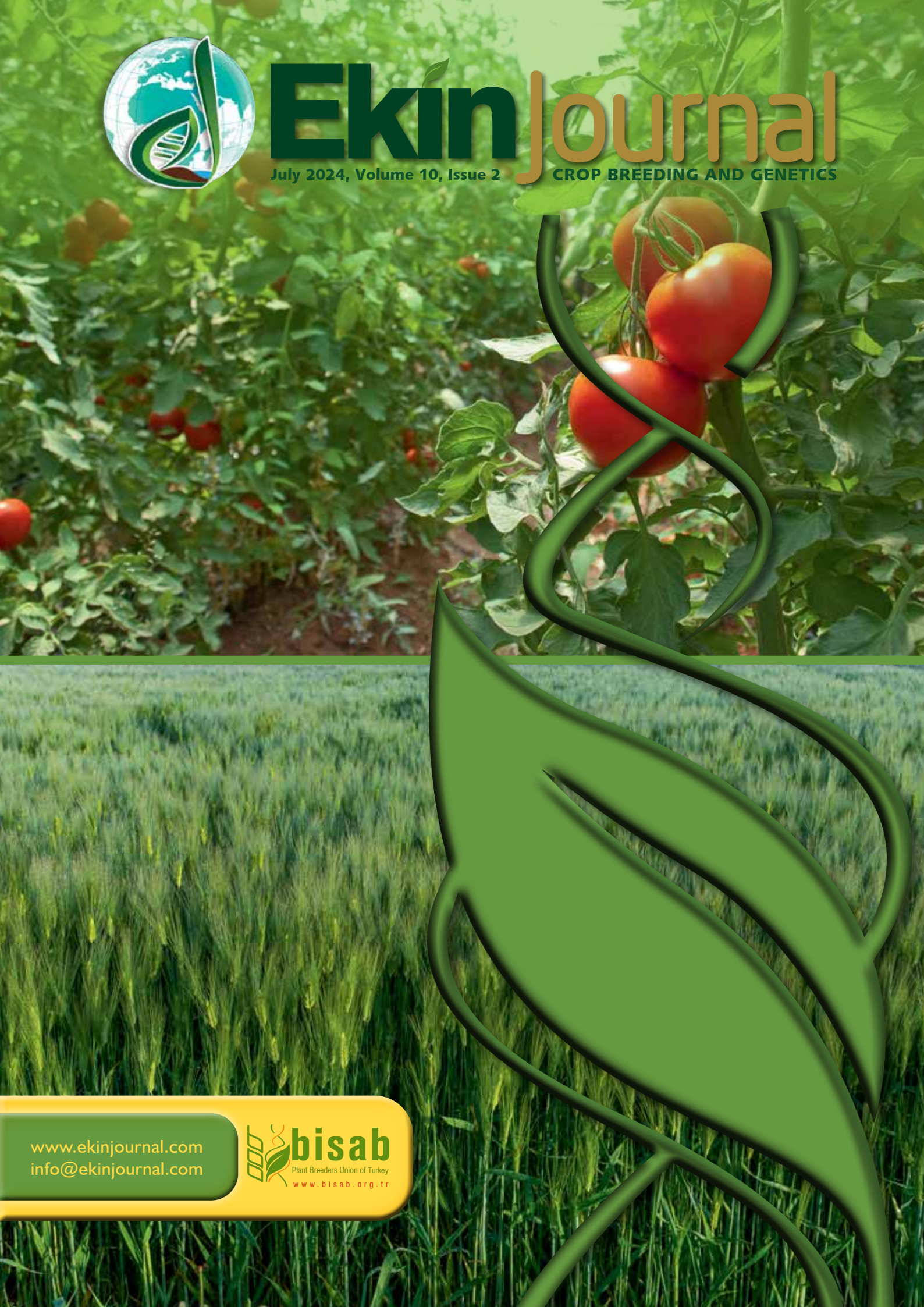

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Ekin Journal CONTENTS
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Variability among Varieties for Response to Hairamine a Protein Hydrolysate on Stem Strength and Grain Yield Attributes in Wheat (*Triticum aestivum* L.)

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ABSTRACT

Hairamine a protein hydrolysate obtained from human hair is a growth promoter as a biostimulant. A field experiment was conducted at the Research Farm of the Faculty of Agriculture, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala (133207) during the *Rabi* season of 2023-2024 to evaluate the response of three wheat varieties DBW 222, WH 1270 and DBW 303 to Hairamine for its effect on grain yield and its attributes. The experiment was conducted in randomized block design with three replications. Three foliar sprays of Hairamine 4 ml/litre of water were applied at 30, 60, 90 days after sowing. Observations were recorded on 5 randomly selected plants from each plot for plant height, chlorophyll content, nitrogen content, stem girth, stem strength, angle of declination, number of grains per spike, test weight, grain yield and bacterial population. The rhizospheric soil was analysed for microbial colonies in control and Hairamine treated plots. Significant increase in various plant characters and microbial colony number was observed in Hairamine treated plots in all the three varieties. The grain yield and stem strength were enhanced by 14.5% and 25.4%, respectively. Therefore, Hairamine application can be recommended as potential bio stimulant for increasing grain yield and lodging resistance in wheat under climate change.

Keywords: Hairamine, hydrolysate, plant growth promoter, bio-stimulant, lodging

Introduction

Wheat (*Triticum aestivum* L.) is the world's principal and commercially important food crop. It belongs to the grass family Poaceae. It provides 21% of the food calories, 20% of the protein and 55% of the carbohydrates in the diet. In comparison to other cereals, wheat grain has greater protein content (12%) and a fairly good niacin and thiamine content. Global wheat consumption has increased in the past four decades to around 781 million tonnes annually and accounts for approximately 25 per cent of worldwide protein supply. In India, the area under wheat crop is 30.46 million hectares with the production of 112.18 million tonnes (2022- 23). Major wheat-growing states in India are Uttar Pradesh, Punjab, Haryana, Rajasthan, Madhya Pradesh, Gujarat and Bihar. The area under wheat cultivation in Haryana (2022-23) was 2.36 million hectares with the production of 12.0 million tonnes (Indiastat, 2023)

