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CONTENTS Pages

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Books: Steel, R.G.D. and J.H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2. ed. New York: McGraw-Hill Publ. Company.

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THE NUTRITIONAL AND CHEMICAL CONTENT OF *Atriplex nitens* SEEDS GROWN UNDER RAINFALL AND NONFERTILIZE CONDITIONS

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ABSTRACT

***Atriplex nitens* Schkuhr plant, which can grow under rainfall conditions without any fertilizer application and can produce high amounts of seeds, have been seen as an alternative feed resource in animal nutrition. However, no previous studies have been found revealing the feed quality content of the seeds with and without bracteole. For this purpose, a study was planned according to factorial experimental design in Randomized Complete Block Design with 3 replications. In this study carried out for 2 years (2021-2022) in Igdir arid conditions, crude protein (CP), dry matter (DM), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), raw ash (RA), dry matter digastiblity (DMD), metabolize enery (ME), dry matter intake (DMI) and relative feed values (RFV) of seeds with and without bracteole were determined and compared. Additionally, the effect of different row spacings (22.5, 45.0 and 67.5 cm) on the quality of the seeds with and without bracteole was also tested. As a result of the study, all feed quality characteristics examined were found to be important in terms of seed types and it was determined that the desired feed quality values (the highest DMD, DMI, CP, ME, RFV and the lowest ADF, DM, NDF, RA and ADL) were more suitable in seed without bracteole. Only CP, NDF and ADL were found to be important in terms of inter-row spacing, and these values increased with increasing inter-row spacing. In addition to the plant having a high seed yield per unit area, considering the seed feed quality values examined in this study, it showed that the seeds with and without bracteole can be used as a good alternative roughage and concentrated feed source, respectively.**

Keywords: Alternative feed, Feed value, Seeds with and without bracteole, Plant density

INTRODUCTION

For a profitable livestock farming, feed must be provided cheaply. Because in animal production, although it varies depending on animal breeds, approximately 70% of production costs go to feed expenses (Harmansah, 2018). In this sense, meadow-pastures and forage crops cultivation areas within field agriculture are among the primary sources that provide cheap and quality feed material to animals. However, changes in climate due to increasing global warming in the last century have increased the pressure on natural resources (such as soil, water, pasture and forest), causing an increase in marginal areas, a decrease in species diversity and density, a weakening of vegetation covers, and a decrease in the amount and quality of grass produced (Temel and Sahin, 2011; Yavuz et al., 2020). As a result, the desired animal product performances cannot be obtained from farm animals that are exposed to inadequate and unbalanced nutrition, or animal husbandry becomes unprofitable due to additional feed costs. For this reason, scientists and producers have in search of alternative feed sources to meet the roughage deficit. In this sense, xerophyte and halophyte species that can grow in areas that are out of production due to salinity and drought have been seen as an important advantage.

The genus *Atriplex* contains more than 260 species, most of which are salt-tolerant, adapted to the arid and semi-arid regions of the Earth, especially Europe, Asia, Africa, Australia and North America (Kadereit et al., 2010; Tan and Temel, 2012). One of these species is *Atriplex nitens*, which can easily grow in extreme climate and soil conditions. It can produce medium quality (11.05% CP, 57.9% NDF, 60.5% DMD and 98.9 RFV) and high amounts of roughage (138.1 t ha^{-1} of fresh hay and 37.5 t ha^{-1} of hay) per unit area without any fertilizer application (Keskin and Temel, 2022; Temel and Keskin, 2022a; Temel et al., 2022a; Temel et al., 2022b). These results showed that the hay material produced by *Atriplex nitens* can be used as an alternative roughage resource in animal nutrition. It has also been reported that its seeds with high secondary compounds are widely used in the health and food sectors (Acar et al., 2017; Rinchen et al., 2017; Kadioglu et al., 2022).

Atriplex nitens Schkuhr, an annual herbaceous plant, is a heterocarpic species which produces three types of fruits that differ in ecological and morphology properties

(Mandák and Pyšek, 2005). The plant grown without any fertilizer application can produce high amounts of seed (18.4 t ha^{-1}) and stem (39.3 t ha^{-1}) material in dry conditions (Keskin et al., 2023). These values can reach 30.6 t seed yield and 49.7 t stem yield per hectares under irrigated conditions (Temel and Keskin, 2022b). In addition, 8.9-13.2 t seed and 21.7-39.7 t stem yields per hectares were obtained from *Atriplex nitens* grown in 22.5, 45.0 and 67.5 cm inter-row spacings in dry conditions without applying fertilizer, depending on the inter-row spacing (Temel et al., 2024). Considering these high seed yields obtained from extreme growing conditions, *Atriplex nitens* has been seen as a great advantage both in terms of bringing the abandoned areas into production due to drought and salinity and in providing a feed source for animals. However, no study has been found that reveals the feed quality properties of *Atriplex nitens* seeds for use as a feed resource in animal nutrition.

The aim of the study was to determine some nutritional and chemical composition contents of seeds with and without bracteole obtained from Atriplex nitens grown in different inter-row spacings under nonfertilize and rainfall conditions.

MATERIALS AND METHODS

The research was carried out in two stages: field and laboratory conditions. Field studies were established for two years (2021 and 2022) in Igdir Province (39° 55′43″ N, 44° 05′ 41″ E), which has the driest climate of Turkey. According to the long-term average (1978-2020) of the study area, the total amount of precipitation was 176.2 mm, the relative humidity was 47.7% and the average temperature was 19.0 ºC (MGM, 2023). In 2021 and 2022, when the research was conducted, average temperature, the total rainfall and relative humidity values of the region were measured as 21.4-20.2 ºC, 159.8-137.5 mm and 44.5-45.6%, respectively. These results showed that the amount of precipitation in 2022 during the development period of the plants was lower than the average of 2021 and long-term. Soil samples were taken from the study area (0-30 cm depth) before sowing and analyzed (Kacar, 2012). The results are presented in Table 1.

Table 1. Some chemical and physical properties of soils of research area.

	Saturation (%)	pH	Total salt (%)	$CaCO_3$ (%)	Org. matter (%)	P_2O_5 (t ha^{-1})	K_2O (t ha^{-1})
2021	57.00	7.65	0.17	3.63	0.75	0.46	0.36
2022	68.00	7.89	0.20	4.30	0.51	0.36	0.32
Class	Clay loam	Slightly alkaline	Slightly saline	Calcareous	Very little	Little	Sufficient

The study was established according to factorial experimental design in Randomized Complete Block Design with three replications under rainfall conditions. In the research, different inter-row spacings (22.5 cm, 45.0 cm and 67.5 cm) were evaluated as factors. There were 12 rows in the plots with 22.5 cm inter-row spacing, 6 rows in 45.0 cm inter-rows and 4 rows in 67.5 cm inter-rows. Accordingly, the area of each plot was planned as 8.1 m^2 (2.70 x 3.0 m). The seeds were sown by hand in the lines made with a marker at a distance of 10 cm in rows and a depth of 4 - 5 cm. Since climate and soil conditions differ from year to year, sowings were carried out on 21.03.2021 in the first year and on 27.03.2022 in the second year. In the current study, no irrigation, fertilizer, insecticide or herbicide was applied to the plants. Only the weeds that appeared between the plots and blocks were controlled by hand pulling and hoeing. Seed harvests were made from 10 randomly selected plants within the plot, when 75% of the fruits on the plant turned yellow (Temel and Keskin, 2022b).

In laboratory studies, threshing processes and feed quality analyzes of the seeds were carried out. Following harvest, the plants brought to the laboratory were kept on the benches for 4-5 days to ensure that they dried thoroughly. After drying, the plants in all treatments were threshed and the fruits were separated from the stems. In this way, seeds with bracteole were obtained (Figure 1a). To obtain seeds without bracteole, seeds with bracteole were removed by rubbing with the hand (Figure 1b).

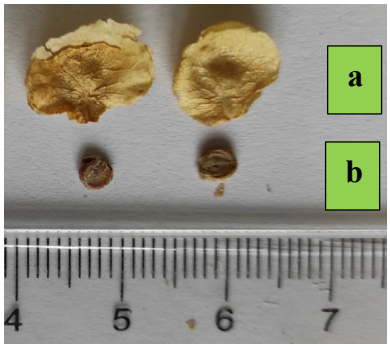

Figure 1. Seeds with bracteole (a) and seeds without bracteole (b)

Then, the seeds with and without bracteole were ground separately in a mill to pass through a 1-mm screen and were made ready for chemical analysis. To determine the dry matter, 3 g of ground sample was dried in a drying oven set at 105 ºC until it attained a constant weight (AOAC, 2020). Total nitrogen amounts were determined according to the Micro Kjeldahl method, and then this value was multiplied by the coefficient of 6.25 to calculate the percent crude protein ratios. For raw ash, 1 gram of ground sample was placed in porcelain crucibles and burned for 8 hours in a muffle furnace set at 550 ºC. Following the burning, the crucibles taken out of the furnace were kept in the desiccator for 2 hours and then the raw ash ratios were determined by weighing (AOAC, 2020). Neutral detergent fibre (NDF%), acid detergent fibre (ADF%) and acid detergent lignin (ADL%) contents were determined on the ANKOM fiber analyzer device using the method developed

by Van Soest et al. (1991). Dry matter digestibility (%) and relative feed value (Boman, 2003), dry matter intake (%) and digestible energy (Mcal kg^{-1}) (Sheaffer *et al*., 1995) and metabolize energy (Mcal kg^{-1}) content (Khalil *et al*., 1986) of the samples were determined using the following equations developed by researchers.

$$DMD = 88.9 - (0.779 \times ADF) \quad (1)$$

$$DE = 0.27 + 0.0428 \times DMD \quad (2)$$

$$ME = 0.821 \times DE \quad (3)$$

$$DMI = 120 / NDF \quad (4)$$

$$RFV = (DMD \times DMI) / 1.29) \quad (5)$$

In the JMP (5.0.1) statistical package program (SAS Institute, 2003, Cary, NC, USA), the results obtained from the current study were subjected to analysis of variance according to factorial experimental design in Randomized Complete Block Design repeatedly over the years. Comparison of significant means was made according to the $LSD_{(0.05)}$ test.

RESULTS AND DISCUSSION

In this article, some feed quality characteristics of *Atriplex nitens* seeds grown in different inter-row spacings under nonfertilize and rainfall conditions were examined. Seed yield characteristics, which are a part of the current study, were determined by Temel et al. (2024) and it was reported that high seed and stem yields were obtained per unit area (Table 2). These results have shown that the *Atriplex nitens*, which can grow in extreme conditions, can be an important alternative feed resource.

Table 2. Mean seed and stem yields obtained from different inter-row spacings in *Atriplex nitens**.

Years	Seed yield (t ha^{-1})			Mean of	Stem yield (t ha^{-1})			Mean of
	22.5	45.0	67.5	years	22.5	45.0	67.5	years
2021	13.72	11.49	9.18	11.47	34.76	24.97	19.36	26.36
2022	12.57	10.09	8.69	10.45	44.64	28.45	21.13	32.41
Mean of inter-rows	13.15	10.79	8.93	10.96	39.70	26.71	21.74	29.38

* Temel et al. (2024).

Variance analysis results, F values and significiances regarding the feed quality characteristics of *Atriplex nitens* seeds grown in different inter-row spacings are given in Table 3. When Table 3 was examined, it was found that there was a significant difference between the years in terms of CP, NDF, DMI and EFV, between the row spacings in terms of CP, NDF and ADL and between seed types in terms of all quality values examined. And also it was found that year x seed type interaction and inter-row spacing x seed type interactions were significant in terms of NDF and RA, respectively.

Table 3. The mean squares values of the examined quality parameters according to factorial experimental design in Randomized Complete Block Design.

SV	df	DMR	CP	RA	NDF	ADF	ADL	DMD	DMI	ME	RFV
Y	1	4.43 $^{n.s.}$	21.95**	0.11 $^{n.s.}$	81.78**	4.07 $^{n.s.}$	1.97 $^{n.s.}$	2.48 $^{n.s.}$	1.75*	0.00 $^{n.s.}$	7178*
R	2	19.4 $^{n.s.}$	17.62**	0.91 $^{n.s.}$	9.80*	4.01 $^{n.s.}$	2.06*	2.42 $^{n.s.}$	0.76 $^{n.s.}$	0.00 $^{n.s.}$	4031 $^{n.s.}$
YxR	2	2.21 $^{n.s.}$	0.73 $^{n.s.}$	0.09 $^{n.s.}$	0.72 $^{n.s.}$	0.05 $^{n.s.}$	0.14 $^{n.s.}$	0.03 $^{n.s.}$	0.12 $^{n.s.}$	0.00 $^{n.s.}$	487 $^{n.s.}$
T	1	321**	926**	380**	3961**	875**	62**	531**	420**	0.66**	18572**
YxT	1	0.18 $^{n.s.}$	0.89 $^{n.s.}$	0.45 $^{n.s.}$	67.5 $^{n.s.}$	0.65 $^{n.s.}$	0.64 $^{n.s.}$	0.40 $^{n.s.}$	0.53 $^{n.s.}$	0.00 $^{n.s.}$	1525 $^{n.s.}$
RxT	2	8.58 $^{n.s.}$	0.01 $^{n.s.}$	2.30**	3.21 $^{n.s.}$	0.58 $^{n.s.}$	0.03 $^{n.s.}$	0.35 $^{n.s.}$	0.11 $^{n.s.}$	0.00 $^{n.s.}$	874 $^{n.s.}$
YxRxT	2	2.34 $^{n.s.}$	0.06 $^{n.s.}$	0.06 $^{n.s.}$	0.46 $^{n.s.}$	0.14 $^{n.s.}$	0.01 $^{n.s.}$	0.08 $^{n.s.}$	0.13 $^{n.s.}$	0.00 $^{n.s.}$	588 $^{n.s.}$

*P< 0.05, **P< 0.01, n.s.; non-significant, SV: sources of variation, df: degree of freedom, Y: year, R: Inter-row T: seed type DMR: dry matter rate, CP: crude protein, RA: raw ash, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, DMD: dry matter digestibility, DMI: dry matter intake, ME: metabolizable energy.

Dry matter and crude protein rates

When Table 4 is examined, the dry matter ratio of seeds with bracteole was found to be higher than seeds without bracteole. The fact that the amount of fruiting bracteole with low moisture content is high in the seeds with bracteole may have caused this. As a matter of fact, in a study conducted on barley, it was stated that hulled grains had a higher dry matter ratio than hulless ones (Salem et al., 2023).

In the study, it was determined that the crude protein content of seeds without bracteole (26.98%) was higher than that of seeds with bracteole (16.84%) (Table 4). This may be due to the presence of fruiting bracteoles rich in cell wall substances (cellulose, hemicellulose, lignin) in seeds with bracteole. Because fruiting bracteoles contain less intracellular substances (sugar, protein and fat) than endosperm (nutrient tissue). As a matter of fact, in studies conducted on different species, it was stated that naked grains had higher crude protein content than hulled seeds (Biel et al., 2009; Abdel-Haleem and Awad, 2015).

Additionally, Wright et al. (2002) found that the protein content of naked *Atriplex hortensis* seeds varied between 25.7-28.3% and that these values were compatible with current research results. On the other hand, crude protein contents of some concentrated feeds (corn grain: 8.47%, barley grain: 12.23%, wheat grain: 13.43%) widely used in animal nutrition were revealed by Kurt et al. (2022) and the values obtained in the current study were found to be higher. In addition, when the crude protein contents determined in the current study were compared according to roughage quality standards, it was seen that seeds with bracteole were in the high quality group and seeds without bracteole in the best quality group (Rivera and Parish, 2010).

Table 4. Means of dry matter and crude protein ratios of seeds with and without bracteole obtained from different inter-row spacings.

Years	Inter-rows (cm)	DM rate (%)		Mean of years	CP rate (%)		Mean of years
		SB	SWB		SB	SWB	
2021	22.5	93.31	88.93	89.86	16.26	26.23	22.69 a
	45.0	92.91	87.93		17.87	27.48	
	67.5	92.52	83.56		19.19	29.10	
2022	22.5	92.58	87.89	89.16	14.92	25.27	21.13 b
	45.0	92.01	85.92		15.98	26.51	
	67.5	91.62	84.92		16.79	27.28	
Mean of seed types		92.49 a	86.52 b		16.84 b	26.98 a	
Mean of inter-rows		22.5 cm	45.0 cm	67.5 cm	22.5 cm	45.0 cm	67.5 cm
		90.68	89.69	88.16	20.67 c	21.96 b	23.09 a
$LSD_{(0,05)}$		T: 1.87			Y: 0.61, R: 0.74, T: 0.61		

Means with different letters are statistically significant. Y: year, R: Inter-row T: seed type, SB: seed with bracteole, SWB: seed without bracteole, DM: dry matter rate, CP: crude protein.

Crude protein content of the seeds varied between 20.67-23.09% according to row spacing, and it was observed that the protein content increased with increasing row spacing (Table 4). In addition, the crude protein rate of the seeds was found to be higher in 2021 compared to 2022. This may be due to the fact that the plants grown both in wide row spacing and in 2021 (in the months corresponding to the period when the seeds mature) benefit more from the environmental conditions (rainfall, light, nutrients, etc.) and produce the plumper and larger seeds. Regarding the subject, in a study conducted on *Atriplex nitens*, it was reported that plants grown in a year (2021) when the climatic conditions were suitable and in a wide row spacing produced seeds with a higher 1000 grain weight (Temel and Keskin, 2022b; Temel et al., 2024). Because larger seeds can generally have more nutritive tissue or intracellular non-structural carbohydrates (protein, sugar, starch and fat). As a matter of fact, in studies conducted on different plants, it has been reported that crude protein rates increase as 1000 grain weight increases depending on species and varieties (Tan and Temel, 2019; Hicks et al., 2022).

Raw ash and neutral detergent fibre rates

Considering Table 5, it was determined that RA (11.27%) and NDF (32.28%) contents of seeds with bracteole were higher than those of bracteole-peeled seeds (RA: 4.77% and NDF: 11.40%). This may be due to the fact that seeds with bracteole contain a high amount of fruiting bracteole rich in cell wall substances. Because the cell walls that form the fruiting bracteoles are thicker and richer in structural carbohydrates (cellulose, hemicellulose) and ash content (Zhao et al., 2015; Han and Hendek Ertop, 2022). Regarding the subject, Wright et al. (2002) determined the ash content of naked *Atriplex hortensis* seeds as 3.5%. Additionally, in studies conducted on different species, it has been stated that hulled grains have higher cellulose, hemicellulose, lignin and ash contents than hulless grains (Choi et al., 2011; Saleem et al., 2023).

Table 5. Means of raw ash and neutral detergent fibre contents of seeds with and without bracteole obtained from different inter-row spacings.

Years	Interrows (cm)	RA rate (%)		Mean of years	NDF rate (%)		Mean of years
		SB	SWB		SB	SWB	
2021	22.5	11.33	4.28	7.97	33.62	10.94	23.40 a
	45.0	11.16	4.62		35.58	11.54	
	67.5	10.82	5.59		36.56	12.13	
2022	22.5	11.50	3.92	8.08	28.42	11.11	20.38 b
	45.0	11.44	4.77		28.94	11.21	
	67.5	11.38	5.46		31.15	11.47	
Mean of seed types		11.27 a	4.77 b		32.28 a	11.40 b	
Mean of inter-rows		22.5 cm	45.0 cm	67.5 cm	22.5 cm	45.0 cm	67.5 cm
		7.76	8.00	8.31	21.02 b	21.82 ab	22.83 a
$LSD_{(0,05)}$		T: 0.43, R x T: 0.74			Y: 1.04, R: 1.27, T: 1.04, Y x T: 1.47		

Means with different letters are statistically significant. Y: year, R: Inter-row T: seed type, SB: seed with bracteole, SWB: seed without bracteole, RA: raw ash, NDF: neutral detergent fibre.

The values of NDF content obtained in this study showed that both seeds with and without bracteole were included in the best quality class according to roughage quality standards (Rivera and Parish, 2010). In another study, the NDF contents of wheat bran, corn, barley and wheat grains, which are preferred as concentrated feed sources, were determined as 47.15%, 24.56%, 43.64% and 29.79%, respectively (Kurt et al., 2022). According to these results, it was observed that the NDF (except corn and wheat grain) contents of seeds with and without bracteole were lower. In addition, it was determined that the raw ash contents obtained from both seed types were higher than the raw ash content of wheat bran (2.93%), corn (1.57%), barley (3.04%) and wheat (2.52%) grains determined by Kurt et al. (2022).

In the study, it was determined that the NDF content of the seeds increased with increasing inter-row spacing and also that the seeds had a higher NDF content in 2021 (Table 5). This may be due to the fact that plants grown in wide row spacing and in 2021, when the environmental conditions were suitable, produced larger seeds and larger seeds had a higher amount of fruiting bracteole. As a matter of fact, Mandák and Pyšek (2005) reported that *Atriplex sagitata* sown at low density had larger fruits and larger fruits had more fruiting bracteole. In addition, in a study conducted with different oat varieties, it was revealed that the husk ratio increased in seeds with a weight of 1000 grains (Sobayoglu and Topal, 2019). Therefore, it is thought that a high husk or fruiting bracteole ratio increases the NDF content of the seeds.

Although the raw ash content of seeds with bracteole did not change according to inter-row spacing, it was observed that the raw ash content of bracteole-peeled seeds increased with increasing inter-row spacing (Figure 2a). This caused the row spacing x seed type interaction to be significant. When evaluated in terms of year x seed type interaction, the NDF content of seeds without bracteole did not change according to years while the NDF content of seeds with bracteole increased in 2021 (Figure 2b). This may have been caused by the fact that seeds with bracteole had a higher fruiting bracteole ratio due to suitable climatic conditions in 2021 (Temel et al., 2024). As a matter of fact, seeds with a high hull ratio may contain higher amounts of extracellular substances such as cellulose, hemicellulose and lignin (Saleem et al., 2023).

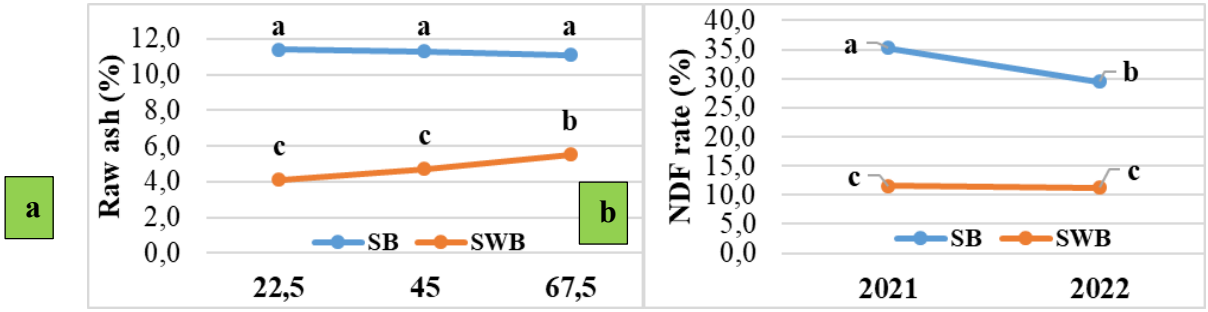

Figure 2. Effects of inter-row x seed type interaction on raw ash (a) and year x seed type (b) on NDF rate. Plots followed by the different letters are statistically significant, SB: seed with bracteole, SWB: seed without bracteole, NDF: neutral detergent fibre.

Acid detergent fibre and acid detergent lignin rates

While ruminant animals can partially digest the ADF, one of the cell wall components, Due to their digestive systems, they cannot digest lignin (ADL). Therefore, it is not desired that ADF (more than 45%) and ADL (more than 20%) contents are high since they negatively affect the digestibility of DM and NDF of the feed. In addition, the ADF and ADL rates of seeds without bracteole were found to be lower than those of seeds with bracteole (Table 6). Accordingly, the ADF content of seeds with and without bracteole was determined as 19.22% and 9.36%, and the ADL content was determined as 5.97% and 3.36%, respectively. These results showed that both seed types were of the best quality according to roughage quality standards (Rivera and Parish, 2010). This difference between seed types might be due to the absence of fruiting bracteoles in seeds with bracteole. Since the fruiting bracteoles contain a higher amount of cell wall components (Hoije et al., 2005; Zhao et al., 2015).

In this study, ADL content increased as inter-row spacing increased. The highest ADL content was determined in the wide row spacing (67.5 cm) (Table 6). This can be caused by the fact that plants growing in wide inter-row spacing produce larger seeds. Because larger seeds can generally have a higher husk rate (Mandák and Pyšek, 2005; Sobayoglu and Topal, 2019). As a matter of fact, in many species, it was determined that the part of the seed containing the most lignin was the husk (Choi et al., 2011; Abdel-Haleem and Awad, 2015).

Table 6. Means of acid detergent fibre and acid detergent lignin rate of seeds with and without bracteole obtained from different inter-row spacings.

Years	Inter-rows (cm)	ADF rate (%)		Mean of years	ADL rate (%)		Mean of years
		SB	SWB		SB	SWB	
2021	22.5	19.27	8.93	14.63	5.82	2.95	4.90
	45.0	19.84	9.20		6.41	3.36	
	67.5	19.96	10.57		6.78	4.06	
2022	22.5	18.24	8.49	13.96	5.27	2.91	4.43
	45.0	18.85	9.14		5.69	3.30	
	67.5	19.17	9.85		5.86	3.56	
Mean of seed types		19.22 a	9.36 b		5.97 a	3.36 b	
Mean of inter-rows		22.5 cm	45.0 cm	67.5 cm	22.5 cm	45.0 cm	67.5 cm
		13.73	14.26	14.89	4.24 b	4.69 ab	5.07 a
$LSD_{(0,05)}$		T: 0.85			R: 0.61, T: 0.49		

Means with different letters are statistically significant. Y: year, R: Inter-row T: seed type, SB: seed with bracteole, SWB: seed without bracteole, ADF; acid detergent fibre, ADL: acid detergent lignin.

Dry matter digestibility and dry matter intake

Dry matter intake (DMI) indicates the amount of dry matter consumed by animals, while dry matter digestibility (DMD) refers to the part of dry matter digested by animals. Therefore it is desired that the values are high in a feed. Considering the DMD and DMI obtained from the research (Table 7), it was seen that *Atriplex nitens* seeds were in the best quality group according to quality standards (Rivera and Parish, 2010). In the current study, DMD and DMI were found to be higher in bracteole-peeled seeds than seeds with bracteole. Additionally, the highest DMI was determined in 2021 (Table 7). This is due to the fact that ADF and NDF are lower in seeds without bracteole and in 2022. Because DMD and DMI are calculated by using ADF and NDF values, respectively (Boman, 2003).

Table 7. Means of dry matter digestibility and dry matter intake of seeds with and without bracteole obtained from different inter-row spacings.

Years	Inter-rows (cm)	DMD (%)		Mean of years	DMI (%)		Mean of years
		SB	SWB		SB	SWB	
2021	22.5	73.89	81.95	77.51	3.57	11.05	6.94 b
	45.0	73.44	81.73		3.37	10.44	
	67.5	73.35	80.67		3.28	9.95	
2022	22.5	74.69	82.29	78.03	4.23	10.81	7.38 a
	45.0	74.22	81.78		4.15	10.71	
	67.5	73.97	81.23		3.90	10.51	
Mean of seed types		73.93 b	81.61 a		3.75 b	10.58 a	
Mean of inter-rows		22.5 cm	45.0 cm	67.5 cm	22.5 cm	45.0 cm	67.5 cm
		78.20	77.79	77.31	7.41	7.17	6.91
$LSD_{(0,05)}$		T: 0.67			Y: 0.43, T: 0.43		

Means with different letters are statistically significant. Y: year, R: Inter-row T: seed type, SB: seed with bracteole, SWB: seed without bracteole, DMD: dry matter digestibility, DMI: dry matter intake.

Metabolizable energy and relative feed value

When Table 8 was examined, it was found that the metabolizable energy (ME) and relative feed value (RFV) of seeds without bracteole were higher than those of seeds with bracteole. This was due to the fact that bracteole-peeled seeds had low ADF ratio and high DMD. As a matter of fact, ME and RFV are calculated by using the ADF and NDF values (Khalil et al., 1986). According to this calculation, ME and RFV of feeds with high NDF and ADF content are low, and vice versa, they are high (Kutlu, 2008). According to NRC (2007), the daily amounts of ME required for survival of ruminants with a live weight of 50 kg is 1.91 Mcal. Accordingly, it has been observed that seeds with and without bracteole has ME content that can provide the daily live weight gain in addition to the survival requirement of ruminants. It was also observed that RFV obtained from the study was much higher than the values that should be found in a quality forage. As a matter of fact that according to roughage quality standards, it was reported fact that feeds with a RFV of over 151 were in the best quality class (Rivera and Parish, 2010).

Table 8. Means of metabolizable energy content and relative feed value of seeds with and without bracteole obtained from different inter-row spacings.

Years	Inter-rows (cm)	ME (Mcal kg^{-1})		Mean of years	Relative feed value		Mean of years
		SB	SWB		SB	SWB	
2021	22.5	2.82	3.10	2.95	204.52	701.81	428.05 b
	45.0	2.80	3.09		192.07	661.35	
	67.5	2.80	3.06		186.62	621.91	
2022	22.5	2.84	3.11	2.96	244.74	689.58	456.29 a
	45.0	2.83	3.10		238.77	679.07	
	67.5	2.82	3.07		223.48	662.09	
Mean of seed types		2.82 b	3.09 a		215.03 b	669.30 a	
Mean of inter-rows		22.5 cm	45.0 cm	67.5 cm	22.5 cm	45.0 cm	67.5 cm
		2.97	2.96	2.94	460.16	442.82	423.52
$LSD_{(0,05)}$		T: 0.02			Y: 26.29, T: 26.29		

Means with different letters are statistically significant. Y: year, R: Inter-row T: seed type, SB: seed with bracteole, SWB: seed without bracteole, ME: metabolizable energy.

CONCLUSION

In this study, it was founded that bracteole-peeled seeds had higher desired feed quality values than seeds with bracteole in terms of the feed quality characteristics. The results showed that the nutritional contents of seeds without bracteole were higher than the nutritional values of some cereal grains, widely used as a concentrated feed source in animal nutrition. In addition, the nutritional values of seeds with bracteole were determined to be of the best quality according to roughage quality standards. In the study, with increasing row spacing, it was determined that the crude protein, NDF and ADL contents of the seeds increased. However, it has been observed that the quality values determined in different inter-row spacings do not restrict the use of seeds as a roughage resource. As a result, it has been shown that the seeds with bracteole produced by the *Atriplex nitens* plant, which can grow under arid and unsoiled conditions, can be used as an alternative roughage source for the feed quality characteristics examined. Besides, it was concluded that bracteole-peeled seeds can be used as a concentrated feed resource in the nutrition of farm animals. It was also thought that the secondary compounds of the seeds and their inclusion levels in animal rations in future studies would be useful to reveal.

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EFFECTS OF PLANT DENSITY ON MICRONUTRIENT UPTAKE IN SUNFLOWER (*Helianthus annuus* L.) VARIETIES

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ABSTRACT

The objective of this study was to determine the effects of plant density per unit area on micro nutrients (Iron, Copper, Zinc, Manganese and Boron) uptake of some sunflower genotypes. Three sunflower varieties (Sanay MR, Oliva CL and LG5543 CL) were used as genetic material and three different plant densities: 40800, 57100 and 95200 plants/ha (sowing spacing; 0.35 x 0.70, 0.25 x 0.70 and 0.15 x 0.70 m, respectively). Field experiments were conducted in a split plot design with three replications. According to the results, the micronutrient concentrations as well as the seed and oil yields and partly also oil content increased significantly as the plant density increased. For all analyzed micronutrients (Fe, Cu, Zn, Mn and B), the highest concentration has been obtained with 95200 plant ha-1. Micronutrient elements as well as seed and oil yields differed according to plant density and cultivars. Among the varieties, LG 5543 CL more effected by plant density had the highest micronutrient concentration, seed yield and oil yield. As a result, a high plant density (95,200 plant ha) with the highest micronutrient content and also the highest seed and oil yield could be recommended for Mediterranean environments with a semi-humid climate. However, optimum plant density was found differently according to varieties and years.

Keywords: cultivar, micro nutrients, plant density, sunflower

INTRODUCTION

Sunflower (Helianthus annuus L) has a wide range of adaptation, and is grown as an important oil crop in America, Europe, Asia, Africa and Australia. It was first used by natives in North America as a dyestuff and as an additive to breads. Spanish travelers first cultivated the seeds they collected from North America in the 1850s as ornamental plants. Then it spread by sea from Spain to Italy, Egypt, Afghanistan, China and India. Sunflower, which was brought to Russia in the 18th century, was used here for the first time as an oil plant. With the widespread use of hybrid seeds in sunflower plants, noticeable improvements have been made in high yield, quality and resistance to diseases and pests. In general, its cultivation has become widespread due to its adaptability to all kinds of soil and environmental conditions (Rauf et al., 2017). While the average area of sunflower cultivation in the world was 7.4 million hectares between 1955-59, today this area has tripled and reached 25 million hectares. In the same period, world sunflower production increased approximately 3.9 times and exceeded 20,57 million tons (Hellal et al. 2019; Mansour et al. 2020a, 2020b; FAO, 2023). There has been an increase in cultivation and production areas both in our country and in the world in recent years, since all plant organs can be evaluated and the pulp, stem and table residues remaining after oil is extracted are used as fuel.

Sunflower is an important oil plant after palm, soybean and canola due to its high oil (% 40-47), protein (% 20-27), calcium and vitamin (A, D and E) content. (Skoric et al. 2008; Jabeen and Ahmad, 2011; Iqbal et al. 2014; Li et al. 2017; Diovisalvi et al. 2018; Primo et al. 2018). Approximately 90% of the sunflower seeds produced in the world are processed for oil, and sunflower is the 3rd in the world oilseed production. (MAF, 2020). 12% of the consumable vegetable oil produced in the world is sunflower oil. Besides, linoleic type sunflower oil contains % 60-75 linoleic acid and % 10-30 oleic acid. The ratio of saturated fatty acids (palmitic, stearic, arachidic) is around % 11-12 (Colak et al. 2020).

In order to obtain high yield and quality, optimum growing conditions must be provided in sunflower. If optimum conditions cannot be provided, at the beginning of the main factors limiting growth and development in sunflower, mineral nutrients with a rate of 60% items are coming. Dry matter production in sunflower is high during vegetative and especially generative development period. As a result, the need for nutrients increases from the vegetative development period. Especially in this period, feeding of sunflower plants with microelements enhances

the processes of assimilating macro-elements (Domaratskyi, 2021). Micronutrients have significant role on the growth, photosynthesis translocation, seed formation, pollen grain germination, protein and amino acid synthesis, stigma receptivity of oil seed crops. Consequently, micronutrients increase yield and quality in oilseeds (Sher et al. 2021). As a micro nutrient, zinc (Zn) is an essential element for hormone stimulation, chlorophyll formation, protein mechanism, lipid metabolism, carbohydrate synthesis an activity of enzymes. Besides, Zn has a significant role on biomass production by effecting RNA-DNA synthesis and decreasing lipid peroxidation and protein oxidation. (Balafrej et al. 2020; Ozyigit et al. 2021) Boron (B) is needed for growth of burgeons, root tips and leaves. According to previous studies, it has been dedicated that B effects fatty acid concentration and seed protein (Ayaz et al. 2021). Similarly, iron (Fe) plays a very important role in plant growth due to the basic components in physio-biochemical cells (Kim and Guerinot, 2007; Tripathi et al. 2018). It also plays an important role in the biosynthesis of chlorophyll and in the cells of different enzyme functioning (Khobra et al. 2014). In addition, Fe basically retains catalase and peroxidase activities, which are part of the antioxidant defense shield (Kumar et al. 2010). In plants, copper is essential for the capacities of enzymes involved in carbonate assimilation, ATP components, photosynthesis, molybdenum cofactor (Moco) biogenesis, redox reactions (electron transfer chain), lignin biosynthesis, and others (Kumar et al. 2021).

The relationship between plant density and micronutrient uptake is nuanced and can depend on various factors, including the specific nutrient, plant species, soil conditions, and overall management practices. While high plant density can influence micronutrient uptake positively in some situations. High plant density often leads to increased competition for resources such as water, sunlight, and nutrients. This competition can stimulate more efficient nutrient uptake mechanisms in some plants. Higher plant density generally means more roots in a given area. This increased root density can enhance the exploration of the soil for micronutrients, potentially improving nutrient uptake (Bejandi et al. 2012). Besides, the effect of plant density on soil pH can affect micronutrient availability. Changes in pH can increase the solubility and accessibility of some micronutrients in soil, which can affect their uptake by plants. Its well-known that different plant species have varying nutrient requirements and uptake mechanisms. Some plants may respond positively to higher plant density in terms of micronutrient uptake. It has also been observed that these different responses may vary in different varieties of the same plant (Heidari et al. 2008).

The number of plants per unit area is one of the factors that have the greatest effect on unit area yield. The number of plants per unit area is provided by the distances intra-rows and between rows applied in planting. Since the distance between rows is adjusted in accordance with the working width of the hoeing machines so that cultural operations can be carried out easily, this width is generally applied in the range of 60-70 cm. For this reason, different plant Density are obtained by keeping the spacing between rows constant and changing the distance intra-rows. Optimum plant density may vary according to the climate and soil conditions of the region, as well as according to the variety used. Undoubtedly, plant density per unit area also affects the nutrient and micronutrient uptake of plants. In many studies, researchers stated that planting frequency has important effects on yield and quality factors in sunflower (Emam and Awad, 2017; Fakirah at all. 2017; Ozkan, 2019; Gul and Ada, 2019; Alpman and Sinan, 2020).

Based on the above information, this study aimed to determine the effect of different plant density on micronutrient uptake of oil sunflower varieties for Mediterranean environments with a semi-humid climate.

MATERIALS AND METHODS

Experiment design

This field research was conducted at Bursa Uludag University Faculty of Agriculture Application and Research Centre during spring seasons in both 2017 and 2018. Field experiments were conducted in a split-plot design with three replications in both years. Varieties were main plots and plant density subplot. Main plots were set as 25,2 m x 6 m and subplots as 2,8 m x 6 m and 4 rows were planted for each plot All statistics of the research were obtained from plants in the middle two rows. Plants in the outermost rows were considered as edge effects and were not taken into consideration. Three sunflower varieties (Sanay MR, Oliva CL and LG5543 CL) were used as genetically material and three different plant density: 40800, 57100 and 95200 plants ha^{-1} (sowing spacing; 0.35 x 0.70 m, 0.25 x 0.70 m and 0.15 x 0.70 m, respectively).

Soil and climate characteristics of the trial sites

Soil physical and chemical characteristics are shown in Table 1. As can be seen from the table, the farm soil in the BUU Faculty of Agriculture Agricultural Research and Application Center, where the experiments were carried out, contains some heavy-textured material rich in lime and clay (Katkat et al. 1985). There is no salinity problem. It contains Na^{+} 0.26 me/100g, K^{+} 0.92 me/100g, Mg^{++} 10.20 me/100g, Ca^{++} 30.42 me/100g (Aksoy et al. 2001). Rich in potassium and phosphorus, poor in organic matter.

In the first year (2017), a total precipitation of 255.4 mm was recorded during the sunflower vegetation period (March-August). In 2018, a total of 282.4 mm precipitation occurred. More precipitation occurred in the second year of the experiment compared to the first year. When looking at the average temperature for many years, it is obvious that there is no serious temperature difference between the trial years. Climatic data for the growing season are given in Table 2. When the precipitation totals for many years are examined, it is seen that almost the same amount of precipitation was received as in 2018, the second trial year.

