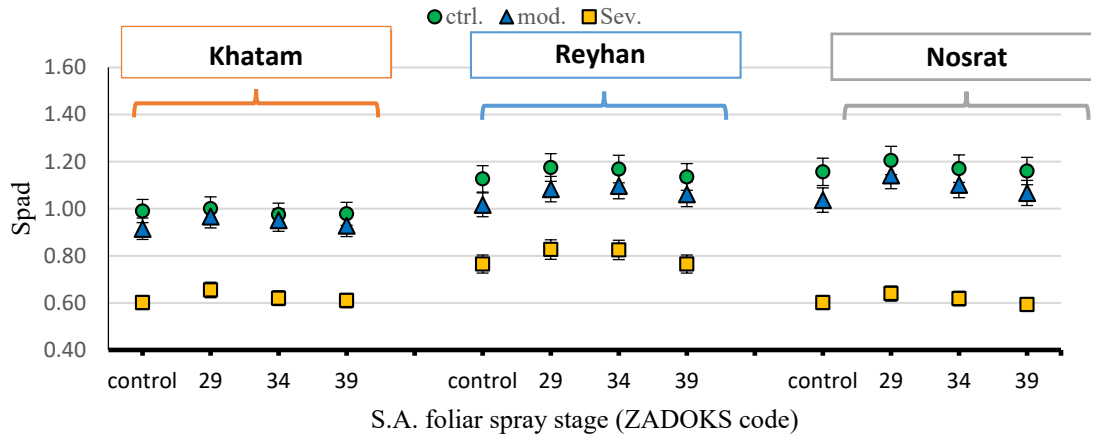

Figure 3. Triple interaction of investigated factors on the concentration of Spad. Columns with at least one letter in common do not have a statistically significant difference (Duncan 5%)

The lowest reduction of chlorophyll a and b in moderate stress conditions belonged to cultivar Khatam with an average of 4.5%. The lowest level of chlorophyll a reduction under severe stress conditions belonged to cultivar Reyhan with an average of 24.8%. Also, the highest chlorophyll b content with an average of 0.58 mg. g-1 fresh weight of leaves was obtained from cultivar Khatam under control conditions. The chlorophyll b content of this cultivar was the highest among the cultivars studied under moderate stress conditions. Under severe stress conditions, the highest amount of chlorophyll b belonged to cultivar Reyhan. Under severe stress conditions, the highest chlorophyll b content was obtained with SA foliar application at ZGS29 stage. However, it was not significantly different from the flag leaf emergence stage in the severe stress conditions (Figure 4). The highest chlorophyll content in each stress condition belonged to the SA foliar application at ZGS29 stage. In addition, the highest chlorophyll b content was obtained from the foliar application at the stage of ZGS29 in cultivar Nosrat with an average concentration of 0.42 Mg. g-1 fresh weights of leaves (Figure 4).

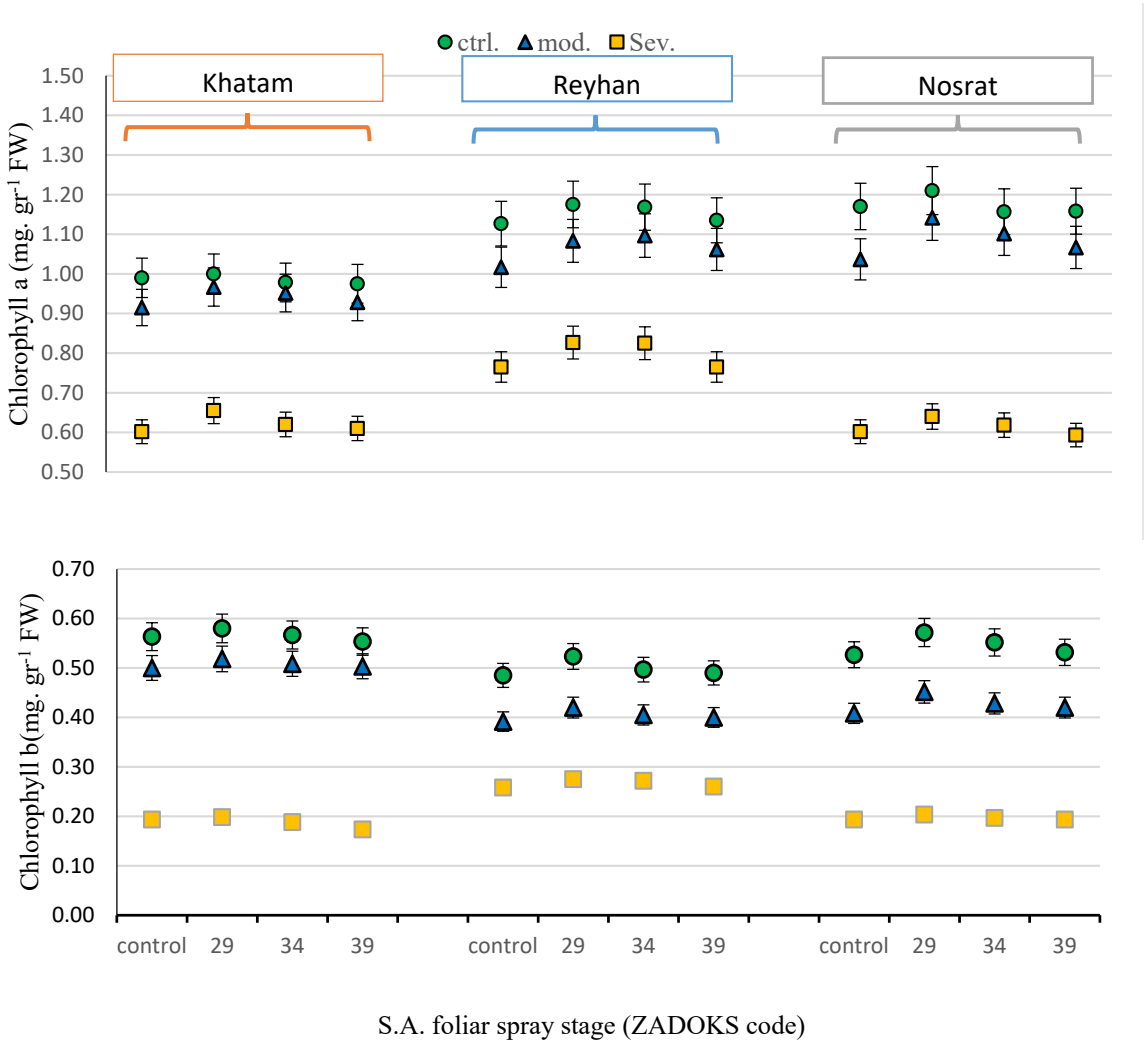

Figure 4. Triple interaction of investigated factors on the concentration of chlorophyll a and chlorophyll b Columns with at least one letter in common do not have a statistically significant difference (Duncan 5%)

The reduction in chlorophyll content in low irrigation conditions may be due to the reduction in chlorophyll synthesis and its destruction (Nouri et al., 2020). Indeed, with drought stress, the amount of photosynthesis decreases and the chlorophyll index decreases (Stone et al., 2003). The researchers examined the effect of drought stress on the activity of antioxidant enzymes in barley plants. The chlorophyll content decreased with the aging of the plant and under the impact of drought stress. Drought stress reduced plant yield, leaf area, chlorophyll content, and overall plant growth (Zhu et al. 2004). Also, Pirnajmedin et al. (2020) have shown that Mild and intense drought stress conditions led to depression in photosynthetic pigments including chlorophyll a and b, total chlorophyll contents and carotenoids. Salicylic acid acts as an antioxidant in drought stress conditions and

prevents pigment damage, especially chlorophyll. Based on the reports, salicylic acid improves photosynthesis in drought stress by preventing damage to chlorophyll (Khan et al., 2003; Pirnajmedin et al., 2020).

Catalase activity, Peroxidase activity and Proline content

ANOVA revealed that the means of drought stress (DS), cultivar (C) and salicylic acid (SA) were significant ($p \leq 0,05$ or $p \leq 0,01$) in catalase activity, peroxidase activity and proline content (Table 5). Catalase activity increased significantly under stress conditions in the cultivars that were able to tolerate stress (Figure 5). The highest catalase activity was obtained in cultivar Reyhan with an average of 9.80 U mg-1 protein.The highest catalase activity was obtained from the interaction of cultivar Reyhan with foliar application in ZGS39. Foliar application in ZGS39 increased catalase activity by 40.3, 27.2 and 17.4% under normal, moderate and severe stress conditions compared to non-foliar treatment (Figure 5).