In modern agriculture, along with fungicides, herbicides and insecticides, various products classified as plant growth stimulants are also used (Calvo et al., 2014). In the era of technology, the use of bio-stimulators is very important. The bio-stimulators affect plant productivity by increasing metabolism, accelerating the absorption nutrients and contributing to their redistribution in the plant body. Bio stimulant Hairamine being rich in organic nitrogen, organic carbon, calcium, amides and amino acids, reduces the need of fertilizers and increases plant growth, develops

resistance in plants against abiotic stresses. In small concentration, this substance is efficient in favouring good performance of the plants' vital processes and allowing higher yield. In addition, bio stimulants applied to plants enhance nutrients use efficiency, abiotic stress tolerance and plant quality traits. (De Vasconcelos et al., 2019). This bio-stimulant is used in wheat field and it has been shown to have positive effect on grain yield and its attributes (Du Jardin, 2015). Different wheat varieties respond differently to bio-stimulant such as Hairamine. Bio-stimulants contribute to better seed germination and induce biological activity of plants. These products are also safe for the environment and contribute to sustainable, high-output low-input crop productions.

Rhizospheric conditions determine root growth and nutrient uptake which ultimately contribute to plant growth and grain yield in wheat. Rhizospheric condition are in turn determined by colony forming units of favourable rhizobacteria. Root exudates determine the multiplication and growth of rhizobacteria. Bio-stimulants are known to enhance root exudates including carbohydrates, vitamins, amino acids and enzymes, which serve as food for growing rhizobacteria thus bio stimulants are used for in rhizosphere towards better plant growth (Nguyen et al., 2019). This study was conducted to examine the effects of foliar application of Hairamine on plant growth traits, stem strength, flexibility, grain yield, components and rhizosphere activity in three wheat varieties to explore the possibility of infusing lodging resistance and sustainably increases yield potential.

Materials and Methods

A field experiment was conducted during winter (*Rabi*) season of 2023-2024 at research farm of Faculty of Agriculture, Maharishi Markandeshwar (deemed to be University), Mullana, Ambala, Haryana (133207) is situated at 30.243228°N latitude, 77.061692°E longitude, altitude of 264 meters above sea level. The average summer and winter temperature was around 38°C and 12°C, respectively. The soil of experimental site was sandy loam in texture, well-drained, had an alkaline reactivity (pH 7.13), low in nitrogen, medium in phosphorus with a conductivity of 0.89d/Sm. The experiment was conducted in factorial randomised block design with three replication and two factors comprising three wheat varieties "DBW 222 (V_1)", "WH 1270 (V_2)" and "DBW 303 (V_3)" (Factor A) and two treatments including T_1 Control (recommended dose of fertilizers) and T_2 Hairamine (100% recommended N, P, K doses through synthetic fertilizers and Hairamine Foliar Spray 4 ml/litre of water) (Factor B). The recommended dose of fertilizer was applied as 150 kg/ha Nitrogen (N) through urea + DAP, 60 kg/ha Phosphorus (P) through DAP and 60 kg/ha Potassium (K) through MOP + 25 kg zinc sulphate/ha. Nitrogen fertilizers were applied in split doses i.e., 1/3 of the nitrogen fertilizer along with the full doses of phosphate and potash at the time of sowing; the remaining nitrogen was supplied evenly after the first and second irrigations. Hairamine was applied as foliar spray thrice (4 ml Hairamine per litre water) at 30, 60, 90 days after sowing. The data was recorded on five randomly selected plants from each treatment for different characters *viz.,* chlorophyll content, nitrogen content, stem girth, stem strength, angle of declination, plant height, number of grains/spikes, test weight (1000 grain weight), grain yield. Mean data for each character was statistically analysed for analysis of variance and critical differences among treatments and control using OP STAT® (Sheoran, et al., 1998).

To analyse the population of rhizospheric bacteria, soil samples were collected from two plots, with one serving as a control and the other treated with Hairamine. These soil samples were aseptically taken from a depth of 10-15 cm, then air-dried in the laboratory and sieved through a 2 mm sieve. The bacterial population in the soil was assessed using the serial dilution technique on Nutrient Agar Media (NAM, Himedia). In this method, a soil suspension was prepared by mixing 1.0 g of soil with 9 ml of sterile distilled water. Subsequently, soil suspension was serially diluted from 10^{-1} to 10^{-4} by transferring 1 ml of suspension from one tube to the next (Rajkhowa, et al., 2000). Next, 0.1 ml of soil suspension from the 10^{-2} and 10^{-3} dilutions was aseptically transferred onto Petri plates containing Nutrient agar media, gently spread with a glass spreader, and then incubated at 37°C for 24 hours. Bacterial populations were assessed after 24-48 hours of incubation. Each colony appearing on the plate was considered one colony forming unit (CFU), with the number of colonies formed on the Petri plate divide by its dilution factor to determine the population per gram of soil (Waksman, 1927; Nazir, 2007). This procedure was repeated thrice on the 3rd, 4th, and 5th days following Hairamine foliar spray.

Results

The results obtained from the present investigation for different characters in response to Hairamine and control treatments are summarised in Table 1.

Plant height (cm): The plant height is measured by normal ruler scale at fully grown stage (end of month March) with grains. The plant height (Table 1, Fig. 1) was compared to control and significant increase was

observed in plants treated with protein hydrolysate formulation (Kumar, et al., 2023). Among two applied treatments, T_1(control) showed significantly lower plant height in three wheat varieties DBW 222 (92.94 cm), WH 1270 (93.14 cm), DBW 303 (92.94 cm) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher plant height in three wheat varieties DBW 222 (102.47 cm), WH 1270 (99.01 cm) and DBW 303 (102.01 cm) at maturity stage. Variety DBW222 showed highest response (10%) for plant height.

Chlorophyll Content (spad): Among two applied treatments, T_1 (control) showed significantly lower chlorophyll content in flag leaf (Table 1, Fig. 2) in three wheat varieties DBW 222 (37.72 spad), WH 1270 (32.66 spad), DBW 303 (38.53 spad) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher chlorophyll content in flag leaf in three wheat varieties DBW 222 (43.61 spad), WH 1270 (40.99 spad) and DBW 303 (43.46 spad) at maturity stage. Variety DBW 222 showed highest response (15%) for chlorophyll content.