Cultural practices

Sowing was done in the first half of April in both years. Hand hoeing was done twice for weed control and soil ventilation. Experiments were carried out in dry conditions.

Harvest was done in September in both years. In order to prevent nutrient deficiencies in the plants and to ensure the optimum development of the plants 84 kg ha^{-1} N (15-15-15), 50 kg ha^{-1} P and 50 kg ha^{-1} K was applied on the plant rows in the all experimental area. Then, when the plants were at the 6-8 leaf stage, 25 kg of nitrogen was used.

Table 1. Soil properties of experimental area

Properties	Quantities	Properties (mg kg^{-1})	Quantities
Texture	Clay	Sodium (Na)	134
pH	7,86	Potassium (K)	224
EC (mS cm^{-1})	0,32	Magnesium (Mg)	346,8
Lime (% $CaCO_3$)	2,25	Calcium (Ca)	8367
Organic matter (%)	1,74	Iron (Fe)	8,67
Total nitrogen (N) (%)	0,12	Copper (Cu)	1,39
Available phosphorus (P) (mg kg^{-1})	17,65	Zinc (Zn)	1,69
		Manganese (Mn)	21,37
		Boron (B)	1,19

Table 2. Climatic data for the growing season

	Avg. Temperature (°C)			Avg. Humidity (%)			Monthly Precipitation (mm)		
Months	**2017**	**2018**	**Long Term Avg.**	**2017**	**2018**	**Long Term Avg.**	**2017**	**2018**	**Long Term Avg.**
March	10,8	13,7	8,6	71,7	71,4	67,7	24,2	66,0	66,1
April	13,2	16,2	13,0	67,8	68,5	66,1	38,2	14,6	66,0
May	18,3	20,4	17,4	75,0	75,3	62,0	80,2	92,6	43,4
June	23,0	23,6	22,5	70,0	70,0	57,8	75,2	59,4	36,5
July	25,7	25,9	24,8	55,3	62,1	56,2	15,0	15,8	17,7
August	25,8	26,4	24,5	66,0	63,5	57,3	5,0	2,0	13,8
September	23,2	22,3	20,2	61,0	68,7	63,8	17,6	32,0	40,8
Total	**140**	**148,5**	**131**	**466,8**	**479,5**	**430,9**	**255,4**	**282,4**	**284,3**
Average	**20**	**21,2**	**18,7**	**66,6**	**68,5**	**61,6**	**36,4**	**40,3**	**40,6**

Plant analysis

Representative grains were collected on day 130, washed by tap and distilled water than dried at 70 °C until they reach a constant weight. Grain samples were grounded and acid digested in 3 mL of %65 nitric acid (HNO_3) and d 3 mL of 35% hydrogen peroxide (H_2O_2) in microwave system (Berghof MWS 2, Germany) (Celik et al. 2017). Fe, Cu, Zn, Mn and B concentrations of grains were determined with ICP-OES (Inductively coupled plasma optical emission spectrometry) (PerkinElmer Optima 2100 DV) (Hansen et al. 2013). Dry weights and Fe, Cu, Zn, Mn and B concentrations were multiplied for calculation of grain micro element contents.

Statistical analysis

The data obtained from the experiments were statistically analyzed according to the split plot design in randomized blocks with 3 replications. The effects of the varieties, plant Density and variety x plant density interaction were evaluated at the 0.05 and 0.01 probability levels using the F-test. The F-protected least significant difference (LSD) was calculated at the 0.05 probability level according to Steel and Torrie (1960). Variance analyses (ANOVA) for all data were analyzed by JMP 9.0.2. software to determine the significance of treatments and their interactions. In addition, Person's correlation coefficient values were calculated using the MINITAB (version 19) software to determine the degree of pairwise relationships between seed yield, yield components, and macro and micro nutrients uptake in hybrid sunflower varieties. On the other hand, the relationship between seed yield (dependent variable) and independent variables such as some yield components and some macro and micro nutrients was evaluated by multiple stepwise regression analysis.

RESULTS AND DISCUSSION

The effect of different sunflower cultivars with different plant density rates on some micro-nutrients concentrations are shown in Table 3**.** According to table, micronutrient (Fe, Cu, Zn, Mn, B) uptake of cultivars were significantly increased by increasing plant density in the two experimental years. In addition, the highest values were obtained with 95200 plant ha^{-1} and the lowest values were obtained with 40800 plant ha^{-1} plant density in both years. Same results have been described in many previous studies. Jaswinder et al. (2019) dedicated that high plant density increases the nutrient uptake. The same researchers reported that, increasing nutrient uptake might be due to better established root system, translocation of nutrients from soil, transport of nutrients to seed which led to better yield.

Table 3. Effects of plant density and variety on micro elements of sunflower in 2017 and 2018

	Plant density (plant ha^{-1})					
	95200		57100		40800	
	2017	2018	2017	2018	2017	2018
Cultivars	**Fe (mg kg^{-1})**					
Sanay MR	25,09 a	23,71 a	21,58 c	20,83 bc	20,16 d	18,78 de
Oliva CL	21,71 c	19,92 cd	25,08 a	23,34 a	21,93 bc	20,46 bc
LG 5543 CL	25,45 a	23,99 a	23,11 b	21,65 b	18,91 d	17,54 e
Mean	**24,09 A**	**22,54 A**	**23,26 B**	**21,94 A**	**20,33 C**	**18,93 B**
	Cu (mg kg^{-1})					
Sanay MR	2559,28 a	2485,74 a	1914,31 bc	1858,77 bc	1692,92 de	1643,65 de
Oliva CL	1911,28 bc	1855,68 bc	2566,35 a	2491,56 a	1813,74 cd	1761,19 cd
LG 5543 CL	2597,46 a	2522,17 a	1979,99 b	1922,35 b	1556,92 e	1511,93 e
Mean	**2356,01 A**	**2287,86 A**	**2153,55 B**	**2090,89 B**	**1687,86 C**	**1638,92 C**
	Zn (mg kg^{-1})					
Sanay MR	623,02 a	604,70 a	399,11 b	388,10 b	298,34 d	287,75 d
Oliva CL	328,70 cd	319,38 cd	603,22 a	586,36 a	220,59 e	214,17 e
LG 5543 CL	584,65 a	568,16 a	366,46 bc	355,79 bc	195,80 e	190,30 e
Mean	**512,13 A**	**497,41 A**	**456,26 B**	**443,42 B**	**238,24 C**	**230,74 C**
	Mn (mg kg^{-1})					
Sanay MR	61,04 ab	59,08 ab	38,19 c	37,09 c	27,37 e	26,84 e
Oliva CL	31,40 de	30,49 de	61,94 a	60,14 a	21,18 f	20,43 f
LG 5543 CL	57,40 b	55,42 b	35,04 cd	33,97 cd	18,00 f	17,48 f
Mean	**49,95 A**	**48,33 A**	**45,05 B**	**43,73 B**	**22,08 C**	**21,58 C**
	B (mg kg^{-1})					
Sanay MR	3,69 a	3,58 ab	2,11 b	2,04 c	1,39 d	1,34 e
Oliva CL	1,77 c	1,72 d	3,79 a	3,71 a	1,16 de	1,13 ef
LG 5543 CL	3,54 a	3,44 b	1,98 bc	1,93 cd	0,92 e	0,90 f
Mean	**3,00 A**	**2,91 A**	**2,63 B**	**2,56 B**	**1,15 C**	**1,12 C**

Fe and Cu concentrations of sunflower varieties were observed between 17,54 mg kg^{-1} - 25,45 mg kg^{-1} and 1511,93 - 2597,46 mg kg^{-1} respectively depending on plant density. The highest Fe and Cu concentrations were found in LG 5543 CL which was cultivated as 95200 plant ha^{-1} for both experiment years. In addition, the lowest values were obtained with LG 5543 CL and 4800 plant ha^{-1} plant density.

As can be seen from Table 2, the Zn concentration decreases as the plant density decreases, the highest Zn concentration was obtained at 95200 plant ha^{-1}. Between the cultivars, while the highest Zn concentration was observed as 623,02 mg kg^{-1} in Sanay MR and as 584,7 mg kg^{-1} in LG 5543 CL which planted as 5200 plant ha^{-1}, the lowest concentration was observed in as 190,30 mg kg^{-1} with LG 5543 CL which planted as 4800 plant ha^{-1} (Table 3).

Mn and B concentrations of sunflower varieties were observed between 17,48 mg kg^{-1} – 61,94 mg kg^{-1} and 0,90 – 3,79 mg kg^{-1} respectively depending on plant density. Unlike the aforementioned elements; the highest Mn and B concentrations were obtained as 61,94 and 3,79 mg kg^{-1} respectively in Oliva CL with 57100 plant ha^{-1} but, the lowest values were obtained as 17,48 and 0,90 mg kg^{-1} respectively in LG 5543 CL with 40800 plant ha^{-1} plant density (Table 3). As a result, 95200 plant ha^{-1} was the optimal plant density for Fe, Cu, Zn uptake while, 57100 plant ha^{-1} was the optimal for B and Mn uptake of sunflower.

The effect of different sunflower cultivars with different plant density rates on some quality properties and yield of sunflower are shown in Table 4. As can be seen from the table, quality parameters as seed yield, oil content and oil rate are significantly increased by increasing plant density in the two experimental years. On the contrary, head diameter of sunflowers was significantly decreased with plant density. Maximum head diameter was observed between 15,59 and 23,86 cm according to plant density. For both experiment years the maximum head diameter was measured with 40800 plant ha^{-1} plant density in Sanay MR cultivar. In addition, the minimum head diameter observed in Oliva CL cultivar with 95200 plant ha^{-1} plant density in both experiment years. Allam and Galal, (1996); Salehi and Bohrani, (2000); Al-Thabet, (2006) dedicated that decreasing in plant density was led to an increase in head diameter which results are similar with our study. Several studies have reported that with increasing plant density decreases head diameter but increasing plant density enhances the seed yield (Johnson et al. 2010; Jahangir et al. 2006; Panhwar et al. 2017). Modanlo et al. (2021) reported that, with increasing plant density yield and oil yield have effected positively but, head diameter, plant height, 100-grain yield and grain yield per plant were decreased with increasing plant density.

Table 4. Effects of plant density and variety on some quality properties and yield of sunflower in 2017 and 2018

	Plant density (plant ha^{-1})					
	95200		57100		40800	
	2017	2018	2017	2018	2017	2018
Cultivars	**Head diameter (cm)**					
Sanay MR	19,54 bcd	18,05 bcde	17,13 cd	16,28 de	23,86 a	23,10 a
Oliva CL	16,53 d	15,59 e	19,86 bc	19,87 bc	21,10 ab	21,10 ab
LG 5543 CL	18,13 bcd	17,23 cde	19,93 bc	18,95 bcd	20,76 ab	20,77 ab
Mean	**18,07 B**	**16,95 B**	**18,97 B**	**18,36 B**	**21,91 A**	**21,65 A**
	Seed Yield (kg ha^{-1})					
Sanay MR	328,96 f	298,36 g	356,42 e	331,42 ef	343,63 e	313,25 fg
Oliva CL	405,65 b	386,50 b	386,64 c	365,86 bc	370,33 d	357,48 cd
LG 5543 CL	481,03 a	450,83 a	369,74 d	339,68 de	346,03 e	323,60 ef
Mean	**405,21 A**	**378,56 A**	**370,93 B**	**345,65 B**	**353,33 C**	**331,44 C**
	Oil content (%)					
Sanay MR	43,76 c	44,40 d	45,45 b	46,50 b	42,49 d	43,26 e
Oliva CL	44,21 c	45,24 c	45,55 b	46,28 b	43,40 cd	44,61 cd
LG 5543 CL	45,59 b	46,42 b	47,40 a	48,55 a	43,86 c	44,66 cd
Mean	**44,52 B**	**45,35 B**	**46,13 A**	**47,11 A**	**43,25 C**	**44,18 C**
	Oil Yield (kg ha^{-1})					
Sanay MR	143,92 e	132,43 g	161,96 c	154,13 de	146,02 e	135,53 fg
Oliva CL	179,37 b	174,90 b	176,13 b	169,35 bc	160,72 c	159,43 cd
LG 5543 CL	219,33 a	209,31 a	175,26 b	164,92 bcd	151,76 d	144,51 ef
Mean	**180,87 A**	**172,21 A**	**171,11 B**	**162,80 B**	**152,83 C**	**146,49 C**

Yield and oil yield of sunflower cultivars have effected positively with increasing plant density. Yield was observed between 313,25 and 481,03 kg ha^{-1} according to plant density and oil yield was observed between 135,53 - 219,33 kg ha^{-1}.

Maximum seed yield and oil yield were obtained with 95200 plant ha^{-1} plant density in LG 5543 CL cultivar but, minimum values were observed in Sanay MR cultivar with 40800 plant ha^{-1} plant density for both experimental years. Unlike these parameters, maximum oil content of sunflower was obtained with 57100 plant ha^{-1} plant density. It was measured between % 42,49 and % 47,40. LG 5543 CL cultivar which provided the highest grain and oil yield in 95 200 plant ha^{-1} Density, also achieved the highest Fe, Cu, Zn and B uptake at the same plant density. These results revealed that high uptake of micronutrients in the plant provides high seed and oil yield. Therefore, many studies were found that fertilization with these micronutrients increases the yield and quality of sunflower (Khurana and Chatterjee, 2001; Gawande et al. 2022; Baraich et al. 2016; Pattanayak et al. 2017; El-Din Mekki, 2015). Similar to yield parameters, maximum and minimum oil content values were found in LG 5543 CL and Sanay MR cultivars respectively. Findings on oil content in previous studies have shown different results at varying plant densities (Modanlo et al. 2021; Partal, 2022). This is thought to be because seed from higher plant density may have a thinner pericarp with a slightly higher oil content. In addition, the percentage of oil of sunflower seeds depends on the ratio of the percentage of shell and oil content in the kernel (Rao and Reddy, 1985; Sabo and Pepo, 2007; Namvar et al. 2012). As the distance between the rows increased, in other words, as the plant density decreased, low seed yields is obtained in these plots due to the low plant numbers per unit area. The best seed yields per hectare is measured in narrow row sowing distances (Alpman and Sinan 2020). Our results are supported by findings of some previous studies which indicating that increase plant density significantly increase seed yield (Beg et al. 2003, Robinson 1976, Barros et al. 2004; Sabo and Pepo, 2007; Weiss, 2000; Wade and Foreman, 1988). On the contrary some researchers dedicated that the seed yield was not affected by increasing plant densities explaining that the optimum plant density for seeds yield depends on the cultivar and environment (Majid and Schneiter, 1987; Pala, 1992; Barros et al., 2004).

The results showed that as the plant density increased, the uptake of micronutrients such as Fe, Cu, Zn increased, as well as the seed and oil yield and oil content. Increasing plant density provided an increase in seed and oil yield and partially also oil content by increasing the micronutrient uptake in the plant. Khurana and Chatterjee (2001) reported that zinc fertilization had a positive effect on sunflower and increased seed yield of sunflower and seed oil by application of zinc. Because of Zn having important role in carbohydrate and nitrogen metabolism, fruit and root development. In addition, Zn is essential in amino acid and RNA - DNA synthesis, by involving in in bio-synthesis of plant hormones, Indole Acetic Acid (IAA) and variety of enzymes. Gawande et al. (2022) dedicated that when Zn and Fe concentrations increases in sunflower, number of filled seed is also increased thus the yield of sunflower is enhanced. The same researchers also indicated that this increase in dry matter and yield in sunflower is due to higher photosynthetic rate of plants, which depends upon number of functional leaves, plant height, and dry matter

accumulation in plants. In many studies, researchers have explained this increase in yield is because zinc and iron have a very important role especially in protein and auxin metabolism and that they are essential for many enzyme activations (Rao et al. 2020; Farzanian et al. 2010).

The results of the research showed that micronutrient uptakes, seed and oil yield, and partly also oil contents in sunflower vary according to plant density and cultivars. Our findings revealed that the plant density to be applied in planting and the variety to be preferred are important in order for the plants to obtain higher micronutrient uptakes and to reach higher yield and quality.

Since the uptake of micronutrients such as Fe, Cu and Zn increases as the plant density increases, these micronutrients must be sufficiently present in the soil in areas where high plant Density are applied. For high yield and quality in sunflower, these micronutrients, which are deficient in the soil, should be applied in the form of fertilizer. Thus, Milev (2015) proved that sunflower reacted to multicomponent mineral fertilizers with increased yield and its quality. Baraich et al. (2016) showed that foliar fertilization (8 Zn + 0.75 B + 0.30 Fe kg ha−1) significantly improved the sunflower yield and its components. Pattanayak et al. (2017) showed that sunflower positively responded to combined NPK, zinc and boron fertilization. Kandhro et al. (2021) proved the beneficial interaction of Zn fertilization and plant irrigation on sunflower yielding. In addition, they indicated the need to select an appropriate variety for the habitat conditions. Li et al. (2018) demonstrated that foliar zinc fertilization had a positive effect on the yielding of sunflower, even when the content of this element in the soil was sufficient. Mekki (2015) believed that boron was important in fertilizing sunflower seeds. In a greenhouse experiment, he showed that the deficiency of this micronutrient in the soil reduced both the size and quality of the crop. Al-Amery et al. (2011) proved that boron fertilization reduced the number of empty achenes, which resulted in a significant increase in yield.

In the study, positive and significant relationships were found between seed and oil yields and the uptake of some micronutrients. Pearson correlation coefficients describing pairwise relationships between yield components and some micronutrients of the tested hybrid sunflower cultivars are shown in **Table 5.** Positive and significant correlation were observed between SY and Fe ($r = 0.447$, $p \leq 0.01$), Cu ($r = 0.416$, $p \leq 0.01$), Zn ($r = 0.292$,$p \leq 0.05$), Mn ($r = 0.297$, $p \leq 0.05$), B ($r = 0.326$, $p \leq 0.05$), Mg ($r = 0.326$, $p \leq 0.05$) and HD ($r = - 0.347$, $p \leq 0.01$). Also, the pairwise relationships between oil yield and the same micronutrient elements gave similar results. Similarly, significant and positive correlations were observed between OC and Fe ($r = 0.283$, $p \leq 0.05$), Cu ($r = 0.267$, $p \leq 0.05$), Zn ($r = 0.277$, $p \leq 0.05$), Mn ($r = 0.284$, $p \leq 0.05$), B ($r = 0.94$, $p \leq 0.05$), Mg ($r = 0.288$, $p \leq 0.05$) and HD ($r = - 0.423$, $p \leq 0.01$). In contrast, the correlations between grain yield, oil yield and oil ratio and micronutrients were not statistically significant. Micronutrients, both present in the plant and applied externally to the plant, have an increasing effect on yield and quality. Anuprita et al., (2005) detected that application of micronutrients increase the uptake of all the plant nutrients and enhance the mechanism against disease and pest thus consequently improve yield and plant growth. Iron (Fe) is also one of the important nutrient involved in the formation of chlorophyll and light reaction of electron transport chain and thus can enhance the growth and yield of crop (Kakar et al., 2000; Tariq and Mott, 2006). The use of boron has increased the vegetative and reproductive growth of the sunflower (Asad *et al*.,2003). Boron is involved in cell wall synthesis, maintenance, sugar translocation and membrane integrity and its requirement is higher for seed production than vegetative production (Dordas and Brown, 2001). Alipatra et all (2018) reported that sunflower seed yield showed highest significant positive correlation with TNU (total N uptake, r = 0.670**), closely followed by TBU (Total B uptake, r = 0.669**), TSU (Total S uptake, r = 0.662**), TPU (Total P uptake, r = 0.618**) and TKU (Total K uptake, r = 0.561**).

Table 5. Correlation coefficients between seed yield and macro and micronutrients uptake in hybrid sunflower genotypes (based on mean values of two years)

Traits	Fe	Cu	Zn	Mn	B	HD	SY	OC	OY
Fe	1,0000	0,9240**	0,8516**	0,8531**	0,8596**	-0,2584	0,4466**	0,2827*	0,447**
Cu		1,0000	0,9609**	0,9645**	0,9733**	-0,286*	0,4161**	0,2673*	0,416**
Zn			1,0000	0,9979**	0,9942**	-0,294*	0,2916*	0,2774*	0,292*
Mn				1,0000	0,9976**	-0,290*	0,2974*	0,2836*	0,297*
B					1,0000	0,3265*	0,2881*	0,2941*	0,326*
HD						1,0000	-0,347**	-0,423**	-0,35**
SY							1,0000	0,2289	1,00**
OC								1,0000	0,2289
OY									1,0000

Notes:Fe = Iron, Cu = Copper, Zn = Zinc, Mn = Manganese, B = Boron, HD = Head diameter, SY = Seed yield, OC = Oil content, OY = Oil yield.

A multiple regression model was used to evaluate the relationships between seed yield (dependent variable) and macro and micro nutrients and some yield components (independent variables). According to multiple regression analysis, the appropriate regression model was determined as follows:

Seed yield = 189.7 + 1.527 total Cu uptake** - 0.429 total Zn uptake** - 4.767 Head diameter*

The stepwise regression equation showed that an independent variable like total Cu uptake had positive significant relationships but negative significant total Zn uptake and head diameter with seed yield (dependent variable). In a similar study, Alipatra et al. (2018) found that independent variables like total N uptake, plant height, available N, weight of filled seeds/capitulum and seed index (100-seed weight) had positive significant relationship with seed yield (dependent variable). The results of this study and previous studies have revealed that there is a positive and significant relationship between grain and oil yields and some macronutrients, micronutrients and some yield components in sunflower, but there may be changes in macro and micronutrient elements that may affect grain yield depending on the conditions in which the research is conducted.

CONCLUSION

In sunflower, which is an important oil plant, the effects of plant density per unit area and cultivar differences on the micronutrient uptake, yield and quality of the plant were investigated. The results revealed that micronutrient uptakes, seed and oil yield, and partly also oil contents in sunflower vary according to plant density and cultivars. Increasing plant density provided an increase in seed and oil yield and partially also oil content by increasing uptake of micronutrient such as Fe, Cu, Zn in the plant. In study, maximum seed yield and oil yield were obtained with 95200 plant ha^{-1} plant density in LG 5543 CL cultivar but, minimum values were observed in Sanay MR cultivar with 40800 plant ha^{-1} plant density for both experimental years. The conclusion that can be obtained from this study is to suggest a high plant density (95200 plant ha) and a suitable cultivar in order to achieve high micronutrient uptake in sunflower and hence high seed and oil yield and partly high oil content in Mediterranean environments with a sub-humid climate. The study has evaluated the most commonly used planting densities in the literature. In future research, higher and lower plant densities should be considered to further support and enrich the literature. The results of our study have demonstrated a positive impact on yield and micronutrient uptake at higher densities. However, how yield and nutrient element uptake will be affected when density is further increased should be determined in future studies.

LITERATURE CITED

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THE EFFECT OF IRRIGATION MANAGEMENT, MUNICIPAL WASTE COMPOST AND NITROGEN FERTILIZER ON SEED YIELD, QUALITY AND SOME PHYSIOLOGICAL TRAITS OF PEANUT (*Arachis hypogaea* L.)

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ABSTRACT

Present study was conducted to investigate the effect of municipal waste compost and nitrogen fertilizer on yield and some physiological traits of peanut under both irrigation and no-irrigation conditions. A split-split plot experiment was conducted based on a randomized complete block design with three replications and performed in two years (2018 and 2019). The main plot consisted of irrigation at two levels of with and without irrigation. Compost treatment (at two levels of application of 5 t ha^{-1} and no application) was considered as a subplot. Nitrogen fertilizer (at four levels of 0.0, 20.0, 40.0 and 60.0 kg of pure nitrogen per ha) was considered as a sub-sub plot. The results showed that the application of 5 t ha^{-1} compost significantly increased carotenoids and rate of kernel production by 16.1% and 15.2%, respectively. In the interaction of irrigation and compost, the highest seed protein and seed yield were obtained in two levels of compost and irrigation conditions. In both years, the highest seed yield was observed in the treatments of irrigation and application of 40 and 60 kg N ha-1. Besides, the application of compost along with 40 and 60 kg N ha^{-1} resulted in a significant increase in seed yield. In the second year compared to the first year, under no-irrigation and no-application of nitrogen, seed yield was 21% higher. It seems that application of 40 kg ha^{-1} N along with 5 t ha^{-1} of municipal waste compost in peanut cultivation can be effective in improving the physiological traits and seed yield, especially under no-irrigation.

Keywords: Drought stress, Chlorophyll content, Seed oil, Seed protein

INTRODUCTION

Increasing world population and demand for more food production has reduced the organic matter content of agricultural lands due to continuous cultivation and limited sources of organic matter do not meet the growing demand for fertilizer (McDonagh et al., 2001). Therefore, the use of materials such as agricultural waste, industrial and municipal wastes as sources of supply organic matter is expanding. However, in order to reduce their environmental hazards, some of these wastes need to be decomposed and detoxified before they can be used in agriculture (Sirousmehr et al., 2014). Doing this process changes the nature of the waste and the formation of a new material that called compost. The use of compost is effective on economic and environmental factors such as reducing transport and landfill costs, complying with environmental legislation, reducing the use of mineral fertilizers and improving the properties of agricultural soils (Hargreaves et al., 2008). Applying compost improves the soil structure, strengthens the mineral content of the soil and allows the soil to retain moisture longer. Compost can hold water two to six times more than its volume of water, preventing it from volume of water, preventing it from being wasted (Agassi et al., 2004). Compost in heavy soils improves soil porosity and improves soil aeration. It also acts like a sponge in light soils and largely preventing leaching by retaining water and nutrients (Waqas et al., 2014). The use of compost in agriculture can increase plant growth and yield through its effect on increasing plant water use efficiency and nutrient release (Governog et al., 2003; Tepecik et al., 2022; Tepecik et al., 2023). In addition, compost enhances the activity of soil microorganisms and contributes to its fertility and prevents soil erosion by forming stable aggregates (Doan et al., 2015). Compost has the ability to increase the fertility of rainfed fields due to its organic composition and high water holding capacity (Ozturk and Yildirim, 2013; Doan et al., 2015; Abbott et al., 2018

Nitrogen deficiency, both directly and indirectly, has always been considered as a limiting factor in plant growth (Moshki et al., 2017). Nitrogen is directly involved in production of new cells, production of nitrogen compounds in cells, production of enzymes and cell membrane components of the cells and also has an indirect effect on also has an indirect effect on leaf area growth and plant growth (Arshadi and Asgharipour, 2011). Nitrogen deficiency symptoms in most plants appear as yellowing or pale leaves (chlorosis), especially in the lower leaves of the plant. Under severe nitrogen deficiency conditions, these leaves turn completely yellow and drop from the plant (Taiz and Zeiger, 2010). Some studies have shown that nitrogen deficiency reduces the water potential and also increases the abscisic acid of the leaves, which may be effective in the aging process of the leaves (Gardner et al., 1988; Haidari et al., 2023). Ichie et al. (2002) reported that among the macronutrients and essential elements for plants, nitrogen is the most important element for growth.

Photosynthetic pigments such as chlorophyll are the most important factors in the photosynthetic capacity of plants, because they directly affect the rate and amount of photosynthesis and biomass production (Nouri et al., 2020). These compounds, in addition to trapping the radiation energy of the sun and transferring it to the photosynthetic system (in form of antenna pigments, in the energy funnel complex), are also considered part of the plant's antioxidant system, contributing to the destruction of reactive oxygen species and effective factors in oxidative stress (Taiz and Zeiger, 2010; Inze and Montagu, 2000).

Peanut (*Arachis hypogaea* L.) is one of the sources of edible oil supply in the world and an important crop in Guilan province in Iran. This product has an effective role in promoting the economic prosperity of this province. In 2018, the area under peanut cultivation in Gilan province was 2860 ha and the largest share in the production of this product belonged to farmers in Astana region with 9529 tons of dry pods from 2507 ha (Anonymous, 2019). Peanut is not very drought tolerant and insufficient water supply is one of the factors limiting its yield. In other words, successful peanut cultivation and production requires adequate water supply during the growing season (Reddy et al., 2003). However, the sensitive stage of peanuts to water availability is from flowering to the end of the pod filling period (i.e. reproductive stage) and drought stress during the vegetative growth stage, compared to reproductive stage, has little effect on seed yield (Kumar et al., 2010). Therefore, it seems that peanut planting might be accomplished without irrigation in areas with adequate rainfall and a match between rainfall distribution and peanut reproduction stage. It is presumed that by applying compost which can increase the water use efficiency, a positive step can be taken to produce peanuts without irrigation.

Although legumes can provide some of the required nitrogen through their ability to biologically fix nitrogen (Denison and Kiers, 2011), studies demonstrated that nitrogen application at early growth stages is useful and even necessary, as nodules have not yet formed and the amount of starter nitrogen in the soil would be low (Salvagiotti et al., 2008). It is also advisable to use nitrogen fertilizers at the final stages of crop growth along with the formation of seeds, because seeds are very strong reservoirs of nitrogen accumulation and the amount of fixed nitrogen may not meet the needs of seeds (Silvia and Frantisek, 2012). Sugut et al. (2013) showed that the application of 200 kg N ha^{-1} resulted in the highest yield of fruits and seeds and increased the amount of nitrogen and protein content of peanut seeds. Accordingly, the present study aimed to investigate the effect of municipal waste compost and nitrogen fertilizer on yield and quality and physiological characteristics of peanut plants under both irrigation and no-irrigation conditions.

MATERIALS AND METHODS

This research was carried out in a farm located in Parkapasht village of Astaneh Ashrafieh city in Guilan province (37°18’N, 49°52’E, 2 m a.s.l.). The experimental design was Randomized Complete Block Design (RCBD) arranged in split plots with three replications, carried out in 2018 and 2019 and subjected to a combined analysis. In this experiment, the main plot consisted of two levels of irrigation, with and without irrigation. Compost application treatment (at two application levels of 5 t ha^{-1} and control) as a subplot and nitrogen fertilization treatment (at four levels of 0.0, 20.0, 40.0 and 60.0 kg of pure nitrogen per ha) as a sub-subplot. The homogeneity of the variance of the experimental errors was ensured by means of the Bartlett test before the performance of the combined data analysis.

Before land preparation, the soil was randomly sampled from six points at a depth of 0 to 30 cm and its physical and chemical properties investigated (Table 1). The texture of soil at the experimental site was silty. After soil preparation, planting took place in the third week of May in both years of the experiment. Before sowing, the physical and chemical properties of the municipal waste compost were analysed (Table 2) and then applied according to the determined rate in the respective treatments. Nitrogen was applied as urea fertiliser in two stages (half before planting and half one month after germination) according to the specified rate. Each plot consisted of six planting rows of 5m in length with a distance between rows of 0.6 m. Row spacing and planting depth were 20 and 6 cm, respectively, and plant density was considered to be 8.3 plants per m^2.

After planting, all field operations such as irrigation and application of nitrogen fertilizer (in the respective treatments), and weed control were applied equally in all treatments. In both years, for irrigation treatment, the amount of irrigation water was monitored by installing a volume meter in the field. Irrigation was applied eight times and in total of 2400 m^3 water was used. The amount of rainfall during the growing season in the two years of the experiment was 138 and 219 mm, respectively (Table 3).

Table 1. Soil characteristics of the experiment site

Year of experiment	pH	EC (dS m^{-1})	K (mg kg^{-1})	P (mg kg^{-1})	Total N (%)	OC (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	S (%)	Ca (mg kg^{-1})
2018	7.32	0.60	195	12.0	0.11	1.08	98.6	3.2	2.2	38.1
2019	7.29	0.55	218	10.8	0.09	0.81	39.4	2.1	0.14	42.1

Table 2. Characteristics of used compost in the experiment

Year of experiment	EC (dS m^{-1})	Na (%)	K (%)	P (%)	Total N (%)	OC (%)	Humidity (%)
2018	2.35	0.60	0.72	1.25	1.58	20.31	18.77
2019	2.75	0.50	0.31	1.60	1.66	21.32	18.14

Table 3. Meteorological information of the experiment site during the growing season

Months of growing season (from planting up to harvest)	Year of experiment	Rate of rainfall (mm)	Mean of humidity (%)	Mean of temperature (°C)
May	2018	12.0	75	19.0
	2019	61.3	77	19.1
June	2018	16.4	74	23.4
	2019	1.3	72	25.0
July	2018	14.0	74	28.4
	2019	54.6	76	26.7
August	2018	88.6	78	27.2
	2019	22.1	76	25.9
September	2018	7.0	77	25.1
	2019	79.7	83	23.3

In both years, in the middle of flowering stage, the amount of chlorophyll *a*, *b* and carotenoids of the leaves were determined using a spectrophotometer (Lichtenthaler and Wellburn, 1983). First, 0.1 g of leaves was ground with four ml of 80% acetone in a porcelain mortar and the resulting solution was centrifuged for 5 min at 3000 rpm. Adsorption of the supernatant was then measured using a model A model 2100 spectrophotometer at wavelengths of 646, 663 and 470 nm was used to determine the amount of chlorophyll and carotenoids. 80% acetone was used for resetting. The contents of chlorophyll a and b and of carotenoids (in mg/g fresh leaf weight) were calculated using equations 1, 2 and 3.

Eq 1: $Chl_a = 12.25A_{663} - 2.79A_{646}$

Eq 2: $Chl_b = 21.21A_{646} - 5.1A_{663}$

Eq 3: $carotenoid = \frac{1000A_{470} - 1.8chla - 85.02chlb}{198}$

In these equations: A_{646}, A_{663} and A_{470} are the light absorption at wavelength of 646, 663 and 470 nm, respectively.

In the lower half of each plot, used for yield assessment, the crop is harvested following removal of the weeds, threshed and seed separated for seed yield measurement. To calculate the rate of seed production, 200 g of fully ripe and dried pods were selected from each plot. A digital balance was then used to determine the weight of the shell, pods and seeds from this 200 g sample. The seed percentage was determined from the ratio of seed weight to pod weight. The amount of nitrogen in the seed was determined by titration after distillation using an automatic Kajeldahl system, and then seed protein was determined as the product of nitrogen multiplied by 5.46 (Smart, 1994). Seed oil was extracted by the Soxhlet method (Smart, 1994). Harvesting was carried out in the last week of September in both years of the experiment. The data were analysed using SAS software and the means were compared using the Duncan test.

RESULTS AND DISCUSSION

Chlorophyll a

The interaction effect of irrigation, compost and nitrogen on peanut chlorophyll *a* content was significant (Table 4). The highest amount of chlorophyll *a* was observed in treatment with irrigation and application of 5 t. ha^{-1} compost and 60 kg N ha^{-1} at the rate of 11.1 mg g^{-1} FW. In other treatments, the amount of chlorophyll *a* was less than 11 mg g^{-1} FW (Table 5). In both compost levels and irrigation conditions, the amount of chlorophyll *a* was significantly higher than in same levels of compost and

non-irrigation conditions. The amount of chlorophyll an also increased significantly with increasing nitrogen use at different compost and irrigation levels. The lowest amount of chlorophyll *a* was also observed in treatment of no-irrigation and no-application of compost and nitrogen. The amount of chlorophyll *a* in this treatment did not even reach seven mg g^{-1} FW (Table 5). It seems that water scarcity has a negative effect on the process of chlorophyll a synthesis and this has reduced the amount of it. According to scientific reports, measuring the concentration of chlorophyll as an indicator of the strength of the main sources of photosynthesis (leaves) in the plant is a well-known and reliable method. (Nouri et al., 2020; Arshadi et al., 2021). Karami et al. (2020) reported a decrease in the amount of chlorophylls in Amaranth (*Amaranthus hypochondriacus*) along with reducing the soil moisture availability and increasing the intensity of drought stress. The decrease in chlorophyll content is caused by its increased degradation, destroying the photosynthetic pigment structure and lacking conditions for chlorophyll synthesis. In another study, Nikolaeva et al (2010) investigated the effects of drought stress on chlorophyll content and antioxidant enzyme activity in leaves of three wheat cultivars and found that chlorophyll content increased at the beginning of the drought stress and then decreased during the stress. In addition, this study showed that applying compost at irrigation levels resulted in a significant increase in chlorophyll a compared to no application. It appears that by providing water and nutrients necessary for chlorophyll synthesis (such as nitrogen), compost application was able to increase its levels. Research has shown that nitrogen is part of the chlorophyll molecule and that it may increase chlorophyll synthesis if it is available (Taiz and Zeiger, 2010). Arshadi et al. (2021) reported a significant increase in chlorophyll a in chickpea with the combined application of rhizobial and mycorrhizal biofertilisers and attributed this to the availability of elements required for chlorophyll a synthesis (particularly nitrogen) by rhizobia and mycorrhiza.

Table 4. Variance analysis results for studied traits of peanut

SOV	DF	Mean squares					
		Chlorophyll *a*	Carotenoids	Seed yield	Seed oil	Protein of seed	Rate of Kernel production
Year (Y)	1	1.66 ns	32.90 ns	63860 ns	117 ns	85.70 ns	84.40 ns
Replication * Y	4	13.61	49.70	16012	139	13.10	15.60
Irrigation (I)	1	128.70 **	204 **	2317574 **	1600 **	263 **	222 ns
Y × I	1	0.00 ns	18.90 ns	28635 **	0.04 ns	0.10 ns	0.37 ns
Error (a)	4	0.116	15.6	4001	0.145	1.85	194
Compost (C)	1	25.81 **	150 **	586875 **	2.10 **	68.00 **	1820 **
Y × C	1	0.00 ns	18.00 ns	5400 ns	0.40 ns	0.02 ns	0.04 ns
I × C	1	3.93 **	57.00 ns	148208 **	0.66 **	20.50 **	0.04 ns
Y × I × C	1	0.00 ns	19.60 ns	8702 ns	0.08 ns	0.14 ns	0.04 ns
Error (b)	8	0.08	18.61	5864	0.13	1.26	85.50
Nitrogen (N)	3	3.28 **	46.82 ns	484472 **	15.32 **	101 **	15.41 **
Y × N	3	0.00 ns	18.91 ns	9475 *	0.04 ns	0.06 ns	0.15 ns
I × N	3	0.16 **	17.82 ns	22875 **	6.96 **	0.22 ns	1.04 ns
C × N	3	0.18 **	22.83 ns	20186 **	4.07 **	1.76 ns	1.47 ns
Y × I × N	3	0.00 ns	18.50 ns	8964 *	0.05 ns	0.04 ns	0.15 ns
Y × C × N	3	0.00 ns	18.92 ns	2159 ns	0.07 ns	0.06 ns	0.26 ns
I × C × N	3	0.68 **	16.31 ns	6210 ns	4.77 **	2.97 ns	5.93 ns
Y × I × C × N	3	0.00 ns	18.40 ns	1074 ns	0.03 ns	0.04 ns	0.26 ns
Error (c)	48	0.01	18.90	2386	0.08	1.23	2.18
CV (%)	-	1.51	25.91	3.53	0.63	4.99	2.39

*ns, * and **: non-significant, significant in 5% and 1% level, respectively*

Carotenoids

The effect of irrigation on peanut carotenoids was significant (Table 4). Irrigation caused a significant increase of 18.9% in amount of peanut leaves carotenoids compared to droughty condition (Figure 1). These results are in agreement with the findings of other researchers (add references). Karimi et al. (2020) also stated in their reports, a significant decrease in carotenoids during the decrease of soil moisture.

The effect of the compost on the carotenoids of the peanut was significant (Table 4). Applying 5 t ha^{-1} of compost significantly increased (16.1%) the amount of

carotenoids in peanut leaves (Figure 2). It seems that the application of compost, due to its ability to release nutrients gradually (Governog et al., 2003), can be effective in providing the necessary substrate for carotenoid synthesis in the peanut plant. In general, carotenoids are isoprenoid molecules that are studied in the form of groups such as hydrocarbon carotenes, such as lycopene and beta-carotene, or xanthophylls (Taiz and Zeiger, 2010).