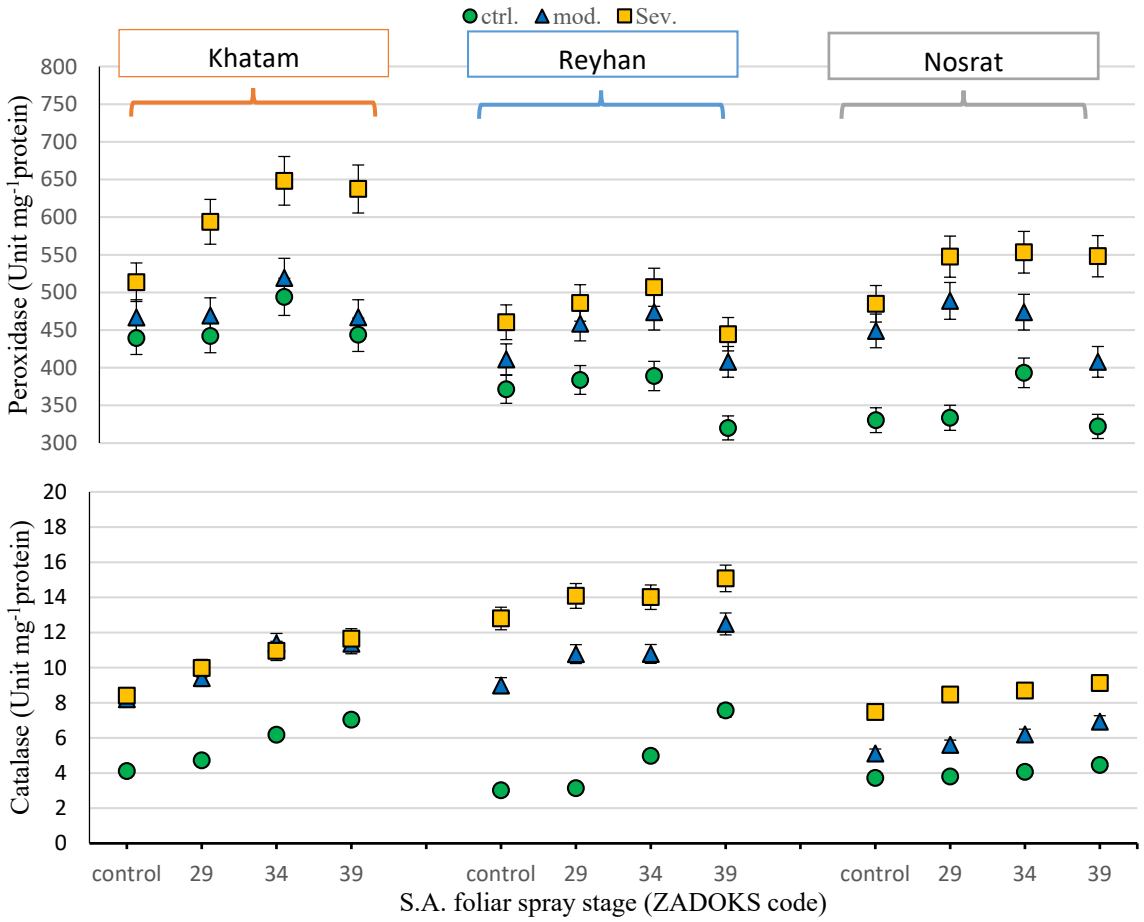

Figure 5. Triple interaction of investigated factors on the concentration of Peroxidase and Catalase Columns with at least one letter in common do not have a statistically significant difference (Duncan 5%)

There was an increase in peroxidase activity in the cultivars under stress conditions (Figure 1). The highest peroxidase activity was obtained in cultivar Khatam with an average of 511.2 U mg^{-1} proteins. Peroxidase activity increased in the severe stress treatment compared to the control in cultivars Khatam (31.5%), Reyhan (29.6%) and Nosrat (54.7%).The highest peroxidase activity was obtained from the interaction of cultivar Khatam with foliar application at ZGS34 (Figure 5).

The highest proline levels were found in the Reyhan cultivar, averaging 13.99 mmol g^{-1}. The application of regular irrigation in Khatam, Reyhan and Nosrat cultivars reduced the proline content by 46.2, 66.6 and 52.4% respectively compared to the severe stress treatment. This index's lowest and highest values were obtained for all SA levels in control, moderate and severe stress, respectively. The proline content of Reyhan cultivar decreased with the delay in foliar application. However, this trend increased in Khatam and Nosrat. The three-way interaction of the studied factors showed that the highest proline content in the severe stress treatment was obtained in the Reyhan cultivar and no leaf application with a mean of 15.08 mmol/g fresh weight of the plant (Figure 6). However, in the normal irrigation treatment, the highest proline content was obtained from the foliar application treatment at ZGS29, in all the cultivars studied.

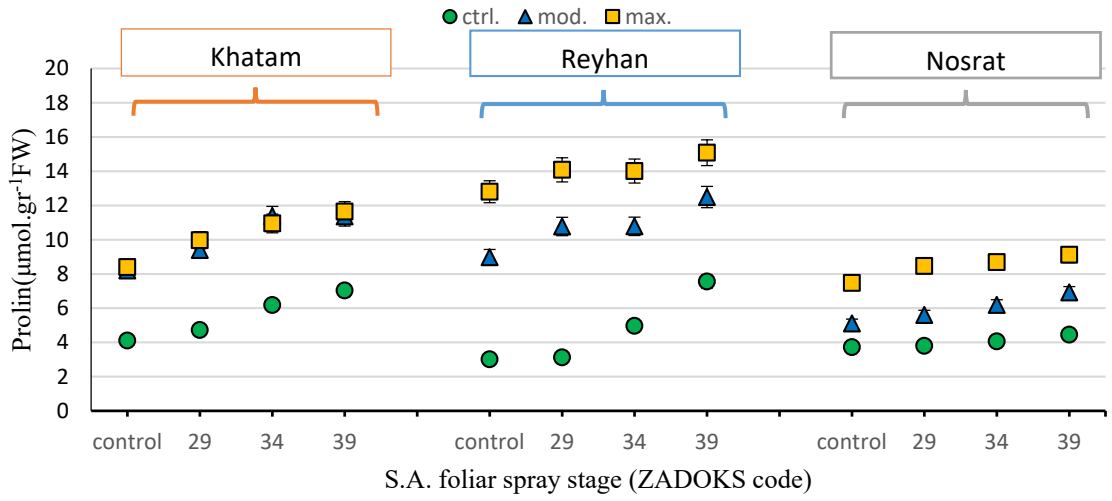

Figure 6. Triple interactions of investigated factors on proline concentration Columns with at least one letter in common do not have a statistically significant difference (Duncan 5%)

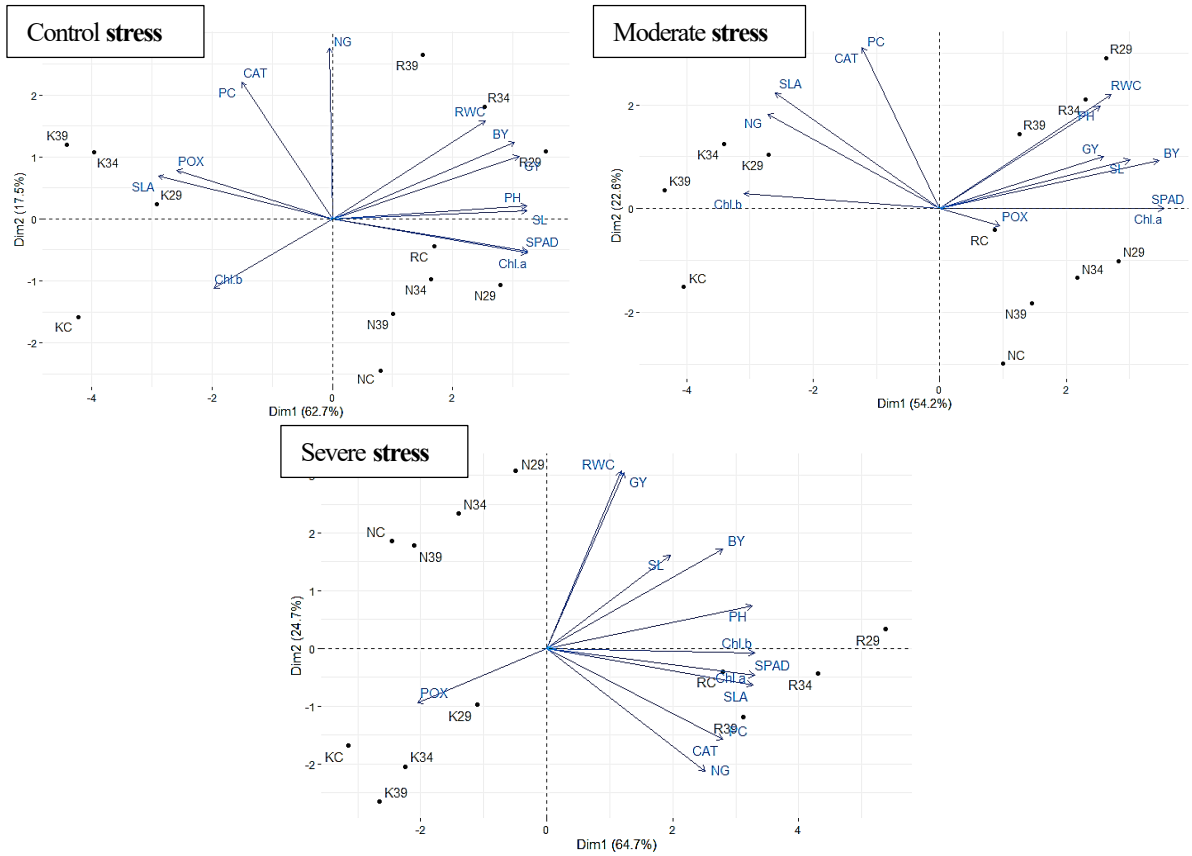

Figure 7. Principal Component Analysis (PCA) of data for all characteristics of plants under the different Irrigation. Plant Height (PH), Spike Length (SL), Number of Grains Spike^{-1} (NG), Biological Yield (BY), Grain Yield (GY), Leaf Chlorophyll Index (SPAD), Chlorophyll a (Chl a), Chlorophyll b (Chl b), peroxidase activity (POX), catalase activity (CAT), and Proline Concentration (PC).