Nitrogen Content (%): Among two applied treatments, T_1(control) significantly lower nitrogen content in flag leaf (Table 1, Fig. 3) was found in three wheat varieties DBW 222 (15.52%), WH 1270 (14.41%), DBW 303 (14.61%) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher nitrogen content in flag leaf in three wheat varieties DBW 222 (16.78%), WH 1270 (17.07%) and DBW 303 (17.63%) at maturity stage. Variety DBW 303 showed highest response (20%) for nitrogen content.

Stem girth (mm): Among two applied treatments, T_1 (control) significantly lower stem girth (Table 1, Fig. 4) was found in three wheat varieties DBW 222 (0.49 mm), WH 1270 (0.45 mm), DBW 303 (0.47 mm) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher stem girth in three wheat varieties DBW 222 (0.53 mm), WH 1270 (0.51 mm) and DBW 303 (0.53 mm) at maturity stage. Variety DBW 222 and DBW 303 showed highest response (8% and 12%) for stem girth.

Stem strength (g): Among two applied treatments, T_1 (control) significantly lower stem strength (Table 1, Fig. 5) was found in three wheat varieties DBW 222 (403.3 g), WH 1270 (375.3 g), DBW 303 (366.6 g) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher stem strength in three wheat varieties DBW 222 (476 g), WH 1270 (484.6 g) and DBW 303 (473.3 g) at maturity stage. Variety WH 1270 showed highest response (29%) for stem strength.

Angle of declination (degree): Among two applied treatments, T_1 (control) showed significantly lower angle of declination (Table 1, Fig. 6) in three wheat varieties DBW 222 (132.3°), WH 1270 (131.6°), DBW 303 (135.3°) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly larger angle of declination in three wheat varieties DBW 222 (144.6°), WH 1270 (143°) and DBW 303 (144.6°) at maturity stage. Variety DBW 222 and DBW 303 showed highest response (9% and 6%) for angle of declination.

Number of grains/spike: Among two applied treatments, T_1 (control) significantly lower number of grains/spike (Table 1, Fig. 7) was found in three wheat varieties DBW 222 (45.4 grains), WH 1270 (45.8 grains), DBW 303 (45.6 grains) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher number of grains/spike three wheat varieties DBW 222 (51.3 grains), WH 1270 (51 grains) and DBW 303 (51.6 grains) at maturity stage. Variety DBW 303 showed highest response (13%) for number of grains/spike.

Test weight (1000 grain weight): Among two applied treatments, T_1 (control) significantly lower test weight (Table 1, Fig. 8) was found in three wheat varieties DBW 222 (25.53 g), WH 1270 (24.93 g), DBW 303 (25.73 g) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher test weight in three wheat varieties DBW 222 (31.43 g), WH 1270 (31.13 g) and DBW 303 (31.73 g) at maturity stage. Variety DBW 303 showed highest response (23%) for test weight.

Grain yield (q/ha): Among two applied treatments, T_1 (control) showed significantly lower grain yield (Table 1, Fig. 9) in three wheat varieties DBW 222 (41.53 q/ha), WH 1270 (40.93 q/ha), DBW 303 (41.73 q/ha) at maturity stage. Whereas, T_2 (Hairamine) recorded significantly higher grain yield in three wheat varieties DBW 222 (47.43 q/ha), WH 1270 (47.13 q/ha) and DBW 303 (47.73 q/ha) at maturity stage. Variety DBW 303 showed highest response (14%) for grain yield.

Bacterial population: The rhizospheric soil, the area surrounding plant roots, serves a crucial function in promoting plant growth. In our present study, we investigated the colony-forming units (CFU) of bacteria present in the rhizospheric soil of both Hairamine-treated and untreated fields (Fig.10, 11). Our observations revealed that in the treated field, the CFU count was 282 x 10^{-2}, whereas in the control group, it was 125 x 10^{-2}. These results suggest that the application of Hairamine leads to a significant increase in bacterial population around the roots. This augmentation in bacterial presence likely contributes to overall plant growth and subsequent yield enhancement.

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Discussion

Comparative evaluation of control (T_1) and foliar application of Hairamine (T_2) treatments, revealed significant positive differences between two treatments in all the three wheat varieties DBW 222, WH 1270, DBW 303 for various parameters of plant growth (plant height, chlorophyll content, nitrogen content), stem sturdiness (stem strength, stem girth, angle of declination) and grain yield parameters (grains/spike, test weight and grain yield).

The utilization of Hairamine as a bio-stimulant in wheat cultivation has demonstrated numerous beneficial effects on various growth and yield parameters. The findings of the study consistently demonstrated the positive impact of Hairamine as bio-stimulant on wheat plants. From an agronomic standpoint, the utilization of Hairamine as bio stimulant offer several advantages. Foliar application of Hairamine resulted in higher chlorophyll content, nitrogen content, stem strength, stem girth and angle of declination depicting stem flexibility.

This suggest that Hairamine can be sustainably used to infuse lodging resistance in wheat which is relevant in inclement weather condition due to climate change. This could be due to constituent profile of Hairamine having high organic carbon, nitrogen, calcium, amides and amino acids.

The enhanced growth parameters observed in treated plants can lead to higher yield, increased weight of 1000 seeds, improved quality and greater economic returns. Such advantages of bio-stimulant application have been reported earlier for Hairamine in wheat (Behl, et al., 2023, Kumar et al., 2023), cotton (Kumar, et al., 2021), banana, (Kumar, et al., 2021),

In conclusion, the foliar application of Hairamine as bio-stimulant in wheat cultivation has the potential to revolutionize the agricultural landscape by promoting sustainable practices and maximizing crop productivity. This ecofriendly approach offers a sustainable alternative to conventional method of farming, promoting soil health, crop productivity and environmental conservation.

Table 1. Effect of foliar application of Hairamine on yield and yield attributes in wheat varieties.