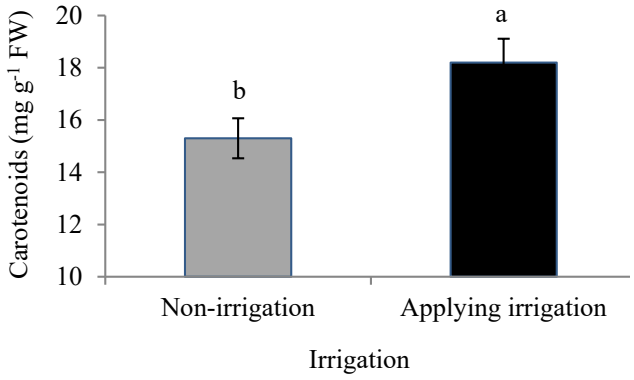

Figure 1. Effect of irrigation on carotenoids of peanut.
Means that have a common letter, have not significantly different together at 5% based on Duncan test.

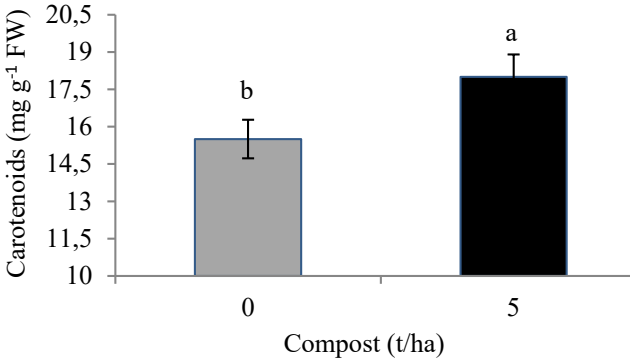

Figure 2. Effect of compost on carotenoids of peanut.
Means that have a common letter, have not significantly different together at 5% based on Duncan test.

Seed yield

The interaction effect of irrigation and application of compost on the seed yield of peanut was significant (Table 4). The treatment of irrigation and application of 5 t ha^{-1} compost at the rate of 1576 $kg.ha^{-1}$ produced the highest seed yield. This treatment was statistically grouped with the irrigation and no compost treatment (Table 6). Seed yield was significantly higher in both compost levels and irrigated conditions compared to the unapplied and irrigated treatments. The lowest seed yield was also observed in the treatment without irrigation and without applying compost, so that the seed yield in this treatment was less than 1110 kg ha^{-1} (Table 6).

The interaction effect of compost and nitrogen on peanut seed yield was significant (Table 4). That is, the highest seed yield was obtained in two treatments of applying 5 t ha^{-1} compost and applying 40 and 60 kg N ha^{-1}, and it was only in these two treatments that the seed yield reached more than 1550 kg ha^{-1}. The evaluation of the results of the comparisons of means showed that the seed yield in the treatment with 5 t. ha^{-1} compost and 60 kg N/ha was about 10% higher than in the same conditions with nitrogen fertilizer and no compost (Table 7). In general, each level of nitrogen fertilizing in the conditions of application of 5 t ha^{-1} of manure, compared to their similar levels in the conditions of no application of manure, showed significantly higher seed yield (Table 7). The treatment of no application of compost and nitrogen fertiliser had the lowest seed yield of 1160 kg ha^{-1} (Table 7). It seems that application of compost with nitrogen fertilizer can significantly increase the yield of peanut because of its positive effects such as gradual release of nutrients (Governog et al., 2003) and increased soil moisture storage (Waqas et al., 2014).

Year, irrigation and N interactions on peanut seed yield were significant (Table 4). In both years, the highest seed yield was assigned to irrigation treatments and application of 40 and 60 kg N ha^{-1}, and only under these treatments the obtained seed yield was more than 1600 kg ha^{-1}. In the other treatments the seed yield was less than 1530 kg ha^{-1} (Table 8). In both years, the highest seed yield was assigned to irrigation treatments and application of 40 and 60 kg N ha^{-1}, and only under these treatments the seed yield obtained was more than 1600 kg ha^{-1}. This is probably due to higher precipitation during the growing season in the second year than in the first year (Table 3). In both years, however, the negative effect of water shortage was reduced by nitrogen application under non-irrigated conditions (Table 8). The role of nitrogen fertiliser in the reduction of the negative effects of moisture deficiency has been reported by other researchers (Tran et al., 2014). Studies have shown that cell growth is strongly dependent on water availability and maintenance of cell turgor, and that reducing turgor pressure reduces the rate of cell growth and development (Khalid et al., 2019). It appears that the negative effects of the lack of moisture on the peanut can be compensated to some extent by the application of nitrogen fertilizer in areas such as Gilan. Nitrogen availability can be effective in producing more dry matter and achieving higher yields by having a positive effect on chlorophyll and plant protein synthesis and plant leaf development (Arshadi and Asgharipour, 2011).

Table 5. Mean comparisons of interaction of irrigation, compost and nitrogen on Chlorophyll a and seed oil of peanut

Irrigation	Compost (t ha^{-1})	Nitrogen (kg ha^{-1})	Chlorophyll *a* (mg g^{-1} FW)	Seed oil (%)
Non-irrigation	0	0	6.7 p	41.9 i
		20	7.1 o	42.6 h
		40	7.4 n	43.6 g
		60	7.5 m	42.8 h
	5	0	8.4 l	43.3 g
		20	8.5 k	41.8 i
		40	8.7 j	42.0 i
		60	7.9 i	44.5 f
Applying irrigation	0	0	9.7 g	48.9 e
		20	9.8 f	50.7 c
		40	10.0 e	51.7 b
		60	10.2 d	51.7 b
	5	0	9.5 h	49.8 d
		20	10.6 c	50.8 c
		40	10.9 b	52.5 a
		60	11.1 a	51.8 b

Means that have a common letter, have not significantly different together at 5% based on Duncan test.

Table 6. Mean comparisons of interaction of irrigation and compost on seed yield and seed protein of peanut

Irrigation	Compost (t ha^{-1})	Seed yield (kg ha^{-1})	Seed protein (%)
Non-irrigation	0	1109 c	19.2 c
	5	1344 b	21.8 b
Applying irrigation	0	1498 a	23.5 a
	5	1576 a	24.2 a

Means that have a common letter, have not significantly different together at 5% based on Duncan test.

Table 7. Mean comparisons of interaction of compost and nitrogen on seed yield of peanut

Compost (t.ha^{-1})	Nitrogen (kg ha^{-1})	Seed yield (kg ha^{-1})
0	0	1160 e
	20	1256 d
	40	1356 c
	60	1443 b
5	0	1233 d
	20	1450 b
	40	1555 a
	60	1603 a

Means that have a common letter, have not significantly different together at 5% based on Duncan test.

Table 8. Mean comparisons of interaction of year, irrigation and nitrogen on seed yield

Year	Irrigation	Nitrogen (kg ha^{-1})	Seed yield (kg ha^{-1})
2018	Non-irrigation	0	920 h
		20	1144 g
		40	1270 ef
		60	1401 cd
	Applying irrigation	0	1367 d
		20	1507 b
		40	1618 a
		60	1623 a
2019	Non-irrigation	0	1114 g
		20	1235 f
		40	1305 e
		60	1424 c
	Applying irrigation	0	1385 cd
		20	1526 b
		40	1630 a
		60	1643 a

Means that have a common letter, have not significantly different together at 5% based on Duncan test.

Seed oil

The analysis of variance showed that the interaction of irrigation, compost and nitrogen on the amount of seed oil was significant (Table 4). The highest amount of seed oil was observed in the treatment of irrigation and compost application and 40 kg N ha^{-1}, and only in this treatment the amount of seed oil reached more than 52%. This was followed by irrigation and compost application and 60 kg N ha^{-1}. In the other treatments, seed oil was less than 51% (Table 5). The amount of seed oil in different levels of compost and nitrogen fertilizer application and doing irrigation conditions was significantly higher than the same levels of compost and nitrogen fertilizer in non-irrigation conditions. Especially, each levels of nitrogen fertilizer in conditions of applying irrigation, compared to their similar levels in the conditions of non-irrigation, showed a significantly higher amount of seed oil (Table 5). The lowest seed oil content was observed in the treatment that did not irrigate and did not apply composting and N fertilizing, with seed oil content in this treatment not even reaching 42% (Table 5). Although temperature is reported to be the most important environmental factor affecting seed oil production in oilseed crops (Damian et al., 1998; Dragicevic et al., 2015), water availability seems to have a significant effect on oil production and transfer to peanut seeds. Scientific research indicates that the synthesis of oil and its accumulation in peanut seeds strongly depend on the photosynthetic material produced during the period of 5-12 weeks after flowering, and most of the photosynthetic compounds produced during this period are used for oil synthesis and its transport (Fageria, 2009). Therefore, the occurrence of drought stress during this period can be effective in reducing the amount of seed oil in peanut, as the availability of moisture during this period is important for photosynthesis and the conversion of photosynthetic products into oil and its transfer to the growing seed.

Seed protein

The interaction effect of irrigation and compost on peanut seed protein was significant (Table 4). Thus, the highest seed protein was observed in the two treatments of irrigation and no compost application and application of 5 t. ha^{-1} compost, and in these two treatments the amount of seed protein reached more than 23.4% (Table 6). In other words, in both compost levels under irrigated conditions, seed protein reached more than 23.4%. On the other hand, in the non-irrigated conditions (Table 6), the seed protein in both composts did not even reach 22%. The treatment with no irrigation and no compost also had the lowest seed protein. Thus, the seed protein in this treatment was less than 20% (Table 6). However, in the conditions of doing irrigation, there was no significant difference between the two levels of compost treatment in terms of seed protein; But in the condition of no irrigation, the use of compost caused a significant increase in seed protein compared to the condition of not using it (Table 6). Under drought stress conditions, protein is usually degraded, which is probably due to the formation of some amino acids by protein degradation in response to drought stress (El-Sabagh et al., 2019). In the present study, reduced seed protein was quite evident when not irrigated. However, compost application under no-irrigation could significantly improve seed protein content. This is probably due to the high moisture holding capacity of compost (Waqas et al., 2014). However, the amount of seed protein was still significantly lower with no irrigation and compost application compared to different levels of irrigation.

The effect of nitrogen fertilizer on the amount of peanut protein was significant (Table 4). The amount of seed protein increased significantly with increasing N application. The highest amount of seed protein was obtained under 60 kg N ha^{-1} with a rate of 23.9% (Figure 3). The lowest amount of seed protein (less than 19.3%) was observed with no nitrogen fertilizer (Figure 3). Considering the important role of nitrogen in protein molecule structure (Taiz and Zeiger, 2010), the effect of reduced nitrogen availability on seed protein synthesis is obvious and increasing nitrogen availability may improve peanut seed protein levels. These results are consistent with the findings of other researchers. A significant increase in peanut seed protein during the application of nitrogen fertilizer was reported by Sugut et al. (2013).

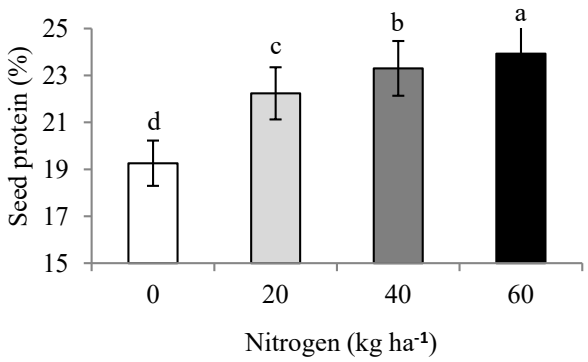

Figure 3. Effect of nitrogen on seed protein of peanut
Means that have a common letter, have not significantly different together at 5% based on Duncan test.

Rate of kernel production

The effect of compost on the rate of peanut kernel production was significant (Table 4). Applying 5 t ha^{-1} of compost resulted in a significant increase of 15.2% in the rate of kernel production (Figure 4). Apparently, compost application is effective in allocating more nutrients to rate of kernel production of peanut due to its ability to absorb and retain water and nutrients (Governog et al., 2003) and gradual release of nutrients (Waqas et al., 2014). The effect of nitrogen fertilization on the rate of kernel production of peanut was significant (Table 4). The rate of kernel production also increased when the amount of nitrogen applied was increased from zero to 60 kg N ha^{-1}. However, between 40 and 60 kg N ha^{-1} there was no significant difference. On this basis, the lowest rate of kernel production was observed in the no nitrogen fertilizer treatment (Figure 5). These results agree with those reported by other researchers. Abdzad Gohari et al. (2018) also found in their research that with an increase in the amount of nitrogen consumption from zero to 60 kg per ha, the rate of kernel production increased and with a further increase in the amount of nitrogen from 60 to 90 kg per ha, the percentage rate of kernel production decreased significantly.

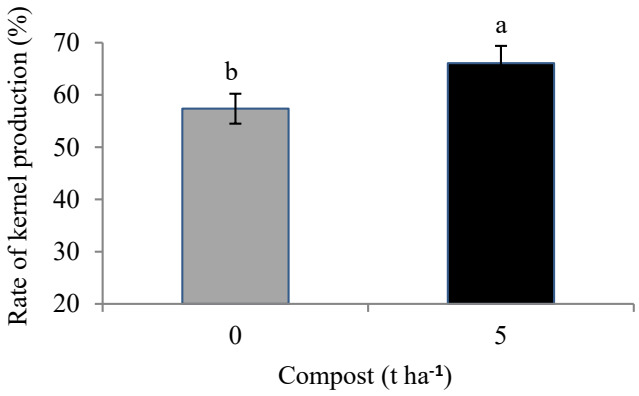

Figure 4. Effect of compost on rate of kernel production of peanut
Means that have a common letter, have not significantly different together at 5% based on Duncan test.

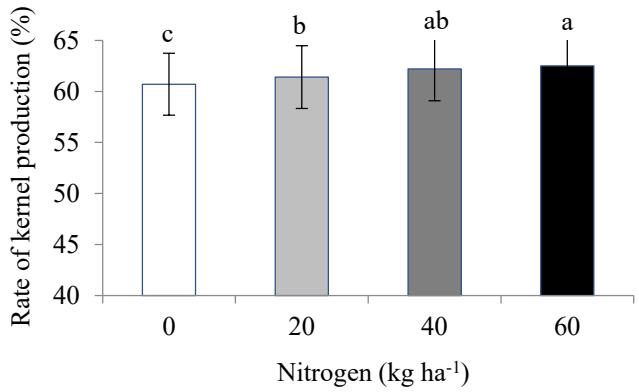

Figure 5. Effect of nitrogen on rate of kernel production of peanut
Means that have a common letter, have not significantly different together at 5% based on Duncan test.

CONCLUSIONS

Nitrogen application compensated to some extent for the negative effects of drought stress on the physiological traits studied, based on the results of the present study. However, for some traits, such as seed yield, there was no significant difference between 40 and 60 kg N ha^{-1}. This is probably due to the ability of the peanut plant to biologically fix nitrogen. In other words, the peanut plant's nitrogen requirements up to 40 kg N ha^{-1} are probably supplied by the nitrogen fertilizer and the remaining plant requirements are met by biological fixation. Furthermore, the application of municipal waste compost reduced the negative effects of water stress under drought stress conditions and improved physiological characteristics and seed yield under no-stress conditions. Therefore, it seems that in order to improve the physiological characteristics and seed yield of peanut, the application of 40 kg N ha^{-1} together with 5 t ha^{-1} of municipal waste compost can be effective.

CONFLICT OF INTERESTS

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

STATEMENTS AND DECLARATIONS

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DETERMINATION OF YIELD AND FATTY ACID CONTENTS OF DIFFERENT CAMELINA (*Camelina sativa* L. Crantz) GENOTYPES

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ABSTRACT

Grain yield and fatty acid components of camelina (*Camelina sativa* L. Crantz) are largely unknown in the Eastern Mediterranean. For this reason, a two-year field experiment was carried out with three replicates in Randomized Complete Block Design to determine the yield performances and fatty acid components of 33 camelina genotypes in Mediterranean climate conditions. In the study, in addition to grain yield and agronomic characteristics, oil quality parameters palmitic acid, stearic acid, oleic acid, linoleic acid, and erucic acid were analyzed. It was determined that genotype 28 (3120 kg ha^{-1}) gave good results in terms of yield, followed by genotype 9 (2735 kg ha^{-1}) and 1 (2651 kg ha^{-1}). These genotypes are genetically drought-resistant. Besides, 28 (3.09 %), 9 (2.66 %) and 1 (2.73 %) are the preferred genotypes for the Eastern Mediterranean due to their two-year mean erucic acid content based on the 5% EU residue limit for erucic acid in edible oils. It has been concluded that in regions where the Mediterranean climate prevails and drought stress begins to be seen, camelina cultivation can be done with natural rainfall.

Keywords: Camelina genotypes; Mediterranean climate; grain yield; oil quality; erucic acid

INTRODUCTION

Camelina (*Camelina sativa* L. Crantz) is an annual oil plant from the *Brassicaceae* family (Kurt and Seyis, 2008) and it is tolerant to drought and high temperatures and has greater spring freezing tolerance than canola (Putnam et al., 1993; Angelini et al., 1997; Blackshaw et al., 2011; Yildirim and Onder, 2016; Katar and Katar, 2017). Camelina was first cultivated in the Neolithic Age and was used as an oil plant during the Iron Age, spanning areas from the ancient Roman Empire to the steppes of Southeast Europe and Southwest Asia (Putnam et al. 1993; Subasi et al., 2022). Production of camelina in Europe declined as canola production increased (McWay, 2008). In addition, since Camelina has the potential to be produced with natural rainfall conditions in the winter in the Mediterranean climate zone, it can be considered an oil plant that does not require irrigation or can be grown with very limited irrigation in winter conditions in drought regions. Although the camelina is not selective in terms of soil requirements and has low agricultural requirements, plant yield varies depending on genetic factors, environmental conditions and responses to agricultural practices (Zahuski et al., 2020). In Mediterranean climate conditions, polyunsaturated fatty acid production and α-linolenic acid content increase due to warmer weather during autumn planting and seed filling in the plant. In addition, the dry and hot weather condition that occurs during the seed development period negatively affects the enzymes and greatly affect the oil content. In cultivation whose product pattern is based on cereals, crop diversity can be achieved by sowing camelina in winter and yield increases are observed with the increase in biomass and seed weight. It is also important in mitigating the effects of drought conditions or low rainfall conditions (Zahuski et al., 2020; Angelini et al., 2020).

Camelina seeds are processed into edible oil with a high content of omega-3 fatty acids, as well as into high-protein feed for cattle, fish, poultry, and pigs. Camelina also has numerous industrial applications, including in the production of biofuels and bioproducts such as bioplastics for packaging (Zahuski et al., 2020). While the seeds of summer camelina varieties contain 42% oil, this rate can reach 45% in winter varieties (Kurt and Seyis, 2008). Karvonen et al. (2002) reported that camelina oil (*Camelina sativa*–derived oil) is a good source of α-linolenic acid compared to other edible oils. Altogether 36% to 40% of its fatty acid content consists of α-linolenic acid, an n-3 fatty acid of plant origin. Protein and cellulose are among the important chemical quality criteria of camelina seed. The crude protein in camelina

seeds varies between 18-22% and the crude cellulose ratio varies between 11-15%. Camelina seed contains high vitamin E (25.8-28.2 mg/100 g), making it a strong source of antioxidants (Reenberg, 1994). Camelina breeding studies started at the beginning of the 21st century and considering the significant differences in terms of seed yield and yield-related characteristics, there is a significant potential for the development of camelina through breeding studies (Zahuski, 2020). In recent years, the suitability of camelina varieties with an erucic acid content of less than 1% for human nutrition has been proven in laboratory tests (Toncea et al., 2013). However, there is only one registered camelina variety (Arslanbey) in Turkey and this variety was obtained by selection method under the climate conditions of Central Anatolia (Ankara ecological conditions), where cold winter conditions prevail. It has been reported that the average grain yield is 2350 kg ha^{-1} of this genotype (Katar, 2013). Therefore, although camelina cultivation will be carried out in different geographies, there is no chance of choosing a higher-performing variety in coastal areas close to the Mediterranean. This situation may limit the efficiency (Sevilmis et al., 2019) and camelina increases the biological diversity of arable land. This requires evaluating a diverse group of camelina genotypes for adaptability, production, and oil quality. Identification of well-adapted and high-yielding camelina genotypes will help increase the genetic diversity of Camelina (Zahuski, 2020). However, such studies are lacking in the region. Therefore, this study was conducted on camelina genotypes of diverse origins and morphology for adaptability, seed yield, and fatty acid contents under Mediterranean climate conditions.

MATERIALS AND METHODS

The 33 camelina genotypes, used in this study, were obtained from the gene bank of the United States Department of Agriculture (USDA) in 2017 (Table 1).

Table 1. Information about the camelina genotypes was obtained from the United States Department of Agriculture (USDA) for use in studying adaptation in Turkey

Genotype	Accession information	Origin	Genotype	Accession information	Origin
1	Ames31231	Georgia	**22**	PI 650147	Sweden
2	Ames31232	Georgia	**23**	PI 650148	Denmark
4	PI 258367	Russia	**24**	PI 650149	Germany
7	PI 304270	Sweden	**25**	PI 650150	Denmark
8	PI 304271	Sweden	**26**	PI 650151	Sweden
9	PI 311735	Poland	**27**	PI 650152	Germany
10	PI 311736	Poland	**28**	PI 650153	Russia
11	PI 597833	Denmark	**29**	PI 650154	Russia
13	PI 633193	Germany	**30**	PI 650155	Poland
14	PI 633194	Germany	**31**	PI 650156	Russia
15	PI 650140	Germany	**35**	PI 650160	Russia
16	PI 650141	USA	**36**	PI 650161	Russia
17	PI 650142	Denmark	**37**	PI 650162	Poland
19	PI 650144	Denmark	**38**	PI 650163	Russia
20	PI 650145	Germany	**40**	PI 650165	Rusya
21	PI 650146	Sweden	**42**	PI 650167	Polonia

Research area soil properties

The research was carried out at Ege University, Faculty of Agriculture, experimental area in Izmir 2019-2020 and 2020-2021. Although the altitude of the field is 10 m, the experimental area has a heavy soil structure with clay-silt soil at 0-20 cm depth and clay-loamy structure at 20-40 cm depth (Ilker, 2017).

Climate characteristics of the research area

Long-term climate data for the test site was given in Table 2, and climate data during the growing period of camelina was given in Table 3.

Table 2. Climate data for Bornova location based on long-term average (2013-2022)

Parameter	November	December	January	February	March	April	May
Monthly Min. Temperature (°C)	1.8	-2.2	-4.7	-2.0	-1.9	2.6	8.5
Monthly Average Temperature (°C)	14.9	10.4	8.9	11.0	12.9	17.1	22.1
Monthly Max. Temperature (°C)	28.9	23.9	23.8	27.5	28.0	33.0	39.0
Monthly Average Relative Humidity (%)	66.6	68.8	67.0	65.9	64.2	61.7	56.1
Monthly Total Precipitation Average (mm)	60.06	79.92	**153.62**	95.14	65.50	35.89	36.83

Table 3. Climate Data during the growing period of camelina for Bornova in 2019, 2020 and 2021.

Parameter	Year/Month	November	December	January	February	March	April	May
Monthly Average Temperature (°C)	2019	16.9	11.3	8.7	9.8	13.2	16.3	21.9
	2020	14.3	12.4	8.3	10.8	13.5	16.4	21.6
	2021	15.6	11.2	10.6	11.1	11.1	16.7	22.9
Monthly Total Precipitation Average (mm)	2019	58.2	73.4	369.3	106.3	37.8	66.1	12.6
	2020	2.2	126.0	37.5	76.6	83.0	56.1	55.2
	2021	51.9	178.3	213.5	138.0	98.0	25.4	0.6

Sowing, Maintenance and Harvest

Seeds were sown manually in November 2019 and November 2020 in 3 m long, 3-row plots. The rows are 20 cm apart and the seed is 10 cm apart down the row with 3 replications, according to the Randomized Complete Block Design. After plant emergence was observed, thinning was carried out to 93 maintained plants in each plot.

Over the years, fertilizer (15-15-15 NPK) was applied at 100 kg per hectare, as basal dose at sowing into the soil whereas 100 kg of urea fertilizer (46%) per hectare was applied as topdress fertilizer. Weeds were controlled twice (March-May) manually and with a hoeing machine between rows. Pesticides were not applied during the experiment. Since there was not enough rain, sprinkler irrigation was applied after the planting process and the emergence took place. After this stage, the irrigation water needed by the plant during the vegetative growth period was provided by natural rainfall conditions. Plants reaching maturity were harvested by hand from the soil surface.

Morphological and yield-related traits

Harvesting was done manually in June 2020 and 2021, waiting for all genotypes to mature. Plant height, first lateral branch height, and number of capsules per plant were measured on five randomly selected plants representing each plot. The average of the measurements was calculated and documented for each genotype.

The number of seeds in 10 randomly selected capsules representing each plot was measured and calculated and their averages were taken. Threshing was carried out after harvest. The number of seeds in the capsule was recorded. Then, seed samples were taken in five replications for thousand-grain weight, thousand grains were counted and their weights were measured on a precision scale. Grain yield was obtained by harvesting the plants in the plot at three replications and then converted to kg ha^{-1}.

Fatty acids (%)

The percentages of fatty acids in Camelina oils were determined by using gas chromatography. Samples, ground to approximately 30-50 g, were placed into Erlenmeyer flasks and covered with 100-150 ml of hexane, a lipid solvent. The flasks were then sealed with cotton and shaken in a shaker at a medium speed (~200 rpm) for 12 hours. After this process, the oil in the samples forms a solution with hexane, which is then filtered into a beaker using glass wool. The solvent was then removed from the solution to obtain raw oil (Basoglu, 1986; Koyuncu, 1996). Before fatty acid analysis, esterification was applied to the raw oil samples (Anonymous, 2000). In this process, 0.5 g of raw oil sample was placed into a 50 ml Falcon tube, and then 1 ml of 2 N methanolic KOH solution and 7 ml of n-hexane were added. The mixture was centrifuged at 4500 rpm for 30 minutes to clarify the upper phase. The upper phase containing fatty acid methyl esters was transferred into special glass bottles for injection into gas chromatography. Using an automatic sampling apparatus, 1 µl of samples was automatically taken and injected into the device. A capillary column (60 m x 0.25 mm i.d., 0.20 µm film thickness) was used for determining the oil composition. During the analysis, peaks were identified by calculating the peak's time and area, and the results were given as percentages of fatty acids. Based on the observed peaks, the amounts of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), alpha-linolenic acid (ALA) (C18:3), and erucic acid (C22:1) contained in Camelina oil were determined as percentages (%).

Statistical analysis

In terms of the examined characteristics, a combined analysis of variance over the years was performed to determine whether there was a difference between the general averages of each genotype and application years, and the average values between genotypes and years were compared according to the LSD test (Steel and Torrie, 1960). Variance analyses and LSD tests were performed with the TOTEMSTAT package program (Acikgoz et al., 2004).

RESULTS

Grain Yield (kg ha^{-1})

Except for plant height, the year × genotype interaction was statistically important. The yield performances of genotypes varied under climatic conditions in different growing seasons (Table 4). This was probably due to climatic conditions that varied from year to year.

Table 4. Results of combined analyses of variance over two years for the morphological characteristics of camelina genotypes.

		Mean Square Values					
Sources of Variation	**DF**	**Yield**	**Plant height**	**First lateral branch height**	**No. of capsules per plant**	**No. of seeds per capsule**	**Thousand-grain weight**
Year (A)	1	103732.4 **	2081.6 **	5699.4 **	143194.0 **	29.8 ns	0.139 **
Error 1	4	4236.2	10.5	53.8	2711.4	10.7	0.003
Genotype (B)	32	11686.8 **	537.4 **	282.3 **	10674.0 **	13.3 **	0.203 **
Year × Genotype (A × B)	32	1856.5 **	105.3 ns	169.9 **	3644.0 **	3.44 **	0.030 **
Error	128	993.5	86.8	16.1	553.7	1.32	0.005

* and ** indicate significance at 0.05 and 0.01 levels of probability, respectively. ns; not significant

The highest grain yield among camelina genotypes in both years was obtained from genotype 28 originating from Russia (3120 kg ha^{-1}). This was followed by genotype 9 originating from Poland and number 1 originating from Georgia (2735 kg ha^{-1} and 2651 kg ha^{-1} respectively) (Table 5). The average yield in 2021 was 1288 kg ha^{-1}. Genotypes 27, 10, 35, and 43 were among the five genotypes that showed the lowest seed yield performance in both years. Although ecological factors resulting from the difference in harvest years cause some decrease in seed yields, these genotypes maintained their high yield potential compared to other genotypes. Besides, in the 2021 growing season, which is the second year of the experiment, the mean grain yield of 2021 decreased in all genotypes due to less rainfall in April and almost no rainfall in May. Despite this, genotypes 28 and 9, which were determined as high yield in 2020, showed the highest performance in terms of grain yield in 2021, when water stress occurred. Therefore, it can be inferred that these genotypes have a strong genetic structure in terms of grain yield in Mediterranean climate coastline water stress conditions.

Table 5. Mean grain yields and plant height for 2020-2021.

Grain Yield (kg ha^{-1})					**Plant Height (cm)**				
2020		**2021**		**Two years**	**2020**		**2021**		**Two years**
Genotype No	**G. Yield**	**Genotype No**	**G. Yield**	**Mean**	**Genotype No**	**P. height**	**Genotype No**	**P. height**	**Mean**
1	**2651 AB**	**1**	**1921 AB**	**2286**	**1**	76.9	**1**	77.4	77.1 A-F
2	1780 D-H	**2**	1675 A-D	1727	**2**	87.4	**2**	87.4	**87.4 A**
4	1418 G-K	**4**	1330 C-I	1374	**4**	80.5	**4**	86.6	83.5 A-E
7	1647 E-H	**7**	944 H-L	1295	**7**	69.3	**7**	67.9	68.6 FG
8	2262 BCD	**8**	1426 B-H	1844	**8**	78.8	**8**	84.9	81.8 A-E
9	**2735 AB**	**9**	**2018 A**	**2376**	**9**	69.1	**9**	80.9	75.0 C-F
10	613 M	**10**	556 KL	584	**10**	31.5	**10**	41.1	36.3 H
11	2061 C-F	**11**	1573 A-G	1817	**11**	81.3	**11**	78.9	80.1 A-E
13	1876 C-G	**13**	1142 E-J	1509	**13**	57.9	**13**	80.6	69.2 FG
14	1696 E-H	**14**	1287 C-I	1491	**14**	85.5	**14**	81.8	83.6 A-E
15	1337 H-K	**15**	1136 E-J	1236	**15**	65.7	**15**	84.1	74.9 C-F
16	1484 G-K	**16**	1338 C-I	1411	**16**	72.0	**16**	80.9	76.4 B-F
17	2319 BC	**17**	1355 C-I	1837	**17**	79.1	**17**	73.7	76.4 B-F
19	2011 C-F	**19**	1761 ABC	1886	**19**	84.1	**19**	84.1	84.1 A-E
20	2015 C-F	**20**	1635 A-E	1825	**20**	69.1	**20**	80.4	74.7 C-F
21	1774 E-H	**21**	1178 D-J	1476	**21**	64.7	**21**	84.0	74.3 D-F
22	1870 C-G	**22**	1793 ABC	1831	**22**	69.6	**22**	85.3	77.4 A-F
23	1599 F-I	**23**	1057 H-K	1328	**23**	80.2	**23**	74.1	77.1 A-F
24	1356 H-K	**24**	1212 D-I	1284	**24**	71.3	**24**	90.7	81.0 A-E
25	1677 E-H	**25**	1431 B-H	1554	**25**	82.5	**25**	86.9	84.7 A-D
26	1074 JKL	**26**	1232 D-I	1153	**26**	81.6	**26**	79.0	80.3 A-E
27	464 M	**27**	670 JKL	567	**27**	84.9	**27**	85.9	85.4 A-C
28	**3120 A**	**28**	**2040 A**	**2580**	**28**	65.0	**28**	82.5	73.7 EF
29	1906 C-G	**29**	966 H-L	1436	**29**	83.7	**29**	88.4	**86.0 AB**
30	1830 D-H	**30**	1062 G-K	1446	**30**	83.9	**30**	85.6	84.7 A-D
31	1628 E-I	**31**	1321 C-I	1474	**31**	82.3	**31**	92.4	**87.3 A**
35	988 KL	**35**	861 I-L	924	**35**	76.6	**35**	80.2	78.4 A-F
36	2137 CDE	**36**	1219 D-I	1678	**36**	71.1	**36**	86.9	79.0 A-F
37	1922 C-G	**37**	1205 D-I	1563	**37**	79.2	**37**	87.7	83.4 A-E
38	1572 F-J	**38**	1122 F-J	1347	**38**	76.3	**38**	86.4	81.3 A-E
40	1893 C-G	**40**	926 H-L	1409	**40**	78.1	**40**	91.4	84.7 A-D
42	1747 E-H	**42**	1593 A-F	1670	**42**	80.7	**42**	84.9	82.8 A-E
43	1134 IJK	**43**	506 L	820	**43**	66.3	**43**	56.9	61.6 G
Mean	1745		1288	1516	**Mean**	74.7		81.2	77.9
LSD: 51.12					LSD: 10.68				

Plant height (cm)

In terms of plant height, the year × genotype interaction was insignificant, that is, the plant height performances of genotypes did not differ under climatic conditions in different growing seasons (Table 4). Genotype 2 originating from Georgia (87.4 cm) and genotype 31 originating from Russia (87.3 cm) showed the highest average plant height in camelina. These genotypes were followed by genotype 29 (86.0 cm) originating from Russia, which is in the EU group. The lowest average plant height was obtained from genotype number 10 (36.3 cm), originating from Poland (Table 5). This is due to the difference in genotype characteristics.

First lateral branch height (cm)

The year × genotype interaction was significant for the first lateral branch height (Table 4). This feature is very important in camelina plants, as in many cultivated plants. The lower the first lateral branch height in plants that are machine-harvested, the greater the risk of seed loss during the harvest of the plant. In the first year, the highest first lateral branch height was shown by genotype 26 (55.7 cm) originating from Sweden, and genotype 14 (55.1 cm) from Germany, and these genotypes were followed by genotype 25 from Denmark in the EU group. The average first lateral branch height was found to be 35.7 cm. When the first lateral branch height of 2021 is examined, genotype 23 (64.2 cm) ranks first, followed by genotype 29 (57.0 cm) in group B. In the second year, the first lateral branch height was found to be 46.1 cm. Genotype 10 showed the lowest first lateral branch height in both years. At the same time, this genotype has the lowest plant height, and due to this property, it is the last genotype that can be preferred in terms of suitability for machine harvesting. Plant height and first lateral branch height do not interact with each other. Although genotypes 26 and 23 showed the highest first lateral branch height performance in 2020 and 2021, their plant heights were close to the average and they were not among the genotypes with the highest plant height (Table 6).

Table 6. First lateral branch height (cm) and number of capsules per plant data for 2020-2021.

First lateral branch height (cm)					**No. of capsules per plant**				
2020		**2021**		**Two years**	**2020**		**2021**		**Two years**
Genotype No	**First lateral branch height (cm)**	**Genotype No**	**First lateral branch height (cm)**	**Mean (cm)**	**Genotype No**	**No. of capsules per plant**	**Genotype No**	**No. of capsules per plant**	**Mean**
1	36.6 F-I	**1**	33.0 J	34.80	**1**	**296.2 A**	**1**	**184.6 A**	240.40
2	47.7 BC	**2**	47.7 D-H	47.70	**2**	185.4 D-G	**2**	**182.0 A**	183.70
4	34.7 G-J	**4**	43.4 G-I	39.00	**4**	134.4 I-K	**4**	139.0 B-G	136.70
7	30.8 I-L	**7**	34.3 J	32.50	**7**	233.8 B	**7**	98.8 H-J	166.30
8	42.4 C-F	**8**	49.4 C-G	45.90	**8**	231.9 B	**8**	146.6 A-F	189.25
9	32.1 I-K	**9**	42.0 HI	37.05	**9**	240.9 B	**9**	152.2 A-E	196.55
10	17.4 N	**10**	19.3 K	18.35	**10**	59.7 M	**10**	62.5 JK	61.10
11	46.4 CD	**11**	44.6 E-I	45.50	**11**	228.2 BC	**11**	158.0 A-C	193.10
13	22.1 MN	**13**	50.3 C-F	36.20	**13**	188.1 D-G	**13**	117.0 D-H	152.55
14	**55.1 A**	**14**	46.2 D-I	50.65	**14**	193.4 C-F	**14**	116.0 E-H	154.70
15	43.8 C-E	**15**	50.5 C-F	47.15	**15**	147.1 E-J	**15**	130.1 B-H	138.60
16	36.4 F-J	**16**	45.6 E-I	41.00	**16**	208.2 B-E	**16**	131.4 B-H	169.80
17	39.4 E-H	**17**	49.8 C-G	44.60	**17**	**246.0 B**	**17**	122.6 C-H	184.30
19	41.2 D-G	**19**	41.2 I	41.20	**19**	167.6 F-I	**19**	161.8 AB	164.70
20	25.3 LM	**20**	48.7 D-G	37.00	**20**	241.4 B	**20**	**164.2 AB**	202.80
21	32.6 I-K	**21**	48.3 D-H	40.45	**21**	166.8 F-I	**21**	116.4 D-H	141.60
22	43.7 C-E	**22**	47.0 D-I	45.35	**22**	110.0 J-L	**22**	122.9 C-H	116.45
23	30.5 I-L	**23**	**64.2 A**	47.35	**23**	136.1 H-K	**23**	132.2 B-H	134.15
24	34.8 G-J	**24**	55.3 BC	45.05	**24**	172.3 E-I	**24**	158.5 A-C	165.40
25	**54.0 AB**	**25**	50.8 B-E	52.40	**25**	111.9 J-L	**25**	100.9 G-I	106.40
26	**55.7 A**	**26**	46.9 DI	51.30	**26**	67.4 M	**26**	104.9 G-I	86.15
27	26.8 K-M	**27**	52.5 B-D	39.65	**27**	106.0 KL	**27**	114.0 F-H	110
28	28.1 LM	**28**	41.1 I	34.60	**28**	**288.8 A**	**28**	162.1 AB	225.45
29	39.2 E-H	**29**	**57.0 B**	48.10	**29**	213.6 B-D	**29**	107.0 G-I	160.30
30	33.3 H-K	**30**	55.4 BC	44.35	**30**	236.9 B	**30**	112.0 F-H	174.45
31	35.0 G-J	**31**	55.8 BC	45.40	**31**	153.6 G-I	**31**	136.6 C-H	145.10
35	30.0 J-L	**35**	45.2 E-I	37.60	**35**	89.4 LM	**35**	71.2 I-K	80.30
36	35.0 G-J	**36**	48.1 D-H	41.55	**36**	229.3 BC	**36**	148.4 A-F	188.85
37	32.2 I-K	**37**	46.3 D-I	39.25	**37**	174.0 E-H	**37**	135.0 B-H	154.50
38	32.2 I-K	**38**	44.2 F-I	38.20	**38**	159.0 F-I	**38**	122.5 C-H	140.75
40	30.2 I-L	**40**	44.7 E-I	37.45	**40**	239.0 B	**40**	111.2 F-H	175.10
42	25.2 LM	**42**	48.4 D-H	36.80	**42**	232.5 B	**42**	154.3 A-D	193.40
43	27.4 K-M	**43**	34.2 J	30.80	**43**	110.3 J-L	**43**	46.8 K	78.55
LSD: 6.51					LSD: 38.1				

No. of capsules per plant

One of the most important characteristics affecting yield is the number of capsules (fruits) in the plant. In the camelina, the fruit is in capsule form (Karayel et al., 2021). It was determined that the year × genotype interaction is important. This interaction between

genotype and environment, particularly due to water stress experienced in the second year, may have led to differentiation in the adaptation abilities of genotypes to adverse conditions (Table 4). In 2020, the highest number of capsules was obtained from genotypes 1 (296 capsules/plant) and 28 (289 capsules/plant). These genotypes were followed by genotype number 17. In the 2nd year of the experiment, genotypes 1 and 2 showed the highest capsule number performance, followed by genotype 20. In the second year of the experiment, genotypes 1 and 28 gave statistically the highest number of capsules. It was determined that these were followed by genotype number 20. The lowest number of capsules in 2020 was observed in genotypes 10, 26, and 35. The number of capsules directly affects the yield. Genotypes 10 and 35, which show the lowest number of capsules, are among the genotypes with the lowest grain yield in terms of grain yield performance. In addition, the fact that genotypes 1 and 28 maintained their superiority in terms of the number of capsules in the plant and grain yield even under poor environmental conditions, although the number of capsules in the plant decreased in the second harvest year, proves this idea (Table 6).