A series of secondary metabolites, especially amino acids, accumulate in plants when they are exposed to stress conditions. Amino acids as precursors of protein compounds play a significant role in plant growth and development metabolism (Trovato et al., 2021). Based on the results of the present experiment, Application of SA decreased the adverse effects of drought stress by elevation of photosynthetic pigments and could enhance

barley yield and drought tolerance in Case Study genotypes.

Proline as an amino acid plays a significant role in modulating drought stress. This role is due to maintaining osmotic potential, inhibiting reactive oxygen species, and finally preventing oxidative stress in plants (Verbruggen and Hermans, 2008). The increase of proline content along with the increase of environmental stresses such as drought is considered a strategy to protect the plant. Plants reduce the water potential by increasing organic compounds such as sugars and proline in the cell and through osmotic regulation. They provide the possibility of absorbing more water from environments under low water conditions (Ashraf and Foolad, 2007), so proline content in the tolerant line became more sensitive. Proline content was identified as the best physiological index for indirect selection of drought-tolerant genotypes in this present study these results are in agreement with the results reported by Barati et al. (2020).

A principal components analysis was conducted to evaluate the relationships between traits under drought stress treatments (Figure 7). As illustrated in the figure, the first and second components in control, moderat and serve stress accounted for approximately 62.7% and17.5%, 54.2% and 22.5%, and 64.7% and 24.7%, respectively. Approximately, all associations between characters have been affected by irrigation levels. Furthermore, most parameters were integrally occupied with high correlation with Reyhan cultivar in all drought stress specially in serve stress level, POX was associated with the Khatam (Figure 7).

CONCLUSIONS

The bush height decreased with the severity of drought stress. Drought stress also reduced grain yield and other yield-related components. Drought stress increased leaf proline. This increase in its concentration in the barley plant under drought conditions is a kind of defense mechanism for mitigating the effects of stress on plant cells. Spraying the leaves with salicylic acid increased the proline in the leaves and reduced the destructive effects of the stress. The chlorophyll a and b concentration also increased at the end of flag leaf emergence and 50% of stem emergence. The highest level of chlorophyll a was measured in the cultivar Reyhan and the highest level of chlorophyll b was measured in the cultivar Khatam. The application of salicylic acid compensated the effects of deficit irrigation on photosynthetic pigment traits, relative leaf water content, spike length, plant height, and number of grains per spike, biological yield, and grain yield. Salicylic acid generally increased grain yield, this positive effect was strongly correlated with foliar application time. Salicylic acid foliar application at the end of tillering stage had the highest yield among other foliar treatments. Generally, based on the results obtained, salicylic acid foliar application can improve plant tolerance against drought stress and reduce its complications by increasing the growth indices and affecting the biochemical characteristics of the studied barley cultivars. Among the investigated cultivars, grain yield of Reyhan cultivar was higher than other cultivars. It seems that this cultivar's superior yield is due to its superiority of the number of grains and spike length among the studied cultivars according to the studied yield components. The basil cultivar with the highest percentage of relative moisture in the flag leaves had the highest drought tolerance among the cultivars studied. The highest proline concentration was observed in the Reyhan cultivar, indicating greater drought stress tolerance than other cultivars. This result opens new perspectives in economics, the application of plant hormones, and plant breeding for crop production in deficit-irrigation conditions. Furthermore, it seems that more investigations are required in order to elucidate the physiological and biochemical mechanisms of drought tolerance in barley and in fact Our results demonstrate the potential of Timely use of salicylic acid for the protection and optimization of cereal crop yields under Deficit-Irrigation Condition.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest or personal relationships.

STATEMENTS AND DECLARATIONS

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IN VITRO CLONAL MICROPROPAGATION OF IZMIR OREGANO (*Origanum onites* L. cv. "Ceylan-2002")

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ABSTRACT

In this study, which was carried out to develop an *in vitro* clonal micropropagation procedure in elite cultivar of *Origanum onites* L. cv Ceylan-2002, node explants were subjected to pre-washing and different sterilization methods. The highest level of sterilization efficacy was achieved in sterilization trials using diverse durations and commercial bleach doses, with a 100% success rate. Mean regenerated shoots number per explant (0.13), mean shoot length (0.12 cm), and mean leaf number (0.87) were achieved in MS medium+2 mg l^{-1} BAP+0.3 gl^{-1} activated carbon after four weeks of culture. The highest propagation coefficient (3.40) was obtained in the 4th subculture. In the rooting experiments the highest mean stem thickness value (2.02 mm) and the highest mean shoot length (10.02 cm) were obtained in media containing 1.0 g l^{-1} activated carbon. The rooted plantlets (100%) were acclimatized with a survival success rate of 16.25%. Essential oil analysis of well-developed rooted plantlets was performed using GC-MSD. The highest essential oil content (2.00%) was determined in regenerated shoots from MS medium+2 mg l^{-1} IBA+1 g l^{-1} activated carbon. The highest thymol ratio (65.33%) was found in regenerated shoots from ½ MS media supplemented with 0.5 mg l^{-1} IBA+1 g l^{-1} activated carbon. The highest carvacrol ratio (6.82%) identified in regenerated shoots was obtained from MS media, including 2.0 mg l^{-1} IBA+1 g l^{-1} activated carbon.

Keywords: *Origanum onites* L., Ceylan-2002, *in vitro*, clonal micropropagation, essential oil analysis.

INTRODUCTION

Oregano (*Origanum* spp.) is a herbaceous plant belonging to the Lamiaceae family, and it includes many aromatic and medicinal plants. Türkiye, Chile, Peru, Mexico, Greece, Israel, Albania, Indonesia, and Egypt are the most important producers of the oregano plant, which has 40 species worldwide and 35 species in Turkey (Goleniowski et al., 2003; Atilabey et al., 2015; Tunca and Yesilyurt, 2017). The most exported oregano species are *Origanum onites* L., *Origanum vulgare* subsp. *hirtum*, *Origanum minutiflorum*, *Origanum majorana,* and *Origanum syriacum* var. *bevanii* (Bozdemir, 2019).

Oregano is used as a spice and is obtained from *Origanum* species. Although carvacrol and thymol are the predominant secondary metabolites in Origanum species, concentration of these compounds varies considerably between species (Oluk and Cakir, 2009; Bayram et al., 2010; Sevindik et al., 2017). These compounds are effective against cancer cells through some cell collapse, as well as protecting the cell membrane. *O. onites* L. contains rosmarinic acid, γ-terpinene, γ-cimene, α-terpinene and α-pinene compounds with carvacrol and thymol (Atar and Colgecen, 2019). Carvacrol is a natural essential oil with high importance in aromatic compounds. It has been demonstrated to possess a range of beneficial properties, including antioxidant, antimicrobial, anticancer, antidiabetic, cardioprotective, antiobesity, and hepatoprotective effects, as well as antiaging properties (El-Gengaihi et al., 2006; Silva et al., 2012; Memar et al., 2017; Imran et al., 2022). Thymol is known for its antioxidant, antispasmodic, antimicrobial, and anti-inflammatory effects (Memar et al., 2017). The amount of carvacrol and thymol compounds is an important factor in determining the price of oregano trade (Ozyazici and Kevseroglu, 2019).

Although oregano is a perennial herb, its commercial life is about seven years. Traditional production methods include seeds and cuttings (Sokat, 2021). However, in this method, the desired quality and standard production may not be achieved due to foreign fertilization (Bahtiyarca Bagdat, 2006; Colak Esetlili and Cakici, 2010). The collection of oregano plants from the natural flora leads to the extinction of many plant species, the loss of natural gene resources of these plants, and the collection of non-purpose material. Given the numerous challenges

associated with the conventional production of oregano, the development of alternative biotechnological methods is necessary for the large-scale cultivation of oregano species and the attainment of standardized products with consistent quality. (Gungor and Bayraktar, 2005; Bahtiyarca Bagdat, 2006; Tokul, 2015; Sonmez, 2019).

Plant tissue culture is the *in vitro* aseptic culture of cells, tissues, organs, and their components in solid or liquid media under defined physically and chemically controlled conditions (Thorpe, 2006; George and Manuel, 2013). Micropropagation provides rapid production of high-quality, disease-free plant materials bearing the characteristics of the species in a limited area in a short time. Plants can reproduce anywhere under controlled environmental conditions throughout the year, regardless of season and weather (George and Manuel, 2013). Techniques applied for clonal propagation of plants are generally time-consuming and labor-intensive (Datta et al., 2017).

Within the framework of this study, experiments were carried out to ensure the surface sterilization of the oregano cultivar 'Ceylan-2002', belonging to *Origanum onites L.*, and to investigate the effect of the presence of activated carbon (AC) in the medium content on the darkening after sterilization. It was tried to determine the effects of plant growth regulators (PGRs) in different types (BAP, IBA, and NAA) and doses on shoot multiplication in sterilized explants. Experiments were set up in MS and ½ MS media containing different IBA doses for rooting from shoots. The acclimatization of rooted plantlets was attempted. In addition, chemical analyses were made in micropropagated plants to determine the content of essential oils.