Parameters	V_1T_1	V_1T_2	V_2T_1	V_2T_2	V_3T_1	V_3T_2	Critical difference		
							Factor A (Variety)	Factor B (Treatment)	Interaction (A X B)
Plant height (cm)	92.94	102.47	93.14	99.01	92.94	102.01	0.296	0.241	0.418
Chlorophyll content (spad)	37.72	43.61	32.66	40.99	38.53	43.46	3.171	2.589	NS
Nitrogen content (%)	15.52	16.78	14.41	17.07	14.61	17.63	NS	1.345	NS
Stem girth (mm)	0.49	0.53	0.45	0.51	0.47	0.53	NS	0.022	NS
Stem strength	403.3	476	375.3	484.6	366.6	473.3	NS	49.947	NS
Angle of declination	132.3	144.6	131.6	143	135.3	144.6	NS	2.493	NS
Number of grain/spike	45.4	51.3	44.8	51.0	45.6	51.6	0.032	0.026	0.045
Test weight	25.53	31.43	24.93	31.13	25.73	31.73	0.014	0.011	0.019
Grain yield (q/ha)	41.53	47.43	40.93	47.13	41.73	47.73	0.03	0.025	0.043

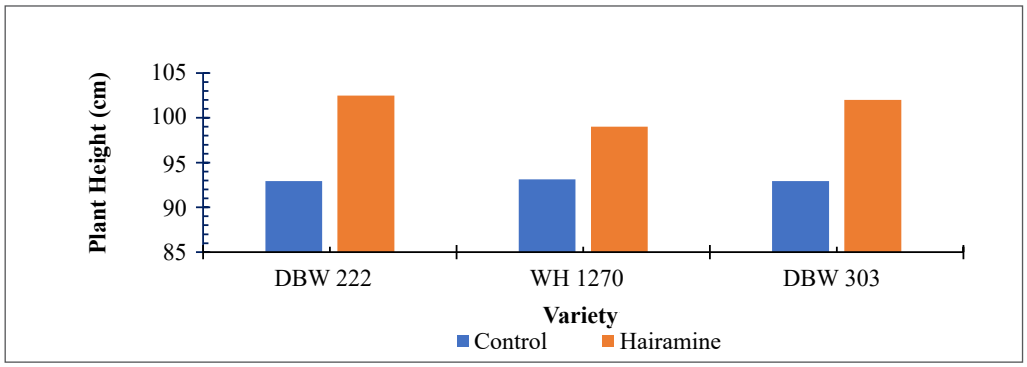

Figure 1. Effect of Hairamine on plant height in wheat

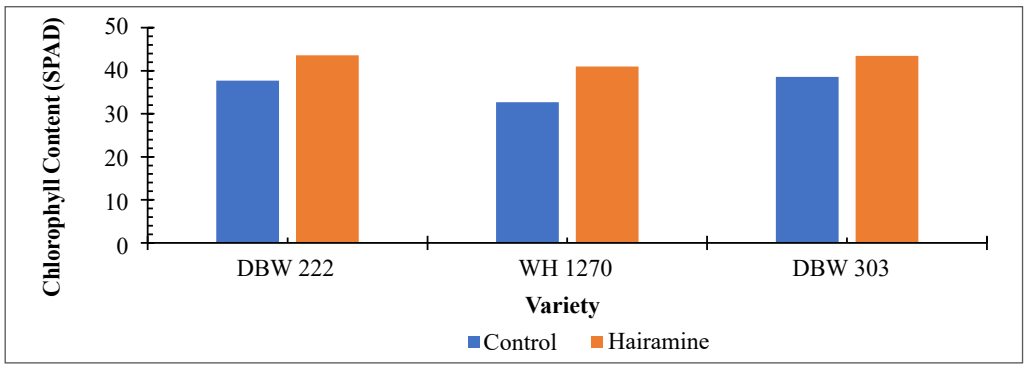

Figure 2. Effect of Hairamine on chlorophyll content in wheat

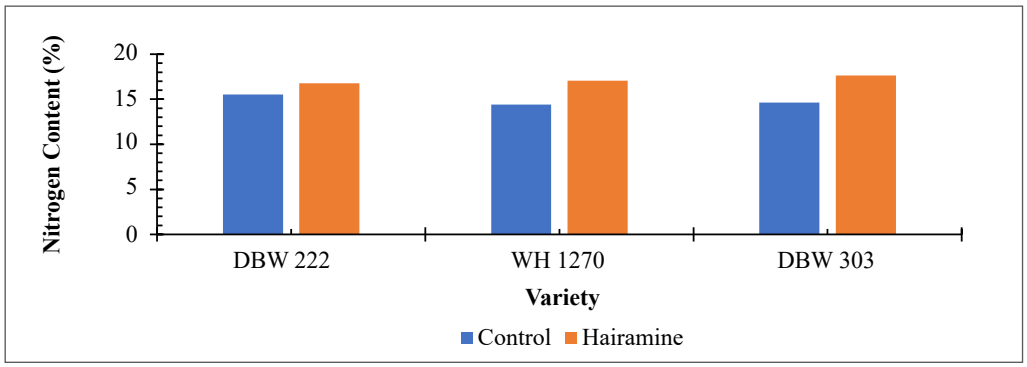

Figure 3. Effect of Hairamine on nitrogen content in wheat

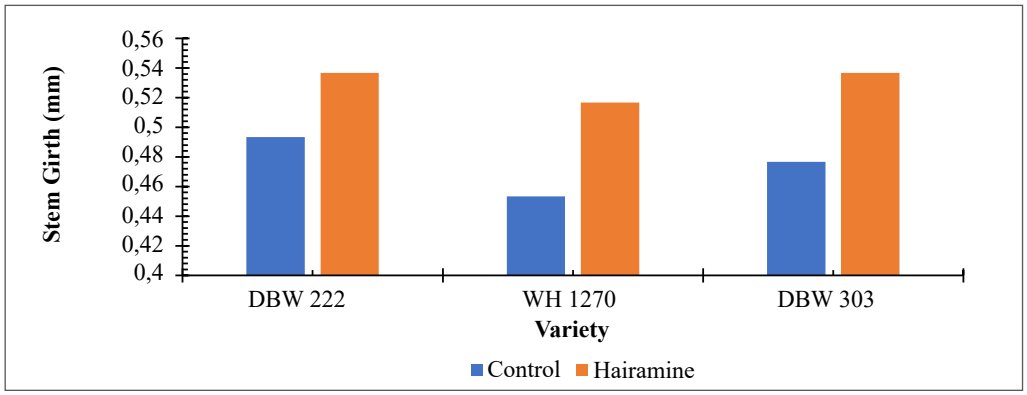

Figure 4. Effect of Hairamine on stem girth in wheat

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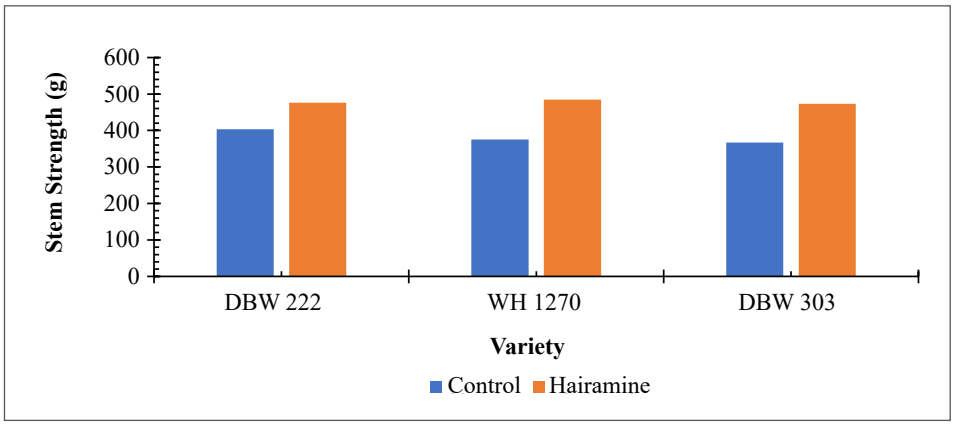