No. of seeds per capsule

One of the important properties affecting the yield is the number of grains in the capsule (Sevilmis and Bilgili, 2019). It has been determined that the year × genotype interaction is important, that is, the performance of genotypes in the number of grains in the capsule varies under climatic conditions in different growing seasons (Table 4). In 2020, the highest number of grains in the capsule was obtained from genotype no. 19 (14.4) and this genotype was followed by genotype no. 35, from Russia, and genotype no. 22, originating from Sweden. In 2021, genotype no. 35 (15.4) exhibited the highest grain number performance in the capsule, and this genotype was followed by genotypes no. 14 and 16. Although the number of grains in the capsule is important among the yield components, it is not sufficient on its own. For example, although genotype 35, originating from Russia, is one of the three highest-performing genotypes in both years in terms of the number of grains in the capsule, it is seen to be in the background when evaluated in terms of yield (Table 7).

Table 7. Number of seeds per capsule and thousand-grain weight (g) data for 2020-2021.

No. of seeds per capsule					**Thousand grain weight (g)**				
2020		**2021**		**Two years**	**2020**		**2021**		**Two years**
Genotype No	**No. of seeds per capsule**	**Genotype No**	**No. of seeds per capsule**	**Mean**	**Genotype No**	**Thousand grain weight (g)**	**Genotype No**	**Thousand grain weight (g)**	**Mean**
1	9.4 L-O	**1**	11.3 F-L	10.35	**1**	1.07 D-F	**1**	1.04 E-H	1.06
2	12.3 B-F	**2**	12.2 C-I	12.25	**2**	0.87 I-M	**2**	0.84 J-L	0.86
4	12.4 B-E	**4**	11.3 F-L	11.85	**4**	0.95 G-K	**4**	0.94 G-J	0.95
7	8.9 M-O	**7**	11.6 F-K	10.25	**7**	0.88 I-M	**7**	0.93 H-J	0.91
8	10.5 F-N	**8**	10.8 H-M	10.65	**8**	1.04 E-H	**8**	1.02 F-H	1.03
9	10.6 E-M	**9**	12.8 B-G	11.70	**9**	1.19 C	**9**	1.16 B-D	1.18
10	10.7 D-M	**10**	9.6 L-N	10.15	**10**	1.08 C-F	**10**	1.02 F-H	1.05
11	11.3 D-J	**11**	13.0 B-F	12.15	**11**	0.89 I-M	**11**	0.84 J-L	0.87
13	11.6 D-I	**13**	11.3 F-L	11.45	**13**	0.98 F-J	**13**	0.95 G-J	0.97
14	11.0 D-L	**14**	14.6 AB	12.80	**14**	0.91 I-M	**14**	0.85 J-L	0.88
15	11.8 C-G	**15**	11.5 F-L	11.65	**15**	0.86 K-M	**15**	0.85 J-L	0.86
16	9.8 I-O	**16**	**14.6 AB**	12.20	**16**	0.81 MN	**16**	0.78 KL	0.80
17	11.7 D-H	**17**	14.0 A-C	12.85	**17**	0.90 I-M	**17**	0.87 I-K	0.89
19	**14.4 A**	**19**	13.8 A-D	14.10	**19**	0.93 H-L	**19**	0.89 I-K	0.91
20	10.3 G-N	**20**	12.0 D-I	11.15	**20**	0.91 I-M	**20**	0.93 H-J	0.92
21	12.6 A-D	**21**	12.7 B-G	12.65	**21**	0.95 G-L	**21**	0.90 I-J	0.93
22	**14.0 AB**	**22**	13.8 A-D	13.90	**22**	**1.37 B**	**22**	1.17 BC	1.27
23	11.1 D-L	**23**	12.0 D-I	11.55	**23**	1.18 CD	**23**	0.75 L	0.97
24	10.6 E-M	**24**	11.0 G-L	10.80	**24**	0.83 L-N	**24**	0.78 KL	0.81
25	9.9 H-N	**25**	13.5 B-E	11.70	**25**	**1.69 A**	**25**	**1.20 B**	1.45
26	10.6 E-N	**26**	9.8 K-N	10.20	**26**	**1.68 A**	**26**	**1.35 A**	1.52
27	9.96 H-N	**27**	10.4 I-N	10.18	**27**	0.50 O	**27**	0.63 M	0.57
28	11.6 D-I	**28**	13.7 A-D	12.65	**28**	1.04 E-H	**28**	1.05 D-G	1.05
29	11.0 D-L	**29**	9.8 K-N	10.40	**29**	0.91 I-M	**29**	1.02 F-H	0.97
30	8.7 N-O	**30**	10.0 J-N	9.35	**30**	0.98 F-I	**30**	1.06 C-F	1.02
31	13.7 A-D	**31**	12.6 C-H	13.15	**31**	0.87 J-M	**31**	0.85 J-L	0.86
35	**14.0 AB**	**35**	**15.4 A**	14.70	**35**	0.88 I-M	**35**	0.90 IJ	0.89
36	9.5 J-O	**36**	8.6 N	9.05	**36**	1.09 C-F	**36**	1.07 C-F	1.08
37	9.4 K-O	**37**	8.7 N	9.05	**37**	1.31 B	**37**	1.15 B_E	1.23
38	10.6 E-N	**38**	10.8 H-M	10.70	**38**	1.05 E-G	**38**	0.94 G-J	1.00
40	8.0 O	**40**	9.0 MN	8.50	**40**	1.11 C-E	**40**	1.06 C-F	1.09
42	11.3 D-K	**42**	11.8 E-J	11.55	**42**	0.74 N	**42**	0.97 F-I	0.86
43	11.7 D-H	**43**	12.3 C-H	12.00	**43**	1.04 E-H	**43**	0.98 F-I	1.01
LSD: 1.87					LSD: 0.115				

Thousand-grain weight (g)

In the study, it was determined that the year × genotype interaction was important, that is, the thousand-grain weight performances of genotypes varied under climatic conditions in different growing seasons (Table 4). With an average thousand-grain weight of 1.01 g, in 2020, the highest thousand-grain weight was obtained from genotypes 25 and 26. These genotypes were followed by genotypes 22 and 37. In 2021, genotype 26 (1.35 g) exhibited the highest thousand grain weight performance. Genotypes 25 and 26 had the highest thousand-grain weight in both harvest years, and differences between the two genotypes were observed against the other 31 genotypes. In the second year, the values of thousand grain weights were lower (Table 7).

Palmitic Acid (C16:0) (%)

In the study, it was determined that the year × genotype interaction was important, that is, the palmitic acid performances of genotypes varied under climatic conditions in different growing seasons (Table 8).

Table 8. Palmitic acid and stearic acid values for 2020-2021

Palmitic acid (%)					Stearic acid (%)				
2020		2021		Two years	2020		2021		Two years
Genotype No	Palmitic acid	Genotype No	Palmitic acid	Mean	Genotype No	Stearic acid	Genotype No	Stearic acid	Mean
1	5.26 M-P	**1**	5.13 P-S	5.20	**1**	2.46 G-I	**1**	2.44 F-I	2.45
2	5.60 D-I	**2**	5.49 H-K	5.55	**2**	2.22 LM	**2**	2.28 JK	2.25
4	5.19 O-P	**4**	5.16 O-S	5.18	**4**	2.13 M	**4**	2.24 KL	2.19
7	5.58 E-I	**7**	5.69 C-G	5.64	**7**	2.33 J-L	**7**	2.31 JK	2.32
8	5.74 C-E	**8**	5.79 B-D	5.77	**8**	2.23 LM	**8**	2.25 KL	2.24
9	5.80 BC	**9**	5.75 B-F	5.78	**9**	2.60 A-E	**9**	2.53 C-F	2.57
10	5.57 F-I	**10**	5.62 D-H	5.60	**10**	2.24 LM	**10**	2.24 KL	2.24
11	5.62 D-H	**11**	5.60 E-H	5.61	**11**	2.48 E-I	**11**	2.45 F-H	2.47
13	5.38 J-M	**13**	5.34 K-N	5.36	**13**	2.29 KL	**13**	2.28 JK	2.29
14	5.50 H-K	**14**	5.51 H-J	5.51	**14**	**2.70 A**	**14**	**2.65 AB**	2.68
15	5.69 C-F	**15**	5.71 B-G	5.70	**15**	2.64 A-D	**15**	2.50 D-G	2.57
16	5.67 C-G	**16**	5.38 J-N	5.53	**16**	2.65 A-C	**16**	2.65 A-C	2.65
17	5.59 E-I	**17**	5.81 BC	5.70	**17**	**2.68 A**	**17**	2.45 F-H	2.57
19	5.36 K-N	**19**	5.23 N-R	5.30	**19**	**2.69 A**	**19**	2.57 A-E	2.63
20	5.21 N-P	**20**	5.27 M-P	5.24	**20**	2.63 A-D	**20**	2.50 D-G	2.57
21	5.45 I-L	**21**	5.42 I-M	5.44	**21**	2.55 C-H	**21**	2.59 A-D	2.57
22	5.33 L-O	**22**	5.28 M-P	5.31	**22**	2.53 D-H	**22**	2.46 E-H	2.50
23	5.14 P	**23**	5.11 P-S	5.13	**23**	2.55 C-G	**23**	2.58 A-D	2.57
24	5.52 G-K	**24**	5.59 F-H	5.56	**24**	2.56 B-G	**24**	2.54 B-F	2.55
25	5.10 PR	**25**	5.03 S	5.07	**25**	2.40 I-K	**25**	2.34 H-K	2.37
26	5.37 J-N	**26**	5.35 J-N	5.36	**26**	**2.67 AB**	**26**	2.55 B-F	2.61
27	**6.00 A**	**27**	**6.10 A**	6.05	**27**	2.31 KL	**27**	2.27 JK	2.29
28	5.76 CD	**28**	5.80 BC	5.78	**28**	2.31 KL	**28**	2.33 I-K	2.32
29	5.57 F-I	**29**	5.32 L-O	5.45	**29**	2.43 H-J	**29**	**2.67 A**	2.55
30	5.57 F-I	**30**	5.60 E-H	5.59	**30**	2.55 C-H	**30**	2.52 D-F	2.54
31	5.84 A-C	**31**	5.07 RS	5.46	**31**	2.53 D-H	**31**	2.14 L	2.34
35	5.54 F-J	**35**	**5.86 B**	5.70	**35**	2.48 F-I	**35**	2.50 D-G	2.49
36	4.94 RS	**36**	5.16 O-S	5.05	**36**	2.64 A-D	**36**	2.64 A-C	2.64
37	4.93 S	**37**	5.22 N-R	5.08	**37**	2.26 L	**37**	2.30 JK	2.28
38	5.52 G-K	**38**	5.46 G-K	5.49	**38**	2.43 H-J	**38**	2.39 G-J	2.41
40	5.62 D-H	**40**	5.62 E-H	5.62	**40**	2.27 L	**40**	2.24 KL	2.26
42	**5.95 AB**	**42**	5.76 B-E	5.86	**42**	2.59 A-F	**42**	2.60 A-D	2.60
43	5.50 H-K	**43**	5.57 G-I	5.54	**43**	2.29 KL	**43**	2.26 K	2.28
LSD: 0.166					LSD: 0.119				

In both years of the experiment, the highest palmitic acid value belonged to genotype 27 originating from Germany. The genotypes with the lowest palmitic acid content in the first year are genotypes 37 and 36. In the second year, genotypes 25 and 31 occurred (Table 8).

Stearic Acid (C18:0) (%)

It has been determined that the year × genotype interaction is important, that is, the stearic acid performances of genotypes vary under climatic conditions in different growing seasons (Table 11). The stearic acid content obtained from camelina genotypes was 2.46% on average in the first year, and the highest palmitic acid values were obtained from genotypes 14, 19, and 17. It is clearly understood that genotypes 14, 16, and 36 are the genotypes with the highest and similar stearic acid values in both harvest years, regardless of ecological factors. Similarly, it was observed in many genotypes such as genotypes 4, 8, and 10 in terms of low stearic acid value. Therefore, it is thought that stearic acid content is

independent of ecological conditions and may be a genotypic property (Table 8).

Oleic Acid (C18:1) (%)

It was determined that the year × genotype interaction is important, that is, the oleic acid performances of genotypes vary under climatic conditions in different growing seasons (Table 11). The average oleic acid value in the first year is 15.41%, and the highest oleic acid value belongs to genotype 16 (17.87%). Genotype 16 was followed by genotypes 35 (16.92%) and 42 (16.88%). In the second year, genotype 16 (18.91%) was observed as the genotype containing the highest oleic acid value. Genotype 16 was followed by genotype 42 (16.94%). In line with the findings, it was observed that genotype 16 was the genotype with the highest oleic acid content in both harvest years, regardless of ecological factors, and was visibly separated from other accessions (Table 9). In the second year of the experiment, due to the lack of expected rainfall in April and May, increases in oleic acid percentages were detected in these three genotypes (16, 35, 42) and other genotypes in the second year.

Table 9. Oleic acid and linoleic acid values in 2020-2021 (%).

Oleic acid (%)					Linoleic acid (%)				
2020		2021		Two years	2020		2021		Two years
Genotype No	Oleic acid	Genotype No	Oleic acid	Mean	Genotype No	Linoleic acid (%)	Genotype No	Linoleic acid (%)	Mean
1	14.68 K-O	**1**	16.19 CD	15.44	**1**	17.41 G-I	**1**	17.80 I-J	17.61
2	15.08 I-M	**2**	15.03 G-J	15.06	**2**	17.10 I-K	**2**	17.12 K-L	17.11
4	14.96 I-M	**4**	15.04 G-J	15.00	**4**	**19.45 B**	**4**	19.37 C	19.41
7	16.04 D-G	**7**	15.63 D-G	15.84	**7**	16.93 I-L	**7**	17.00 KL	16.97
8	14.93 I-M	**8**	14.92 H-L	14.93	**8**	16.82 J-M	**8**	16.75 LM	16.79
9	14.04 OP	**9**	14.26 L-N	14.15	**9**	**20.49 A**	**9**	**20.94 A**	20.72
10	14.82 J-N	**10**	14.79 I-M	14.81	**10**	18.30 D-F	**10**	18.14 G-I	18.22
11	15.52 G-I	**11**	15.36 E-I	15.44	**11**	17.79 F-H	**11**	17.81 IJ	17.80
13	15.45 G-J	**13**	15.47 E-H	15.46	**13**	16.73 K-M	**13**	16.81 LM	16.77
14	16.43 B-E	**14**	16.47 BC	16.45	**14**	17.87 E-G	**14**	18.17 F-I	18.02
15	16.25 B-F	**15**	15.45 E-I	15.85	**15**	17.33 G-J	**15**	18.39 E-H	17.86
16	**17.87 A**	**16**	**18.91 A**	18.39	**16**	16.43 L-N	**16**	16.31 M-N	16.37
17	15.56 G-I	**17**	14.92 H-L	15.24	**17**	17.24 I-K	**17**	17.90 H-J	17.57
19	16.10 C-G	**19**	16.59 B-C	16.35	**19**	16.90 I-L	**19**	16.66 LM	16.78
20	16.34 B_E	**20**	16.44 BC	16.39	**20**	16.29 MN	**20**	16.96 KL	16.63
21	16.76 BC	**21**	16.58 BC	16.67	**21**	16.93 I-L	**21**	17.42 JK	17.18
22	14.93 I-M	**22**	15.09 F-J	15.01	**22**	16.90 I-L	**22**	17.02 K-L	16.96
23	15.57 F-I	**23**	15.61 D-G	15.59	**23**	16.02 N	**23**	16.02 NO	16.02
24	15.51 G-I	**24**	15.39 E-I	15.45	**24**	17.18 I-K	**24**	16.98 K-L	17.08
25	13.62 P	**25**	13.71 N	13.67	**25**	15.43 O	**25**	15.70 O	15.57
26	14.51 M-O	**26**	14.54 J-M	14.53	**26**	16.19 N	**26**	16.36 MN	16.28
27	14.54 L-O	**27**	14.46 J-M	14.50	**27**	17.24 I-K	**27**	17.05 KL	17.15
28	12.72 R	**28**	12.89 O	12.81	**28**	**20.03 A**	**28**	**20.31 B**	20.17
29	15.21 I_L	**29**	15.75 D-F	15.48	**29**	18.51 D	**29**	18.45 E-G	18.48
30	15.89 E-H	**30**	15.93 C-E	15.91	**30**	18.64 D	**30**	18.68 D-F	18.66
31	15.35 H-K	**31**	14.80 H-M	15.08	**31**	18.40 DE	**31**	17.13 KL	17.77
35	**16.92 B**	**35**	15.38 E-I	16.15	**35**	18.60 D	**35**	18.46 D-G	18.53
36	16.72 B-D	**36**	16.55 B-C	16.64	**36**	16.96 I-L	**36**	17.08 KL	17.02
37	15.91 E-H	**37**	15.95 C-E	15.93	**37**	17.29 H-J	**37**	18.04 G-I	17.67
38	14.21 N-P	**38**	14.15 M-N	14.18	**38**	18.30 D-F	**38**	18.36 F-H	18.33
40	15.02 I-M	**40**	15.00 G-K	15.01	**40**	18.80 CD	**40**	18.90 C-E	18.85
42	**16.88 B**	**42**	**16.94 B**	16.91	**42**	19.29 BC	**42**	19.00 CD	19.15
43	14.46 M-O	**43**	14.34 K-N	14.40	**43**	18.50 D	**43**	18.54 D-G	18.52
LSD: 0.683					LSD: 0.538				

Linoleic Acid (C18:2) (%)

It was determined that the year × genotype interaction is important, that is, the linoleic acid performances of genotypes vary under climatic conditions in different growing seasons (Table 11). The average linoleic acid value in 2020 is 17.64%, and the highest linoleic acid values belong to genotypes 9 (20.49%) and 28 (20.03%). These genotypes were followed by genotype number 4 (19.45%). In 2021, the average linoleic acid value of all genotypes was 17.74%, and the genotype containing the highest linoleic acid value was again genotype 9. In line with the findings, it was observed that genotypes 9 and 28 were the genotypes with the highest linoleic acid content in both harvest years, regardless of ecological factors, and were visibly separated from other accessions. A similar situation is also valid for genotypes 25 and 23, which show the lowest linoleic acid value. In both crop years, they ranked last by maintaining the lowest linoleic acid content, and similarly, genotypes 16 and 26 were among the 5 genotypes with the lowest linoleic acid content in

both years. Therefore, the linoleic acid content is independent of ecological conditions. It is thought that linoleic acid content will not show significant differences according to years and may be a genotypic feature (Table 9).

Alpha-linolenic acid (ALA) (%)

Since the genotype x year interaction is insignificant, evaluations for this quality parameter were made over both years and two-year averages (Table 11). In 2020, the average a-linolenic acid value was 36.97%, and the highest a-linolenic acid value was obtained from genotype no. 2 originating from Georgia, with an average of 39.45%. In 2021, the average a-linolenic acid value of all genotypes was 37.24%, and the highest a-linolenic acid value was gained from genotype number 8, which is of Swedish origin (39.63%). Based on the two-year average, it was determined that genotypes 8, 2, and 13 were the genotypes with the highest α-linolenic acid content, regardless of ecological factors, and were visibly separated from other accessions. A similar situation is valid for genotypes 42 and 28, which show the lowest α-linolenic acid value (Table 10). Therefore, it is thought that the a-linolenic acid content is independent of the annual changes in the ecological conditions of Mediterranean regions.

Table 10. Alpha-linolenic acid and erucic acid values in 2020-2021

Alpha-Linolenic Acid (%)					**Erucic acid (%)**				
2020		**2021**		**Two years**	**2020**		**2021**		**Two years**
Genotype No	**Alpha-Linolenic Acid**	**Genotype No**	**Alpha-Linolenic Acid**	**Mean**	**Genotype No**	**Erucic acid**	**Genotype No**	**Erucic acid**	**Mean**
1	38.54	**1**	37.62	38.07 D-H	**1**	2.78 E-J	**1**	2.67 G-L	2.73
2	39.45	**2**	39.15	**39.29 AB**	**2**	2.81 D-I	**2**	2.78 F-H	2.80
4	37.66	**4**	37.66	37.65 F-J	**4**	2.76 F-J	**4**	2.71 F-K	2.74
7	38.61	**7**	38.85	38.73 A-D	**7**	2.72 F-K	**7**	2.76 F-I	2.74
8	39.42	**8**	39.63	**39.52 A**	**8**	2.91 D-F	**8**	2.88 C-F	2.90
9	35.57	**9**	34.91	35.23 RS	**9**	2.56 K-O	**9**	2.75 F-J	2.66
10	38.56	**10**	38.58	38.57 B-E	**10**	2.84 D-H	**10**	2.86 D-G	2.85
11	38.20	**11**	38.32	38.25 C-F	**11**	2.52 L-O	**11**	2.72 F-K	2.62
13	39.07	**13**	38.86	38.96 A-C	**13**	2.75 F-K	**13**	2.78 F-H	2.77
14	35.97	**14**	36.01	35.98 N-R	**14**	2.59 J-N	**14**	2.60 H-L	2.60
15	36.93	**15**	37.67	37.29 H-K	**15**	2.97 DE	**15**	2.54 K-M	2.76
16	36.18	**16**	36.52	36.34 M-P	**16**	2.47 N-P	**16**	2.36 M-O	2.42
17	37.65	**17**	38.80	38.22 C-F	**17**	2.61 I-N	**17**	2.60 H-L	2.61
19	36.67	**19**	37.95	37.30 H-K	**19**	2.46 N-P	**19**	2.66 G-L	2.56
20	36.73	**20**	38.11	37.41 G-J	**20**	2.70 G-L	**20**	2.66 G-L	2.68
21	35.18	**21**	36.37	35.77 O-R	**21**	2.88 D-G	**21**	2.67 G-L	2.78
22	35.90	**22**	36.41	36.15 M-P	**22**	**3.33 B**	**22**	3.05 CD	3.19
23	37.91	**23**	37.67	37.78 E-I	**23**	2.65 H-N	**23**	2.78 F-H	2.72
24	36.86	**24**	37.01	36.93 J-M	**24**	2.60 J-N	**24**	2.55 J-M	2.58
25	37.95	**25**	38.48	38.21 C-G	**25**	**3.68 A**	**25**	**3.49 A**	3.59
26	36.71	**26**	37.13	36.91 J-M	**26**	**3.39 B**	**26**	**3.28 B**	3.34
27	38.83	**27**	38.35	38.58 B-E	**27**	2.74 F-K	**27**	2.60 H-L	2.67
28	34.86	**28**	34.73	34.79 S	**28**	3.19 BC	**28**	2.99 C-E	3.09
29	38.34	**29**	37.66	38.00 D-H	**29**	2.23 RS	**29**	1.88 P	2.06
30	36.00	**30**	36.35	36.17 M-P	**30**	2.50 M-P	**30**	2.31 NO	2.41
31	35.91	**31**	38.46	37.18 I-L	**31**	2.61 I-N	**31**	2.78 F-H	2.70
35	35.34	**35**	36.03	35.68 P-R	**35**	2.30 P-S	**35**	2.52 K-M	2.41
36	36.12	**36**	36.32	36.21 M-P	**36**	2.39 O-R	**36**	2.48 L-N	2.44
37	36.59	**37**	36.60	36.59 K-N	**37**	2.69 G-M	**37**	2.61 H-L	2.65
38	36.56	**38**	36.49	36.52 K-O	**38**	3.01 CD	**38**	3.06 C	3.04
40	35.80	**40**	35.60	35.69 PR	**40**	2.76 F-J	**40**	2.82 E-G	2.79
42	33.73	**42**	34.04	33.88 T	**42**	2.16 S	**42**	2.24 O	2.20
43	36.25	**43**	36.69	36.46 L-P	**43**	2.83 D-H	**43**	2.79 E-H	2.81
LSD: 0.803					LSD: 0.204				

Erucic Acid (C22:1) (%)

Year × genotype interaction was important for Erucic acid, that is, the erucic acid performances of genotypes varied under climatic conditions in different growing seasons (Table 11). The average erucic acid value in the first year was 2.73%, and the highest erucic acid value was genotype 25 originating from Denmark with a rate of 3.68%. Genotype 25 was followed by genotypes 26 and 22 of Swedish origin in group B. In 2021, the average erucic acid value of all genotypes was 2.70%, and genotype 25 of Danish origin (3.49%) was observed as the genotype containing the highest erucic acid value. In line with the findings, it was revealed that genotypes 25 and 26 were the genotypes with the highest erucic acid content,

regardless of ecological or other environmental factors, and were visibly separated from other accessions. A similar situation is also valid for genotypes 42 and 29, which show the lowest erucic acid value. Therefore, it is thought that erucic acid content is independent of environmental and climate conditions, erucic acid content will not show significant differences between years and may be a genotypic property (Table 10).

Table 11. Variance analysis table for fatty acids in camelina genotypes.

		Mean Square Values					
Sources of Variation	**DF**	**Palmitic acid**	**Stearic acid**	**Oleic acid**	**Linoleic acid**	**Afa-Linolenic acid**	**Erucic acid**
Year (A)	1	0.010 ns	0.034 ns	0.002 ns	0.335 ns	2.441 ns	0.041 ns
Error 1	4	0.001	0.002	0.084	0.105	0.883	0.025
Genotype (B)	32	0.244 **	0.090 **	4.440 **	5.377 **	7.255 **	0.356 **
Year × Genotype (A × B)	32	0.036 **	0.011 **	0.252 **	0.159 **	0.469 ns	0.025**
Error	128	0.007	0.004	0.117	0.073	0.323	0.010

* and ** indicate significance at 0.05 and 0.01 levels of probability, respectively. ns; not significant.

DISCUSSION

Grain yield results from the first year of the study are consistent with those of Katar (2013) and Solis et al. (2013) who are relatively parallel to the results reported. Camellina yield is higher in different countries: Austria (1.91 t ha^{-1}), Canada (1.05 t ha^{-1}), Chile (1.41 t ha^{-1}), Denmark (1.82 t ha^{-1}), Germany (1.88 t ha^{-1}) and in Italy (2.25 t ha^{-1}). Yield-related traits in camelina genotypes were significantly affected by environmental conditions, allowing the identification of potentially good candidates for increasing seed yield. Zahuski et al. (2020) indicated that seed yield was significantly affected by climate and environmental conditions, and thousand-seed weight was also affected in the study conducted during the spring vegetation period in Poland. Besides, Angelini et al. (2020) reported that they investigated the feasibility of camelina cultivation under natural rainfall conditions in their study conducted under Mediterranean ecological conditions (Central Italy), they compared 7 different camelina varieties in spring and autumn planting times. As a result of this study, they reported that higher grain yield was obtained from autumn plantings and according to two-year data, the grain yield value of 3400 kg ha^{-1} was reached in the second year from the genotype named V3, the two-year average of this genotype was 2650 kg ha^{-1} and the general average was 1900 kg ha^{-1}. Arslan et al. (2014) who obtained similar results to our study, reported that the grain yield varied between 875-1811 kd ha^{-1} in the first year and 1066 - 4198 kg ha^{-1} in the second year as a result of a two-year study in Ankara ecological conditions. On the other hand, Kose et al. (2017) reported 8.20 kg ha^{-1} and Gesch (2014) 7.43 kg ha^{-1} grain yields. Comparing our results, the grain yield values reported by these two researchers are quite low. It is estimated that the main reason for these differences is due to variations in genotypes and ecological conditions of the location where the research was conducted.

The results obtained from plant height are similar to those of Kose et al. (2017) and Narmamatov (2021). The highest plant height (103.5 cm) in camelina genotypes was achieved from autumn sowings. Plant height plays an important role in determining the harvest index, and an increase in plant height generally reveals a decrease in the harvest index value (Angelini et al., 2020). Similarly, it was observed that camelina genotypes gave higher plant height in autumn sowings (Tuncturk et al., 2019).

The first lateral branch heights obtained from the research were found to be higher compared to the results of (Yalinkilic et al., 2022) who reported 16.85-36.40 cm. There are significant differences between genotypes in terms of first lateral branch height. The main reason for this difference is due to climatic conditions and comes from genotypic differences. Although the height of the first lateral branch does not have a direct effect on grain yield, it is important in terms of suitability for machine harvesting.

The numbers of capsules obtained from the research are based on Karayel et al. (2021) and Agegnehu and Honermeier (1997) are relatively similar to the results. However, Kose et al. (2017) (14.8 capsule/plant), Gore (2021) (27.5-70.5 capsule/plant) and Yılmaz et al. (2019) (62.9 capsules/plant) are different from the results reported. It is thought that differences between growing conditions, ecological conditions and genotypes are effective in the differences in observed results.

Each capsule of the camelina plant contains an average of 8-16 seeds (Kurt and Seyis, 2008). The results obtained from the research (11.44) are largely similar to the average results reported by Yildirim and Onder (2016) (13.83-16.67), although no additional phosphorus fertilization was applied. The main reason for the differences in the number of seeds in the capsule of the genotypes used in the research is genotypic differences and environmental factors between harvest years.

Two-year average thousand kernel weight data (0.99 g) was obtained from Yildirim and Onder (2016) (0.82-1.06 g), Tuncturk et al. (2019) (0.94 g) and Marquard and Kuhlmann (1986). The weight of a thousand grains in camelina generally varies between 0.8-1.6 g (Kurt and

Seyis, 2008). The difference between the results reported by the aforementioned researchers and the results obtained in this study may be due to the locations and climatic conditions, but also to the fact that different genotypes were tested.

Similar palmitic acid values were reported by Kiralan et al. (2018) and Zubr and Matthaus, (2002). However, the findings obtained by Šípalová et al (2011) differ greatly from the average values of 6.9-11.0% reported in their research. This difference may be due to the different genotypes used, the fact that the compared research was conducted as a pot experiment, and the fertilization program applied.

The average stearic acid values obtained from the research (2.43-2.46%) are similar to the results obtained by Kurt and Gore (2018) (2.43-2.77%) and Campbell et al. (2013) (2.6%). Kıralan et al. (2018) are partially similar to the results obtained (2.70%).

It is known that, in oilseed crops, heat during seed development greatly affects the conversion of carbohydrates to lipids and may explain herein the differences noticed in oil content (Angelini et al., 2020). Similar to this study, the two-year average oleic acid values obtained as a result of the research (15.4%) are compared to Kıralan et al. (2018), Campbell et al. (2013) and Kurt and Gore (2018) were found to be close to the results obtained.

Average linoleic acid values (17.64-17.74%). Kurt and Gore (2018), (16.0-20.3%), Campbell et al. (2013) (18.2%) and are largely similar to the results obtained by Kıralan et al. (2018) (17.66%).

In a study conducted in Central Anatolia, where the winter season is quite cold and snowy, it was reported that linolenic acid contents were obtained much lower than in our study (Katar and Katar, 2017). Average a-linolenic acid values were similar to some studies conducted (Marquard and Khulmann, 1986; Zubr and Matthaus, 2002). However, these results are consistent with those of Campbell et al. (2013) (28%), it was derived that the minimum a-linolenic acid value obtained in both years was higher.

Erucic acid (%) results are similar to earlier studies (Kuzmanović et al., 2021; Marquard and Khulmann, 1986; Basoglu, 1986). However, Campbell et al. (2013) are quite different from the average erucic acid value of 4%. Although the amounts of erucic acid contained in the genotypes used in the research vary slightly from year to year, there is no significant change in their rankings depending on the amount of erucic acid contained in the genotypes in both years.

CONCLUSION

The yield performances of camelina genotypes, which are not well known in the Eastern Mediterranean, were evaluated under Mediterranean climate conditions, it was observed that the yield value of genotype number 28 (3120 kg ha^{-1}) gave promising results. This was followed by genotypes 9 (2735 kg ha^{-1}) and 1 (2651 kg ha^{-1}) in both years. It was determined that these genotypes had a genetic structure that was more tolerant to drought in terms of grain yield in the Mediterranean climate coastline. On the other hand, genotypes 2, 7, 10, 27, 35, 40 and 43 are not at the desired level in Bornova/Izmir conditions. Erucic acid, a monounsaturated fatty acid, is known to be harmful to human health. All genotypes used in this study were within the limits suitable for human consumption in terms of erucic acid content in edible oil (Vetter et al., 2020) and all genotypes used in the study were of quality that can be used as cooking oil. It has been understood that Camelina cultivation can be done with natural rainfall conditions in regions where the Mediterranean climate prevails, where drought stress has begun to be seen, and it is also concluded that promising genotypes with high oil quality can be evaluated in plant breeding to combine yield and quality.

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INTEGRATION OF NOVEL SSR MARKERS INTO THE LENTIL (*Lens culinaris* Medik.) GENOME

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ABSTRACT

The development of simple sequence repeat markers (SSRs) for lentils has played a pivotal role in enhancing the comprehension of the lentil (*Lens culinaris* Medik.) genome through genetic mapping. The study aimed to determine the relative positions of newly developed microsatellites to the lentil genome using an F_7-derived recombinant inbred lines (RIL) population of 71 individuals developed from a cross between Eston and PI320937. Molecular analysis was performed with 100 newly developed lentil SSR markers and a linkage map was constructed using MapMaker/EXP 3.0b and MapChart 2.2 software. Among the 100 SSR markers, 12 markers exhibited polymorphism, 54 markers were identified as monomorphic, and 34 markers remained unamplified. While 10 out of the 12 polymorphic markers successfully integrated into two linkage groups, covering a cumulative length of 19.2 cM, two markers remained unlinked. Linkage group-1, comprised of 8 markers, spanned 4.8 cM, and linkage group-2 extended over a length of 14.4cM with two markers. Despite only partially representing 2 out of the 7 chromosomes in the lentil genome, this map holds promise for future mapping studies. Through the addition of markers, it could facilitate marker-assisted selection and the identification of QTLs associated with specific agronomic traits.

Keywords: Linkage map, Microsatellites, Polymorphism, Recombinant Inbred Lines, Lentil.

INTRODUCTION

Lentil (*Lens culinaris* Medik.), documented as one of the oldest legumes ever cultivated is an annual autogamous cool season legume with a diploid chromosome number of 2n =2x =14 and a ~4.2 Gb genome size (Bett and Cook, 2016). The global production area of lentil spans 5.01 million hectares and yields 6.53 million tons of lentils, with leading producers including Canada, India, Australia, Türkiye, USA, Nepal, Syria, Bangladesh, and China (FAOSTAT 2022). In addition to contributing 35-53% starch, 23-31% protein, and 18% fiber to the human diet, its low cholesterol establishes it as a nutritious and health-conscious choice (Devos, 1998). Moreover, the nitrogen fixation from root nodulation, combined with its capability for carbon sequestration, underscores the significant role lentils play in enhancing soil fertility and optimizing crop management practices (Quereshi et al., 2010).

Currently, the advancement of lentil improvement is hindered by the limited developed molecular tools for genomic analyses (Gupta et al., 2012). This underscores the pressing need for increased development of molecular markers that can hasten the accurate applicability of biotechnological approaches to enhance marker assisted selection (MAS) in lentil breeding. Despite the reported lower genetic variation in lentil compared to other plants, the use of microsatellites remains unparalleled in maximizing polymorphism for the creation of high-density maps that facilitate the identification of QTLs and target genes (Sonante and Pignone, 2001; Saha et al., 2010).

Microsatellites, also known as SSRs or short tandem repeats (STR), consist of 1-6 nucleotides that are unevenly distributed throughout the entire prokaryotic and eukaryotic genomes (Asp et al., 2007). Utilizing microsatellites in lentil has immensely permitted analysis of linkage and agronomical traits due to their co-dominance inheritance, high polymorphic rate, transferability and information content, locus specificity, reproducibility, relatively simple and safe detection procedure (Begna and Yesuf, 2021). Despite the impediment to the use of microsatellites in crop breeding and molecular studies due to their high cost of development, they are still considered ideal for map construction (Avise, 2012). While the initial lentil genetic maps were constructed using morphological markers (Zamir and Ladizinsky, 1984), isozyme markers (Tadmor, 1987), and DNA-based markers (Havey and Muehlbauer, 1989), the significant improvement in lentil genome mapping started with using SSR, AFLP, ISSR and morphological loci (Duran, 2004). Subsequently, a series of maps followed (Hamwieh et al., 2005; Tullu et al., 2008; Andeden et al., 2015; Verma et al., 2015; Dikshit et al.,

2016; Kumar et al., 2018; Singh et al., 2019; Kahraman et al., 2019; Gupta et al. 2023; Topu et al. 2023.). Simultaneously, other molecular tools were utilized in lentil mapping studies (ITAP (intron-targeted amplified polymorphic) (Phan et al., 2007), ISSR (Rubeena and Taylor, 2003; Tanyolac et al., 2010), SNPs (Ates et al., 2016; Bhadauria et al. 2017, Vijayan, et al., 2017; Sudheesh, Rodda, et al., 2016; Temel et al., 2014, Ates et al., 2018).

Given the current global climate change crisis resulting in the escalation of biotic and abiotic stresses of disease, drought, floods, and salinity (Jain et al., 2023), it becomes crucial to innovate strategies to mitigate their effects on lentil cultivation on a genetic basis. Because of the foregoing, increasing the marker repertoire by availing novel SSRs is essential for creating detailed lentil maps. Linkage maps based on gene recombination are classical tools for genetic analyses that enable the localization of targeted genomic regions and hence primal to lentil trait development. This research aimed to pinpoint the positions of novel genomic SSR markers on the genome that will later be used for gene identification on respective chromosomes. This will in turn contribute to effective plant breeding, MAS and further mapping studies of major lentil genes.

MATERIALS AND METHODS

Plant Materials and DNA Extraction

An F_7-derived LR39 RIL mapping population of 71 individuals developed from the cultivated lentil parents "Eston" × "PI320937" by the University of Saskatchewan, Canada was used. This population was developed to ascertain the genetic background of resistance to anthracnose (*Colletotrichum lentis*) in PI320937 (Tullu et al., 2003). Genomic DNA was isolated from fresh 3-week-old seedlings at the Erciyes University, Genome and Stem Cell Center, Türkiye by Lefort et al. (1998). The quantity and quality of the extracted DNA was determined using the NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and 1% agarose gel electrophoresis.