MATERIALS AND METHODS

Materials

In this study, node explants of 'Ceylan-2002' cultivar belonging to *Origanum onites* L. species registered by Ege University Faculty of Agriculture, Field Crops Department in Izmir were used as starting material.

Methods

Surface sterilization

After the shoots were collected from the field and separated from their leaves, the node parts were cut and prepared as explants. The explants were pre-washed for five min in water with detergent and then under running water for 10 min. In all trials, in the first step, explants were shaken in 70% EtOH for 1 min. Afterward, the explants were kept in commercial bleach (containing 5% NaOCl) at different doses (15, 20 and 30%) for different times (15-20 min). They were rinsed three times with sterile distilled water. Six different methods were tested for the surface sterilization of node explants (Table 1). After sterilization procedures, node explants were cultured in MS (Murashige and Skoog, 1965) media containing (AK0,3) and without (AK0) 0.3 g l^{-1} of AC. Culture vessels were maintained in a photoperiod of 16 hours light/8 hours dark, at 24±2°C and 3500 lux light intensity.

Table 1. Surface sterilization trials

Sterilization technique[a]	Commercial bleach doses (%)	Application time (min)
A	15	15
B	15	20
C	20	15
D	20	20
E	30	15
F	30	20

[a]: 70% EtOH in each sterilization technique was applied for 1 min.

Shoot regeneration

After the optimum sterilization protocol was determined, sterilized node explants were cultured in shoot regeneration media (KS) containing different PGRs, doses, and combinations, using MS as a basal medium. MS medium without PGR was used as the control group in the experiment (Table 2).

Table 2. Shoot regeneration treatments[a]

Culture medium- Codes	PGRs (mg l^{-1}/L)		
	BAP	IBA	NAA
KS1	-	-	-
KS2	-	0.5	-
KS3	-	1	-
KS4	-	2	-
KS5	0.5	-	-
KS6	1	-	-
KS7	2	-	-
KS8	0.5	0.5	-
KS9	0.5	1	-
KS10	0.5	2	-
KS11	1	0.5	-
KS12	2	0.5	-
KS13	1	1	-
KS14	1	2	-
KS15	2	1	-
KS16	2	2	-
KS17	-	-	0.1
KS18	2	-	0.1
KS19	-	-	0.2
KS20	2	-	0.2

[a] MS medium was used as the main medium in all experiments. 3 g l^{-1} gelrite was added as gelling agent. In addition, 0.3 g l^{-1} AC was added to all trials.

In vitro rooting and acclimatization

Regenerated shoots were cultured in MS and ½ MS media diversified with 0.3 g l^{-1} and 1 g l^{-1} AC and different IBA doses (0. 0.5, 1,0. 2,0 mg l^{-1}) for rooting experiments (Table 3). Eighty rooted plantlets (five plantlets from each medium) of Ceylan-2002 cultivar obtained from 16 different rooting media *in vitro* conditions were selected and acclimatized. The roots of the oregano shoots, carefully removed from the culture vessels, were cleaned by washing them with water to remove the gelling agent residues. Oregano plantlets were transferred to plastic cups containing peat, the bottom of which was pierced with a needle. After watering plantlets, each cup was covered with a perforated nylon bag. Transplanted plantlets were kept in 16 hours light/8 hours dark photoperiod, 3500 lux light intensity, and 24 ± 2ºC temperature conditions. After the acclimatization process, the bags on the plastic cups were periodically removed to adapt to the outdoor conditions, and the plantlets were aerated and watered a little. On the 18th day, the bags were completely removed, and the plantlets were stored for 12 days in laboratory conditions under a photoperiod of 16 hours light/8 hours dark, a light intensity of 3500 lux, and a temperature of 24±2ºC. After the 30-day acclimatization period, the shoots' survival rates (%) were recorded.

Table 3. *In vitro* rooting media

Culture medium- Codes	Basal medium	AC (g l^{-1})	IBA (mg l^{-1})
KK-1	MS	0.3	0
KK-2			0.5
KK-3			1
KK-4			2
KK-5	½ MS		0
KK-6			0.5
KK-7			1
KK-8			2
KK-9	MS	1	0
KK-10			0.5
KK-11			1
KK-12			2
KK-13	½ MS		0
KK-14			0.5
KK-15			1
KK-16			2

Essential oil analysis

Essential oil components analysis of well-developed rooted plantlets of Ceylan-2002 cultivar obtained from seven different rooting media *in vitro* conditions was carried out with GC-MSD in Ege University Drug Development and Pharmacokinetic Research and Application Center R&D Laboratories (ARGEFAR). The essential oil rate was determined by hydro-distillation using the Clevenger apparatus. A total of 50 g fresh leaves of *in vitro* plantlets were used for essential oil analysis.

Statistical analysis

All *in vitro* treatments were applied in a randomized plot design with three replications. The data obtained from the applications were evaluated using the Minitab 17 (Minitab®, LLC, Pennsylvania, USA, 2015) program.

RESULTS

Surface sterilization

The percentage (%) of sterile explants obtained as a result of applied sterilization methods is shown in Table 4. Observations were made four weeks after the explants were cultured. In addition, the problem of hyperhydricity was observed in the study, and observations were conducted to determine its effect (Table 4). Observations regarding hyperhydricity were made four weeks after explants were cultured. An examination of the sterile explant percentage values revealed that the CV values were generally less than 10%. When examined in this context, it shows that the data is consistent.

Table 4. Percentage of sterile explants obtained from different surface sterilization methods applied to node explants and of explants with hyperhydricity (%)[a]

Surface sterilization technique	Percentage of sterile explant (%) ± SE	CV(%)	Percentage of explants showing hyperhydricity (%) ± SE
A	43.33±3.33 c	13.32	56.67±3.33 a
B	100.00±0.00 a	0.00	26.67±3.33 b
C	80.00±0.00 ab	0.00	10.00±10.00 b
D	100.00±0.00 a	0.00	13.33±3.33 b
E	90.00±5.77 ab	10.00	6.67±3.33 b
F	100.00±0.00 a	0.00	6.67±3.33 b

Source	*F value*	*p-value*	*F value*	*p-value*
Sterilization techique	66.40	0.000	14.57	0.000

[a]Applications were made in 3 replications, and 10 explants were used for each replication. The differences between the mean values shown with different letters in the same column are significant at the P≤0.01 level according to the Tukey multiple comparison test. SE: Standard Error CV: Coefficiency of variation

Micropropagation

The mean number of regenerated shoots from node explants of the Ceylan-2002 cultivar was determined four weeks following the culture. This result is presented in Table 5 (Fig 1). Node explants belonging to 'Ceylan-2002' cultivar obtained from shoot regeneration experiments were subcultured nine times in KS-7 medium. Propagation coefficients calculated over the sum of node explants obtained from subcultures performed at four week intervals are given in Table 6. In the study, the mean leaf number, root number, and root length values per explant were also determined. No root formation was observed in the 1st and 2nd subcultures. The study revealed that as the number of subcultures increased, a small number of multiple shoots occurred in the node explants, particularly in subcultures 6-9 (Table 6, Fig 2). In the experiments where the effects of the number of subcultures were determined, the CV value was calculated in all experiments except root regeneration. When the multiplication coefficient values were examined, all values except the 3rd subculture were <20%, and it was seen that the data were consistent. In the values of the mean number of shoots obtained per node, shoot length obtained per explant, and number of leaves obtained per explant, the CV value was determined as <20% except for the 2nd subculture (Table 6).

Table 5. Some micropropagation parameters determined in MS media containing different types and doses of PGRs [a]

Media	Mean regenerated shoots number per explant±SE	Mean shoot length (cm)±SE	Mean number of leaves ±SE
KS-1	0.10±0.06	0.03±0.02	0.50±0.32
KS-2	0.03±0.03	0.02±0.02	0.30±0.30
KS-3	0.07±0.07	0.02±0.02	0.27±0.27
KS-4	0.10±0.06	0.05±0.03	0.73±0.55
KS-5	0.00±0.00	0.00±0.00	0.00±0.00
KS-6	0.07±0.07	0.02±0.02	0.37±0.37
KS-7	0.13±0.07	0.12±0.09	0.87±0.44
KS-8	0.00±0.00	0.00±0.00	0.00±0.00
KS-9	0.03±0.03	0.02±0.02	0.13±0.13
KS-10	0.00±0.00	0.00±0.00	0.00±0.00
KS-11	0.00±0.00	0.00±0.00	0.00±0.00
KS-12	0.00±0.00	0.00±0.00	0.00±0.00
KS-13	0.00±0.00	0.00±0.00	0.00±0.00
KS-14	0.00±0.00	0.00±0.00	0.00±0.00
KS-15	0.00±0.00	0.00±0.00	0.00±0.00
KS-16	0.00±0.00	0.00±0.00	0.00±0.00
KS-17	0.03±0.03	0.02±0.02	0.13±0.13
KS-18	0.00±0.00	0.00±0.00	0.00±0.00
KS-19	0.00±0.00	0.00±0.00	0.00±0.00
KS-20	0.00±0.00	0.00±0.00	0.00±0.00

[a]Applications were made in 3 replications, and 10 explants were used for each replication. SE: Standard Error

Table 6. Micropropagation parameters obtained in the experiments carried out to determine the effect of the subculture numbers [a]