Figure 5. Effect of Hairamine on stem strength in wheat

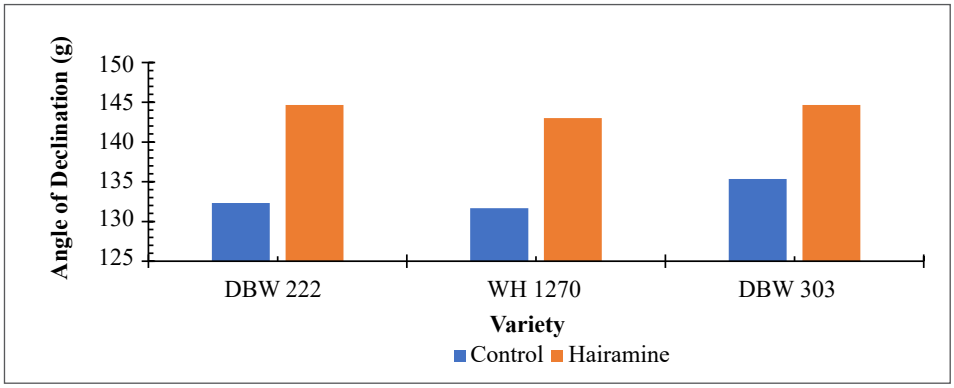

Figure 6. Effect of Hairamine on angle of declination in wheat

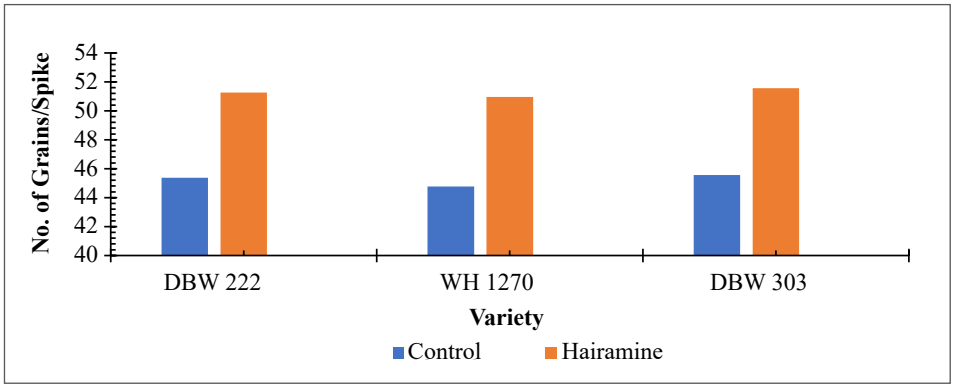

Figure 7. Effect of Hairamine on no. of grains/spike in wheat

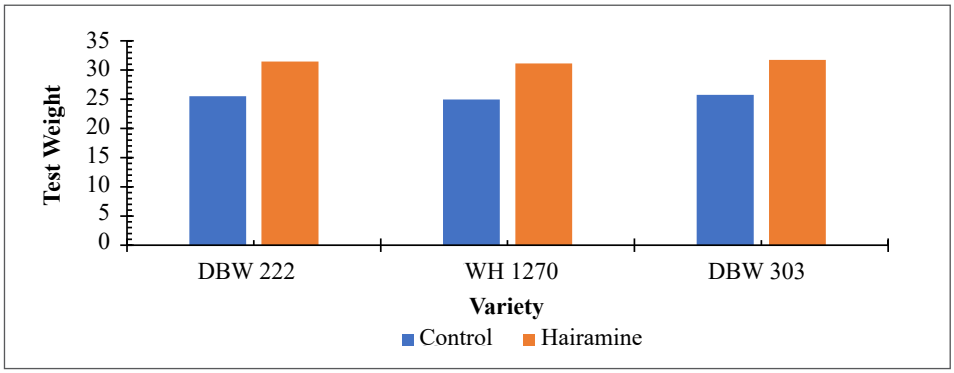

Figure 8. Effect of Hairamine on test weight in wheat

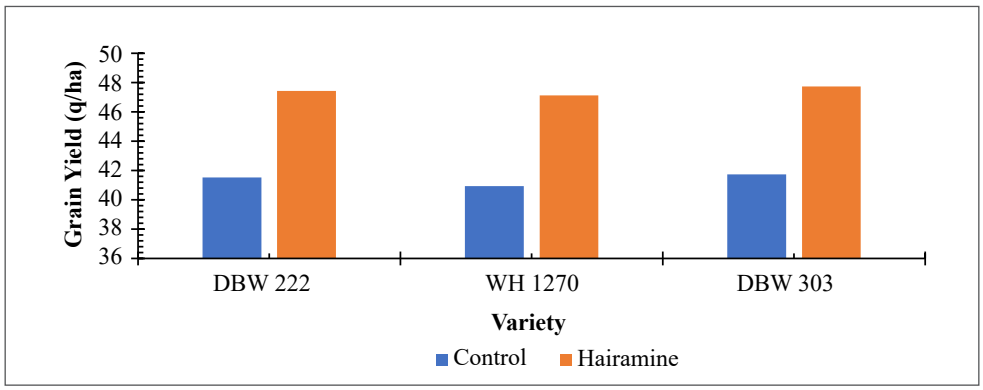

Figure 9. Effect of Hairamine on grain yield in wheat

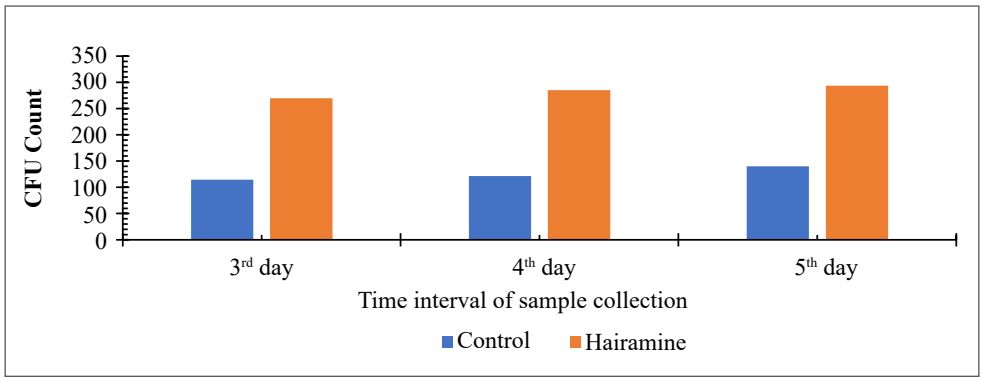

Figure 10. Effect of Hairamine on bacterial population of wheat

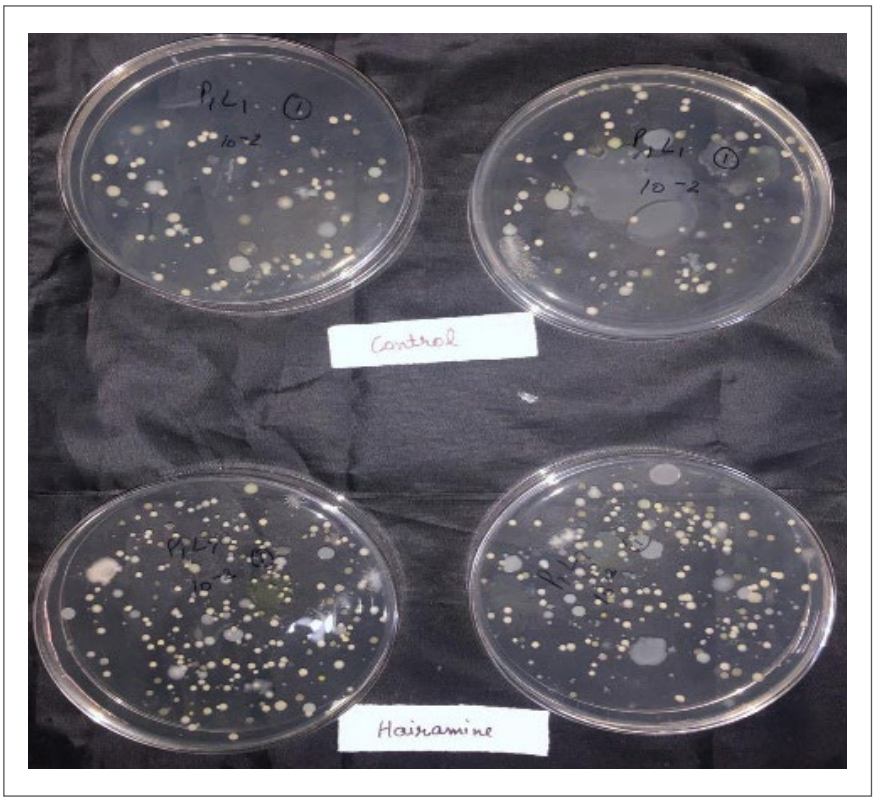

Figure 11. Petri plates sowing CFU from rhizospheric soil

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Changes for Grain Yield and Spike Characters in Early Segregating Generations of Bread Wheat Crosses

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ABSTRACT

The study was carried out to evaluate 20 bread wheat cross populations in F_2, F_3 and F_4 segregating generations, and to determine promising cross combinations with high performance for spike characteristics and grain yield. The five parents of the crosses were also evaluated along with the populations over the years. The ranges of mean value across populations were 8.66-10.94 cm for spike length, 41.08-54.96 number of grains per spike, 2.07-2.50 number of grains per spikelet, 1.89-2.46 g for grain weight per spike, 1.89-2.43 for spike density, 69.8-75.85% for spike index and 3592-5478 kg ha^{-1} for grain yield on average. These ranges were larger for all traits than the across generations indicating that there is sufficient variation for spike traits in the populations studied. Considering the statistical significance among the generations for the investigated spike traits, selection will be more efficient in as early generation as possible for the number of grains per spike, the number of grains per spikelets. Though it is not statistically significant the highest spike length and spike index values were obtained in the F_2 generation means that it may be appropriate to select in early generation for these traits keeping in mid the hybrid vigor may be affecting the traits of interest. It was concluded that it would be more appropriate to make the selection for the remaining spike characters after the F_2 generation. Among the parents used in crosses, Sana, Pehlivan and Krasunia cultivars showed their high performance in spike characteristics, and it might be plausible to use them as parents in wheat breeding studies to increase yield through spike characteristics. The Sana/Flamura 85, Sana/Krasunia, Pehlivan/Sana, Bezostaja1/Krasunia and Krasunia/Pehlivan were noted as the most promising crosses for spike characteristics.

Keywords: Bread wheat, reciprocal hybrids, spike characteristics, variation, generation

Introduction

A significant increase in the production of cereals, which are the most important crop plants, in the next decades, is of critical importance due to the increasing global population. This is particularly difficult as it is anticipated that key manageable resources, which are important components of crop production, will not increase (Connor and Mínguez, 2012) and available land will likely decrease (Albajes, et al., 2013).