PCR Reactions

A total of 100 genomic SSR markers (Bakir and Kahraman, 2019; Demir and Bakir, 2022; Bakir et al., 2023), developed from enriched genomic libraries of AC and AG repeats in *Lens culinaris* cv. Kafkas, were used. The PCR amplification was performed in a final volume of 15 ul in the presence of 15 ng of genomic DNA, 10 pmol of each primer (Forward & Reverse), 2 mM of $MgCl_2$, dNTP (0.5 ul), Buffergreen (10X), 0.35-unit Taq DNA polymerase (Thermo Scientific, Waltham, MA, USA). The PCR program consisted of an initial step of 3 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 50-66 °C, 2 min at 72 °C and a final extension of 10 min at 72°C. The PCR reaction was checked in 3% metaphor agarose to evaluate the polymorphism of the SSR markers in the parents.

Successfully amplified polymorphic markers in parents were used to ascertain polymorphism according to Schuelke (2000) using an M13-tailed primer (M13 universal sequence (−21), TGT AAA ACG ACG GCC AGT) that was 5'- tagged with fluorophores ROX, HEX or 6-FAM to enhance multiplexing and added to the 5' end of each forward primers. The total reaction mixture was 15 ul comprising of 15 ng of genomic DNA, 10 pmol of each primer (Forward & Reverse), 2 mM of $MgCl_2$, dNTP (0.5 ul), Buffer green (10X), 0.1 µM labeled M13 (-21) universal primer, 0.35-unit Taq DNA polymerase (Thermo Scientific, Waltham, MA, USA). The amplification program consisted of an initial step of 3 min at 94°C, followed by 35 cycles of 1 min at 94 °C, 1 min at 50-66 °C, 2 min at 72 °C, followed by 8 cycles of 1 min at 94 °C, 1 min at 53 °C, 2 min at 72 °C, and a final extension of 10 min at 72 °C. In a ratio of 1:1:2 for HEX, 6-FAM or ROX respectively, amplicons were grouped in threes and mixed with 9.5 µl Hi-Di™ formamide (Applied Bio-systems, Foster City, CA, USA) and 0.5 µl GeneScan-600 LIZ size standards (Applied Bio-systems, Foster City, CA, USA). The final mixture was run through a denaturation process at 95 °C for 5 min, immediately chilled on ice and then electrophoresed on the Applied Bio-systems Prism 3500-Genetic Analyzer System (Applied Bio-systems, Foster City, CA, USA). The fragment size was determined using GENEMAPPER software v5.0 (Applied Bio-systems, Foster City, CA, USA).

Linkage Mapping

The chi-square (χ^2) goodness of fit test was used to calculate the presence of segregation distortion at each locus ($p < 0.05$) by comparing the observed segregation ratios to the 1:1 expected Mendelian ratios of RIL populations and a genetic linkage map was constructed using the MAPMAKER/EXP V.2.0 program (Lander et al., 1987). In determining the genetic linkage between two random markers, the linkage criteria of LOD = 3 score was used and the Kosambi mapping function was used to convert recombination frequencies into genetic distances (Kosambi, 2016). Mapchart software V.2 was then used to visualize the output (Voorrips, 2002).

RESULTS AND DISCUSSION

The RIL population derived from "Eston" × "PI320937" lentil parents was used in other studies to construct a consensus map using 9,793 DArT markers, identify QTLs associated with earliness, plant height using (AFLP, SSRs and RAPD markers), QTL analysis for selenium uptake (4 SSRs, and 1,780 SNPs), resistance to anthracnose (*Colletotrichum lentis*) and ascochyta blight (*Ascochyta lentis*) (RAPD and AFLP markers) and stemphylium blight resistance (*Stemphylium botryosum*) (Tullu et al., 2002; Tullu et al., 2006; Tullu et al., 2008; Podder, 2012; Ates et al., 2016; Ates et al., 2018; Ates, 2019).

SSR Analysis

In the tested population, 12 SSR markers that accounted for 12% of the total 100 tested markers were found

polymorphic. A proportion of these same markers were tested on 24 lentil cultivars (Bakir and Kahraman, 2019), 23 lentil cultivars (Demir and Bakir, 2022), and 10 lentil cultivars (Bakır et al., 2023). However, higher polymorphic rates of 58.4%, 26.6% and 48.5% respectively were observed. In a like manner, Kahraman et al. (2019) reported dissimilar polymorphic rates for the same SSR primers used by Duran et al. (2004), Hamwieh et al. (2005), and Rajesh et al. (2008) yielding polymorphic rates of 20%, 6.45% and 34.85%, respectively. Whereas Kahraman et al. (2019) used WA8649041 × Precoz, mapping populations from ILL 5588 x L 962-16-1 and *Lens culinaris* ssp. *culinaris* cv. Lupa × *L. culinaris* ssp. *orientalis* Boiss. (BG 16880) were used by Hamwieh et al. (2005) and Duran et al. (2004), respectively. This could suggest population genetic makeup as the source of the significant polymorphic dissimilitude. With the source of the population's genetic makeup being parental combination, polymorphism could be affected by either interspecific or intraspecific combinations (Sari et al., 2023) https://doi.org/10.1038/s41598-023-37268-w). Although generally in the genus *Lens* Mill., intersubspecific populations produce higher polymorphic rates (Tahir and Muehlbauer 1994). The subspecies combination of the Eston × PI320937 (cultivated lentil × cultivated lentil) was found similar to several studies. Jha et al. (2017) reported a polymorphic rate of 5.79% from an intraspecific cross of *Lens culinaris* ssp. *culinaris* (WA8649090 × Precoz), while Rubeena et al. (2003) observed a rate of 19.2% in an F_2 intraspecific population (ILL5588 × ILL7537). Additionally, Phan et al. (2007) found a rate of 15.7% from 626 ITAP markers tested on the cultivars Digger (ILL5722) and Northfield (ILL5588) parents of an F_5 RIL population as well as Radhika et al. (2007) observed polymorphism of 9.5% in JV (JG62 × Vijay) and 11.6% in VI (Vijay × ICC4958) intraspecific $F_{8:9}$ RIL populations of chickpea. Other similar studies include Septiningsih et al. (2012) and Koyama et al. (2001) that used a mapping population from a combination of indica × indica parents in rice. Septiningsih et al. (2012) reported 10.5% polymorphism out of the 1,074 SSR markers used. Besides the parental crosses, polymorphism could be attributed to the plant's mode of reproduction (cross- or self-pollinated), type of mapping population (RILs, F_2, BC, DH, NILs) and the type of markers used for genotyping (Vaillancourt and Slinkard, 1993; Eujayl et al., 1998).

Genetic Mapping

Segregation distortion analysis showed nine markers (75%); Lc_Mcu5, Lc_Mcu6, Lc_Mcu14, Lc_Mcu19, Lc_Mcu20, Lc_Mcu45, Lc_Mcu70, Lc_Mcu87 skewed towards the genotype Eston and Lc_Mcu26 towards the genotype P1320937 (Table 1). A 1:1 normal segregation was exhibited by three markers (Lc_Mcu79, Lc_Mcu47 and Lc_Mcu2). All the polymorphic markers were used in the mapping to eliminate the loss of any genetic information that could be linked to distorted markers (Takumiet et al., 2013, Kirungu et al., 2020). Although segregation distortion depends on the specific cross of parents (intra- or interspecific), in comparison; Eujayl et al. (1997) observed 83.3% in lentil, Eujayl et al. (1998) 26.6% in a RIL population, 14% by Rubeena et al. (2003) in lentil, (9.5% SSRs and 17.8% AFLP in a lentil RIL population) Hamwieh et al. (2005), 48% Tanyolac et al. (2010), 5.6% Ates et al. (2018) and 38.4% Winter et al. (2000) in *Cicer* sp. The underlying factors of this phenomenon could be selective elimination and gametic selection which includes preferential fertilization and pollen tube competition (Paterson et al., 2000).

Table 1. Chi-square test (χ^2) for segregation distortion of genomic SSRs in RIL population

		Genotype				
Marker	Genetic characteristic	A/A	B/B	A/B	χ^2	Direction of distortion
Lc_Mcu2	codominant	43	27	1	3.61	-
Lc_Mcu5	codominant	42	0	25	35.66**	Eston
Lc_Mcu6	codominant	49	20	0	12.18**	Eston
Lc_Mcu14	codominant	46	0	25	38.60**	Eston
Lc_Mcu19	codominant	22	2	46	35.94**	Eston
Lc_Mcu20	codominant	36	2	32	31.14**	Eston
Lc_Mcu26	codominant	2	69	0	63.22**	P1320937
Lc_Mcu45	codominant	44	0	3	41.38**	Eston
Lc_Mcu47	codominant	25	23	2	0.16	-
Lc_Mcu70	codominant	22	0	48	39.82**	Eston
Lc_Mcu79	codominant	27	39	3	2.21	-
Lc_Mcu87	codominant	38	13	9	11.76**	Eston

** :Significant at the 0.05 probability level

A total of 10 markers accounting for 83.3% of the polymorphic markers were mapped (Figure 1). Despite the low polymorphic rate, the majority of the markers were mapped with only two markers Lc_Mcu2 and Lc_Mcu26 (16.7%) unlinked. A similar scenario was reported by Gupta et al. (2012) and Andeden et al. (2015) that observed 4.43% and 18% polymorphism but mapped 82.35% and 79% of the polymorphic markers, respectively. Additionally, Singh et al. (2021) successfully genotyped RILs with only eight polymorphic markers out of 389 SSRs (2.05%) and revealed markers LcSSR440 and LcSSR606 that co-segregated with rust resistance. A comparison with

Gupta et al. (2012) - 66.7%, Verma et al. (2015) - 33.3% and Kahraman et al. (2019) - 56.5% found this mapping rate higher, but to the contrary, lower than Saha et al. (2013) and Qureshi et al. (2010) with 100% and 91.5%, respectively.

This linkage map (Figure 1) which covers a total length of 19.2 cM is one of the smallest microsatellite maps created. Albeit the consideration of having the same number of LGs as the haploid chromosome numbers of the species under study is ideal, this study only had two of the seven expected LGs. The insufficient number of polymorphic markers coupled with the small mapping population could be a reason for this. This is not only accentuated by Ferreira et al. (2006) that an insufficient mapping population number produces inaccurate ordering of loci on LGs and/or imprecise fragmentation but also by Pootakham et al. (2015) that asserts the negative effects of few markers on the calculation of marker order. Therefore, increasing marker density could decrease the number of unlinked markers to attain better coverage of the genome (Gupta et al., 2012).

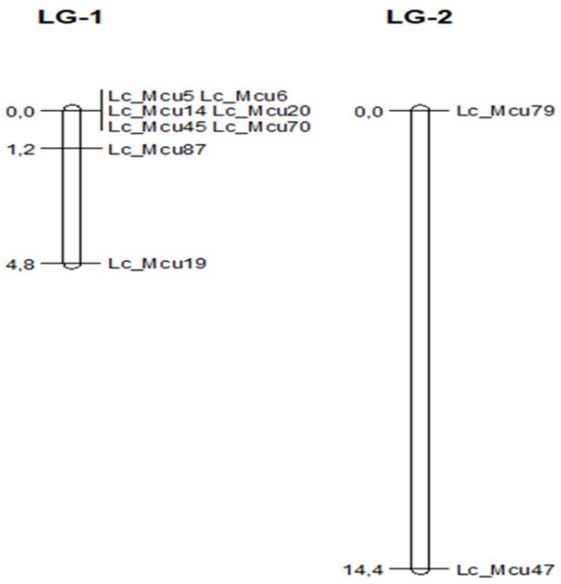

Figure 1. Linkage group 1 (LG-1) that included 8 markers and Linkage group 2 (LG-2) mapped two markers.

Using RIL populations in mapping is purposed to increase linkage breakdown since the probability of recombination is two-fold higher than in F_2 or BC_1 populations (Biswas et al., 2010). However, an appraisal of the linkage map (Figure1) depicts an uneven distribution of markers to the distal points of the LGs indicating a difference in the crossing-over frequency on chromosomes as the possible cause for the observed marker density. The markers are situated at the telomeres which are recombination-suppressed regions that have an extremely low recombination rate than the regions of the centromeres (Tanksley et al., 1992).

CONCLUSION

In spite of the low number of genomic SSRs used for constructing the linkage map in this study, we envisage that the partial coverage of two out of the seven chromosomes of the lentil genome could still be used for further mapping studies. Owing to the profound applicability of microsatellites, addition of new markers or an amalgamation with other lentil maps is imperative for its future utilization in breeding and crop advancement strategies. Besides enabling complete genome coverage by saturation with markers, using a larger mapping population will counteract rare recombination events caused by the presence of centromeric heterochromatin or limited recombination at the telomeres and in turn produce a fine map that will be used as a genetic framework for future qualitative and quantitative trait analysis for lentil.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DETERMINATION OF FEED QUALITY CHARACTERISTICS OF SOME SILAGE MAIZE (*Zea mays* L.) HYBRIDS CULTIVATED IN EASTERN MEDITERRANEAN CONDITIONS

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ABSTRACT

The research was carried out in Kahramanmaras Eastern Mediterranean Transition Zone Agricultural Research Institute (DAGTEM) in the main crop growing season of 2019 and 2020 in a Randomized Complete Block Design with three replications to determine the silage quality characteristics of 20 different silage maize hybrids (Macha, Ranger, Simon, AS160, Dracma, DS0224, DKC6442, Colonia, Inove, Antex, Everest, Torro, 73may81, Kilowaatt, Klips, PR31Y43, 30B74, DKC7240, C955, and Gladius) as main crop. The study showed that silage quality varied significantly for maize hybrids and the region's climatic conditions in different years. According to the two-year average results, the dry matter content (T_{60}), dry matter recovery, crude protein content, crude ash content, starch content, NDF content, ADF content, ADL content, pH value, and forage yield were between 28.43-32.59%, 90.11-97.69%, 6.01-7.44%, 4.93-7.52%, 18.88-27.04%, 44.28-54.69%, 23.85-30.30%, 2.00-3.62%, 3.84-3.90, and 51343.5-79920.5 kg ha^{-1}, respectively, and C955, Everest, and PR31Y43 were the hybrids that potential silage nutritional value of them was prominent.

Keywords: Maize varieties, starch, silage quality, *forage yield.*

INTRODUCTION

Maize variety is widely grown as silage feed in the world and in Türkiye due to its high dry matter production per unit area, higher energy content compared to other forages, suitability for mechanization, ability to be easily mixed into rations, and relatively high consumption by dairy cattle (Fernandez et al., 2004). Maize is considered among the easily ensiled plants, mainly thanks to its chemical composition (NRC, 2001). However, commercially available silage maize varieties today are generally considered silage crops only because they yield high green forage. No seed company specializes only in silage maize cultivars. Most of the seed need for silage hybrid maize production is met by non-silage varieties (Ozata et al., 2012). Companies generally produce and market the seeds of grain maize, silage maize, and even other products. In fact, of course, it is unnecessary to find a company that will only produce maize for silage.

However, the resulting seed production genotypes are quickly registered as silage cultivars due to the need for more practical and applicable parameters in silage maize breeding and registration. In order to register any maize variety as silage today in Türkiye, it is sufficient to know the green grass yield, hay yield, and protein ratio. This situation is not favourable in terms of silage feed quality.

Many studies have been conducted on the effect of varieties on silage quality. In these studies, two or three varieties were generally used, and inoculation was examined together with other factors such as silo opening time or chopping size. For example, Sheaffer et al. (2006) used four different varieties to examine the effects of maize varieties and nitrogen fertilization. Two of these varieties were low in lignin content and brown midrib (BMR); one was a variety with abundant leaves, and the other was a standard hybrid. Researchers have determined that BMR varieties have low green forage yield. However, due to their high digestibility properties, animal milk production increased in response to unit feed consumption, and the amount of produced milk per unit area of forage maize was similar. Researchers have reported that BMR hybrids have high neutral detergent fiber (NDF) digestibility. However, dry matter yield is relatively low. Therefore, they have milk production potential similar to that of standard hybrid varieties.

Similarly, Nennich et al. (2003) reported that the variety with abundant leaves, improved for increasing feed quality and animal performance, differed from standard varieties regarding feed quality and milk production of ruminants. In the study conducted with three varieties, the researchers found significant differences between the varieties in terms of NDF digestibility. The fact that there are differences

even among a few varieties indicates that the varieties will have different silage properties and feed quality characteristics. On the other hand, maize silage typically contains between 25-35% starch and 40-50% NDF on a dry matter (DM) basis (Ferraretto et al., 2015a). Mertens (2003) reports that maize silages contain 36-54% NDF and 8.3-9.3% protein on a DM basis. As can be seen, there is a wide variation in the chemical structure of maize arising from growing conditions and genotypic characteristics.

This study was conducted to determine the silage quality characteristics of silage maize varieties supplied by different companies in Eastern Mediterranean ecological conditions and to identify a readily determinable and effective silage maize breeding parameter.

MATERIALS AND METHODS

The research was conducted in 2019 and 2020 at the Kahramanmaras ecological conditions under a Randomized Complete Block Design with three replications. Macha, Ranger, Simon, AS160, Dracma, DS0224, DKC6442, Colonia, Inove, Antex, Everest, Torro, 73may81, Kilowaatt, Klips, PR31Y43, 30B74, DKC7240, C955 and Gladius silage maize varieties were used crop materials. The commercial companies from which the varieties were obtained, as well as their maturity periods and FAO maturity groups, are given in Table 1.

Table 1. Hybrids used, commercial companies from which they were supplied, and FAO groups

Hybrids	Companies	FAO Groups	Hybrids	Companies	FAO Groups
Macha	Polen	580	Everest	May	680
Ranger	Polen	600	Torro	Polen	680
Simon	Polen	600	73MAY81	May	700
AS160	Agromar	600	Gladius	Sygenta	800
Dracma	Syngenta	630	Kilowatt	KWS	700
DS0224	Agromar	630	Klips	KWS	700
DKC6442	Monsanto	650	PR31Y43	Pioneer	700
Colonia	Agromar	650	30B74	Pioneer	720
Inove	Syngenta	650	DKC7240	Monsanto	750
Antex	Syngenta	650	C955	Monsanto	800

According to the climate data for the period when the research was carried out, it was determined that the average temperatures of the second year of the study were above the long-term average, and the total amount of precipitation in both years was well below the long-term data (Table 2). Silage maize requires a minimum of 6-13 °C for germination (Sanchez et al., 2014) and more than 600 mm of rainfall during the vegetation period (Haarhoff et al., 2019) or needs irrigation when the rainfall is not enough. It can be seen from Table 2 that the average temperature values were at the desired level for maize, but it was not possible to grow maize without irrigation.

Table 2. Some climate data for the research years and long-term

		Months					
Climate data	**Year**	**May**	**June**	**July**	**August**	**September**	**Sum/Average**
Rainfall (mm)	2019	18.50	0.30	0.10	0.10	1.5	20.50
	2020	8.20	0.00	0.00	0.00	0.00	8.20
	Long Term	41.20	8.40	3.30	2.20	11	66.10
Average Temperature (°C)	2019	15.90	24.50	29.00	29.50	26.3	25.04
	2020	23.45	25.49	30.49	29.65	28.75	27.56
	Long Term	20.30	25.30	25.10	28.40	25.00	24.82
Relative humidity (%)	2019	47.20	46.90	47.20	47.7	41.2	46.04
	2020	43.30	49.00	44.60	40.95	42.86	44.14
	Long Term	54.95	49.67	51.90	48.76	45.42	50.14

Long-term covers the year range of 1930-2021

Some physical and chemical properties of the research area soils are given in Table 3. As seen in Table 3, the trial soils are slightly alkaline and have high $CaCO_3$ content. Soil organic matter is 1.86% in the 0-30 cm depth, where the effective root system is located, 1.91% in the 0-60 cm depth, and can be classified as moderately rich in organic matter. It was determined that the soil of the trial area was sufficient in terms of usable potassium and moderate in terms of phosphorus for maize growth.

Table 3. Some physical and chemical properties of the research soil.

Soil depth(cm)	pH	$CaCO_3$ (%)	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Organic matter (%)
0-30 cm	7.53	26.26	46.5	398.7	1.86
30-60 cm	7.52	26.12	42.7	652.4	1.91

This main crop planting was done with a parcel drill in 5 m long parcels, with 70 cm row spacing and 20 cm row spacing for each hybrid in 2019 and 2020. Until the plants reached 40-45 cm tall, weed control was carried out mechanically. During the experiment, irrigation was done using the sprinkler method at needed intervals. Hybrids used in the research reached harvest maturity at different times since each belonged to different maturity groups and vegetation periods were divergent. For this reason, hybrids' dry matter content was considered the essential criterion for harvest, and mowing was done when crops had an average DM content of 32-34%. The harvest stage generally coincides with the period when the grain's milk line decreases to 2/3 of the grain (Loucka et al., 2018). Plants were shredded in a plant shredder machine to a theoretical length of 2-3 cm to make silage. For each parcel, 0.5 kg of material with three parallel was placed in unique plastic bags, automatically sealed after removing 99.9% of the O_2 using a vacuum device (Ferraretto et al., 2015b). Silages were opened after 60 days, and silage quality characteristics were determined.

Approximately 100 g of samples were taken from each silage package after 60 days of fermentation. The samples were dried at 78°C until their weight was constant, then weighed, and the dry matter ratio before (T_0) and 60 days after (T_{60}) ensiling were calculated. Dry matter recovery (DMR), which explains the differences of forage DM mass in the silo day 0 and on the day that the silo opened, was calculated by dividing DM (T60) to DM (T0) and multiplying by 100 as described by da Silva et al., 2020. The dried material obtained here was used for chemical analysis after grinding at a 1 mm sieve. Cell wall components (NDF, ADF, and ADL) contents (%) were determined using the Ankom Fiber Analyzer (Fiber Analyser, ANKOM brand, A220 model) (Van Soest et al., 1991). The samples' nitrogen (N) content was determined using the Kjeldahl method. Crude protein was calculated using N x 6.25 (AOAC, 1990). Starch contents of silages were determined using polarimetry according to Evers' Polarimetric Method (International Organization for Standardization, 1997).

RESULTS AND DISCUSSIONS

The DM content of fresh material of silage maize hybrids varied between 30.30-35.59%, and there was no statistical significance among the DM contents (Table 4). The DM ratio of fresh forage before ensiling is one of the most critical factors affecting the silage's aerobic stability, fermentation, and quality. The DM content for a quality maize silage was determined by Kilic (1986) as between 30-35%, by Basmacioglu and Ergul (2002) as between 25-35%, and by Mohd-Setapar et al. (2012) between 25-40%. In the present study, it can be assumed that the DM contents of maize hybrids were at the desired level, as many researchers reported regarding silage quality. Unlike the DM content of fresh forage before ensiling, differences among DM of resulting silages opened on the 60th day were statistically significant. The highest DM content (32.59%) in resulting silages was determined in the C955 hybrid, followed by the Everest (32.46%). In comparison, Colonia (28.43%) hybrids have the lowest DM content ($P<0.05$), and ten hybrids, which have the highest DM content at T_{60}, were in the same statistical group due to relatively higher LSD value. When the average values were considered, it was determined that the DM content of samples taken before and after ensiling in the first year was higher than that of the second year ($P<0.01$).

Table 4. Dry matter content (%) of T_0 and T_{60} silages and dry matter recovery (%) (DMR) values.

	DM (T_0) (%)			DM (T_{60}) (%)			DMR (%)		
Hybrids	**2019**	**2020**	**Average**	**2019**	**2020**	**Average**	**2019**	**2020**	**Average**
Macha	34.21	30.16	32.19	33.38	28.55	30.97^{A-E}	97.58	94.90	96.24
Ranger	33.44	33.90	33.67	30.76	30.72	30.74^{A-F}	92.33	90.92	91.63
Simon	33.88	35.94	34.91	31.53	31.16	31.35^{A-D}	93.19	87.03	90.11
AS160	33.92	30.46	32.19	30.49	27.67	29.08^{DEF}	89.96	91.37	90.67
Dracma	33.00	33.24	33.12	30.36	28.09	29.23^{C-F}	91.87	85.34	88.61
DS0224	33.69	32.40	33.05	30.51	28.99	29.75^{C-F}	90.54	90.85	90.70
DKC6442	34.34	32.40	33.37	32.36	29.17	30.77^{A-F}	94.24	90.66	92.45
Colonia	32.42	28.18	30.30	30.06	26.80	28.43^{F}	92.80	95.26	94.03
Inove	32.47	32.53	32.50	30.91	29.09	30.00^{C-F}	95.11	89.93	92.52
Antex	34.08	31.56	32.82	31.86	29.18	30.52^{B-F}	93.49	89.65	91.57
Everest	34.62	36.55	35.59	33.37	31.55	32.46^{AB}	96.34	87.47	91.91
Torro	32.95	33.34	33.15	31.03	30.27	30.65^{A-F}	94.15	91.21	92.68
73May81	33.00	32.76	32.88	30.11	31.85	30.98^{A-E}	91.66	97.16	94.41
Kilowaatt	34.63	30.30	32.47	34.02	29.29	31.66^{ABC}	98.23	96.81	97.52
Klips	33.75	29.38	31.57	32.26	27.52	29.89^{C-F}	95.72	93.84	94.78
PR31Y43	33.05	28.29	30.67	30.37	28.53	29.45^{C-F}	91.97	98.74	95.36
30B74	34.36	28.77	31.57	31.24	25.94	28.59^{EF}	91.07	90.48	90.78
DKC7240	34.48	27.88	31.18	32.58	26.12	29.35^{C-F}	94.50	93.83	94.17
C955	34.91	31.56	33.24	34.23	30.94	32.59^{A}	98.05	98.01	98.03
Gladius	33.70	29.27	31.49	32.85	28.65	30.75^{A-F}	97.54	97.84	97.69
Average	33.75^{A}	31.47^{B}		31.71^{A}	28.95^{B}		94.02	92.56	
CV (%)	9.24			7.00			5.87		
LSD	Hybrid: ns Year: 1.10** Year x hybrid: ns ns: not significant			Hybrid: 2.44* Year: 0.77** Year x hybrid: ns			Hybrid: ns Year: ns Year x hybrid: ns		

The DM obtained from the silage of maize hybrids were higher than the values reported by Degirmenci (2000) (25.00-25.90%), compatible with the findings reported by Deniz et al. (2001) (26.49-37.37%) and Basaran et al. (2017) (%28.36-34.58), while lower than the values submitted by Arslan et al. (2016) (44.42%) and Akdemir et al. (1997) (36.13-39.89%). In addition to various characteristics, climatic conditions, and cultural practices are also influential in determining the DM content of maize hybrids. Year, hybrid effect, and year x variety interactions were not statistically significant concerning dry matter recovery (DMR) values. DMR of the hybrids varied between 90.11% for Simon and 98.03% for C955 hybrids (Table 4). Even though there were no significant differences among DMR values of hybrids, considering that silages are prepared with great effort and cost, the approximately 7% difference between the highest and lowest DMR is essential in the business economy.

As can be seen from Table 5, crude protein contents of silage maize varieties varied between 6.01-7.44% depending on the hybrids (P<0.01); the PR31Y43 variety had the highest crude protein value, insignificantly followed by Simon with 7.37% and Everest varieties with 7.32%, while the lowest crude protein ratio was acquired from Colonia hybrid with a value of 6.01%. The PR31Y43 silage maize hybrid came to the fore in the study regarding CP content, which is vital for feeding the value of silage. Considering the year effect, CP content ranged from 6.65 to 7.23%, and the average protein value obtained in the second year was significantly higher (P<0.01) than in the first year. It was determined that the CP content of Ranger, Simon, Antex, AS160, and some other hybrids increased in the second year, whereas that of Colonia, Klips, and Gladius hybrids decreased in the second year, and this caused a year x hybrid interaction (P<0.01) (Figure 1). Oz et al. (2012) reported that the CP contents of 50 different silage maize lines varied between 7.09-9.82%. Similarly, Ozata and Kapar (2017) reported that the silage crude protein ratio of different silage maize varieties ranged from 5.62 to 9.06% in the first year and from 5.22 to 7.81% in the second year, supporting the CP results of the present study.

A year and hybrid effects, as well as year x hybrid interaction, were found to be statistically significant (P < 0.01) in terms of the ash content of the silages opened on the 60th day of fermentation (Table 5). The ash rates of different maize hybrids changed between 4.93 and 7.52%; the highest ash content was obtained from the Antex variety with 7.52%, while the lowest values were obtained from Kilowaatt with 4.93% and from 73May81 with 4.99%. It was determined that the average ash content in the second year of the study was higher than in the first year. This difference can be explained by the fact that the plants remained green at harvest due to lower temperatures in the first year. As crop maturing progresses, the leaf/stem ratio decreases, and accordingly, there is a decrease in CP and crude ash values (Akbay et al., 2022). The hybrids used responded differently depending on the years in terms of their ash content, causing a year x variety interaction. For example, the ash content of the DKC7240 hybrid, which was 5.87% in the first year, increased by 34.07% in the second year and reached 7.87%, whereas the raw ash content of the Torro hybrid, which was 6.47% in the first year, decreased by 8% and fell to 5.95% in the second year. In other words, it was determined that the varieties showed different reactions regarding raw ash content depending on the years. The ash values obtained from the current research are compatible with the values of 6.31-8.27% reported by Geren (2000), 4.18-6.91% reported by Erdal et al. (2009), and 5.7-7.1% reported by Seydosoglu and Saruhan (2017).

Table 5. Crude protein (%), crude ash (%), and starch (%) contents and statistical groups.

	CP (%)			Ash (%)			Starch (%)		
Hybrids	**2019**	**2020**	**Average**	**2019**	**2020**	**Average**	**2019**	**2020**	**Average**
Macha	5.99^{kl}	6.68^{c-k}	6.34^{EF}	7.28^{a-d}	7.19^{a-e}	7.24^{AB}	26.51	24.81	25.66^{AB}
Ranger	6.44^{f-k}	7.84^{ab}	7.14^{A-D}	6.51$^{b-ı}$	6.49$^{b-ı}$	6.50^{B-E}	27.37	24.05	25.71^{AB}
Simon	6.83^{b-k}	7.91^{a}	7.37^{AB}	5.16^{j-n}	5.40$^{ı-n}$	5.28^{HIJ}	21.30	21.90	21.60^{BCD}
AS160	6.71^{c-k}	7.57^{a-e}	7.14^{A-D}	6.15^{d-m}	6.45^{c-j}	6.30^{C-F}	18.67	19.26	18.97^{D}
Dracma	6.26^{h-l}	7.14$^{a-ı}$	6.70^{B-F}	5.40$^{ı-n}$	5.93^{e-m}	5.67^{E-J}	24.12	27.25	25.69^{AB}
DS0224	6.68^{c-k}	7.61^{a-d}	7.15^{A-D}	5.41$^{ı-n}$	6.39^{c-k}	5.90^{D-I}	18.78	18.97	18.88^{D}
DKC6442	6.04^{jkl}	7.64^{a-d}	6.84^{A-E}	5.80^{f-m}	6.32^{c-l}	6.06^{C-H}	24.97	24.40	24.69^{ABC}
Colonia	6.69^{c-k}	5.32^{l}	6.01^{F}	5.41$^{ı-n}$	5.43$^{ı-n}$	5.42^{F-J}	21.36	23.36	22.36^{A-D}
Inove	6.53^{e-k}	7.30^{a-g}	6.92^{A-E}	6.42^{c-k}	6.89^{a-h}	6.66^{A-D}	29.69	24.38	27.04^{A}
Antex	6.63^{d-k}	7.93^{a}	7.28^{ABC}	7.39^{a-d}	7.64^{abc}	7.52^{A}	27.97	22.75	25.36^{AB}
Everest	7.06^{a-j}	7.58^{a-d}	7.32^{AB}	6.13^{d-m}	7.36^{a-d}	6.75^{A-D}	22.77	25.58	24.18^{ABC}
Torro	6.15$^{ı-l}$	7.71^{abc}	6.93^{A-E}	6.47^{b-j}	5.95^{e-m}	6.21^{C-G}	18.05	25.24	21.65^{BCD}
73May81	6.90^{a-k}	7.46^{a-f}	7.18^{ABC}	5.11^{k-n}	4.86^{mno}	4.99^{IJ}	20.55	25.94	23.25^{A-D}
Kilowaatt	6.97^{a-k}	7.24^{a-h}	7.11^{A-D}	4.36^{no}	5.50$^{ı-n}$	4.93^{J}	26.36	23.96	25.16^{ABC}
Klips	7.55^{a-e}	7.24^{a-h}	7.40^{AB}	5.58^{h-n}	6.97^{a-g}	6.27^{C-G}	19.65	21.24	20.45^{CD}
PR31Y43	7.50^{a-e}	7.38^{a-g}	7.44^{A}	5.01^{l-o}	5.92^{e-m}	5.46^{F-J}	19.03	23.92	21.48^{BCD}
30B74	6.39^{g-k}	6.76^{c-k}	6.58^{C-F}	5.76^{g-m}	7.77^{ab}	6.76^{A-D}	22.34	24.95	23.65^{A-D}
DKC7240	6.05^{jkl}	7.49^{a-e}	6.77^{A-E}	5.87^{f-m}	7.87^{a}	6.87^{ABC}	23.79	23.03	23.41^{A-D}
C955	6.23^{h-l}	6.61^{d-k}	6.42^{DEF}	3.79^{o}	7.11^{a-f}	5.45^{F-J}	25.84	23.73	24.79^{ABC}
Gladius	7.37^{a-g}	6.23^{h-l}	6.80^{A-E}	4.31^{no}	6.39^{c-k}	5.35^{G-J}	25.74	26.39	26.07^{AB}
Average	6.65^{B}	7.23^{A}		5.67^{B}	6.49^{A}		23.24	23.76	
CV (%)	9.24			13.35			17.72		
LSD	Hybrid: 0.74** Year: 0.24** Year x Hybrid: 1.04** ns: not significant			Hybrid: 0.93** Year: 0.30** Year x Hybrid: 1.32**			Hybrid: 4.79* Year: ns Year x hybrid: ns		

Maize grains are of great importance as starch sources in silage maize. The starch content of the grain varies depending on the maturity period and reaches its highest value during the dough formation period (Hill, 1993). The starch contents of maize hybrids ensiled during the dough formation period varied between 18.88-27.04%. Approximately half of the energy value of maize silage comes from its starch content. For this reason, it is desired that the starch content of maize silo feed should be high to a certain extent. From the results obtained, the Inove (27.04%) variety stands out with its highest starch content; besides, Gladius, Dracma, Macha, Ranger, and Antex varieties have relatively high rates of starch, respectively. For other results, DS0224 (18.88%) and AS160 (18.97%) varieties were determined to have the lowest starch content ($P<0.05$). In the study, the average starch content was determined to be 23.24% in the first year and 23.76% in the second year, and there was no significant difference in starch content between the years. The findings from the present study are parallel to the values (22.0-26.2%) reported by Simsek-Soysal et al. (2022).

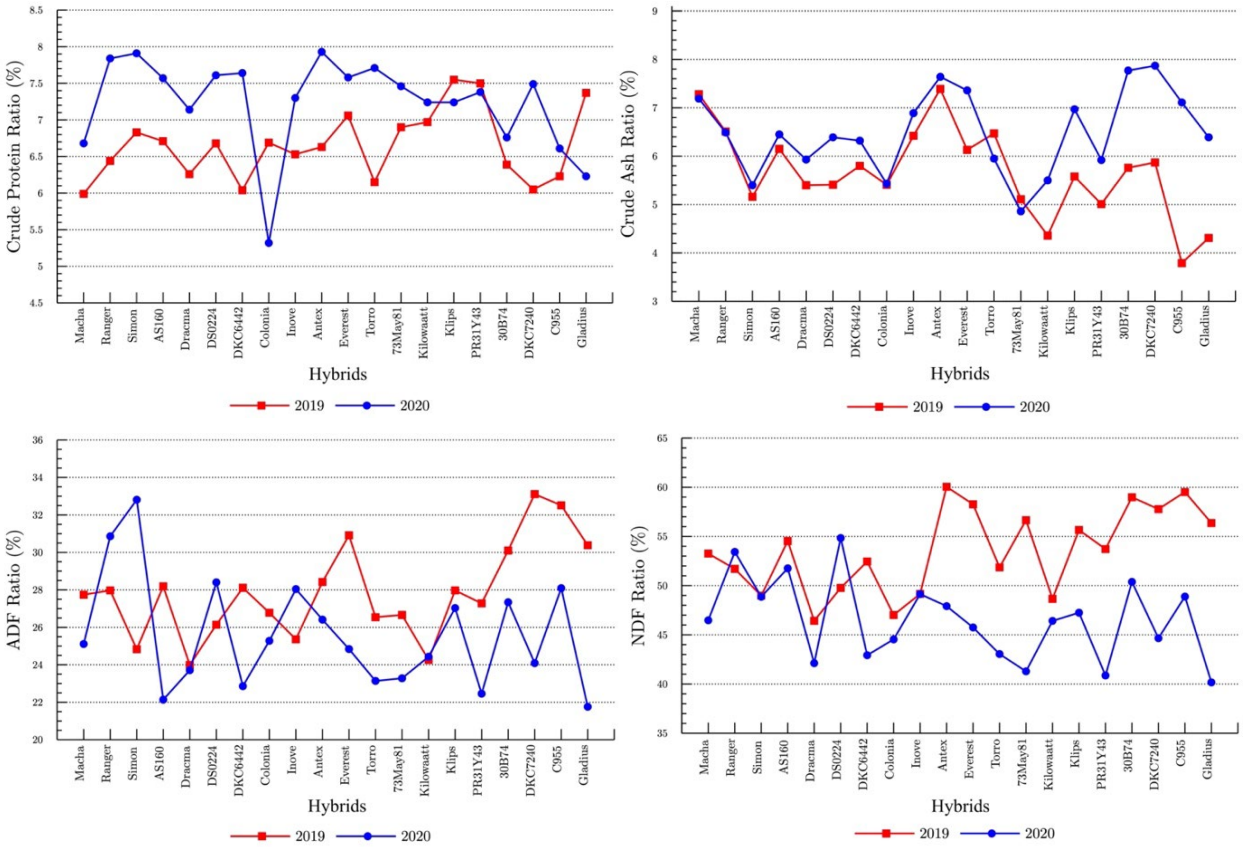

Figure 1. Year x Hybrid interactions for CP, Ash, ADF, and NDF

It was determined that the effects of year and hybrid on the ADF contents of maize silages opened on the 60th day of fermentation were not statistically significant. However, there were differences in the hybrids' responses in both years regarding ADF contents, and the year x hybrid interaction was significant. While the ADF values of the varieties were generally lower in the second year than in the first year, the opposite situation occurred for hybrids such as Ranger and Simon. This interaction can be associated with the year x variety interaction occurring in the plants' crude protein and ash content values. The ADF content of feeds containing high protein is known to be low (Arzani et al., 2006), and there is a negative correlation between them (Mcdonald et al., 1995; Prajapati et al., 2019). Loucka et al. (2018) reported that the ADF ratio of maize varieties should be 30% for a quality silage. Oz et al. (2012) reported that the ADF contents of 50 selected maize lines varied between 26.70-30.76%. Ozata and Kapar (2017) reported that the ADF contents of silo feeds of different maize varieties varied between 24.1-35.9%. The findings obtained from the current study agree with the reports mentioned above.

Table 6. Average ADF (%), NDF (%), and ADL (%) values and statistical groups.