Subculture	Multiplication coefficient±SE	CV(%)	Mean number of shoots obtained per node±SE	CV(%)	Mean shoot length obtained per explant (cm) ± SE	CV(%)	Mean number of leaves obtained per explant ±SE	CV(%)	Mean number of roots obtained per explant±SE	Mean root length obtained per explant (cm)±SE
1rd	1.08±0.08 a	13.32	0.23±0.03 cd	19.89	2.05±0.11 b	9.31	11.15±1.17 ab	18.14	0.00±0.00 c	0.00±0.00
2nd	1. 88±0.21 cd	19.35	0.25±0.06 cd	38.96	1.87±0.35 b	32.79	10.88±2.01 ab	32.05	0.00±0.00 c	0.00±0.00
3rd	2.33±0.38 bc	28.50	0.18±0.01 d	11.11	3.82±0.81 a	36.77	13.85±0.37 a	4.65	0.17±0.09 abc	0.16±0.08
4th	3.40±0.27 a	13.69	0.35±0.02 abc	10.30	1.79±0.07 b	6.87	9.49±1.33 abc	24.21	0.15±0.05 abc	0.21±0.07
5th	2.10±0.08 bcd	6.68	0.35±0.02 abc	8.33	1.87±0.21 b	19.27	7.94±0.14 bc	3.07	0.72±0.33 ab	1.87±0.97
6th	2.23±0.06 bc	4.95	0.34±0.01 bc	4.45	2.51±0.04 ab	2.42	7.89±0.17 bc	3.62	0.81±0.21 a	1.99±0.74
7th	2.73±0.09 abc	6.27	0.43±0.03 ab	11.84	1.95±0.09 b	7.96	6.65±0.29 bc	7.48	0.09±0.02 bc	0.21±0.08
8th	2.96±0.25 ab	14.38	0.47±0.01 a	4.40	1.84±0.12 b	11.14	6.24±0.10 c	2.79	0.14±0.06 abc	0.35±0.18
9th	3.00±0.23 ab	13.11	0.43±0.02 ab	7.53	2.06±0.05 b	3.97	6.72±0.22 bc	5.54	0.29±0.02 abc	0.66±0.11
Source	***F value***	***p-value***	***F value***	***p-value***	***F value***	***p-value***	***F value***	***p-value***	***F value***	***p-value***
Subculture	10.91	0.000	15.11	0.000	4.32	0.005	7,89	0.000	4.82	0.000

[a]Applications were made in 3 replications, and 10 explants were used for each replication. According to the Tukey multiple comparison test, the differences between the mean values shown with different letters in the same column are significant at the P≤0.01 level. SE: Standard Error CV:Coefficient of variation.

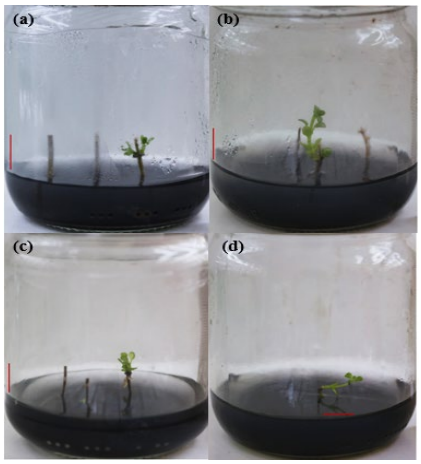

Figure 1. Shoots of Ceylan-2002 cultivar developed in shoot propagation media; a) KS-3, b) KS-4, c) KS-6, d) KS-7. (bar: 1 cm)

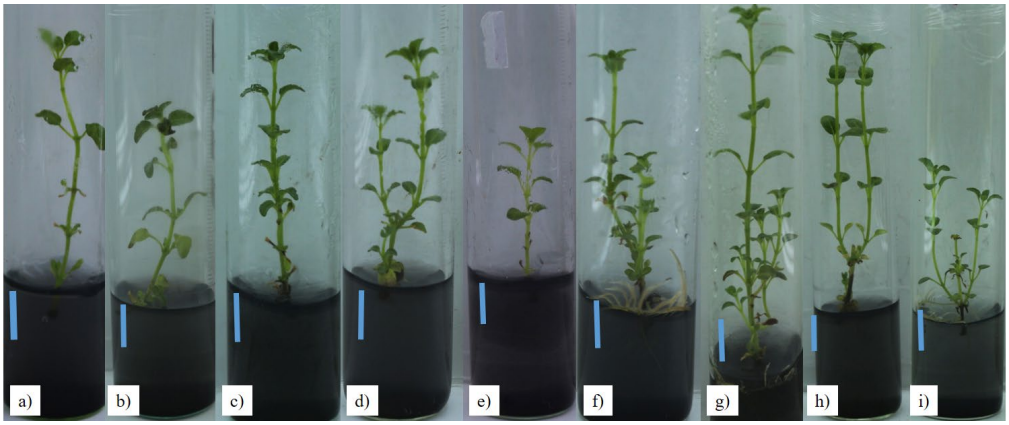

Figure 2. Shoots obtained from the node explants of oregano plants with subcultures in the KS-7 medium; a-i) shoots belonging to subculture numbers (1-9) (bar 1 cm).

In vitro rooting

Rooting is required to acclimatize the shoots produced in vitro conditions. In the studies carried out for this purpose, various parameters related to rooting were tried to be determined. (Table 7 and 8). In addition, the root number values produced *in vitro* were also examined in the study. (Table 7 and 8) Another parameter observed in the study was the root length of regenerated shoots (Table 7, 8, and Fig 3). In the observations made as a result of the culture period of four weeks, it was determined that the amount of AC had a positive effect on stem thickening. The highest mean stem thickness value (2.02 mm) and the highest mean shoot length (10.02 cm) were obtained in media containing 1.0 g l^{-1} activated carbon (Fig 4). When the CV values were examined, it was seen that the majority were <10%, but values >20% were also seen. In applications with <20%, the heterogeneity of the study can be observed due to small sample groups. In the context of plant tissue culture studies, it is a common finding that the CV tends to be high, particularly when considering the culture conditions, including light, temperature, pH, and the type of explant utilized, as well as the inherent physiological differences among plants. (Table 7).

Table 7. Effect of MS and ½ MS media containing different doses of IBA and AC on different root properties[a]

	Root regeneration percentage±SE (%)			Root number per explant±SE (unit)			Mean root lenght±SE (cm)		
		CV (%)			CV(%)			CV(%)	
KK-1	93.33±0.03	6.19		7.47±1.30 ab	30.11		1.82±0.27 ab	25.42	
KK-2	73.33±0.15	34.32		7.63±1.11 ab	25.07		1.87±0.08 ab	7.56	
KK-3	90.00±0.00	0.00		8.47±0.94 ab	19.20		2.16±0.10 ab	8.12	
KK-4	83.33±0.12	24.98	87.50 b	6.47±1.39 ab	37.12	7.32 b	1.67±0.37 ab	38.69	1.81 b
KK-5	90.00±0.06	11.11		7.23±1.03 ab	24.74		1.89±0.23 ab	20.77	
KK-6	93.33±0.03	6.19		8.60±0.25 ab	5.07		1.63±0.38 ab	40.06	
KK-7	96.67±0.03	5.97		6.90±0.87 ab	21.88		1.78±0.07 ab	6.74	
KK-8	80.00±0.10	21.65		5.80±1.61 b	48.06		1.65±0.32 ab	33.74	
KK-9	96.67±0.03	5.97		9.5±0.55 ab	10.04		1.53±0.19 b	20.96	
KK-10	100.00±0.00	0.00		10.67±0.33 ab	5.33		1.77±0.17 ab	17.29	
KK-11	96.67±0.03	5.97		9.13±1.01 ab	19.16		1.77±0.03 ab	3.27	
KK-12	96.67±0.03	5.97	97.92 a	12.03±1.62 a	28.92	9.44 a	1.92±0.08 ab	7.53	2.05 a
KK-13	96.67±0.03	5.97		7.83±0.38 ab	8.50		2.33±0.23 ab	17.32	
KK-14	96.67±0.03	5.97		9.23±1.02 ab	19.08		2.72±0.07 a	4.25	
KK-15	100.00±0.00	0.00		7.03±1.21 ab	29.84		2.42±0.21 ab	14.97	
KK-16	100.00±0.00	0.00		10.10±1.69 ab	28.92		1.98±0.31 ab	11.64	

Source	***F value***	***p-value***	***F value***	***p-value***	***F value***	***p-value***
Medium (M)	0.94	0.339	2.25	0.143	4.98	0.033
AC	12.02	0.002	11.32	0.002	5.30	0.028
IBA	0.84	0.482	0.88	0.461	0.95	0.429
BMxAK	0.48	0.493	0.69	0.414	12.77	0.001
BMxIBA	0.58	0.630	0.43	0.730	0.80	0.502
AKxIBA	1.10	0.365	1.83	0.161	0.78	0.512
BMxAKxIBA	1.25	0.308	0.34	0.795	1.34	0.279

[a]Applications were made in 3 replications, and 10 explants were used for each replication. The differences between the mean values shown with different letters in the same column are significant at the $p \leq 0.01$ level according to the Tukey multiple comparison test. SE: Standard Error CV: Coefficiency of variation