Among the most important crops, wheat is one of the most critical for ensuring the nutrition of the world population: because it is the most widely grown crop in the world and accounts for 30% of global cereal production and 45% of grain nutrition (Charmet, 2011), and is the primary source of protein for the world population, representing 20% of daily intake for developing countries (Braun et al., 2010). It is considered to be a situation that can only be achieved by recovering high rates of genetic gains, but that cannot be easily achieved as there is evidence that genetic gains in yield are much lower than required recently (Reynolds et al., 2012). The actual rate of wheat production in recent years has increased by only 0.5% per year, far less than the 1.4% required to deal with the growing human population (Ray et al., 2013). Therefore, improved wheat production should be provided by further increasing the grain yield per area. The possibility of accelerating the breeding

process will increase with the genetic variation information available for traits determining yield in early generations (Reynolds and Borloug, 2006).

Wheat is one of the plants that plays the most critical role in food the world population. Wheat is the most widely grown plant in the world due to its adaptability. Wheat provides 30% of global grain production and 45% of grain nutrition (Charmet , 2011) and in developing countries, it is the primary source of protein in the world's population, accounting for 20% of the daily intake (Braun et al., 2010). These high rates can be achieved by recovering genetic gains, and there is evidence that increases in wheat yield have been low in recent years, and it is considered that the food needed for nutrition in the future cannot be provided by such an increase (Reynolds et al., 2012). It shows that wheat production has increased by 0.5% in recent years, which is well below the 1.4% increase needed by the increasing world population (Ray et al., 2013). In order to meet the food needs, wheat production must be increased by further increasing the grain yield per unit area. Accelerating the breeding process with genetic diversity information for the traits that determine yield in early generations will increase the probability of increasing yield (Reynolds and Borloug, 2006).

Grain weight per spike, considered the final yield component, is the end point in the development of many components that occur in the early growth stages. Since the grain weight per spike has a direct effect on the harvest index, it makes an important contribution to yield formation. It directly reflects the efficient use of nutrients and their transport to productive parts of the plant (Borojevich, 1983). It was stated that grain yield is influenced by spike characters like spike length, a number of grains per spike and spikelet, grain weight per spike, spike density and spike index. For the improvement of wheat yield, these attributes must be improved for selection so that the yield of the wheat can be increased because these have a strong association with the grain yield (Ahmed et al., 2023). Qu et al. (2009) reported that grain yield was improved with the increasing number of grain spikelets because of the increased spikelet number. Grain yield, which is the primary characteristic considered in wheat breeding, is a complex trait that is controlled by many genes and is highly affected by environmental conditions (Shi et al., 2009; Öztürk et al., 2023). The yield, the final product of many processes, is determined directly and multilaterally by the yield components such as productive spikes per unit area, the number of grains per unit area and the total kernel number per unit area that is the product and grain crop per ear (Arbuzova et al., 2010). Wheat spike characters are a key determinant of multiple grain yield components and a detailed examination of spike traits is beneficial to explain wheat grain yield and the effects of differing agronomy and genetics (Zhou et al., 2021).

Analysis of breeding history also revealed wheat grain yield improvement in the last century was highly associated with an increase in grain number per unit area, which is largely determined by the grain number per spike (Hawkesford et al., 2013). Wheat grain number per spike is determined by the combination of the number of spikelets per spike and the number of grains per spikelet and each wheat spikelet has more than one grain. This makes the wheat spikelet one of the most essential grain yield components (Wolde et al., 2019). Other characteristics that affect the total number of grains are considered as the number of fertile tillers per plant, the number of spikelets per spike and the number of fertile flowers per spikelet.

A combination of length and density, a spike is a source of assimilation considered an essential trait of the yield. Spikes are green and functional with the awns (Sharma and Subehi, 2003). In wheat, all parts of the ear, such as the awn, glume, lemma, palea, pericarp and even peduncle, are capable of photosynthesis, and a significant portion of assimilates are obtained from the photosynthesis of these organs (Wang et al., 2001). Especially the awn plays an important role in the grain-filling stages and contributes to large grain and high grain yield in awn wheat varieties (Li et al., 2006). Various results have been obtained on the contribution of a spike to grain yield as an organ that regulates photosynthesis and respiration in many wheat varieties. It has been reported that it contributes 10-76% (Wang et al., 2001) and approximately 22-45% (Maydup et al., 2010), and these rates are higher than any flag leaf or other leaves.

In this direction, it was aimed to determine appropriate promising bread wheat cross combinations and F_2, F_3 and F_4 generations for grain yield and spike characters at F_2, F_3 and F_4 generations in Tekirdağ ecological condition.

Materials and Methods

Twenty bread wheat population and 5 five bread wheat varieties, which are the parents of the populations were used as genetic material in the study (Table 1). No selection was made in the F_2, F_3 and F_4 generations.

The study was conducted in the experimental area of Namık Kemal University Faculty of Agriculture, Department of Field Crops during three growing seasons using randomized complete block design with four replicates. The trials consist of 4 m^2 (20 cm- spaced 4 rows, 5 m long) plots. Seeding density was 500 seeds

per m^2. The sowing time was at the end of October each year, and a total of 160 kg ha^{-1} N fertilization was applied in the sowing, tillering and booting stages divided equally into three. Weeds were chemically controlled.

In this study, grain yield spike length, number of grain per spike, grain weight per spike, number of grain per spikelet, spike density and spike index were measured in the spike of 10 plants in three consecutive segregating populations (F_2, F_3 and $F_{4)}$. The spike density, grains/spikelets and spike index were calculated as:

$$SD=\frac{\text{Spikelets/spike}}{\text{Spike length}}$$

$$G/SL=\frac{\text{Grains/spike}}{\text{Spikelets/spike}}$$

$$SI=\frac{\text{Grain weight/spike}}{\text{Spike weight}}$$

Combined analyses of variance (ANOVA) across generations for grain yield and some spike traits were performed. The "MSTAT version 3.00/EM" package program is used for statistical analysis. The differences among means for parents and populations for each year were determined by Duncan's New Multiple Range Test.

Results and Discussion

The results of combined analyses of variance (ANOVA) including three consecutive generations for each traits indicated that there were highly significant differences among populations means. However, the mean differences between the generation averages were not significant for spike length, grain weight per spike and spike index. The results of the significance test performed to determine the differences between populations and generation averages for each trait examined are shown in Table 2.