	ADF (%)			NDF (%)			ADL (%)		
Hybrids	**2019**	**2020**	**Average**	**2019**	**2020**	**Average**	**2019**	**2020**	**Average.**
Macha	27.74^{a-h}	25.11$^{d-ı}$	26.43	53.26^{e-j}	46.48^{k-r}	49.87^{B-G}	2.01	2.01	2.01^{DE}
Ranger	27.97^{a-h}	30.86^{a-d}	29.42	51.71^{e-l}	53.43^{b-j}	52.57^{A-D}	2.95	2.28	2.62^{B-E}
Simon	24.83$^{e-ı}$	32.81^{ab}	28.82	48.98^{h-o}	48.87^{h-o}	48.93^{C-G}	3.05	2.37	2.71^{B-E}
AS160	28.19^{a-g}	22.14^{hı}	25.17	54.53^{a-h}	51.76^{e-l}	53.15^{ABC}	4.03	3.20	3.62^{A}
Dracma	23.99^{ghı}	23.71^{ghı}	23.85	46.43^{k-r}	42.12^{p-s}	44.28^{H}	2.48	2.40	2.44^{B-E}
DS0224	26.14$^{d-ı}$	28.40^{a-g}	27.27	49.77^{g-m}	54.84^{a-h}	52.31^{A-D}	2.98	2.23	2.61^{B-E}
DKC6442	28.11^{a-g}	22.86^{ghı}	25.49	52.45^{d-k}	42.93^{o-s}	47.69^{E-H}	2.57	2.63	2.60^{B-E}
Colonia	26.78$^{e-ı}$	25.28$^{d-ı}$	26.03	47.02^{k-r}	44.54^{m-s}	45.78^{GH}	2.73	2.73	2.73^{BCD}
Inove	25.36$^{d-ı}$	28.04^{a-h}	26.70	49.16^{h-n}	49.14^{h-n}	49.15^{C-G}	2.76	2.76	2.76^{BC}
Antex	28.42^{a-g}	26.41$^{d-ı}$	27.42	60.05^{a}	47.92^{i-p}	53.99^{AB}	3.24	2.18	2.71^{B-E}
Everest	30.91^{a-d}	24.84$^{e-ı}$	27.88	58.27^{a-d}	45.75^{l-s}	52.01^{A-E}	2.38	2.36	2.37^{B-E}
Torro	26.54$^{d-ı}$	23.14^{ghı}	24.84	51.86^{e-l}	43.05^{n-s}	47.46^{FGH}	2.19	2.19	2.19^{B-E}
73May81	26.66$^{e-ı}$	23.28^{ghı}	24.97	56.66^{a-e}	41.29^{qrs}	48.98^{C-G}	2.00	1.99	2.00^{E}
Kilowaatt	24.26$^{f-ı}$	24.43$^{e-ı}$	24.35	48.66^{h-o}	46.42^{k-r}	47.54^{FGH}	2.60	1.99	2.30^{B-E}
Klips	27.97^{a-h}	27.03$^{b-ı}$	27.50	55.66^{a-g}	47.25^{j-q}	51.46^{A-F}	2.49	2.55	2.52^{B-E}
PR31Y43	27.28$^{a-ı}$	22.46^{ghı}	24.87	53.72$^{b-ı}$	40.87^{rs}	47.30^{FGH}	2.09	2.15	2.12^{C-E}
30B74	30.10^{a-f}	27.34$^{a-ı}$	28.72	58.99^{abc}	50.38^{f-m}	54.69^{A}	2.65	2.67	2.66^{B-E}
DKC7240	33.11^{a}	24.09^{ghı}	28.60	57.78^{a-e}	44.66^{m-s}	51.22^{A-F}	2.12	2.11	2.12^{CDE}
C955	32.51^{abc}	28.09^{a-h}	30.30	59.52^{ab}	48.89^{h-o}	54.21^{AB}	2.90	2.87	2.89^{B}
Gladius	30.38^{a-e}	21.76^{ı}	26.07	56.37^{a-f}	40.17^{s}	48.27^{D-H}	2.22	2.22	2.22^{B-E}
Average	27.86^{A}	25.60^{B}		53.54^{A}	46.54^{B}		2.62	2.39	
CV (%)	13.71			7.59			25.21		
LSD	Hybrid: ns Year: 1.33** Year x Hybrid: 5.96* ns: not significant			Hybrid: 4.37** Year: 1.38** Year x Hybrid:6.19**			Hybrid: 0.73** Year: ns Year x hybrid: ns		

It was determined that year, hybrid, and year x hybrid interactions were statistically significant regarding NDF contents of resulting silages (Table 6). It was determined that NDF rates varied between 44.28-54.69% depending on the varieties; the highest NDF rate was obtained from the 30B74 variety with 54.69%, followed by the C955 variety, with 54.21%, and the lowest NDF content was obtained from the Dracma with 44.28%. NDF content in any forage refers to all fiber in the plant cell, including hemicellulose, cellulose, and lignin. NDF is a critical quality criterion that gives information about how much feed consumption by ruminants. NDF content of the feed in the ration is desired to be between 27-30%. The study shows that the average NDF rate in the first year (53.54%) was higher than the average NDF rate in the second year (46.54%), and both were above the desired level. This situation can also be associated with the dry matter content. As the plant matures, its dry matter content increases, but digestion becomes difficult (Akbay et al., 2020). Oz et al. (2012) reported that the NDF values of 50 selected maize lines varied between 43.07-57.66%, and similarly, Ozata and Kapar (2017) reported that the NDF rates of silo feeds of different maize varieties varied between 40.8-58.6%. Considering both the current research results and the results of previous studies, the ADF contents in maize silages are higher than the desired level.

Table 7. Average pH and forage yield (kg ha^{-1}) values and statistical groups.

	pH			Forage Yield (kg ha^{-1})		
Hybrids	**2019**	**2020**	**Average**	**2019**	**2020**	**Average**
Macha	4.02	3.83	3.93^{ABC}	51171.1	83564.3	67367.7^{A-E}
Ranger	3.83	3.85	3.84^{C-F}	49905.6	72219.0	61062.3^{B-E}
Simon	3.83	3.74	3.79^{EF}	56227.8	77421.4	66824.6^{A-E}
AS160	3.96	3.85	3.91^{A-D}	58105.6	92100.0	75102.8^{A}
Dracma	3.79	3.71	3.75^{F}	66896.7	84054.8	75475.8^{A}
DS0224	3.92	3.85	3.89^{A-D}	48174.4	79052.4	63613.4^{B-E}
DKC6442	3.90	3.86	3.88^{A-E}	49052.2	84454.8	66753.5^{A-E}
Colonia	3.73	3.84	3.79^{EF}	53427.8	85695.2	69561.5^{ABC}
Inove	3.85	3.78	3.82^{DEF}	57505.6	82842.9	70174.3^{AB}
Antex	3.87	3.86	3.87^{B-E}	63278.9	80292.9	71785.9^{AB}
Everest	3.90	3.94	3.92^{ABC}	51045.6	70723.8	60884.7^{B-E}
Torro	3.93	3.81	3.87^{B-E}	49681.1	67823.8	58752.5^{CDE}
73May81	3.92	3.82	3.87^{B-E}	57826.7	82341.7	70084.2^{AB}
Kilowaatt	3.92	3.87	3.90^{A-D}	53722.2	69709.5	61715.9^{B-E}
Klips	3.91	3.87	3.89^{A-D}	43242.2	82636.9	62939.6^{B-E}
PR31Y43	3.92	3.81	3.87^{B-E}	53846.7	81583.3	67715.0^{A-D}
30B74	3.99	3.72	3.86^{B-E}	43968.9	83802.4	63885.7^{B-E}
DKC7240	3.99	3.90	3.95^{AB}	39111.1	76270.2	57690.7^{DE}
C955	3.96	3.94	3.95^{AB}	34790.0	78226.2	56508.1^{E}
Gladius	3.93	4.02	3.98^{A}	45890.0	78595.2	62242.6^{B-E}
Average	3.90^{A}	3.84^{B}		51343.5^{B}	79920.5^{A}	
CV (%)	2,19			14.84		
LSD	Hybrid: 0.03** Year: 0.1** Year x hybrid: ns ns: not significant			Hybrid:11203.2** Year: 3542.8** Year x hybrid: ns		

One of the most essential criteria used to determine silo feed quality is pH measurement. Whether the wet forage is sufficiently fermented or not may be understood by measuring the pH value of the silo feed. Table 7 shows that year and variety affect the pH value of results from silages belonging to corn varieties (T_{60}) statistically significant ($P<0.01$). In contrast, the variety x year interaction has no meaningful effect on pH values. It is known that the pH value of a good silo feed should be around 3.8-4.0, and the pH values of the silages vary between 3.75-3.98, which is in the range of desired values. Gladius variety has the highest pH value with 3.98, followed by DKC7240 and C955 varieties with insignificant differences, and the lowest pH value is obtained from the Dracma variety with 3.75. It is seen that the pH (T_{60}) values vary between 3.84 and 3.90 according to year, and the highest pH value is obtained from the plantings in the first year. The pH value remained stable over the years in the study due to no significant difference between the values according to the variety x year interaction. The findings related to silage pH from the present study are in agreement with some other studies, such as Alcicek et al. (1997), Geren (2000), and Geren (2001), who reported that it varied between 3.75-4.10, 3.87-4.24 and 4.03-4.15, respectively.

The forage yield of corn hybrids was changed between 56508.1 and 75475.8 kg ha^{-1}, depending on the varieties. The highest forage yield was obtained from Dracma and AS160 hybrids with the values of 75475.8 and 75102.8 kg ha^{-1}, respectively (Table 7), followed by Antex with 71785.9 kg ha^{-1}, Inove with 70174.3 kg ha^{-1} and 73May81 variety with 70084.2 kg ha^{-1}, with no statistical differences. The lowest forage yield was obtained from the C955 variety with 56508.1 kg ha^{-1}, significantly different from the abovementioned ones. There are some other studies on corn varieties, but the variety differed from those used in the present study. For instance, Ozata et al. (2012) reported that forage yields varied between 33405.0-62970.0 kg ha^{-1} and the highest grass yield was obtained from the TTM.2007-145 genotype in the study conducted with corn genotypes under Samsun ecological conditions. Olgun et al. (2012) reported that when the green grass yields of 23 different corn genotypes were compared, the yield varied between 66990-134870 kg ha^{-1} in a study conducted in Eskisehir ecological conditions. In parallel, Seydosoglu and Saruhan (2017) reported that the highest green grass yield in Diyarbakır conditions was obtained from the Burak variety with 103728 kg ha^{-1}, while the lowest green grass yield was obtained from the 31Y43 variety with 60005.0 kg ha^{-1}. It was determined that in the first year of the study, the average forage yield was 51343.5 kg ha^{-1}, while it was 79920.5 kg ha^{-1} in the second year. The yield difference between years may be due to the differences in average temperatures between years because the average temperature of the growing period 2020 was almost 2.5 ºC higher than that of the 2019 year.

CONCLUSION

For a successful silage fermentation, it is desired that the dry matter content during harvest is sufficient and the dry matter loss at resulting silage is low. The study determined that C955 and Everest varieties stood out with their high dry matter content. For a quality silage feed, starch, protein, ash rates, and digestibility are required to be high, while fiber concentration is expected to be relatively low. In the study, PR31Y43, Simon, and Everest hybrids stood out regarding crude protein content, Antex and Macha regarding crude ash content, and Inove, Gladius, and Dracma regarding starch content. However, 73May81 and Macha hybrids' fiber content was lower than other hybrids. The pH values of all hybrids were in the range of desired values, although the forage yield showed further variability. This two-year study conducted with 20 different silage hybrid maize showed remarkable differences in silage feed quality characteristics, the highest potential nutritional value of silage feeds, and the forage yield acquired from PR31Y43 and Antex hybrids under Eastern Mediterranean conditions.

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THE EFFECT OF DIVIDED TOP-DRESSING APPLICATIONS OF DIFFERENT NITROGEN FERTILIZERS ON GRAIN YIELD AND QUALITY TRAITS IN BREAD WHEAT (*Triticum aestivum* L.)

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ABSTRACT

This study was carried out to determine the effect of different divided top-dressing applications of different nitrogen fertilizers on grain yield and quality traits of four bread wheat varieties (Selimiye, Esperia, Gelibolu and Rumeli). The experiments were conducted in a randomized split-plot design with 3 replicates during the 2017 and 2018 growing seasons. In the experiment, varieties were allotted as main plots and top-dressing applications were allotted as subplots. Five different pure nitrogen (N) top-dressing applications were done in the form of urea and calcium ammonium nitrate (CAN) at the beginning of tillering, the end of tillering, the beginning of stem elongation and the end of stem elongation stages. In the study, changes in grain yield, thousand kernel weight, test weight, protein content, wet gluten content, gluten index, Zeleny sedimentation value and delayed sedimentation value were investigated. Gelibolu variety for grain yield, Rumeli and Esperia varieties for grain quality were prominent. The considering the ease of application for grain yield and quality, 2nd application (80 kg ha^{-1} pure N in the form of urea at the beginning of tillering, 40 kg ha^{-1} pure N in the form of urea at the beginning of stem elongation) can be proposed in the years when April and May rainfalls are sufficient, and 3rd application (40 kg ha^{-1} pure N in the form of urea at the beginning of tillering, 40 kg ha^{-1} pure N in the form of urea at the end of tillering, 40 kg ha^{-1} pure N in the form of CAN at the beginning of stem elongation) can be proposed in the years when April and May rainfalls are insufficient.

Keywords: Grain yield, nitrogen, top-dressing, quality traits, *Triticum aestivum* L.

INTRODUCTION

Wheat is a strategic crop plant with the highest cultivation and production both in the world and in our country. Today, wheat is the staple food of about 50 countries in the world due to its wide adaptability, its ability to be grown in many different climatic conditions, the appropriate nutritional value of its grain, and the ease of transportation, storage and processing (Ongoren, 2013).

In the world, 808 million tons of wheat is produced on approximately 219 million ha and in Turkey, 19.7 million tons of wheat is produced on approximately 6.6 million ha (Anonymous, 2022). It is a known fact that the world's population is increasing rapidly, while cultivation areas are rapidly decreasing due to faulty practices in cultural processes such as tillage, irrigation, fertilization, spraying, erosion, industrialization and urbanization. According to the 2015 report of the United Nations Department of Economic and Social Affairs, the world population is estimated to be 9.7 billion in 2050 and 11.2 billion in 2100 (Anonymous, 2015). The only way to produce the wheat needed by the world's population is to increase unit area yields. Unit area yield in wheat is the result of the combined effects of genetic potential of the plant, environmental factors and cultivation techniques (Peterson et al., 1992; Altinbas et al., 2004). Nitrogen fertilization is one of the most effective cultivation technique applications that growers can easily control to increase unit area yield and product quality in wheat (Koc and Genc, 1990). Because it is very important to provide the plant nutrients that wheat needs during the growing season at the required time, in adequate quantities and with suitable methods in order to achieve the desired yield. In the researches, it has been determined that the share of fertilization in increase of grain yield of wheat is over 50% (Colkesen et al., 1993).

Nitrogen fertilizers have high mobility in the soil due to their high solubility. Especially in conditions of high rainfall, their mobility increases and they suffer serious losses as a result of washing in the soil. It is also known that nitrogen is lost from the soil in the form of ammonia gas. This prevents the expected benefits from fertilization (Karacal et al., 1988).

Nitrogen use efficiency in the production of all cereals (wheat, barley, rye, oats, maize, sorghum and rice) in the world is estimated at 33% (Raun and Johnson, 1999). The

remaining 67% of nitrogen is lost in various ways. Low soil organic matter, unbalanced use of rapidly soluble nitrogen fertilizers at unsuitable times and methods, salinity and aridity, inadequate or excessive irrigation with unsuitable irrigation methods, late sowing, low sowing density, inadequate weed control, cultivation of the same crop pattern for many years without taking deep-rooted plants and legumes in crop rotation, few varieties with high nitrogen use efficiency are shown as the reasons for low nitrogen uptake efficiency (Karasahin, 2014). Guo et al. (2019) indicated that nitrogen utilization efficiency and top-dressing significantly impact wheat yield and grain quality.

Especially in recent years, due to global warming and climate change, the irregularity of rainfall during the wheat growing season has attracted attention. This limits the effective utilization of nitrogen top-dressings by the plants and causes the expected grain yield not to be obtained. Therefore, in order to ensure that wheat can benefit from the nitrogen to be applied in the most effective way, it can be considered as a way to reach the desired yield and product quality by dividing the nitrogen in critical growing periods by taking into account the distribution of precipitation during the growing season.

Many studies showed that divided top-dressing applications of N in wheat significantly positive affect grain yield and protein content (Avci, 2007; Dere and Koycu, 2007; Kara et al., 2009; Mutlu, 2021; Zheng et al., 2021; Zhang et al., 2021), 1000-kernel weight (Avci, 2007; Ongoren, 2013; Zhang et al., 2021), wet gluten content (Avci, 2007; Dere and Koycu, 2007; Kara et al., 2009; Zheng et al., 2021), gluten index (Avci, 2007; Kara et al., 2009), Zeleny sedimentation value (Avci, 2007; Kara et al., 2009; Zheng et al., 2021) and delayed sedimentation value (Kara et al., 2009).

Therefore, this research was conducted to determine the effect of different divided-topdressing applications of different nitrogen fertilizers on grain yield and some quality traits of bread wheat in the northwest of Türkiye.

MATERIALS AND METHODS

Selimiye and Gelibolu bread wheat varieties from Thrace Agricultural Research Institute, Esperia bread wheat variety from Tasaco Agriculture Company and Rumeli bread wheat variety from Thrace Agriculture Seed Company were used as plant materials in the research.

This research was conducted in a farmer's field in Meneksesofular Village of Edirne Province (41° 45' 47.4480" N and 26° 38' 27.0960" E, 120 m altitude) during the 2017 and 2018 wheat growing periods. During the both years, a blind experiment was established by sowing sunflower in the experimental areas without any fertilization in the previous year.

Some climatic data of the experimental location during the wheat growth period was recorded as shown in Table 1. When the long-term average data for Edirne Province between October and June were analyzed, it was found that there was an average temperature of 10.5°C, a total of 482.7 mm precipitation and a relative humidity 74.6%. The mean temperature (8.9°C), total precipitation (408.0 mm) and relative humidity (72.8%) in the first year of the experiment were lower than the long-term and the second year (12.1°C; 799.6 mm; 77.3%). Although the total rainfall in the second year of the experiment was much higher than the long-term average, it was noticed that the distribution of rainfall according to months was unbalanced. It is understood that the amount of precipitation received in April and May, when stem elongation, heading and nutrient transportation to the grain, was well below the long-term averages.

Table 1. Some climatic data of the experimental area in the 2017 and 2018 years.

Months	Mean temperature (°C)			Precipitation (mm)			Relative humidity (%)		
	2016-17	2017-18	Long-term	2016-17	2017-18	Long-term	2016-17	2017-18	Long-term
October	14.3	13.6	14.1	44.4	135.2	52.9	69.5	77.1	81
November	0.7	9.5	8.5	3.2	71.6	72.4	72.9	75.7	80
December	0.7	7.4	4.2	3.2	119.6	61.7	72.9	85.1	82
January	-1.9	4.3	2.8	67.8	55.6	48.1	83.7	88.1	81
February	5.3	5.7	4.2	43.4	101.8	46.9	80.0	89.5	77
March	10.2	8.9	7.6	51.0	145.6	52.2	73.0	88.8	73
April	12.5	16.6	12.8	65.6	3.0	51.0	63.1	61.3	68
May	17.9	20.3	17.9	85.0	18.8	56.0	65.4	64.0	67
June	21.2	22.6	22.3	44.4	148.4	41.5	74.4	66.4	62
Average/Total	**8.99**	**12.1**	**10.5**	**408.0**	**799.6**	**482.7**	**72.8**	**77.3**	**74.6**

Source: Meteorological Station Data, Edirne-Turkiye

The soil characteristics between 0 and 30 cm deep in the experimental areas were given in Table 2. In the first and second year, the experimental areas were border fields with similar soil structures. It was determined that the soils of the experimental areas were clay-loam, slightly alkaline, salt-free and low in organic matter

The experiment was laid out in split-plots design with 3 replicates during the 2017 and 2018 seasons. In the experiment, bread wheat varieties were allotted as main

plots and top-dressing applications were allotted as subplots. The seeds of varieties were sown by hand in plots consisting of 6 rows of 5 m and each row was 0.2 m apart. Sowing rate was 500 seeds m^{-2}. Studies have shown that 160 kg ha^{-1} of pure nitrogen should be applied in wheat cultivation in the Thrace Region in order to reach the potential wheat yield of the region (Gucdemir, 2006). Thus, 40 kg of the total 160 kg ha^{-1} pure nitrogen to be applied in the experiment was given to all plots as base fertilizer with 20.20.0 compound fertilizer at sowing. The remaining 120 kg ha^{-1} of pure nitrogen was applied manually with urea and calcium ammonium nitrate (CAN) fertilizers as top-dressing fertilizers in 5 different ways as shown in Table 3 considering the growth stages (GS; Zadoks et al., 1974) of wheat and rainfall. In the study, chemical control was applied against weeds and leaf rust diseases. All plots were harvested with a HEGE-125 combine harvester at maturity on July 01, 2017 in the first year and June 14, 2018 in the second year. After harvesting the plots, grain yield (kg ha^{-1}), 1000 kernel-weight (g), test weight (kg hl^{-1}, protein content (%), wet gluten content (%), gluten index (%), Zeleny sedimentation value (ml) and delayed sedimentation value (ml) were determined.

Table 2. Soil characteristics of the experimental areas.

	2017		2018	
Characteristics	**Value**	**Class**	**Value**	**Class**
Texture	-	Clay-loam	-	Clay-loam
pH	7.70	Slightly alkaline	7.14	Slightly alkaline
EC, mm/cm	836.00	Low	800.00	Low
$CaCO_3$, %	3.17	Low	1.59	Low
Organic matter, %	1.38	Low	1.41	Low
Nitrogen (N), %	0.07	Low	0.07	Low
Phosphorus (P), ppm	7.21	Low	16.00	Low
Potassium (K), ppm	284.94	High	399.00	High
Sodium (Na), ppm	8659.43	Sufficient	7848.47	Sufficient
Iron (Fe), ppm	12.28	High	11.98	Medium
Copper (Cu), ppm	1.68	Sufficient	1.48	Sufficient
Zinc (Zn), ppm	1.01	Low	0.96	Low
Manganese (Mn), ppm	8.15	Low	7.65	Low

Table 3. Top-dressing applications.

	Top-dressing application stages			
Applications	**The beginning of tillering (GS 21)**	**The end of tillering (GS 25)**	**The beginning of stem elongation (GS 31)**	**The end of stem elongation (GS 37)**
1st	80 kg ha^{-1} pure N (urea)	-	40 kg ha^{-1} pure N (CAN)	-
2nd	80 kg ha^{-1} pure N (urea)	-	40 kg ha^{-1} pure N (urea)	-
3rd	40 kg ha^{-1} pure N (urea)	40 kg ha^{-1} pure N (urea)	40 kg ha^{-1} pure N (CAN)	-
4th	80 kg ha^{-1} pure N (urea)	-	20 kg ha^{-1} pure N (CAN)	20 kg ha^{-1} pure N (CAN)
5th	40 kg ha^{-1} pure N (urea)	40 kg ha^{-1} pure N (urea)	20 kg ha^{-1} pure N (CAN)	20 kg ha^{-1} pure N (CAN)

After checking the normality of the data distribution, data from each year were analyzed separately after verifying the significant effect of the "year of experiment" (i.e., 2017 and 2018) as an explanatory variable regarding the studied traits. Analysis of variance (ANOVA) for all examined traits were performed according to split-plots design using the JMP statistical software. The means were compared using the LSD (least significant difference) test at the 5% probability level (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The results of analysis of variance for the effects of variety and top-dressing application and their interactions on investigated traits were given in Table 4. Besides, mean values and significance group of variety, top-dressing applications and their interaction were given in Table 5 and Table 6.

Grain yield (kg ha^{-1})

The effect of variety, top-dressing application and variety x top-dressing application interaction on grain yield were statistically significant ($p \leq 0.01$) in both years (Table 4). The mean grain yields of the varieties varied between 5745.6-6116.2 kg ha^{-1} in the first year and 3677.9-4121.2 kg ha^{-1} in the second year (Table 5). The highest mean grain yield was determined in Gelibolu variety in 2017 and in Esperia variety in 2018. In the first experimental year, the mean grain yield of top-dressing applications varied between 5736.2-6070.8 kg ha^{-1}. The highest grain yield was obtained from the 2nd application, followed by the 4th application (5977.4 kg ha^{-1}) in the same statistical group (Table 5). In the second experimental year, grain yield means obtained from top-dressing applications varied between 3737.5-4027.9 kg ha^{-1}. The 3rd application had the highest grain yield value, followed by the 5th application (3981.5 kg ha^{-1}) in the same statistical group (Table 5). In

variety x top-dressing application interaction, the highest grain yield was determined in the 2nd application of Gelibolu variety (6760.4 kg ha^{-1}) in 2017, and in the 3rd application of Esperia (4608.3 kg ha^{-1}) in 2018 (Table 6).

According to results of this study, it is noteworthy that the grain yields obtained in 2017 were considerably higher than the grain yields obtained in 2018. This may be due to the difference in the amount of precipitation received in April and May, especially in stages of stem elongation, heading and grain filling, between the years. In the second year of the experiment, wheat plants may have been under agricultural drought stress.

It was observed that the grain yield performances of the varieties were different depending on the years of the experiment. In the first year of the experiment, Gelibolu variety and in the second year Esperia variety stood out with high grain yield values. Similar to our findings, it was determined that there were significant differences between the grain yields of wheat varieties in many studies (Kahriman and Egesel, 2011; Ongoren, 2013; Aydogan and Soylu, 2017).

In our study, it was determined that the effect of different nitrogen top-dressing applications on grain yield varied depending on years. Similar to our findings, Gokmen et al. (2001) reported that the effect of nitrogen fertilizer application on grain yield varied depending on genotype and year. In the first year of the experiment, 2nd and 4th applications had the highest grain yield. In this year, it is noteworthy that regular rainfall was received in April and May, which include the stages of stem elongation, heading and grain filling of the plants. Especially, in the 2nd application, the nitrogen given in the form of urea, which has a slower release than CAN fertilizer, was lost less and the plants benefited from this nitrogen more effectively. On the other hand, in the second year of the experiment, 3rd and 5th applications had higher grain yield values. In 2018, the total precipitation received in April and May (21.8 mm), which includes the stages of stem elongation, heading and grain filling of the plants, was much lower than the long-term average (107 mm). Under these limited rainfall conditions, unlike the other applications, in the 3rd and 5th applications, especially during the tillering period, the application of top-dressing fertilizer in two equal amounts allowed the plants to utilize nitrogen more efficiently. Thus, it was possible to obtain higher grain yield from these applications compared to the other applications. These results indicate that it would be appropriate to divide the total nitrogen to be given during the tillering period into two equal amounts, one at the beginning of tillering and one at the end of tillering, especially in years with limited rainfall in April-May. Our findings are consistent with the findings of Kara et al. (2009), Allart et al. (2023), Gaile et al. (2023), Hamani et al. (2023), Yao et al. (2023), who reported that grain yield of wheat was significantly affected by different nitrogen top-dressing applications.

1000-kernel weight (g)

In both years of our study, variety and top-dressing application had a significant effect ($p \leq 0.01$) on 1000-kernel weight, while the effect of variety x top-dressing application interaction was not significant (Table 4). The mean 1000-kernel weight of the varieties varied from 38.73 g to 47.09 g in the first year of experiment and 40.55 g to 46.72 g in the second year of experiment (Table 5). In the first year, Gelibolu variety had the highest 1000-kernel weight, followed by Selimiye variety (46.11 g) in the same statistical group. In the second year, the highest 1000-kernel weight obtained from Selimiye variety, followed by Gelibolu variety (43.31 g) (Table 5). The mean 1000-kernel weight of top-dressing applications varied between 42.37-44.62 g in the first year of experiment. The fifth application had the highest 1000-kernel weight, followed by 3rd application with 43.62 g in the same statistical group (Table 5). In the second year of experiment, the mean 1000-kernel weight of top-dressing applications ranged from 41.80 g to 44.10 g. The highest 1000-kernel weight was determined in the 5th application, followed by 3rd application with 43.88 g in the same statistical group (Table 5).

Table 4. The results of analysis of variance for investigated traits in the 2017 and 2018 years

		Mean of squares							
SV	**DF**	**Grain yield**	**1000-kernel weight**	**Test weight**	**Protein content**	**Wet gluten content**	**Gluten index**	**Zeleny sedim. value**	**Delayed sedim. value**
					2017				
Replication	2	488.08	5.23	0.59	0.09	0.82	3.15	0.72	1.22
Variety (V)	3	4194.68**	238.49**	22.73**	7.15**	36.34**	1633.97**	1062.84**	4569.26**
Error-1	6	282.73	2.30	0.46	0.36	0.86	4.04	3.43	1.66
Application (A)	4	2536.94**	9.21**	0.41	2.71**	18.81**	15.19*	196.73**	331.06**
VxA	12	2757.57**	4.09	0.38	0.18	3.38**	105.74**	31.58**	188.53**
Error	32	228.72	2.18	0.39	0.14	0.74	3.96	2.60	1.63
Total	59								
					2018				
Replication	2	195.51	5.53	1.52	0.03	0.92	0.32	5.07	1.95
Variety (V)	3	5527.27**	116.67**	18.44**	13.65**	145.15**	762.20**	807.39**	2118.09**
Error-1	6	57.38	1.56	0.37	0.08	0.27	0.92	4.38	2.37
Application (A)	4	1679.18**	12.17**	0.51	1.50**	12.13**	142.93**	155.92**	87.73**
VxA	12	1356.74**	1.44	0.13	0.12	3.17*	74.31**	14.95*	9.87**
Error	32	75.84	1.15	0.20	0.19	1.15	2.77	7.15	2.62
Total	59								

SV: source of variation; DF: degree of freedom; Sedim.: sedimentation; *, ** significant at 5% and 1% levels, respectively

Table 5. Mean values and significance groups of variety and top-dressing applications for investigated traits in the 2017 and 2018 years

	Grain yield (kg ha^{-1})	1000-kernel weight (g)	Test weight (kg hl^{-1})	Protein content (%)	Wet gluten content (%)	Gluten index (%)	Zeleny sedim. value (ml)	Delayed sedim. value (ml)
Years								
2017	5888.4 a	43.27	78.88 b	13.16 a	30.23 a	84.45 b	49.07 a	33.68 b
2018	3899.7 b	42.93	79.09 a	10.98 b	20.58 b	91.57 a	40.58 b	55.20 a
LSD $_{(P\leq0.05)}$	*46.5*	-	*0.170*	*0.150*	*0.340*	*0.670*	*0.840*	*0.670*
				2017				
Varieties								
Selimiye	5745.6 c	46.11 a	79.10 b	12.49 c	29.90 b	69.93 d	40.87 d	15.13 d
Esperia	5782.7 bc	38.73 c	77.08 c	13.43 b	29.77 b	93.47 a	55.00 b	36.13 b
Gelibolu	6116.2 a	47.09 a	79.61 ab	12.73 c	28.81 c	84.07 c	42.87 c	27.00 c
Rumeli	5909.2 b	41.16 b	79.72 a	14.01 a	32.45 a	90.33 b	57.53 a	56.47 a
LSD $_{(P\leq0.05)}$	*150.23*	*1.355*	*0.609*	*0.537*	*0.828*	*1.795*	*1.654*	*1.151*
Applications								
1st	5909.9 b	42.98 bc	78.68	13.32 a	30.42 bc	83.25 b	48.50 c	29.83 d
2nd	6070.8 a	42.78 bc	78.97	13.30 a	30.20 c	85.42 a	50.00 b	28.42 e
3rd	5747.9 c	43.62 ab	78.80	13.51 a	31.44 a	84.83 a	54.75 a	41.42 a
4th	5977.4 ab	42.37 c	78.79	12.32 b	28.17 d	83.25 b	43.42 d	32.50 c
5th	5736.2 c	44.62 a	79.16	13.34 a	30.94 ab	85.50 a	48.67 bc	36.25 b
LSD $_{(P\leq0.05)}$	*125.76*	*1.226*	-	*0.312*	*0.716*	*1.655*	*1.341*	*1.062*
				2018				
Varieties								
Selimiye	3821.7 c	46.72 a	80.05 a	10.85 c	21.91 b	93.80 c	37.73 c	48.33 c
Esperia	4121.2 a	41.15 c	77.50 c	11.20 b	19.65 c	96.47 a	42.07 b	62.53 b
Gelibolu	3978.3 b	43.31 b	79.28 b	9.78 d	16.76 d	81.00 d	32.60 d	42.27 d
Rumeli	3677.9 d	40.55 c	79.53 ab	12.09 a	24.01 a	95.00 b	49.33 a	67.67 a
LSD $_{(P\leq0.05)}$	*67.68*	*1.116*	*0.542*	*0.255*	*0.464*	*0.855*	*1.869*	*1.376*
Applications								
1st	3824.0 c	42.30 b	79.20	10.88 bc	20.77 b	86.25 c	38.67 b	53.83 c
2nd	3737.5 d	41.80 c	78.85	10.98 b	19.76 c	93.33 a	40.00 b	55.92 b
3rd	4027.9 a	43.88 a	79.37	11.54 a	22.22 a	94.08 a	46.92 a	59.42 a
4th	3928.1 b	42.58 b	78.94	10.57 c	19.83 c	89.92 b	39.17 b	52.25 d
5th	3981.5 ab	44.10 a	79.09	10.93 b	20.34 bc	94.25 a	38.17 b	54.58 bc
LSD $_{(P\leq0.05)}$	*72.42*	*0.891*	-	*0.362*	*0.894*	*1.383*	*2.224*	*1.346*

Values followed by the same letter(s) are not significantly different at the 5% probability level according to LSD test; Sedim.: sedimentation

Table 6. Mean values and significance groups for interaction of variety x top-dressing application in the 2017 and 2018 years

Varieties	Applications	Grain yield (kg ha^{-1})	1000-kernel weight (g)	Test weight (kg hl^{-1})	Protein content (%)	Wet gluten content (%)	Gluten index (%)	Zeleny sedim. value (ml)	Delayed sedim. value (ml)
2017									
	1st	5636.5 f	45.47	78.77	12.70	30.70 d-h	66.33 kl	43.00 fgh	8.67 n
	2nd	5593.8 f	46.67	79.72	12.73	30.47 f-ı	70.33 j	41.00 f-ı	15.00 l
	3rd	5552.0 f	45.33	78.55	12.47	31.10 c-g	63.33 l	40.33 hı	13.67 lm
Selimiye	4th	5992.7 cd	44.93	78.98	11.97	28.70 k	68.00 jk	38.33 ı	12.67 m
	5th	5953.1 cde	48.13	79.48	12.57	28.53 k	81.67 h	41.67 fgh	25.67 ı
	1st	5601.0 f	36.93	77.01	13.57	28.87 jk	95.33 ab	53.00d	30.67 gh
	2nd	5953.1 cde	38.53	76.56	13.70	29.43 h-k	94.00 abc	56.00 c	21.67 k
	3rd	5753.1 def	39.60	77.32	13.97	32.03 cd	96.33 a	65.33 a	62.67 a
Esperia	4th	6089.8 c	38.87	77.23	12.23	27.07 l	97.00 a	48.67 e	32.33 fg
	5th	5516.7 f	39.73	77.26	12.87	31.47 c-f	84.67 gh	52.00 d	33.33 f
	1st	6433.3 b	48.53	79.43	12.67	30.23 f-j	77.33 ı	43.33 fg	25.33 ıj
	2nd	6760.4 a	44.40	79.57	12.97	28.63 k	85.00 g	43.67 f	23.33 jk
	3rd	5592.7 f	48.00	79.40	13.10	29.13 ıjk	91.33 cde	48.33 e	31.67 fg
Gelibolu	4th	6071.9 c	46.00	79.41	12.03	26.37 l	81.67 h	38.33 ı	25.33 ıj
	5th	5722.9 ef	48.53	80.25	12.87	29.70 g-k	85.00 g	40.67 ghı	29.33 h
	1st	5968.8 cde	41.00	79.51	14.37	31.90 cde	94.00 abc	54.67 cd	54.67 de
	2nd	5976.0 cd	41.53	80.00	13.80	32.27 bc	92.33 bcd	59.33 b	53.67 e
	3rd	6093.8 c	41.53	79.92	14.53	33.50 ab	88.33 ef	65.00 a	57.67 bc
Rumeli	4th	5755.2 def	39.67	79.53	13.07	30.53 e-ı	86.33 fg	48.33 e	59.67 b
	5th	5752.1 def	42.07	79.63	14.27	34.07 a	90.67 de	60.33 b	56.67 cd
LSD (P≤0.05)		*251.52*	-	-	-	*1.433*	*3.310*	*2.683*	*2.125*
2018									
	1st	3766.0 ef	45.07	80.27	10.80	21.93 cd	85.33 e	36.33 fgh	47.67 ıj
	2nd	3496.9 ı	45.87	80.00	10.90	22.13 cd	96.33 ab	37.00 efg	50.67 h
	3rd	3615.6 ghı	47.47	79.83	11.10	22.63 bcd	95.00 bc	39.67 efg	50.33 hı
Selimiye	4th	4061.5bc	46.93	80.07	10.33	21.00 def	96.67 ab	39.67 efg	46.67 j
	5th	4167.7 b	48.27	80.10	11.13	21.87 cd	95.67 b	36.00 gh	46.33 j
	1st	3835.4 def	41.33	77.60	11.27	19.87 fgh	95.33 bc	40.67 def	62.67 ef
	2nd	3906.2 de	40.00	77.20	11.27	19.97 efg	94.67 bc	41.00 de	64.00 de
	3rd	4608.3 a	41.87	78.03	11.87	21.73 cde	97.00 ab	50.33 b	68.00 bc
Esperia	4th	4131.2 b	40.27	77.20	10.73	18.47 ghı	96.67 ab	38.67 efg	57.67 g
	5th	4125.0 b	42.27	77.47	10.87	18.20 ghı	98.67 a	39.67 efg	60.33 fg
	1st	3945.8 cd	42.27	79.43	9.50	18.03 ıj	67.67 g	28.67 ı	38.33 l
	2nd	3937.5 cd	42.93	79.03	9.80	14.00 k	87.67 de	32.33 hı	42.67 k
	3rd	4185.4 b	44.67	79.70	10.33	18.10 hıj	89.33 d	40.33 efg	47.67 ıj
Gelibolu	4th	3917.7 cd	42.33	79.00	9.47	16.33 j	73.67 f	29.67 ı	39.33 l
	5th	3905.2 de	44.33	79.23	9.80	17.33 ıj	86.67 de	32.00 hı	43.33 k
	1st	3747.9 fg	40.53	79.53	11.93	23.23 bc	96.67 ab	49.00 bc	66.67 bcd
	2nd	3609.4 ghı	38.40	79.17	11.97	22.93 bc	94.67 bc	49.67 b	66.33 bcd
	3rd	3702.1 fgh	41.50	79.90	12.87	26.43 a	95.00 bc	57.33 a	71.67 a
Rumeli	4th	3602.1 hı	40.80	79.50	11.73	23.50 bc	92.67 c	48.67 bc	65.33 cde
	5th	3728.1 fgh	41.53	79.57	11.93	23.97 b	96.00 ab	45.00 cd	68.33 b
LSD (P≤0.05)		*144.28*	-	-	-	*1.787*	*2.766*	*4.448*	*2.692*

Values followed by the same letter(s) are not significantly different at the 5% probability level according to LSD test; Sedim.: sedimentation

In our study, it was determined that there were significant differences between the 1000-kernel weights of wheat varieties. This may be due to the genetic differences in the response of grain filling time, grain filling rate and grain size of the varieties to ecological conditions (Zheng et al., 2021). In both years of the experiment, Gelibolu and Selimiye varieties were noted with high thousand grain weights.