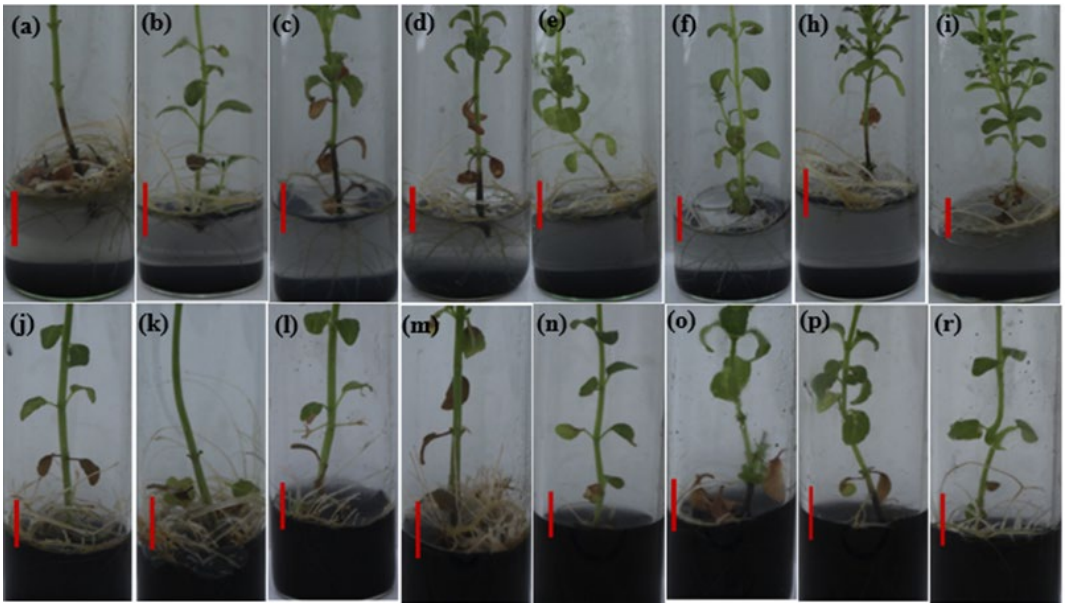

Figure 3. Rooting of regenerated shoots in media containing different doses of IBA and AC; a-r) Root lengths of shoots rooted in KK-1-16 media. (bar 1 cm)

Table 8. Determination of the effect of basic medium and AC on different root properties[a]

Media		Root regeneration percentage (%)		Root number per explant (unit)		Mean root length (cm)	
MS	AK 0.3	85.00 b	91.25	7.51 b	8.92	1.88 b	1.81 b
	AK 1	97.50 a		10.33 a		1.74 b	
½ MS	AK 0.3	90.00 ab	91.25	7.13 b	8.92	1.74 b	1.81 b
	AK 1	98.33 a		8.55 ab		2.36 a	
***p*-value**		0.00		0.00		0.00	

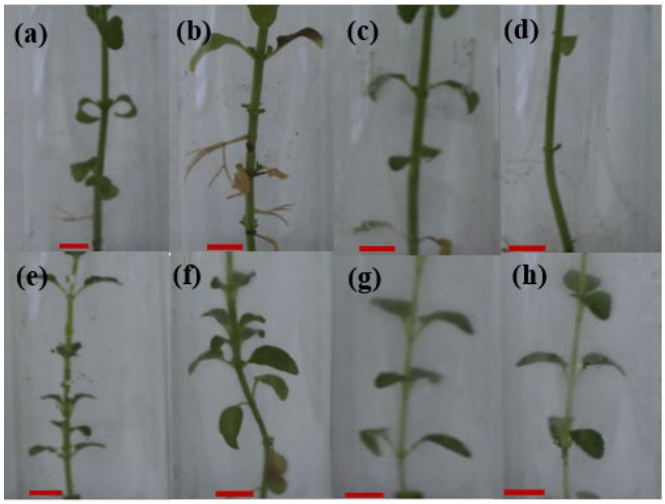

Figure 4. Stem thickening in AC-containing media; a-d) Stem thicknesses in media containing 1 g l^{-1} AC, e-h) Stem thickness in media containing 0.3 g l^{-1} AC (bar 5 mm).

Acclimatization

At the end of the 18th day, the acclimatized plants were kept for 12 days in laboratory conditions with a photoperiod of 16 hours of light/8 hours of darkness, a light intensity of 3500 lux, and a temperature of 24 ± 2°C. The highest survival rate (80%) was recorded from shoots obtained from KK-12 (2 mg l^{-1} IBA + 1g l^{-1} AC) MS medium. The lowest survival rate (0%) was determined in all media containing 0.3 g l^{-1}AC and shoots produced from KK-9 (0 mg l^{-1} IBA + 1 g l^{-1} AC) MS medium. At the end of 30 days, 13 shoots were successfully acclimatized. Acclimatization success was recorded as 16.25% (Fig 5 and 6). In studies carried out for acclimatization, the survival rate of plantlets was 32.50% in media containing 1.0 g l^{-1} AC.

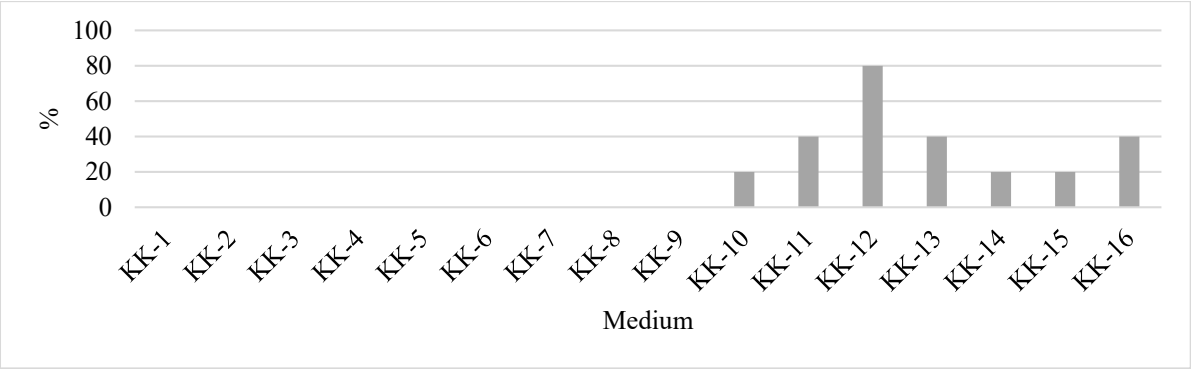

Figure 5. Survival rates after acclimatization

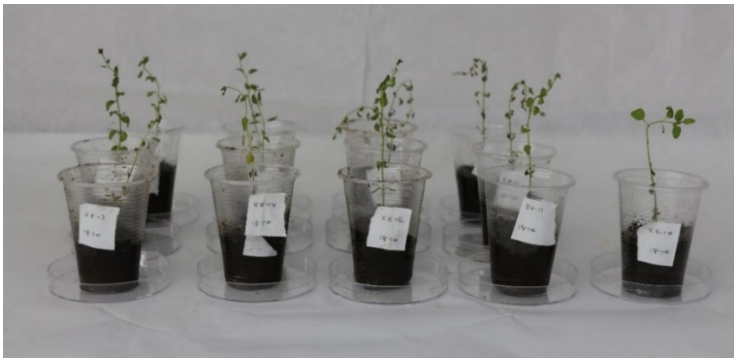

Figure 6. Plants acclimatized after 30 days.

Essential oil analysis

The study also included analyses to determine the essential oil ratios and contents in plants growing *in vitro*. Firstly, the dry matter percentage of the plants was examined prior to the initiation of the analysis. The highest dry matter percentage value (26.30%) was reached in ½ MS medium coded KK-15 containing 1.0 g l^{-1} AC supplemented with 1.0 mg l^{-1} IBA (Table 9).

In the study, the highest essential oil content (2.00 %) was obtained in the KK-12 medium (MS+1 g l^{-1} AC+2 mg l^{-1} IBA). The essential oil's main components were thymol, carvacrol, P-cymene, β-bisabolene, γ-terpinene, α-thujene, (+)-borneol. When thymol and carvacrol values were compared, it was seen that thymol was synthesized at a higher rate. While the thymol content was in the range of 58.32-65.33 %, the carvacrol ratio was in the range of 3.10-6.82 %. While the highest thymol content (65.33 %) was found in KK-14 medium (½ MS+1 g l^{-1} AC+ 0.5 mg l^{-1} IBA), the highest carvacrol content (6.82 %) was obtained in KK-12 medium (MS+1 g l^{-1} AC+2 mg l^{-1} IBA) (Table 10).

Table 9. Mean wet weight (g), dry weight (g), and percentage of dry matter (g) obtained after 4 weeks of culture.

Medium	Wet weight (g)	Dry weight (g)	Percentage of dry matter (%)
KK-9	1.85	0.24	12.97
KK-10	1.17	0.17	14.69
KK-11	1.50	0.20	13.53
KK-12	1.39	0.20	14.25
KK-14	0.28	0.04	14.11
KK-15	0.23	0.06	26.30
KK-16	0.44	0.05	11.28

Table 10. Essential oil contents obtained in different media after 4 weeks of culture (%).

	Medium						
	KK- 9	KK-10	KK-11	KK-12	KK-14	KK-15	KK-16
Essential oil	1.80	1.70	1.56	2,00	1.12	1.10	1.12
α -thujene	2.75	1.97	2.48	2.68	2.17	2.15	2,05
Camphen	0.38	0.28	0.37	0.36	0.36	0.29	0.23
β -pinene	0.14	0.1	0.13	0.14	0.13	0.12	0.11
Sabinene	0.25	0.17	0.22	0.26	0.15	0.18	0.18
Delta-3-caren	0.1	-	0.1	0.09	-	0.08	0.08
β -myrcene	1.74	1.39	1.68	1.77	0.99	1.14	1.13
α- phellandrene	0.25	0.2	0.25	0.26	0.14	0.17	0.17
α -terpinene	1.44	1.16	1.45	1.49	0.86	0.99	0.98
Limonene	0.37	0.29	0.34	0.35	0.3	0.29	0.3
β - phellandrene	0.27	0.2	0.25	0.27	0.2	0.21	0.21
β -ocymene	0.91	0.79	0.98	0.96	0.51	0.51	0.56
γ -terpinene	3.06	2.7	3.26	3.16	1.8	2.19	2.16
P-cymene	6.8	4.87	6.46	6	7.31	6.5	6.91
α -terpinolene	0.14	0.13	0.15	0.14	0.14	0.11	0.1
1-octen-3-ol	0.41	0.26	0.37	0.35	0.32	0.29	0.3
cis-sabinen hydrate	0.7	0.38	0.58	0.84	0.19	0.51	0.56
Linalool	-	-	-	0.15	-	-	-
trans-sabinen hydrate	0.36	0.36	0.35	0.52	0.16	0.36	0.37
trans- α-bergamotene	-	-	-	-	-	0.15	0.14
trans caryophyllene	1.19	1.44	1.36	1.17	0.16	1.15	1,09
terpinen-4-ol	1.17	1,05	1.19	1.07	0.69	1.15	1.11
trans- β -farnesene	-	-	-	-	-	0.1	0.09
α -terpineol	0.15	0.14	0.14	0.15	0.14	0.12	0.12
(+)-borneol	2.28	2.15	2.22	2.2	1.93	1.54	1.38
Germacren-D	1.45	1.47	1.62	1.56	1.58	1.55	1.47
β- bisabolene	6.33	6.82	6.69	6.38	7.09	7.96	7.29
α- amorphene	0.46	0.51	0.43	0.51	0.45	0.57	0.49
betasesqiphellandrene	0.16	0.18	0.16	0.17	0.02	0.19	0.18
cis- α -bisabolene	0.13	0.15	0.15	0.14	-	0.17	0.14
α -cubebene	-	-	-	0.13	-	-	-
Caryophyllene oxide	-	0.11	-	-	0.18	0.12	0.12
α -copaene	-	-	-	-	-	0.15	0.12
(+) epi-bicylosesquiphellandrene	1.12	1.37	1,08	1.36	1.33	1.47	1.19
thymol	60.60	63.91	60.97	58.32	65.33	63.70	64.92
carvacrol	4.68	5,05	4.35	6.82	3.10	3.37	3.38
unknown	0.22	0.27	0.23	0.23	0.27	0.44	0.36

DISCUSSION

İzmir oregano plant has been demonstrated to possess a variety of beneficial properties and biological activities, including antibacterial, antifungal, antimicrobial, antioxidant, antiviral, insecticidal, antioxidant, anti-inflammatory, and antitumor properties. The extensive range of benefits attributed to this plant necessitates further investigation (Koksal et al., 2010; Alekseeva et al., 2020).

In this study, six different surface sterilization methods were applied in order to determine the appropriate sterilization procedure for the node explants. After the sterilization methods were applied, the node explants were cultured in MS media containing (AK0,3) and without (AK0) 0.3 g of AC. In light of the observations recorded at the conclusion of the four-week period, it was determined that the sterilization treatments B, D, and F, which had demonstrated a 100% success rate, were deemed suitable for incorporation into the sterilization protocol. Although no difference was observed in terms of sterilization success between three different treatments, it was decided to use the B method in order to work at lower bleach doses. In the literature, among the studies carried out for the superficial sterilization of node explants of *Origanum* species, there are different sterilization methods (El Beyrouthy et al., 2013; Sevindik et al., 2017; Turker and Hatipoglu, 2018; Grigoriadou et al., 2019; Pandey et al., 2019). In a study carried out in *Origanum vulgare* subsp. hirtum type, sterilization success was determined to be 93% (Iconomou-Petrovich and Nianiou-Obeidat, 1998). Sterilization success obtained in this study was higher than the result of this literature.

In order to determine the parameters affecting hyperhydricity, the effect of sterilization practices on hyperhydricity was investigated first. As a result of the use of AC, the percentage of hyperhydricity decreased from 50% to 41.67%. In the literature, 5 µM salicylic acid has been used to prevent hyperhydricity in *Thymus daenensis* species (Hassannejad et al., 2012). *In vitro* culturing of *O. vulgare* with *Pseudomonas* spp. prevented hyperhydrism (Gogoi and Borua, 2014; Novak and Bluthner, 2020). In addition, 2.0 g l^{-1} AC was used in *Paeonia lactiflora* Pall. plant to prevent hyperhydricity increased the percentage of non-hyperhydric plants from 4.41% to 34.37% (Wu et al., 2011). Although there is no study on the use of AC to prevent hyperhydricity in plants of Origanum species, the outcomes observed in this study align with the existing literature on the effects of AC on hyperhydricity in diverse plant species.

In order to evaluate the effects of various PGRs and their doses on shoot regeneration, node explants were cultured in MS media at 20 different doses. The results of the observations conducted four weeks after the explants were cultured revealed no significant differences in the impact of these PGRs and their various doses on shoot regeneration. Increasing IBA and BAP doses when used alone led to an increase in the percentage of shoot regeneration. A positive effect on shoot regeneration was not observed with BAP and IBA combinations. The rise in NAA doses alone led to a decrease in the percentage of shoot regeneration. No shoot formation was observed in NAA and BAP combinations. In the literature, the highest shoot regeneration (91.67%) in *Origanum vulgare* L. was obtained in MS medium containing 4.0 µM BAP (Pandey et al., 2019). While the percentage of shoot regeneration was 50% in MS medium containing 0.5 mg l^{-1} BAP in the *Origanum heracleoticum* L. plant, this value was observed as 70% in MS medium containing 1.0 mg l^{-1} BAP (Zayova et al., 2019). In another study, the highest percentage of shoot regeneration (100%) in *Origanum vulgare* L was obtained in MS media supplemented with 1.0 mg l^{-1} kinetin and supplemented with 0.25 mg l^{-1} and 0.75 mg l^{-1} chitosan (Premi et al., 2021). In other research, the highest percentage of shoot regeneration (81.56%) from apical meristem explants was obtained in MS medium with 1.5 mg l^{-1} kinetin after germination of seeds of *Origanum onites* L. (Atar and Colgecen, 2019). 'Ceylan-2002' had a low response rate to *in vitro* culture. The results obtained in this study are not similar to those of other studies due to the use of cultivars belonging to different species and cultural methods.

The node explants obtained from the shoot regeneration experiments were subcultured nine times to evaluate the effect of the number of subcultures on the reproduction coefficient. The data obtained regarding the reproduction coefficient are consistent with those reported in the literature. In a study, the highest reproduction coefficient (5.0) was obtained in an MS medium supplemented with 0.4 mg l^{-1} kinetin +0.1 mg l^{-1} NAA in *Origanum syriacum* L. plant (Arafeh et al., 2003). In another study performed on *Origanum majorana* L. plant, the highest multiplication coefficient (4.4) was observed in MS medium supplemented with 1.0 mg l^{-1} BAP. The reproduction coefficient value obtained in this study shows partial agreement with the value obtained in the literature due to using different species and cultivars.

The mean shoot lengths were observed four weeks after culture to evaluate the effect of various PGRs and their doses on the length of the shoots. After the 3rd subculture, the mean shoot length per explant was relatively reduced. In another study, the highest shoot length (2.36 cm) in *Origanum acutidens* (Hand.-Mazz.) Ietswaart was obtained in MS medium containing 1.8 mg l^{-1} BAP + 0.2 mg l^{-1} NAA (Yildirim, 2013). In another study, the highest shoot length (4.38 cm) in *Origanum vulgare* L. was obtained in MS medium supplemented with 0.75 mg l^{-1} chitosan (Premi et al., 2021). In other research, the highest shoot length (3.25 cm) in *Origanum acutidens* (Hand.-Mazz.) Ietswaart was obtained in MS medium with 1.6 mg l^{-1} BAP (Kizil and Khawar, 2017). As in the reproduction coefficient, the reason for the decrease in shoot length may be due to the inhibitory accumulation of PGRs in the medium with the increase in the number of subcultures (Vujović et al., 2012). This finding of this study is similar to these results.

The mean leaf number of regenerated shoots was observed four weeks after culture, allowing for the evaluation of the effect of different PGRs and their doses on leaf number. In a study, the highest mean (13.63) per explant in the shoot propagation study of *Origanum syriacum* L. was obtained in MS medium with 0.5 mg l^{-1} kinetin (Abdallah et al., 2017). Our study is compatible with this study.

No root formation was observed in the first and 2nd subcultures. After the 6th subculture, the number of roots declined sharply. The increase in the number of subcultures leads to physiological changes in plants. In a study about the *Pinus massoniana* Lamb. plant was subcultured 40 times. In long-term subculturing, a decrease in rooting rate was observed after the 20th subculture (Wang and Yao, 2020). The data we obtained are consistent with these studies. In our study, the highest mean number of shoots per node (0.47) was obtained in the 8th subculture. This study found no data on the effect of the number of subcultures on the number of shoots obtained per node, and it is the first research based on such data. The highest propagation coefficient (3.40) was obtained in the 4th subculture.