It was observed that the effect of nitrogen top-dressing applications on thousand grain weight was significant in both experimental years, in this study. In both years of experiment, 3rd and 5th applications had higher thousand grain weight values than other applications. This may be a result of the fact that nitrogen was divided during the tillering period unlike the other applications. Our results are in agreement with previous findings that the effect of different nitrogen top-dressing applications on 1000-kernel weight was significant (Avci, 2007; Ongoren, 2013; Zhang et al., 2021; Zheng et al, 2021; Hamani et al., 2023).

Test weight (kg hl^{-1})

In our study, test weight was significantly affected by variety, but not affected by top-dressing application and variety x top-dressing application interaction in both years (Table 4). In the first year of experiment, the mean value of test weight among varieties ranged from 77.08 kg hl^{-1} to 79.72 kg hl^{-1} (Table 5). Rumeli variety had significantly higher test weight value when compared to other varieties. This variety was followed by Gelibolu variety (79.61 kg hl^{-1}) in the same statistical group. When the results of the second year of the experiment are analyzed, it is seen that the test weight varied between 77.50-80.05 hl^{-1} for the varieties (Table 5). The highest mean test weight value was determined in Selimiye variety, followed by Rumeli variety with 79.53 hl^{-1} in the same statistical group. These results showed that Rumeli variety was prominent in terms of test weight. As it can be seen from Table 5, although the differences between them are statistically insignificant, the 5th application in the first year and the 3rd application in the second year attracted attention in terms of test weight.

The shape and size of the grain, thin or thick pericarp, deep or superficial ventral cavity are the characteristics that affect the test weight (Elgun et al., 2001). In our study, there were significant differences among the varieties for test weight. Similarly, Aydogan and Soylu (2017), and Eser et al. (2020) emphasized that there were significant differences among wheat varieties for test weight. In the present study, it was determined that there was no significant effect of different nitrogen top-dressing applications on test weight in both years. The findings of research for test weight were in agreement with Eser et al. (2020) who found that split dose nitrogen application did not present significant changes among the tested winter wheat varieties.

Protein content (%)

According to the variance analysis results of our study, the effect of variety and top-dressing applications on protein content was significant ($p \leq 0.01$) in both years, while the effect of variety x top-dressing application interaction was not significant (Table 4). In the first year of experiment, the mean protein content of varieties varied from 12.49% to 14.01%. Rumeli variety had the highest protein content, followed by Esperia variety with 13.43% (Table 5). In this year, the mean protein content values obtained from top-dressing applications varied between 12.32% and 13.51% and were grouped in two significance groups. The highest protein content was determined in the 3rd (13.51%), 5th (13.34%), 1st (13.32%) and 2nd (13.30%) applications in the same statistical group, respectively (Table 5). In the second year of experiment, the mean protein content of top-dressing applications varied from 10.57% to 11.54% (Table 5). In this year, similar to the results of the first year, the highest protein content was obtained from the 3rd treatment, followed by the 2nd and 5th applications with 10.98% and 10.93%, respectively.

Protein content in grains is largely influenced by genetics, water and nitrogen availability, biotic and abiotic stresses, and grain-filling duration (Alomari et al., 2023). Considering the years of the study, it is seen that the protein contents obtained in the first year were higher than the second year. This may be due to the difference in the rainfall received in May, which covers the milk development stage of wheat during which proteins are transported to the grains (Table 1). Similar to our results, Kara et al. (2009) determined that the protein content in wheat varied according to years and that the protein content was high in the year with high rainfall in May. In our research, it was determined that the differences between the varieties in terms of protein content were significant in both years. Our findings are similar to the results of Aydogan and Soylu (2017) and Eser et al. (2020), who found that the protein content in grain varied significantly among varieties.

In this study, it was observed that the effect of nitrogen top-dressing applications on protein content was significant in both years. The highest protein content was obtained from the 3rd treatment in both years. In the 3rd application of our study, nitrogen was divided into two equal parts during the tillering stage. Thus, the plants effectively utilized this nitrogen applied in urea form and stored nitrogen in their vegetative organs. Our results are similar to the findings of Avci (2007), Dere and Koycu (2007), Kara et al. (2009), Zheng et al. (2021), Zhang et al. (2021) and Yao et al. (2023) who found that the effect of different nitrogen top-dressing applications on the grain protein content of wheat was significant.

Wet gluten content (%)

In this study, variety, top-dressing applications and variety x top-dressing application interaction had significant effect on wet gluten content in both years (Table 4). The mean wet gluten content of varieties varied from 28.81% to 32.45% in 2017, 16.75% to 24.01% in 2018 (Table 5). Rumeli variety had significantly higher wet gluten content when compared to other varieties in both years. On the other hand, the lowest wet gluten content was obtained from Gelibolu variety in both years (Table 5). When the top-dressing applications in Table 5 are evaluated, it is seen that the wet gluten content varied

between 28.17-31.44% in the first year and between 19.76-22.22% in the second year. The highest wet gluten content in top-dressing applications was obtained from the 3rd application in both years. In variety x top-dressing application interaction, wet gluten content ranged from 26.37% to 34.07% in the first year. The highest wet gluten content value was determined in the 5th (34.07%) and 3rd (33.50%) applications of Rumeli variety (Table 6). In the second year, the mean values of wet gluten content in variety x top-dressing application interaction varied between 14.00% and 26.43%. Similar to the first year, the highest wet gluten content was obtained from the 3rd application of Rumeli variety (Table 6).

Wet gluten content in wheat is an important quality criterion that provides the leavening of dough by retaining the gas formed during the fermentation of dough and has an important and positive relationship with protein content (Egesel et al., 2009). In our study, it is noticed that the values of wet gluten content obtained in the first year were higher than the second year. This may be a result of the high amount of protein transported to the grains in the first year. Similarly, Zheng et al. (2021) reported that wet gluten content varies with years.

It was determined that the wet gluten contents of the varieties used in the study were different in both years. Rumeli variety had the highest and Gelibolu variety had the lowest wet gluten content in both years. This may be due to the different genotypic characteristics of the varieties in terms of quality traits. Our results were similar with the findings of Egesel et al. (2009) and Zheng et al. (2021).

The results of the study showed that the effect of nitrogen top-dressing applications on wet gluten content was significant in both years. The highest wet gluten content was obtained from the 3rd application and the lowest wet gluten content was obtained from the 4th application in both years of experiment. This may be a result of the different protein content obtained from different nitrogen top-dressing applications. Also, the 3rd application had the highest protein content and the 4th application had the lowest protein content in this study. Demirel et al. (2023) found that there was a strong significant positive correlation between grain protein content and wet gluten content. The results of our study for wet gluten content were in accordance with the findings of Avci (2007), Dere and Koycu (2007), Kara et al. (2009), Eser et al. (2020), Zheng et al. (2021) and Gaile et al. (2023), who found that the effect of different nitrogen top-dressing applications on wet gluten content in wheat was significant.

Gluten index (%)

Data in Table 4 indicate that the gluten index is significantly influenced by variety, top-dressing applications and their interaction in both years. The mean values of gluten index for varieties varied between 69.93%-93.33% in the first year, and between 81.00%-96.47% in the second year. In both years, Esperia variety had the highest gluten index value, followed by Rumeli variety (Table 5). Gluten index values obtained from top-dressing applications were different in both years. In the first year, the mean gluten index values for top-dressing applications ranged from 83.25% to 85.50%. The highest gluten index values were determined in 5th (85.50%), 2nd (85.42%) and 3rd (84.83%) applications in the same statistical group (Table 5). In the second year of the experiment, gluten index values obtained from top-dressing applications were between 86.26% and 94.25%. Similar to the first year, the highest gluten index values were obtained from the 5th (94.25%), 3rd (94.08%), and 2nd (93.33%) applications in the same statistical group (Table 5). When the variety x top-dressing application interaction in Table 6 is analyzed, it is seen that the mean gluten index values varied between 63.33% and 97.00% in the first year and between 67.67% and 98.67% in the second year. In the variety x top-dressing application interaction, the highest gluten index values were obtained from the 4th (97.00%) and 3rd (96.33%) applications of Esperia variety in the first year and from the 5th (98.67%) application of the same variety in the second year (Table 6).

Gluten index in wheat is an important quality criterion that shows the ratio of strong proteins in wet gluten and is used to determine the quality of flour. In our study, contrary to protein content and wet gluten content, the mean values of gluten index were generally higher in the second year compared to the first year. This indicates that grains with stronger protein structure were obtained in the second year of our experiment. In the study, it was observed that the varieties had different gluten index values in both years. Esperia and Rumeli varieties had high gluten index values, while Selimiye and Gelibolu varieties had low gluten index values. Our findings are also in accordance with Egesel et al. (2009), who found that gluten index values of wheat varieties were different.

In both years of our study, the significant differences were observed among the top-dressing applications for gluten index. The highest gluten index value was obtained from the 5th application and the lowest from the 1st application. This may be due to the fact that the divided nitrogen applied especially during tillering and stem elongation periods increased the efficiency of nitrogen uptake in plants and thus the proteins accumulated in the grains had a stronger structure. Our results are supported by the results of Avci (2007), Kara et al. (2009), Erekul et al. (2012) who found that different nitrogen top-dressing applications significantly affected gluten index in wheat.

Zeleny sedimentation value (ml)

In our study, Zeleny sedimentation value was significantly affected by variety, top-dressing applications and their interaction in both years (Table 4). The varieties used in this study had Zeleny sedimentation values ranged from 40.87 ml to 57.53 ml in 2017, and from 32.60 ml to 49.33 ml in 2018 (Table 5). The highest Zeleny sedimentation value was determined in Rumeli variety, followed with Esperia variety in both years. The mean Zeleny sedimentation values obtained from top-dressing applications varied between 43.42-54.75 ml in the first year and 38.17-46.92 ml in the second year (Table 5). In both years, the 3rd application had a higher mean Zeleny

sedimentation value than the other applications, followed by the 2nd application (Table 5). When the data given in Table 6 are examined, it is seen that the mean Zeleny sedimentation value in variety x top-dressing application interaction varied between 38.33-65.33 ml in the first year and 28.67-57.33 ml in the second year. In the first year of the experiment, 3rd application of Esperia (65.33 ml) and Rumeli (65.00 ml) varieties had the highest mean Zeleny sedimentation value. In the second year, the highest mean Zeleny sedimentation value was determined in the 3rd application of Rumeli variety (57.33 ml), followed by the 3rd application of Esperia variety (50.33 ml).

Zeleny sedimentation test in wheat is a practical method that provides information about gluten quantity and quality (Evlice et al., 2016). The volume of bread made from flours with high Zeleny sedimentation value is also high. In our study, the mean Zeleny sedimentation values obtained in the first year were higher than the second year. This may be a result of the high protein content obtained in the first year. Our results showed that Rumeli and Esperia varieties, which had higher protein content, wet gluten content and gluten index values than the others, had the highest mean Zeleny sedimentation values. Similar to our findings, Evlice et. al (2016), Egesel et. al (2009) and Eser et. al (2020) reported that Zeleny sedimentation values of wheat varieties were significantly different.

In our study, it was determined that the effects of different top-dressing applications on Zeleny sedimentation value were significant in both experimental years. The 3rd application had the highest Zeleny sedimentation value in both years. This may be related to the high protein content and gluten index values obtained from the 3rd application. Our results agreed with the findings of Avci (2007), Kara et al. (2009) and Zheng et al. (2021), who explained that different nitrogen top-dressing applications significantly affected Zeleny sedimentation value in wheat.

Delayed sedimentation value (ml)

Delayed sedimentation value is usually used to determine sunn pest damage in wheat. In our study, the effect of variety, top-dressing application and their interactions on delayed sedimentation value was significant in both years (Table 4). The delayed sedimentation values obtained from the varieties ranged between 15.13-56.47 ml in the first year and 28.42-41.42 ml in the second year. Rumeli variety had the highest delayed sedimentation value in both years (Table 5). The mean delayed sedimentation values of top-dressing applications varied from 28.42 ml to 41.42 ml in the first year and from 52.25 ml to 59.42 ml in the second year. The highest delayed sedimentation value was obtained from 3rd application in both years (Table 5). As seen in Table 6, the mean delayed sedimentation values obtained from the variety x top-dressing application interaction varied between 8.67-62.67 ml in the first year and 38.33-71.67 ml in the second year. The 3rd application of Esperia variety (62.67 ml) in the first year and the 3rd application of Rumeli variety (71.67 ml) in the second year had the highest delayed sedimentation value.

In our research, delayed sedimentation values obtained in the first year were lower than Zeleny sedimentation values, while delayed sedimentation values obtained in the second year were higher than Zeleny sedimentation values. This indicated that the damage caused by the sunn pest and the wheat stink bug was high in the first year of the experiment.

In our experiment, it was determined that the delayed sedimentation values of the varieties were different in both years. Rumeli variety had the highest delayed sedimentation value in both years. This result shows that Rumeli variety was less affected by the damage of the sunn pest and the wheat stink bug than the other varieties. Similar to our findings, Egesel et al. (2009), Kahriman and Egesel (2011) and Evlice et al. (2016) also reported significant differences among wheat varieties in terms of delayed sedimentation value.

In our study, the effect of different top-dressing applications on delayed sedimentation value was found significant in both years. The 3rd application had the highest delayed sedimentation value in both years. This result shows that in the 3rd application, the damage of sunn pest and wheat stink bug was lower than in the other applications. This may be a result of the high Zeleny sedimentation value obtained from the 3rd application. Because Kara et al. (2009) found a positive and significant relationship between Zeleny sedimentation value and delayed sedimentation value.

CONCLUSIONS

The results obtained in both years showed that the effect of different top-dressing applications on grain yield and quality traits (except test weight) was significant in bread wheat. In the first year of the experiment in which the distribution of precipitation to months was regular, the 2nd and 4th applications stood out in terms of grain yield, and the 3rd and 5th applications were prominent in terms of quality traits. In the second year of the experiment, in which the distribution of precipitation to months was irregular and rainfall was very low especially in April-May, the 3rd and 5th applications stood out in terms of both grain yield and quality traits.

In conclusion, the considering the ease of application for grain yield and quality, 2nd application (80 kg ha^{-1} pure N in the form of urea at the beginning of tillering, 40 kg ha^{-1} pure N in the form of urea at the beginning of stem elongation) can be proposed in the years when April and May rainfalls are sufficient, and 3rd application (40 kg ha^{-1} pure N in the form of urea at the beginning of tillering, 40 kg ha^{-1} pure N in the form of urea at the end of tillering, 40 kg ha^{-1} pure N in the form of CAN at the beginning of stem elongation) can be proposed in the years when April and May rainfalls are insufficient.

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ESTABLISHMENT OF PHENOTYPIC VARIABILITY AND CORRELATIONS OF SEED YIELD AND YIELD RELATED TRAITS IN ALFALFA (*Medicago sativa* L.) CLONAL PROGENIES

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ABSTRACT

The aim of present study was to evaluate phenotypic variability of seed yield and yield-related traits and to establish the relationships among them at eleven alfalfa clonal progenies. The study was conducted in experimental field at Institute of Agriculture and Seed Science Obraztsov chiflik - Ruse under conditions of open pollination (polycross), from 2014 to 2016. The traits plant seed yield, plant height, generative stem number, inflorescence number, pod number, seed number and 1000-seed weight were evaluated. The main effect and second interaction (year, genotype and year x genotype) had a significant influence on all morphological and generative traits. There was wide range of variability for all analized traits between progenies and over study period. PM30 progeny showed superior scores regarding all traits studied and four progenies distinguished with high phenotypic expression of seed yield, pod number, seed number and 1000-seed weight. The seed yield obtained was in range from 2.45 to 3.44 g plant^{-1}. These progenies are valuable germplasm source to be used in further breeding to develop a new synthetic alfalfa variety with stable seed yield. It was found seed yield strongly and positively correlated with plant height (r_p=0.752), pod number per inflorescence (r_p=0.700**) and seed number per pod (r_p=0.611**), which suggest that selection for improving alfalfa seed yield may be performed directly through selection on these three traits.**

Keywords: Alfalfa, clonal progenies, seed yield, variability, correlations.

INTRODUCTION

Common cultivated alfalfa (*Medicago sativa* L.) is an allogamous autotetraploid cross-pollinated perennial species of the family Fabaceae, which means “Best Fodder” in Arabic. Alfalfa has always been and continues to be the most valuable forage legume in world agriculture and a key component of sustainable agricultural systems due to its high yield, excellent forage quality, and ability to improve soil through nitrogen fixation (Bouton, 2012; Naydenova et al., 2022). It is considered as one of the world’s most versatile crops, because its wide adaptation to diverse environmental conditions ranging from burning hot deserts to cool high mountain valleys (Prosperi et al. 2006).

The alfalfa varieties are synthetic populations of wide genetic base (multiple hybridization of different number of selected parental genotypes) and a number of selection schemes have been proposed for their creation (without and with inbreeding, tests of progenies from polycross, topcross, etc.) (Annicchiarico et al., 2015). Developing synthetic varieties through the use of full-sib and half-sib families or clones as parents is a commonly used breeding method in alfalfa (Flajoulot et al. 2005).

Seed yield is an important property for the market success of forage legume varieties (Boelt et al., 2015). Therefore, ability of high seed producing of the new alfalfa varieties is critical in its effective commercial distribution and delivery to the farmers at a competitive price (Torricelli, 2007). According Bolanos-Aguilar et al. (2002), the low seed production is a problem in some alfalfa varieties and the progress in achieving higher seed yield is very limited over years.

The number of studies has been carried out to assess the influence of genetic factors, environmental conditions and the crop management on the seed yield (Andjelkovic et al., 2010; Abd El-Naby et al., 2016; Chen et al., 2016; Avci et al., 2017; Pajcin et al., 2020; Marinova, 2021).

The theoretical seed yield of alfalfa, is 12 t ha^{-1} (Lorenzetti, 1993), but the actual seed yield achieved under the most favourable conditions reaches only 4% of the potential yield (Bolanos-Aguillar et al. 2000). The main causes of low seed productivity of alfalfa are the poor pod set (only 40 to 60 % of the flowers set pods), and the low number of seeds per pod, (usually 3 to 4) (Bodzon, 2016).

Alfalfa seed yield is controlled genetically complex

qualitative characteristic and degree of its phenotypic expression is result of complex interaction between internal (genetic structure of variety) and external (environments, pollinators existence and crop management) factors. Rincker et al. (1988), reported that as quantitative characteristic with complex genetic basis, seed yield depends on the number of seeds per unit area and individual seed weight and ranged from 0 to 2110 kg ha^{-1}. The authors also stated that the seeds produced in the pods and the yield components include pod number per inflorescence, stem number per plant and the plant number per unit area.

The genotypic (GCV) and phenotypic coefficients of variation (PCV) are essential biometric tools and the basis for breeding, because the greater traits variability in a population, the greater the opportunity for selection and improvement of the genotypes for given traits (Rasheed et al., 2023). Typically, the PCV value for a given trait is somewhat higher than the GCV value, demonstrating the influence of the environment in the degree of phenotypic expression of the trait (Monirifar et al., 2011; Ozturk and Yildirim, 2014; Hussain et al., 2021).

In breeding for improved seed yield it is particularly important to determine the effect of generative and morphological characters on seed yield as well as their interrelations (Bodzon, 2016). Knowledge of the relationships between important characteristics makes it possible to improve a greater number of traits simultaneously, especially for those with low genetic variability, in which the success of the selection is achieved by indirect methods (Parihar et al., 2015; Yildirim et al., 2023). The establishment of the relationships between the traits of populations consisting of a different genotypes kept under the same conditions is a basis for establish objective criteria for effective selection (Kosev and Vasileva, 2021).

According Bodzon (2004), breeding for improved seed yield potential of alfalfa should be based on simultaneous selection for several important traits as inflorescence number per stem, pod number per inflorescence, seed number per pod and 1000-seed weight closely related with seed yield.

The objective of present study was to evaluate phenotypic variability of seed yield and yield-related traits between alfalfa clonal progenies and acrross years and to establish the relationships among traits in conditions of open pollination (polycross nursery), for developement alfalfa varieties with high ability of seed producing.

MATERIAL AND METHODS

Plant material and experimental design

The experiment was carried out in experimental field at Institute of Agriculture and Seed Science Obraztsov chiflik – Rousse, Agricultural academy, Bulgaria at 2014-2016. The experimental field is located at 43°48^{I} N latitude 26°02^{I} E longitude and altitude 152 m.

The object of investigation were eleven alfalfa clonal progenies of native origin, developed by vegetative propagation of partly inbred (S_1) superior individual plants (genotypes) on February 2014 in the green house of the Institute. At first the cuttings were rooted in test-tubes of water and then planted in chests of soil. The rooted cuttings were transplanted in the experimental field of the Institute at the end of April.

The polycross nursery was designed as completely randomised block in four replications. The nursery contained total 880 plants, and each clonal progeny was represented by 20 plants in each replication (total of 220 plants). The plants originated from rooted cuttings from each clonal progeny were planted in two 5 - meter long rows with ten plants in each row at a distance of 50 cm between plants and inter-row spacing of 50 cm. For good rooting on the field the plants were immediately watered after planting. The plants were watered total five times in 6-7 days intervals. During growing seasons, the necessary crop management was performed. Phosphorous and potassium in the form of triple superphosphate (400 kg ha^{-1}) and potassium nitrate (600 kg ha^{-1}) were supplied with the ploughing in late autumn before the year of progenies planting. Nitrogen in the form of urea (200 kg ha^{-1}) was applied with the last tillage of experimental in the spring before planting the clonal progenies. Weeds were controlled with hand hoeing as needed throughout the all growing seasons. Pests control was carried out by treatment with appropriate insecticides.

Soil data and climatic conditions during study period

The soil type of experimental site was leached black soil, located on sandy clay. Active soil fertility was characterized by good potassium (33.17 mg 100 g^{-1} soil), insufficient nitrogen (16.84 mg 100 g^{-1} soil) and poor phosphorus (6.15 mg 100g^{-1} soil) nutrient regime. The humus content was low and ranged from 2.03% to 2.17% (for the layer from 0 to 40 cm). The soil reaction was slightly acid (pH - from 5.84 to 5.94).

Weather conditions during study period are presented in Table 1. In the year of alfalfa plants establishment, the total monthly precipitation in all months was close to the long-term average (LTA) (1896-2005) with slight deviations, except for May, when the rainfall amounts was significantly more compare with the LTA (66.1 mm).

In April, May, June and July of the second alfalfa growing season there was significantly less precipitation than the LTA, while in August significantly more precipitation compared with the LTA was recorded. During 2016, in all months, a higher or similar rainfall amounts was recorded compare with the LTA, with the largest precipitation deficit in July (2.2 mm). The total amount of precipitation in the first and second growing season were 478.4 and 406.9 mm, respectively, which is by 94.3 and 22.8 mm more than LTA (384.1 mm). In 2016 the total amount of precipitation (375.4 mm) recorded was below than in the previous two years of study and close to the LTA.

Table 1. Climate data during three growing seasons (2014-2016)

Months	Rainfall (mm)				Temperatures (°C)			
	2014	2015	2016	LTA*	2014	2015	2016	LTA*
March	65.0	61.7	70.0	70.0	9.5	6.5	8.1	5.0
April	64.8	37.2	76.6	50.7	11.9	11.4	14.6	11.4
May	166.7	19.4	98.3	66.1	16.4	18.4	15.9	16.5
June	79.4	65.1	74.2	80.5	19.8	20.1	22.0	20.2
July	67.3	18.8	2.2	67.4	22.6	24.6	24.8	22.5
August	35.2	204.7	54.1	49.4	23.6	23.2	22.9	23.8
September	478.4	406.9	375.4	384.1	17.3	17.37	18.05	16.6
March-September	65.0	61.7	70.0	70.0	9.5	6.5	8.1	5.0

Legend: * LTA (Long term average) - the period from 1896 to 2005 was used

During the year of alfalfa establishment air temperature in all months were close to the LTA. In the second year the mean air temperatures at beginning of the growing season were similar to this in 2014, with mean monthly air temperature deviation in relation to the LTA in May and July +1.9 and +2.10 °C, respectively. The highest mean air temperature was recorded in 2016 (18.05 °C), as in April (14.6 °C), June (22 °C) and July (24.8 °C) is significantly higher than the LTA. The mean monthly air temperatures for the three alfalfa growing seasons (March–August) was 17.6 °C and it was higher by 1°C than the LTA (1896–2005) (16.6°C).

Data collection and statistical analysis

For the study period seven quantitative traits were evaluated of which four generative: seed yield per plant (SYP), pod number per inflorescence (PNI), seed number per pod (SNP) and 1000-seed weight (TSW), and three morphological traits: plant height (PH), generative stem number per plant (GSNP) and inflorescence number per stem (INS). The traits were evaluated during the establishment year, the second and third years.

Plant height (cm) and generative stem number were determined at full pod development stage (green pods) by measuring the length of the stems from soil surface to the tip. Generative stem number was calculated by counting the stems of 10 randomly selected plants for each genotype.

At seed maturity, five plants per progeny on each replicate were randomly sampled. Ten generative stems were selected and their inflorescences were counted to calculate the inflorescence number per stem.

In order to determine the traits pod number per inflorescence and seed number per pod, 20 inflorescences were collected of randomly selected stems. Ten inflorescences were selected and their pods were counted to calculate the number of pods per inflorescence. From the rest 10 inflorescence 10 pods were selected, threshed and their seeds were counted to calculate the seed number per pod. Ten plants per progeny were selected and their pods were threshed. The seeds were cleaned and weighted and the mean seed yield per plant (g) and 1000 seed weight (g) were determined.

Experimental data were processed by the One-way analysis of variance (ANOVA). The significance of differences among clonal progenies were detected by LSD test at 0.01% confidence level. In order to determine the degree of influence of the sources of variation genotype (G), year (Y) and genotype x year (G x Y) interaction on the studied traits a two-way analysis of variance was applied. Variation and correlation analysis was performed to establish the traits variability and the relationships among them. Phenotypic coefficients of variation (PCV) were estimated as a percentage of their corresponding phenotypic standard deviations to the trait grand mean. The magnitude of variation of the traits was determined according to the scale of Mamaev (1973) as follows: very low (up to 7%), low (7.1-12%), moderate (12.1-20%), high (20.1-40 %) and very high (over 40%). The relationships among studied characteristics, expressed by phenotypic correlation coefficients (r_p, %) were determined among all possible combinations of traits. The STATGRAPHICS PLUS software was used.

RESULTS AND DISCUSSION

Data of analysis of variance at evaluation of the alfalfa clonal progenies indicated a different degree of phenotypic expression of seed yield per plant and yield related traits both among clonal progenies and for period of study. The values presented in Table 2 shown that the highest three-year average seed yields of 3.52 and 3.44 g plant^{-1} were obtained in PM30 and JM13, respectively. High seed yield was also recorded at GM27 and SL83, exceeding mean for clonal progenies (2.91 g) by 13.8 and 8.59 %. The seed yield was reported in the literature varied in narrow range from 0.30 to 40 g. The means established in present study corresponded to values obtained by Tlahig et al. (2017), whereas El-Hifny et al. (2019) found mean seed yield per plant of 1.2 g at evaluation of seven alfalfa genotypes under three different sowing dates over the two seasons.

The variability of morphological and generative properties both among studied alfalfa progenies and across years, express by the phenotipic coefficients of variation is presented in Table 2-4.

Table 2. Means, standard divisions and phenotypic coefficients of variability of seed yield among progenies and for study period

Clonal progenies	Seed yield (g plant^{-1})			
	Mean	% to mean for progenies	SD	PCV for study period (%)
SL83	3.16 bc	108.76	0.65	20.64
SL89	2.66 de	91.57	0.21	7.88
SL92	2.50 e	85.96	0.52	20.85
SL99	2.51 de	86.30	0.10	3.80
PM30	3.52 a	121.03	0.68	19.42
PM18	2.87 cd	98.68	1.11	38.56
PM49	2.82 cde	96.84	0.36	12.71
PM65	2.45 de	84.35	0.61	24.95
GM14	2.75 de	94.44	0.65	23.76
GM27	3.31 b	113.80	1.11	33.56
JM13	3.44 ab	118.27	0.84	24.30
Mean	2.91			
$LSD_{0.01}$	0.37			
SD	0.39			
PCV (%)	13.37			

Means followed by same letter in the columns are not significantly different at $p \leq 0.01$

Reported values indicated that seed yield per plant varied moderately among clonal progenies (PCV=13.37%). Data correspond with the results of Basafa and Taherian (2009), who found CV of 13% for seed weight per plant. The authors postolate that phenotypic variability of seed yield among alfalfa genotypes is due to crop management practices and high level of genetic impurity. The values of PCV indicated that clonal progenies exhibited from very low to high variability regarding trait during study period. A large variation in SY among varieties and environments has been reported by Bolanos-Aguilar et al. (2002). The highest stability for SYP in the present study was observed in SL99 (PCV=3.8%). It was found the seed weight per plant varied significantly across years in GM27 (PCV=33.56%).

The power of influence of sources of variation was expressed by η (%). The reported values of 33.16, 20.10 and 16.08% for factors year (Y), genotype (G) and genotype x year (G x Y) interaction, respectively, revealed their significant impact on the degree of phenotypic expression of seed yield (Table 5). Ilic and Dukic (2006) found that the degree of penotypic expression of seed yield is highly influenced by year, as well as by genotype x year interaction when investigated 10 genetically divergent genotypes. A significant influence of the year and the genotype on seed weight per plant was also reported by Lakić et al. (2022) but the authors argued that the genotype x year interaction had no significant effect on the trait.

There were established considerable differences ($p \leq 0.01$) between clonal progenies regarding plant height as they were classified into 7 homogenous groups (Table 3). The means ranged from 67.23 to 53.87 cm. The clonal progenies GM27 and SL83 distinguished with the highest plants and the excesses in values for the trait to the other progenies were statistically significant. It was outlined trend for high genetic potential of JM13 progeny, which ranked third with trait value exceeding by 9% the mean for progenies.

Regarding trait variation among progenies, the PCV value showed that plant height exhibited lower phenotypic variability (PCV<10%) compared to the other studied quantitative traits, with exception of TSW. Data indicated different magnitude of variability for PH during study period, as only SL99 (PCV=3.8%) exhibited low variability. The degree of variation for all other progenies was determined as mederate to high.

Based on analysis of variance, it is evident that the factor G had statistically significant impact on PH (η=36.48%). The analized trait was also considerably influenced by Y (η = 32.88%) and G x Y interaction (η=27.28%) (Table 5).

There were found significant differences in degree of phenotypic expression of the generative stem number per plant (Table 3). The means given in Table 3 indicated that GM14 had the highest ability in stem producing (47.47 stem plant^{-1}) and SL89 the lowest (29.80 stem plant^{-1}). PM65 and PM30 ranked second at reported value of 41.57 and 41.37, respectively.

The coefficient of variation, as a measure of dispersion, determined the variability of GSNP among clonal progenies as moderate (PCV=14.32%). It can be seen that the trait varied considerably (PCV>65%) at all progenies over study period (Table 5). The year, as a source of variation had exclusively strong influence on stems number (η=91.19%). The influence of factors genotype and genotype x year interaction was also statistically significant but much low than year.

Table 3. Means, standard divisions and phenotypic coefficients of variability of morphological traits among progenies and for study period

Clonal progenies	Plant height (cm)				Generative stem number per plant				Inflorescence number per stem			
	Mean	% to mean for progenies	SD	PCV for study period (%)	Mean	% to mean for progenies	SD	PCV for study period (%)	Mean	% to mean for progenies	SD	PCV for study period (%)
SL83	66.73 a	111.22	7.02	10.51	35.27 cd	95.57	25.17	71.37	10.27 b	105.97	4.95	48.22
SL89	58.87 de	98.11	4.41	7.49	29.80 f	80.76	22.45	75.35	10.00 bc	103.22	2.95	29.51
SL92	53.87 h	89.78	6.19	11.48	37.40 c	101.36	28.53	76.29	9.17 de	94.62	4.34	47.30
SL99	55.77 fg	92.94	8.31	14.89	33.60 de	91.06	24.10	71.73	8.30 f	85.67	1.56	18.82
PM30	63.83 c	106.39	11.90	18.64	41.37 b	112.10	29.66	71.70	11.97 a	123.52	3.11	25.98
PM18	57.80 e	96.33	9.51	16.46	35.77 cd	96.93	24.15	67.52	9.50 cd	98.06	4.15	43.69
PM49	55.03 g	91.72	5.42	9.85	36.30 cd	98.37	26.51	73.04	9.30 de	96.00	2.43	26.16
PM65	56.33 f	93.89	6.81	12.09	41.57 b	112.65	29.09	69.98	9.23 de	95.31	1.75	18.96
GM14	59.03 d	98.39	6.79	11.50	47.47 a	128.64	34.86	73.45	8.80 ef	90.84	4.49	51.06
GM27	67.23 a	112.06	5.93	8.82	35.63 cd	96.57	26.00	72.97	9.43 cde	97.37	4.04	42.80
JM13	65.50 b	109.17	6.07	9.26	31.73 ef	86.00	23.80	75.00	10.60 b	109.42	2.52	23.81
Mean	60.00				36.90				10.27			
$LSD_{0.01}$	1.08				2.80				0.65			
SD	4.93				5.25				1.50			
PCV (%)	8.22				14.32				14.56			

Means followed by same letter in the columns are not significantly different at p≤0.01

Table 4. Means, standard divisions and phenotypic coefficients of variability of gnerative traits among progenies and for study period

Clonal progenies	Pod number per inflorescence				Seed number per pod				1000 seed weight (g $plant^{-1}$)			
	Mean	% to mean for progenies	SD	PCV for study period (%)	Mean	% to mean for progenies	SD	PCV for study period (%)	Mean	% to mean for progenies	SD	PCV for study period (%)
SL83	7.20 b	106.21	2.12	29.40	3.70 a	120.07	0.44	11.78	1.92 ab	106.19	0.19	9.62
SL89	6.10 cd	89.99	2.43	39.79	2.50 de	81.13	0.35	13.86	1.69 f	93.49	0.14	8.38
SL92	5.83 d	86.05	1.91	32.72	2.70 cd	87.62	0.53	19.60	1.75 de	96.62	0.13	7.47
SL99	5.80 d	85.56	1.39	23.89	2.97 bc	96.27	0.12	3.89	1.84 c	101.41	0.20	10.62
PM30	8.07 a	119.00	2.30	28.55	3.83 a	124.40	0.72	18.87	1.89 b	104.53	0.19	9.83
PM18	7.20 b	106.21	2.77	38.52	3.10 b	100.60	0.72	23.26	1.79 cd	98.83	0.16	8.71
PM49	6.83 b	100.80	1.83	26.84	2.84 bcd	92.19	0.56	19.58	1.77 d	97.72	0.14	7.77
PM65	6.90 b	101.79	2.91	42.10	2.36 e	76.44	0.04	1.63	1.68 f	92.94	0.27	15.74
GM14	6.70 bc	98.84	2.50	37.28	3.10 b	100.60	0.60	19.35	1.70 ef	93.86	0.18	10.73
GM27	7.10 b	104.74	2.42	34.15	3.07 b	99.52	0.90	29.23	1.96 a	108.21	0.31	15.84
JM13	6.83 b	100.80	1.79	26.19	3.73 a	121.15	1.07	28.64	1.92 ab	106.19	0.03	1.31
Mean	6.78				3.08				1.81			
$LSD_{0.01}$	0.63				0.34				0.05			
SD	0.82				0.55				0.10			
PCV (%)	12.12				17.96				5.61			

Means followed by same letter in the columns are not significantly different at p≤0.01

There were considerable differences among clonal progenies concerning inflorescence number per stem (Table 4). Means for study period shown that PM30 and JM13 distinguished with significantly higher phenotypic expression of the trait than other progenies, with 11.97 and 10.6 inflorescence stem^{-1}, while SL99 was with the lowest one (8.3), at average value for the progenies of 10.27. Abd El-Naby et al. (2016) in a study of 10 genotypes found that INS ranged from 8 to 16 inflorescence stem^{-1}, at mean 11.93.

Table 5. Results of analysis of variance for studied traits.

Trait	Sources of variation	SS	df	MS	F exp.	η (%)	Sign.
SYP	Genotype (G)	51.51	10	5.15	19.46	20.10	**
	Year (Y)	84.99	2	42.49	160.58	33.16	**
	Interaction (G * Y)	41.23	20	2.06	7.79	16.08	**
	Error	78.60	297	0.27			
PH	Genotype (G)	7299.40	10	729.94	322.22	36.48	**
	Year (Y)	6578.86	2	3289.42	1452.08	32.88	**
	Interaction (G x Y)	5458.95	20	272.95	120.49	27.28	**
	Error	672.80	297	2.27			
GSNP	Genotype (G)	7426.33	10	742.63	52.43	4.33	**
	Year (Y)	156559.66	2	78279.83	5526.69	91.19	**
	Interaction (G * Y)	3493.01	20	174.65	12.33	2.03	**
	Error	4206.70	297	14.16			
INS	Genotype (G)	297.02	10	29.70	34.74	9.24	**
	Year (Y)	2201.75	2	1100.87	1287.75	68.49	**
	Interaction (G x Y)	462.18	20	23.11	27.03	14.38	**
	Error	253.9	297	0.86			
PNI	Genotype (G)	133.69	10	13.37	19.65	9.18	**
	Year (Y)	1045.82	2	522.91	768.46	71.79	**
	Interaction (G x Y)	75.24	20	3.76	5.53	5.16	**
	Error	202.10	297	0.68			
SNP	Genotype (G)	74.75	10	7.48	31.89	32.51	**
	Year (Y)	41.41	2	20.70	88.35	18.01	**
	Interaction (G x Y)	44.19	20	2.21	9.43	19.22	**
	Error	69.60	297	0.23			
TSW	Genotype (G)	3.10	10	0.31	60.38	25.04	**
	Year (Y)	3.89	2	1.94	379.15	31.42	**
	Interaction (G x Y)	3.87	20	0.19	37.75	31.26	**
	Error	1.52	297	0.01			

SS - sum of squares; gf - degrees of freedom; MS - variance; F exp. - F experimental; η - degree of influence of the factor;
**significant at 0.01 level

There were established significant differences in degree of trait variation for clonal progenies over study period. The values of PCV ranged from 18.82% (moderate variability) for SL99 to 51.06% (very high) for GM14. Among progenies the trait varied moderately (PCV=14.56%). Pelikán et al. (2014) in a study of 99 alfalfa accessions found high variability (33.1%) of INS. According Bolanos-Aguilar et al. (2000), under a spaced plant design, the number of fertile stems and inflorescences may be more variable, depending on the size of the individual plants while, in dense canopies, the number of fertile stems per unit area is likely to be more stable.

The results indicated significant impact of all sources of variation on the inflorescence number per stem but the variability of the trait was the most influenced by year (η=68.49%). It can be explain by the weather conditions, mainly rainfall and its distribution during flowering - seed set - seeds ripening period (June-August) accros the growing seasons. These results are with accordance by findings of Bolanos-Aguilar et al. (2002) and Karagić et al. (2019).

The results of three-year for pod number per inflorescence shown significant differences between clonal progenies (Table 4). It was found that PM30 had the highest pods per inflorescence (8.07) and SL99 the lowest (5.80), at average value for the progenies of 6.78 pods inflorescence^{-1}. The results are in line whit these reported by Tlahig et al. (2017) who obtained that PNI varied from 5.56 to 8.95 pods inflorescence^{-1}. Karagić et al. (2019) reported a mean of 7.6 pods inflorescence^{-1} and Jevtić et al. (2014) stated values for the trait of 4.74, 7.68 and 6.97 pods inflorescence^{-1} on the lowest, middle and peak inflorescences, respectively. The reported values of PCV determined variability of trait between studied progenies as moderate (PCV=12.12%) and over the study period as very high for PM65 (PCV=42.10%) and high for other progenies. The high trait variability (CV=22.8%) among populations has been demonstrated by Pelikán et al. (2014).

The effects of G, Y and G x Y interaction on pod number per inflorescence were statistically significant (Table 5). It was found that the strongest influence on the pods developemment had year. On the contrary, Lakic et al. (2022) reported significant influence of genotype on NPI in a stuty on within-population variability of seed yield related traits in 10 alfalfa genotypes.

Concerning seed number per pod, data of analysis of variance revealed significant differences ($p \leq 0.01$) among progenies (Table 4). For study period PM30 characterized with the largest amount of seeds (3.83), followed by JM13 (3.73), whereas PM65 had the lowest (2.36). In previous researches has been reported that the number of seed per pod varied from 1.85 to 9.16 seed pod^{-1} (Dordas, 2006), from 5.5 to 5.7 seed pod^{-1} (Rashidi et al., 2009) and from 1.63 to 2.41 seed pod^{-1} (Abasov et al., 2019). Karagić et al. (2019) at studying 20 populations, which represent a part of the European alfalfa core collection, determined mean of 2.6 seed pod^{-1} in year of alfalfa stand establishment. Data shown that between clonal progenies SNP varied moderately (PCV=17.96), but more strongly than other analized traits. It is noticeable that over study period clonal progenies expressed different magnitude of trait variability (from very low to high). The highest stability of trait was established in PM65 (PCV=1.63%).

Data of two-way analysis of variance shown that the seed number per pod was determined more on genetic factors (η=32.51%) than on factors Y and G x Y interaction (Table 5). The results obtained are in agreement with those reported by Abd El-Naby et al. (2016). Avci et al. (2017) reported that the year effect was significant for seed number per pod, whereas row spacing and sowing rate did not significantly affect degree of phenotypic expression of trait during growing seasons.

The values presented in Table 4 indicated the clonal progenies exhibited different potential regarding 1000-seed weight, as the differences between them were statistically significant ($p \leq 0.01$). The highest phenotypic expression of the trait was recorded in GM27 (1.96 g) and the lowest was ascertained in PM65 (1.68 g) with a mean of 1.81 g for progenies. The results correspond with those of Sengul (2006) who reported that 1000-seed weight varied from 1.63 to 2.06 g with a mean of 1.85 g. Tlahig et al. (2017) obtained lower values for the trait from 1.45 to 1.74 g. In term of 1000-seed weight variability data shown that the trait expressed very low variation (PCV=5.61%) among clonal progenies. Data obtained correspond with the results of Iannucci et al. (2002) who reported PCV values less than 12% for TSW. On the contrary, Abbasi et al. (2003) revealed large phenotypic variation of the trait among alfalfa accessions.

Over study period 1000-seed weight exhibited very high stability (PCV=1.31%) in JM13 (Table 5). The trait varied moderately in two progenies and low in the others. It was established that all sources of variation had significant influence on TSW but the impact of factor Y (η=31.42%) and factor G x Y interaction (η=31.26%) was stronger than that of genetic factors. El-Hifny et al. (2019) also found that year (environment) had the stongest effect on TSW than other sources of variation (genotype, sowing date and their interaction).

According Bodzon (2016), the quantitative traits determining seed productivity are polygenically determined and for this reason determining of its effects on seed yield and relationships is decisive for the effectiveness of selection for increased seed yield.

Phenotypic correlation coefficients among analyzed traits are presented in Table 6.The estimate of degree and nature of relationship between analized traits revealed positive and significant phenotypic correlation between seed yield and all generative and morphological traits. Data obtained is in accordance with the results reported by Bodzon (2016) of positive correlation of SYP with 6 generative and 6 morphological traits. There was found weak correlation between seed yield and generative stem number per plant (r_p=0.150). Contrary, Zambrana (1972) reported on high and positive relationship between seed yield and number of fertile stems. Lakić et al. (2022) established positive correlation (r=0.47) between SYP and NPI. Phenotipic correlation coefficient confirmed correlation between SYP and INS (r_p=0.362*). Liatukiene et al. (2009) showed that the weak to medium correlations are more suitable for selecting of parental material.

Table 6. Phenotypic correlation coefficients among 7 characters for the tested genotypes

Traits	SYP	PH	GSNP	INS	PNI	SNP	TSW
SYP	1						
PH	0.752**	1					
GSNP	0.150	-0.165	1				
INS	0.362*	0.630**	-0.066	1			
PNI	0.770**	0.683**	0.241	0.570**	1		
SNP	0.611**	0.802**	-0.303	0.510**	0.290	1	
TSW	0.412*	0.749**	0.151	0.323*	0.661**	0.083	1

*, **significant correlation at 0.05 and 0.01 level of probability, respectively

The plant height was highly and positively correlated with all generative and morphological traits, except for the GSNP (r_p=-0.165). The correlation coefficients revealed a moderate positive correlation of inflorescence number per stem with pod number per inflorescence (r_p=0.570**) as well as with seed number per pod (r_p=0.510**). Furthermore inflorescence number per stem was positively but insignificantly related with 1000-seed

weight (r_p=0.323). There was also found a strong positive relationship between pod number per inflorescence and 1000-seed weight (r_p=0.661**). The correlation between pod number per inflorescence and seed number per pod was weak and statistically no confirmed. Insignificant assosiation between these two traits was established by Bodzon (2016). Khrbeet et al. (2016) found significant negative correlation between TSW and SY (-0.669), SNP (-0.589) and PNI (-0.603). Zhang et al. (2008) also reported a significant negative relationship between SY and TSW.

CONCLUSION

Evaluation of genotypic and morphologic properties of the alfalfa progenies were established that the sources of variation (genotype, year and genotype x year interaction) had a statistically significant influence on all morphological and generative traits analyzed. The degree of phenotypic expression of the traits seed yield per plant and seed number per pod were influenced more on genetic factors than factors Y and G x Y interaction. The factor G x Y interaction had the most significant impact on the 1000-seed weight and plant height.

The largest magnitude of phenotipic variability between clonal progenies was ascertained for seed number per pod (PCV=17.96%) and the lowest for 1000-seed weight (PCV=5.61%).

PM30 progeny showed superior scores regarding all traits studied and JM13, GM27, SL83 and PM18 distinguished with high phenotypic expression of the traits seed yield, pod number per inflorescence, seed number per pod and 1000-seed weight. These progenies were evaluated as a valuable germplasm source to be used in further breeding to develop a new synthetic alfalfa variety with stable seed yield.

It was found seed yield strongly and positively correlated with plant height (r_p=0.752**), pod number per inflorescence (r_p=0.700**) and seed number per pod (r_p=0.611**), wtich suggest that selection for improving seed productive ability in alfalfa may be performed directly through selection on these three traits.

This study would be continued whit testing the polycross progenies propagated by seeds in the polycross nursery, to determine the progenies with the best combining ability, regarding important agro-morphological traits and to be used as parental components in the synthesis of alfalfa synthetic populations.

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IMPROVEMENT OF HIGH AMYLOSE CONTENT IN CH1 RICE VARIETY BY MARKER ASSISTED PSEUDO-BACKCROSS BREEDING

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ABSTRACT

The objective of this research was to introgression of high amylose content into CH1 rice variety by using pseudo-backcrossing breeding .Crossing between CH1 and RD49 was performed to produce F_1 progenies .After that, the progenies will backcross to CH1 to develop BC_1F_1 population .Then, the selected plants from the BC_1F_1 population were continuously selfed to develop BC_1F_2 and BC_1F_3 populations, respectively .For marker assisted selection, the OSR19 DNA marker that is specific to *Wx* gene was used for assisting the selection of plants with high amylose content in foreground selection to choose favorite genotype. Total 67 SSR markers used genetic marker assisted selection was utilized for BC_1F_1 and BC_1F_2 populations .The results showed that selection could be achieved for BC_1F_1 and BC_1F_2 plants having high amylose content and first highest ranking of genetic background similar to recurrent parent exhibited 91 and 97.8 percent, respectively .The marker assisted selection could accelerate in backcross breeding program .The BC_1F_3 seeds of 6 selected lines were planted in rice field for preliminary yield test .It was found that some agronomic characters (Days of 50% flowering, flag leaf length, panicle length, number of tillers per plant and harvest index) and yield of the selected lines were not statistically different from those of CH1 variety and high amylose content as donor parent. Thus, the advantage of the pseudo-backcross method is that it can save not only time and workload, but also the cost of analysis and leaping generations in the normal backcross method. Moreover, genetic similarity results in relatively similar morphological characteristics.

Keywords: CH1, high amylose content, pseudo-backcross breeding, RD49, *Wx* gene

INTRODUCTION

Rice is the main food crop that is consumed within Thailand . In addition, rice is also a very important economic crop for Thailand in 2018 (Saosaovaphak et al., 2022). Yanjie et al. (2018) examined rice with AC ranging from 13 to 20% and found that the correlation of AC with eating quality was positive; this different finding compared to those just cited may be due to the small range of AC in the samples used. Moreover, preference depends on culture (Chen, 2019). For example, it has been reported that consumers in some parts of Vietnam and China prefer low-amylose rice, while people from Philippines, Malaysia, Pakistan and Iran favor intermediate amylose, and high-amylose rice is more popular in Sri Lanka and Myanmar (Calingacion et al., 2014). In Thailand, rice with moderate to high amylose content (AC) is more preferable to eat than rice with low AC (Cruz and Khush, 2000). Qiqnizhan (CH1) rice variety originated from China with high yield potential, new plant type, tall plant and good ability to cross (Rattanapol et al., 2011). AC is one of the most critical criteria determining cooking and eating quality (Juliano, 1971). In fact, AC is primarily a hereditary feature that is governed by the waxy locus (*Wx*) on chromosome 6 (Bao et al., 2008). The *Wx* gene has a polymorphic microsatellite DNA marker with a dinucleotide cytosine-thymine repeat (CT_n) (Bligh et al., 1995).

The novel platform based on pseudo-backcross breeding shortens both multiple foreground selection and background genome recovery. This study proposes a modified form of pseudo-backcrossing to shorten the backcross breeding cycle. Pseudo-backcrossing evolved from tree breeding methods, in which F_1 plants from a

single cross are backcrossed to alternate recurrent parents to avoid inbreeding depression (Bouquet, 1986). Within four years, the entire project was completed in seven cycles consisting of a single backcross, two cycles of pseudo-backcrossing, and three cycles of line fixation (Ruengphayak et al., 2015). To ensure a high recovery of the recurrent parent phenotype after transferring multiple resistance genes using conventional marker assisted selection (MAS), at least three to four backcrosses are required (Suh et al., 2013).

Using conventional breeding methods, the donor segment can remain very large even with many backcross generations. Without marker-assisted background selection, the percentage of recurrent genome content (%RGC) in BC_2 progeny was only 87.5% (Hasan et al., 2015). However, when combined with MAS and phenotypic selection, % RGC in F_2 increased to 93.1% (Wangsawang et al., 2019). Indeed, using genome-wide molecular markers for background screening during backcrossing has been proposed as the most effective method for improving low %RGC (Cho et al., 2020). Although pseudo-backcrossing is the quickest method for gene/QTL pyramiding, it may not be the best breeding platform for creating elite recurrent varieties. However, early-stage marker-assisted genome-wide scanning can be implemented to aid in the reconstruction of favorable genomic backgrounds at the end of the pseudo-backcrossing scheme. Therefore, breeders should consider if they want to shorten the time but incur additional costs or it will take a longer time but cost less than the above method. Finally, the breeders must consider the trade-offs. However, using molecular markers to help in selection may bring additional costs. In an ideal world, new high-throughput, low-cost genome-wide scanning technologies would be used in tandem with skilled breeder selection (Ruengphayak et al., 2015).

The current study's goal was to select new rice lines with high AC using a pseudo-backcrossing breeding method by crossing CH1 with the RD49 rice variety.

MATERIALS AND METHODS

Introgression of Wx^a conferring high AC from RD49 into CH1 by Pseudo-backcross

The pseudo-backcross platform is divided into the RD49, Thai rice with good cooking qualities, a high AC, and higher yields strain was crossed with the rice variety CH1, a new Chinese plant type variety with the potential to provide higher yields (Rattanapol et al., 2011), to develop the F_1 generation as shown in Figure 1. Individual F_1 plants with heterozygous alleles of specific markers were chosen and backcrossed to CH1 using mixed pollen to produce the BC_1F_1 generation. Through marker-assisted selection, the BC_1F_1 progenies were Wx^a/Wx^b allele. The BC_1F_2 generation was created by self-pollinating of the BC_1F_1 progenies with top ten highest ranking of genetic background as similar as CH1. The BC_1F_2 progenies which were homozygous Wx^a/Wx^a allele as RD49 and Wx^a/Wx^b allele were achieved through marker-assisted selection. The BC_1F_3 generation was created by self-pollinating of BC_1F_2 progenies with the highest ranking of genetic background as similar as CH1. Six BC_1F_3 pseudo BILs with Wx^a allele as RD49 were successfully developed.

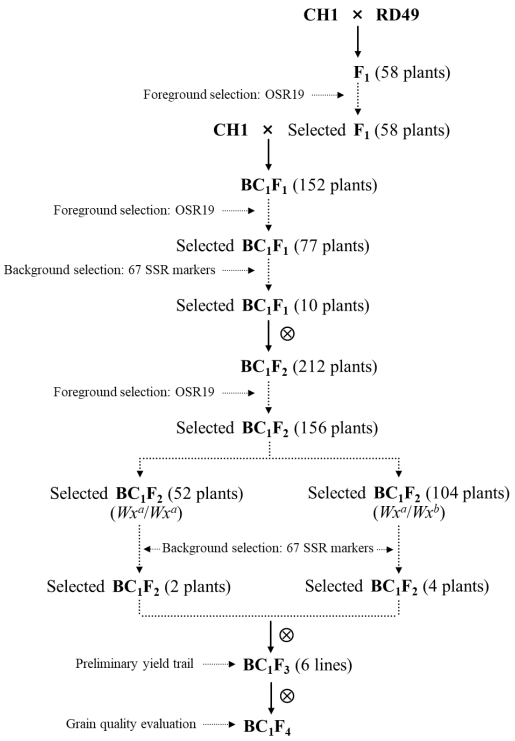


Figure 1. Scheme for the development of high AC using pseudo-backcrossing breeding method through crossing CH1 with the RD49 rice variety.

Genomic scanning of foreground and background

Individual plants carrying the donor allele were identified using marker assisted selection in one backcross generation, BC_1F_2 generation, and BC_1F_3 generation. The SSR marker, OSR19 which contain CT_n alleles of the *Wx* gene was used to identify the plants carrying the favorite *Wx* gene (Akagi et al., 1996). The primer pair OSR19F (5'-CTCTCTCACCATTCCTTCAG-3') and OSR19R (5'-GATCTGAATAAGAGGGGAAAC-3') was developed by Sreewongchai et al. (2014). The plants carrying the band which similar with RD49 were selected. The PCR protocol was following the applied method of Rattanapol et al. (2011).

In a genome-wide survey, the 233 SSR markers from known chromosomal positions distributed evenly across the 12 chromosomes were used to identify the polymorphism between the two parents. The 67 polymorphic SSR markers between the two parents were used for the background profiling in BC_1F_1 generation and BC_1F_2 generation compared with CH1 (Supplementary table 1). The nucleotides of 67 primers reference in McCouch et al. (2002).

Supplementary Table 1. The 67 SSR markers used for genetic background selection in BC_1F_1 and BC_1F_2 populations.

Primer name	Chromosome	Primer name	Chromosome
RM1	1	RM1054	5
RM129	1	RM334	5
RM283	1	RM5371	6
RM472	1	RM400	6
RM405	2	RM125	7
RM71	2	RM501	7
RM475	2	RM25	8
RM106	2	RM433	8
RM526	2	RM444	9
RM573	2	RM105	9
RM208	2	RM3912	9
RM48	2	RM201	9
RM6	2	RM6791	9
RM545	3	RM215	9
RM7	3	RM258	10
RM251	3	RM222	10
RM563	3	RM3717	11
RM16	3	RM332	11
RM416	3	RM536	11
RM85	3	RM287	11
RM3524	4	RM286	11
RM471	4	RM457	11
RM6341	4	RM6440	11
RM518	4	RM206	11
RM551	4	RM224	11
RM241	4	RM19	12
RM317	4	RM247	12
RM280	4	RM83	12
RM559	4	RM277	12
RM7444	5	RM1261	12
RM598	5	RM519	12
RM87	5	RM463	12
RM437	5	RM235	12

Genomic DNA was extracted from fresh frozen leaves of rice plants using the CTAB method with little modification of Murray and Thompson (1980). The PCR amplifications were carried out using a Phire® Plant Direct PCR Kit (Finnzymes; Keilarata, Espoo, Finland), which was designed to amplify DNA directly from rice leaf samples with little modification (Murray and Thompson, 1980). After electrophoresis on 6% polyacrylamide gels, polymorphism in each PCR product was detected using silver nitrate ($AgNO_3$) staining following (Benbouza et al., 2006).

Field experiments and data collection

At Bang Sai, Phra Nakhon Si Ayutthaya, Thailand, agronomic characteristics and yield performance were studied. The experiment was carried out from June to December, 2017. The experiment was designed with three replications using a Randomized Complete Block Design (RCBD). In the experiment, two rice varieties, RD49 and CH1, were used as controls. The seeds of 6 lines and checks were sown in a seed nursery .One-month-old seedlings were then manually transplanted into the rice field with one seedling per hill .Each plot had five rows, each with seven plants, and a planting density of 20 cm between plants (within a row) and 20 cm between rows. On each experimental plot, fertilizing and field management were explained by Wangsawang et al. (2019). Days of 50% flowering (DOF), plant height (PH), flag leaf length (FLL), panicle length (PL), number of tillers per plant (T/P), harvest index (HI), number of panicles per plant (P/P), number of grains per panicle (S/P), number of filled grain per panicle (G/P), 100-grain weight (GW), and total grain weight per plant (W/P) were measured.

Using the STAR 2.0.1 software, an analysis of variance was performed, and Duncan's multiple range test (DMRT) was used for multiple mean comparisons.

Grain quality evaluation

Rice grains from six lines and their parents were harvested when they reached physiological maturity and naturally dried in a greenhouse. Prior to evaluating the grain quality traits, the dried grains were stored at room temperature for one month. Grain samples weighing 100 g were collected from each replicate and combined. The grain quality test was then conducted using 50 g of mixed samples. A mini polisher was used to mechanically deshell grain samples.

The procedures described by (Lanceras et al., 2000) were used to evaluate AC, gel consistency (GC), and gelatinization temperature (GT). The GC was determined by measuring the length of the grain starch slurry in a culture tube of cold gel; the length of the gel, measured from the bottom of the tube to the front of the gel migration, was measured in millimeters one hour later. The longer gel is thought to be softer than the shorter gel. GT is a cooking time indicator. The alkali spreading value (ASV) was used to estimate the GT indirectly; a larger ASV indicates increased alkali spreading and thus a lower GT, whereas a smaller ASV indicates a higher GT.

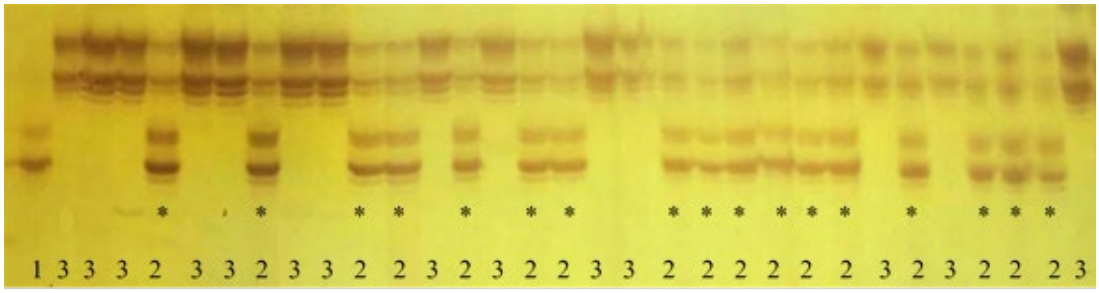
The appearances of the endosperm, as well as the size and shape of the kernel, are morphological characteristics of rice grains. Ten milled rice kernel seeds were measured for length and breadth with vernier caliper, and length/breadth ratios (L/B) were calculated. The appearance of the rice endosperms was determined visually using the procedure described by (Tan et al., 2000).

RESULTS

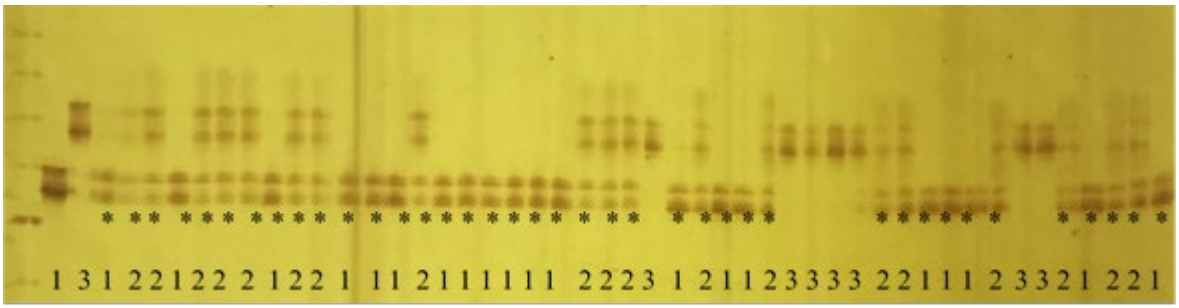
Development of high AC pseudo-backcross inbred lines and marker Assisted Selection

A total of 58 F_1 progenies were produced from the crosses of CH1 and RD49. Each F_1 were backcrossed into CH1 by targeting MAB to generate BC_1F_1 progenies. The BC_1F_1 plants were genotyped using the *Wx* gene specific marker (OSR19). The Wx^a locus for high AC was discovered in RD49. Out of 152 plants, 77 were heterozygous (Wx^a/Wx^b) and 75 were homozygous (Wx^b/Wx^b) as CH1 (Table 1). At a 1:1 ratio, the chi square test was accepted. Hence, only 77 plants were chosen for high AC (Supplementary figure 1). Among 77 plants of BC_1F_1 progenies, 10 plants which had Wx^a/Wx^b allele and highest genetic background similar with CH1 were selected. The 10-BC_1F_1 plants were generated to 212 pseudo- backcrossed BC_1F_2 progenies . Plants carrying the heterozygous Wx^a/Wx^b gene were screened using the OSR19 marker at the seedling stage in the pseudo-BC_1F_2 BILs. The 52 pseudo-BILs were tagged for the homozygous Wx^a/Wx^a gene as RD49, the 104 plants were heterozygous Wx^a/Wx^b, and the 56 plants were homozygous Wx^b/Wx^b gene as CH1 (Table 1 and supplementary figure 2). At a 1:2:1 ratio, the chi square test was accepted. As a result, the 156 pseudo-BILs were examined for genetic background profiling. Using target MAS, 2 fully homozygous (Wx^a/Wx^a) and 4 heterozygous (Wx^a/Wx^b) pseudo-backcrossed BC_1F_2 plants were selected and selfed to generate BC_1F_3 progeny. Finally, six pseudo backcrossed inbred lines (pseudo-BILs) carrying the donor gene's positive Wx^a allele were achieved.

Six pseudo-BC_1F_3 BILs were chosen based on their highest genetic background similarity to CH1 for field testing. Entirely, donor and recipient were intensively crossed and selected for five seasons : two seasons to generate BC_1F_1, another two seasons to generate pseudo backcrossed BC_1F_2 and BC_1F_3 populations, respectively, and one season of selfing to fix the final best- selected pseudo-BC_1F_4 BIL for evaluating grain quality.



Supplementary Figure 1. Example of a DNA band obtained by selecting for the high AC in the BC_1F_1 crossing between the RD49 and CH1 using the OSR19 marker.
Note: 1 is Wx^b/Wx^b as CH1, 2 is Wx^a/Wx^b, 3 is Wx^a/Wx^a as RD49, and * is the BC_1F_1 selected plants.



Supplementary Figure 2. Example of a DNA band obtained by selecting for the high AC in the BC_1F_2 crossing between the RD49 and CH1 using the OSR19 marker.
Note: 1 is Wx^b/Wx^b as CH1, 2 is Wx^a/Wx^b, 3 is Wx^a/Wx^a as RD49, and * is the BC_1F_2 selected plants.

Table 1. The use of the OSR19 marker to segregate alleles associated with AC in the BC_1F_1 and BC_1F_2 population.

Genotype	Number of BC_1F_1 plants	Genotype	Number of BC_1F_2 plants
Wx^a/Wx^a (RD49)	0	Wx^a/Wx^a (RD49)	52
Wx^a/Wx^b	77	Wx^a/Wx^b	104
Wx^b/Wx^b (CH1)	75	Wx^b/Wx^b (CH1)	56
Total	152	Total	212
$\chi^2_{(1:1,0.05)}$ test	0.0313^{ns}	$\chi^2_{(1:2:1,0.05)}$ test	0.0188^{ns}

Remarks: ^{ns} = non-significant.

Genetic background of the pseudo-BILs

Background analysis confirmed the presence of substituted chromosome segments in the 77 BC_1F_1 plants. The study was made using genome-wide molecular markers. The 233 SSR markers were used in the background analysis. Marker polymorphism between CH1 and RD49 was 28.76% (67 markers). Each plant contains an SSR marker that defines the recipient's genetic background, CH1. Ten pseudo-BILs were chosen from BC_1F_1 progenies with the highest genetic similarity to CH1. The average genetic background percentage of the plants ranged from 82.09% (Q-6) to 91.04% (Q-8) (Table 2). The expected background genome recovery for these ten plants was 85.72%. In the study, the Q-8 plant inherited the most genetic background from CH1.

The frequency of CH1 alleles at non-target loci for the individual plant with homozygous introduced gene (Wx^a/Wx^a) derived from RD49 ranged from 94.78) %Q-8-2, Q-8-66, Q-8-72 and Q-8-74 (to 97.01) %Q-8-17 (and the genetic background profiling with Wx^a/Wx^b ranged from 95.52% (Q-8-6) to 97.76% (Q-8-143) after one self-pollination generations BC_1F_2 : pseudo-backcross (Table 2).

Table 2. Genetic background profiling of BC_1F_1 BILs, pseudo-BC_1F_2 BILs with Wx^a/Wx^a and pseudo-BC_1F_2 BILs with Wx^a/Wx^b performed using polymorphic simple sequence repeat markers between CH1 and RD49.

BC_1F_1 BILs	GB (%)	Pseudo-BC_1F_2 BILs (Wx^a/Wx^a)	GB (%)	Pseudo-BC_1F_2 BILs (Wx^a/Wx^b)	GB (%)
Q-8	91.04	Q- 8-17	97.01	Q-8-143	97.76
Q-16	89.87	Q- 8-64	96.27	Q-8-182	97.76
Q-55	87.50	Q- 8-4	95.52	Q-8-145	97.01
Q-18	85.07	Q- 8-82	95.52	Q-8-178	97.01
Q-63	85.07	Q- 8-112	95.52	Q-8-183	97.01
Q-67	85.07	Q- 8-216	95.52	Q-8-128	96.27
Q-68	85.07	Q- 8-2	94.78	Q- 8-15	96.27
Q-38	83.58	Q- 8-66	94.78	Q- 8-28	96.27
Q-20	82.84	Q- 8-72	94.78	Q- 8-73	96.27
Q-6	82.09	Q- 8-74	94.78	Q- 8-6	95.52
Average	**85.72**	**Average**	**95.48**	**Average**	**96.86**

Remarks: GB (%) = Genetic background as recurrent parent (%)

Agronomic and yield performance

The six selected pseudo-BILs were grown under field condition in the BC_1F_3 generation. Tables 3 and 4 showed the results of an analysis of variance (ANOVA) for agronomic and yield performance. Significant differences were found between the two parents for DOF, PL, S/P, G/P, and GW traits. When comparing the pseudo-BILs to the recurrent parent CH1, significant genotype variances for PH, S/P, G/P, and GW were discovered. The pseudo-BILs had mean values ranging from 68.2 to 82 cm (PH), 5.7 to 6.7 (P/P), 189.2 to 290.3 (S/P), 159 to 243 (G/P), and 1.9 to 2.3 g (GW). However, no significant differences in DOF, FLL, PL, T/P, HI, P/P, or W/P were found between the pseudo-BILs and CH1. Six improved lines had mean values ranging from 71.3 to 78.3 days (DFT), 21 to 28.7 cm (FLL), 27.9 to 29.8 cm (PL), 6 to 6.9 (T/P), 0.38 to 0.48 (HI), 5.7 to 6.7 (P/P), and 16.7 to 19.8 g (W/P). Highly stable characters, such as plant height and 100-grain weight, emerged differently from the repeated parent. Plant height has decreased and thousands of plants have increased. Although their genetic similarity is seen in molecular studies, their morphological features appear to be significantly different.

Table 3. Major important agronomic traits of the pseudo-BC_1F_3 BILs compared with parental varieties.

Pseudo-BILs/ Varieties	DOF	PH (cm.)	FLL (cm.)	PL (cm.)	T/P	HI
Q-8-17	77.0 b	68.2 d	23.0	29.1 a	6.7	0.47 a
Q-8-64	71.7 b	71.9 cd	22.9	27.9 ab	6.9	0.48 a
Q-8-143	78.3 b	75.3 bcd	21.0	28.1 ab	6.6	0.43 abc
Q-8-145	72.0 b	79.3 abc	22.1	29.7 a	6.2	0.41bc
Q-8-182	73.7 b	80.9 ab	28.7	29.0 a	6.0	0.38 c
Q-8-183	71.3 b	82.0 ab	25.4	29.8 a	6.4	0.44 ab
Average (line)	74.0	76.3	23.9	28.9	6.5	0.44
CH1	76.0 b	84.3 a	26.7	29.9 a	6.2	0.43 abc
RD49	90.0 a	81.8 ab	18.3	25.4 b	8.3	0.42 bc
Average (parent)	83.0	83.1	22.5	25.4	7.3	0.42
F-test	**	**	ns	*	ns	*
C.V. (%)	5.72	5.79	15.86	5.20	12.42	6.05

Remarks :ns= non-significant; *, = ** significance at 0.05 and 0.01 probability levels, respectively .Means within each column of each agronomic traits followed by the same letter are not significantly different according to DMRT. Days of 50% flowering (DOF), plant height (PH), flag leaf length (FLL), panicle length (PL), number of tillers per plant (T/P) and harvest index (HI).

Table 4. Major important yield component of the pseudo-BC_1F_3 BILs compared with parental varieties.

Pseudo-BILs/ Varieties	P/P[z]	S/P	G/P	GW (g)	W/P (g)
Q-8-17	6.6	189.2 bc	164.3 c	2.1 bc	16.8
Q-8-64	6.7	204.3 bc	165.7 c	2.3 ab	17.4
Q-8-143	6.4	225.7 b	194.6 bc	2.1 cd	16.7
Q-8-145	6.2	213.4 b	159.0 cd	2.0 cd	16.8
Q-8-182	5.7	289.8 a	243.0 a	2.0 cd	17.8
Q-8-183	6.2	290.3 a	236.3 a	1.9 d	19.8
Average (line)	6.3	221.9	193.8	2.1	17.5
CH1	6.2	274.8 a	217.9 ab	2.0 cd	20.3
RD49	8.3	162.3 c	122.9 d	2.4 a	19.1
Average (parent)	7.2	218.6	170.4	2.2	19.7
F-test	ns	**	**	**	ns
C.V. (%)	12.85	10.16	11.14	3.08	18.45

Remarks: ns= non-significant; =** significance at 0.01 probability levels. Means within each column of each yield component followed by the same letter are not significantly different according to DMRT. Number of panicles per plant (P/P), number of grains per panicle (S/P), number of filled grain per panicle (G/P), 100-grain weight (GW), and total grain weight per plant (W/P)

Grain quality

Six pseudo-BILs, the original CH1, and the donor parent, RD49, were tested for three quality traits. Table 5 showed the results of grain quality testing. High AC of the pseudo-BILs ranged from 23.8% (Q-8-183) to 28.8% (Q-8-17). CH1 and RD49 had average AC of 17.4% and 29.1%, respectively. There was no difference in AC between the BILs and RD49. On the other hand, CH1 had intermediate average AC .High AC was perfectly associated with the *Wx* in allele of RD49. CH1 and RD49 had dissimilar ASV of 2.0 and 6.2, and gel consistency (GC) of low and high, respectively. BILs with similar alkali spreading values had mean ASV ranging from 4.7 to 7.0. The GC of pseudo-BILs (Q-8-17, Q-8-64, Q-8-143, Q-8-145, Q-8-182 and Q-8-183) differed as low, low, intermediate, low, intermediate, and intermediate, respectively.

The kernel length and width of CH1 were 7.7 and 1.9 mm, respectively, with an L/W ratio of 4.1. RD49, on the other hand, had kernel length and width of 9.0 and 1.9 mm, respectively, with an L/W ratio of 4.7. There was no significant difference in grain length and width or kernel length and width between the pseudo-BILs and the recipient parent CH1.

Table 5. Comparison of grain quality performance of the pseudo-BC_1F_4 BILs and two parents calculated by combined analysis of variance data in all traits of each experiment.

Pseudo-BILs/ Varieties	AC (%)	ASV (GT)	GC	Kernel size		
				Length (mm)	Width (mm)	L/W
Q-8-17	28.8 a	7.0 a	Low	8.2 bc	1.9 ab	4.4 bc
Q-8-64	27.8 a	6.9 a	Low	8.5 b	1.9 abc	4.5 ab
Q-8-143	24.6 ab	4.7 b	Intermediate	8.1 bcd	1.8 bc	4.5 ab
Q-8-145	24.8 ab	6.10ab	Low	8.0 cde	1.9 abc	4.3 bc
Q-8-182	24.6 ab	5.5 ab	Intermediate	7.8 de	1.9 abc	4.2 c
Q-8-183	23.8 b	5.5 ab	Intermediate	8.1 bc	1.8 c	4.5 ab
CH1	17.4 c	2.0 c	Low	7.7 e	1.9 a	4.1 c
RD49	29.1 a	6.2 a	High	9.0 a	1.9 a	4.7 a
F-test	**	**	-	**	*	**
C.V. (%)	14.85	11.92	-	4.38	4.28	6.33

Remarks :ns= non-significant; *, = ** significance at 0.05 and 0.01 probability levels, respectively. Means within each column of each yield component followed by the same letter are not significantly different according to DMRT.

DISCUSSION

A cross between CH1 and RD49, followed by pseudo-backcross selection, could result in rice lines with high AC accompanying with the goal of improving a favorable variety having favorable genetic background. To best encourage the efficient integration of the *Wx* gene into a CH1, an improved breeding platform based on pseudo backcrossing was developed. Recurrent backcrossing is a traditional breeding method used to transfer alleles from a donor to an elite variety at one or more loci. By six backcrosses process, the expected recurrent parent genome recovery would be 99.2%, which is most similar to improved variety (Wangsawang et al., 2018). However, introducing trait until the end result of a backcrossing program is to obtain lines as similar to the recurrent parent as possible, this approach can be tedious and time-consuming (Hasan et al., 2015). In a nutshell, this modern platform based on pseudo-backcross breeding relates to dual foreground selection and background genome recovery. The entire scheme was completed in two and a half years, consisting of two seasons to generate BC_1F_1, another two seasons to generate pseudo backcrossed BC_1F_2 and BC_1F_3, respectively, and one season of selfing to fix the final best-selected pseudo-BC_1F_4 BILs. This multiple pseudo-backcrossing platform reduces the time required to develop new rice varieties with complex, long-lasting resistance to biotic and abiotic stresses in desirable backgrounds (Ruengphayak et al., 2015).

AC is an important determinant of rice quality. In this study, the Wx^a allele was introduced from RD49 into CH1 via MAS. MAS is a very efficient and cost-effective breeding technology because it is used in most of the steps of the breeding program and greatly increases the success of selecting desirable lines because it directly targets the genotype without the influence of environment and thus speeds up the conventional selection procedures (Collard et al., 2005). Pseudo-BILs had a high AC and agronomic performance comparable to CH1. OSR19 was used in this experiment to identify individuals with the positive allele derived from RD49. All of the BILs with the positive allele of OSR19 had a high AC, indicating that (CT)n repeats were present (Akagi et al., 1996), in these genetic materials, it was able to distinguish between high and intermediate AC. MAS for foreground selection may be especially useful for traits with time-consuming or laborious phenotypic screening processes. It can also be used to select the reproductive-stage traits in seedlings, allowing the best plants for each backcrossing to be identified.

Two pseudo-BC_1F_3 BILs and four pseudo-BC_1F_3 BILs were largely homozygous and heterozygous, respectively in the MAS- based target loci with agronomic traits similar to CH1, having high AC . In BC_1F_1 progenies, background genotype recovery ranged from 82.09% to 91.04%. In the experiment, the Q-8 plant inherited the most genetic background from CH1. This was more or less the expected value, as the recipient genome should theoretically be recovered to 50% in BC_1. The average genetic background percentage in BC_1F_2 progenies ranged from 94.78% to 97.01% as homozygous gene (Wx^a/Wx^a) and 95.52% to 97.76% as Wx^a/Wx^b. The average background genotype recovery rate should be 75% with one time of backcross and self-pollination (BC_1F_2), and that background recovery rate is lower than that of the selected plants in this study because MAS was used to select population in each generation. In addition, the results from this research gave the same results as Cho et al. (2020). This result showed that the pseudo-backcross method with marker assistance can increase the percentage genetic background of plants more than the standard backcross method and skips generations in the normal backcross method.

It is critical that the genes used in MAS and gene introgression do not introduce undesirable traits due to linkage drag (Sun and Mumm, 2015). In addition to high AC, the major positive agronomic traits of RD49 were similar to pseudo BILs. The results showed that there is no negative effect in pseudo BILs after the *Wx* gene introgression. The *Wx* gene was found to be primarily responsible for quantitative GC inheritance (Zhou et al., 2003). The findings of this research showed that *Wx* gene associated with OSR19 had no effect on GC. The ASV of the pseudo-BILs differed significantly from CH1. Lower ambient temperature during the grain filling period may

cause a higher ASV because lower environmental temperatures decreased the relative amount of long amylopectin chains and increased the relative amount of short chains, resulting in a high GT (Fan et al., 2005). Furthermore, high temperatures increased the GT significantly (Zhong et al., 2005). The grain size and shape of the pseudo-BILs matched that of the original CH1.

CONCLUSION

The pseudo-BC_1F_2 BILs used in this study had background genome recovery of 97.76% with the RD49 allele of the Wx^a gene using foreground and background simultaneous selection. This pseudo-backcross design could aid in the introgression of high AC from the RD49 rice variety into the CH1 background, thereby speeding up the backcross breeding program. This study found that the pseudo-backcross method with marker assistance could increase the genetic background percentage of plants than the traditional backcross method. Thus, the advantage of the pseudo-backcross method is that it can save not only time and workload, but also the cost of analysis and leaping generations in the normal backcross method. However, there are still disadvantages of selecting with pseudo-backcross bases SSR markers. The selection of plants in the rice field may have other environmental factors that cause the experimental results to be inaccurate. In the present study, marker assisted pseudo-backcrossing breeding accelerated the development of superior qualities in the genetic background of CH1. The improved CH1 should be immediately useful for Thai farmers and will help farmers to increase their incomes.

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