The effect of AC doses on root regeneration in MS and ½ MS based media was found to be statistically significant. In a study carried out, the highest root regeneration percentage (96%) in *Origanum sipyleum* L. was obtained in MS medium containing 0.5 mg l^{-1} IBA (Oluk and Cakir, 2009). Another study showed the highest percentage of root regeneration (100%) in *Origanum acutidens* (Hand.-Mazz.) Letswaart plant was found in MS media with 0.2 mg l^{-1} NAA + 0.6, 1.2, 1.8, and 2.4 mg l^{-1} BAP (Yildirim, 2013). In another study, the highest root regeneration percentage (91.80%) in *Origanum vulgare* L. was obtained in an MS medium containing 0.6 mg l^{-1} NAA (Oana et al.,

2008). The data obtained in this study are similar to the literature.

The effect of AC dose on the number of roots obtained per explant was found to be statistically significant in MS and ½ MS-based media. An examination of the impact of the medium on the mean root number obtained per explant revealed that MS-based media were found to be effective. Although the effect of IBA doses on the mean root number per explant was not statistically significant, the highest mean root number per explant (9.04) was obtained in media containing 0.5 mg l^{-1} IBA. As a result, when all these data were examined, it was determined that the most effective medium on the mean root number obtained per explant of the regenerated shoots of Ceylan-2002 cultivar was MS-based medium containing 1.0 g l^{-1} AC.

In the literature, the highest mean root length (1.5 cm) obtained in *Origanum syriacum* L. was obtained in an MS medium containing 0.8 mg l^{-1} IAA (Arafeh et al., 2003). In the study we carried out, higher data were obtained. In another study, the highest mean root length (5.5 cm) obtained in the *Origanum sipyleum* L plant was found in MS medium containing 0.5 mg l^{-1} IBA, and the lowest mean root length (1.6 cm) was 1.0 mg l^{-1} IBA (Oluk and Cakir 2009). The results obtained in our study are compatible with Oluk and Cakir (2009), which achieved the highest mean root length in MS medium containing 0.5 mg l^{-1} IBA for *O. sipyleum* L.

This study evaluated the effect of MS and ½ MS-based media with varying doses of AC on stem thickening. It was determined that an increase in AC dose positively affected stem thickness. There is no study in the literature regarding the effect of AC use on stem thickening in oregano plants. However, it was reported that using 10.0 g l^{-1} AC in *Eucalyptus grandis* x *E. urophylla* clones resulted in stem thickening (Jones and Van Staden, 1994). Our results in this study are consistent with Jones and Van Staden (1994) results. In this experiment established to evaluate the effect of MS and ½ MS-based media containing AC at different doses on shoot length, it was determined that the increase in AC doses positively affected shoot length. While the mean shoot length was 4.88 cm in media containing 0.3 g l^{-1} AC, this value increased to 10.02 cm in media containing 1.0 g l^{-1} AC. There is no study in the literature regarding the effect of AC use on shoot length in oregano plants. However, in a study conducted, while the mean shoot length was 0.06 cm in *Thuja occidentalis* L., this value increased to 1.13 cm in the presence of 0.05% (w/v) AC (Nour and Thorpe, 1993). The results we obtained in our study are compatible with the study of Nour and Thorpe (1993).

In studies carried out for acclimatization, the survival rate of plantlets was 0% in media containing 0.3 g l^{-1} AC, while this value increased to 32.50% in media containing 1.0 g l^{-1} AC. In a study carried out by increasing the AC doses from 1.0 g l^{-1} to 5.0 g l^{-1} in Camarosa, Chandler, and Oso Grande strawberry cultivars, the stem diameter values of the plants increased in the acclimatization stage and survival successes in the plantlets rooted in AC media were at the were found as 99% (Adak and Pekmezci, 2011). The findings of the present study are consistent with those of Adak and Pekmezci (2011), who posited that the utilization of AC in various strawberry cultivars enhances the survival rate during acclimatization.

Some of the plants analyzed in terms of the volatile compounds were selected to determine various physiological parameters and to make observations. When wet and dry weight values were examined, higher wet weight values were found in KK-9, 10. 11, and 12 coded MS-based media, while a 5-fold decrease in wet weight values occurred in KK-14, 15, and 16 coded ½ MS-based media. The amount of essential oil obtained during the analysis also showed similar characteristics to this situation.

The highest essential oil content (2.00%) was obtained in MS medium with 2.0 mg l^{-1} IBA; the lowest essential oil content (1.10%) was in ½ MS medium with 1.0 mg l^{-1} IBA in this study. The shoots regenerated from all media had the highest amount of thymol component among the essential oil components. Gurtunca (2011) reported that the essential oil rate of Ceylan-2002 cultivar was determined as 3.43% in 2010 and 4.46% in a study carried out in 2011. In another study, the essential oil ratios of *Origanum onites* L. clones in Bornova and Dikili regions in 2002 and 2003 were between 2.77-4.20% (Avci and Bayram, 2013). In other research, the effect of different water and nitrogen doses on the essential oil composition of Ceylan-2002 cultivar in 2013 was investigated. In the first harvest, it was observed that the amount of thymol varied between 62.45-75.68% and the amount of carvacrol between 5.34-7.56%. In the second harvest, the amount of thymol was between 61.87-69.99%, and the amount of carvacrol was between 5.74-6.83% (Tokul 2015). The data we obtained in our study is compatible with Tokul (2015). Ceylan-2002 cultivar is registered as a thymol-carvacrol type. In this research, the tyhmol ratio was found to be higher than carvacrol. In addition to the composition of mineral and organic compounds and pH value, culture conditions such as temperature, light intensity, and duration are extremely effective factors in the secondary metabolite production of plants grown *in vitro*. In nature, secondary metabolite production is produced by plants in response to environmental stimuli or for defensive purposes. This mechanism can be stimulated by modifying these parameters *in vitro* (Scarpa et al., 2022). In this context in our study, the thymol ratio may have been found to be higher than the carvacrol ratio in oregano plants cultured *in vitro*, where environmental conditions were minimized. Perhaps the thymol ratio may have genetically increased the expression of genes responsible for thymol production by the composition of the medium and the doses of PGRs. In studies on essential oil in *Origanum onites* L. species, carvacrol, thymol, α-thujene, myrcene, camphene, β-pinene, limonene, terpinolene, linalool, α-terpinene, karyopylene oxide, p-cymene, terpinene-4-ol,α-pinene, β-bisabolene, borneol, and α-terpineol were determined as the main components (Tepe et al., 2016). The values obtained as a result of the study were found to be similar to; Kacar

et al. (2006) and Kamatou and Viljoen (2008) for linalool, Kizil et al. (2008) for delta-3-karen, Joshi et al. (2011) for trans-α-bergamoten, Souleles (1991) for trans-β-farnesene, Kizil et al. (2008) and Karık et al. (2021) for β-bisabolene, Aslan-Oz (2017) and Maskovic et al. (2017) for α-cubeben, Karık et al. (2021) for caryopylene oxide and Souleles (1991) for α-copaene. Our study obtained better results than the study carried out by Souleles (1991).

CONCLUSION

Izmir oregano is a plant with high economic value due to its high secondary metabolite content and widespread commercial use. In this study, a micropropagation technique protocol was developed as an alternative method for the production of oregano plants. In the study, the registered local variety Ceylal-2002 was used, and guiding information that can be used to meet the oregano export deficit was presented. Future studies can explore different experiments to reduce hyperhydricity levels. Additionally, elicitation experiments could be performed to enhance secondary metabolite content.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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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.

6. Referee Assignment by the Editor-in-Chief

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.

ASSOCIATION NEWS

EDITOR'S MESSAGE
ACKNOWLEDGEMENT

We would like to extend our heartfelt gratitude to Prof. Dr. Metin B. YILDIRIM for his exceptional dedication and invaluable contributions as the Editor-in-Chief of our journal since its inception. Under his visionary leadership, our journal has achieved remarkable success and reached its current prestigious standing. We are also honored and grateful that he has accepted the "Honorary Editor-in-Chief" title as a testament to his enduring commitment and outstanding service to our journal.

IMPORTANT ANNOUNCEMENT

In 2025, as we celebrate our 30th volume, we will implement several changes to our spelling rules and literature reporting system. Announcements regarding our transition to the APA style will be posted on our website. Additionally, modifications to our page layout are underway.

We are also working to ensure that articles submitted to our journal receive professional English language support, and further details will be shared on our journal's website (www.field-crops.org).

PUBLICATION CONTRIBUTION

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IMPACT FACTOR

The overall impact factor of the Turkish Journal of Field Crops has been increasing steadily such as 1.32 with h-index of 6 in November 2014; 2.01 with h-index of 9 in November 2016; 2.55 with h-index of 11 on November 3, 2017; 3.28 with h-index 13 in November 2018; 3.64 with h-index in June 2019 and 3.91 with h-index 15 in November 2019; 5.47 with h-index 19 in November 2021, 6.12 with h-index 21 in December 2022, 6.72 with h-index 24 in December 2023, 7.38 with h-index 25 in December 2024. Impact factor: Crossref 2023 1.0. Crossref 5 years average 1.4 Quartile Q_3 (WOS) in Agronomy; Q_3 (Scopus).