The important difference between populations for traits indicates the presence of significant variation between populations in which traits are studied and allow breeders to improve these traits through breeding. The grain yield which is the most important economic trait in wheat improvement is a complex quantitative trait controlled by multiple genes and is highly influenced by environmental conditions (Shi et al., 2009). Since non-genetic effects are large (Bernardo, 2003), early generation selection is expected to be ineffective for grain yield. But, screening of segregating populations can give us ideas for future evaluations.

Spike length: Average spike length values in hybrid combinations varied between 8.66-10.94 cm, and between 9.70-10.41 cm in F_2, F_3 and F_4 segregation progeny. Population 17 showed maximum spike length (10.94 cm), followed by population 16 (10.92 cm), and populations 19 and 9 (10.82 and 10.68 cm). The lowest ear length population 1 (8.66 cm) was obtained. Considering the mean values for spike length in hybrid combinations, combinations 17, 16, 18, 9, 15, 14, 6 and 4 were determined as promising.

Number of grains per spike: The number of grains per ear in the hybrid combinations varied in a wide range between 41.08-54.96, and population 16 took the first place in terms of the number of grains per head with 54.96. Population 14 was ranked with the number of grains in 52.89 spikes and populations 7 and 8 were ranked later with the number of grains in 52.64 and 49.98 spikes. The lowest number of grains per spike was obtained in the number 1 combination with 41.12. When the average grain-per-head performance in hybrid combinations is evaluated, combinations 16, 7, 8, 9, 6, 3, 4, 13 and 17 are the most promising ones.

Number of grains per spikelet: The ranges of average values across hybrid combinations were 2.07-2.50 number of grains per spikelet. Segregation generations have been determined as 1.95-2.48 number of grains per spikelet. Population 7 showed the maximum length of a spike (2.50 no). Populations 16, 20 and 8 had a higher number of grains per spikelet (2.44 no) while populations 9 and 4 had a medium number of grains per spikelet (2.42 and 2.38 no). The minimum number of grains per spikelet was revealed by populations 1 and 19 (2.07 no). When the mean performance of average values cross populations was evaluated, the promising cross combinations were numbered combinations of 16, 7, 8, 20, 9, 4, 10 and 6 for the number of grains per spikelet.

Grain weight per spike: The ranges of average values across hybrid combinations were 1.89-2.46 g for grain weight per spike. When the values of the properties examined in the F_2, F_3 and F_4 segregation generations were examined, they changed according to the generations. These values in segregation generations have been determined as 2.04-2.19 g for grain weight per spike. Population 9 showed maximum grain weight per spike (2.46 g). Population 16 had (2.30 g) grain weight per spike while populations 19 and 6 had the medium grain weight per spike (2.29 and 2.28 g). The minimum grain weight per spike was revealed by population 11 (1.89 g). When the mean performance of average values cross populations was evaluated, the promising cross combinations were numbered combinations of 5, 16, 14, 6 and 7 for grain weight per spike.

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Spike density: Spike density is an agronomical important character of wheat. In addition, an optimized spike structure is a key basis for high yields. The spike is an important part of the wheat plant. Cultivating wheat varieties with longer spike lengths (SL) and higher spike density (SD) could increase yield (Faris et al., 2014; Li et al., 2016). The spike density values in the parents and populations used in the study varied between 1.93-2.43 and between 1.93-2.29 in hybrid combinations. While the highest spike density in hybrid combinations was between 2.29 and 7, this value was lower than the parent number 22. In terms of spike density, the combinations 2.26 and 2.25 and 10 and 20 are listed later. The lowest spike length was obtained in the hybrid combination numbered 1.93 and 15, followed by the hybrid number 18 with a spike density value of 1.89. In terms of spike density, the combinations numbered 7.10, 20 and 12 are the most promising, and as the generations progressed, the spike density increased.

Spike index: One of the potential traits to increase grain weight is the spike harvest index, a major component of grain weight, calculated as the ratio of grain weight to spike dry weight (Pradhan et al., 2019). The genetic basis of the spike index is not clearly understood yet and there is little agreement in the literature regarding the effect, phenotypic variability and genotype by environment interaction (GEI) for the spike index of wheat. The spike index value in the examined parents and populations varied between 69.83-75.85%. In terms of the spike index value in 20 hybrid combinations, the combination numbered 17 with 75.85% was in first place, followed by the hybrid combinations numbered 14 and 6 with 75.25% and 75.01. The lowest spike index is 69.83% in hybrid number 20. According to the data obtained, combinations 17, 14 and 6 are the most suitable combinations in terms of spike index.

Grain yield: The highest value in terms of grain yield was obtained in the parent Sana, number 14, 7, 15, 1, 2 and 8 populations were the superior hybrid combinations. On the other hand, populations 3 and 18 give the lowest performance in terms of grain yield.

When the mean performance of average values of cross combinations evaluated, the promising cross combinations were of 14, 7, 12, 9, 11 and 13 for spike density and 14, 6, 5, 7, 13 and 20 for spike index. These results indicate that 19, 2, 7, 13, 6, 14, 4 and 16 combinations may be promising for spike properties. Pradhan et al. (2019) explained that the spike harvest index is an important spike trait since it shows the main components of the grain number and grain weight in wheat, respectively. Islam et al., (1985) stated inclusion of kernel weight in a selection index with grain per spike or spikelet might have been profitable. Bhatta et al., (2019) reported that the spike harvest index value of genotypes varied between 25 and 91%.

Spike index and grain yield were increased from generation F_2 to F_4 for, there were decreases in spike length, the number of grains per spike and spikelet and spike density. Various results have been reported for the selection of yield and yield components in segregation generations. It has been explained that plant characters carrying the desired gene or allele combinations can be easily identified and selected in early generations, preferably in F_1, before reaching homozygosity in late generations (Cristina and Hall, 1995). Sing and Singh, (1997) reported that selection may be effective for seed weight in early-generation F_2, whereas early-generation selections for harvest index, grain yield and dry matter weight are ineffective in common wheat. Rasmussen (1987) explained that delaying selection to a later generation, such as F_4, can lead to the loss of such desired gene combinations. Islam et al., (1985) pointed out that the selection for the grain number per spikelet or grain number per spike may be more effective than for weight per grain and yield *per se* in the F_2 generation.

Current study shows that the highest variation in spike length, number of grains per spike, grain weight per spike and spike index is in the F_2 generation, and as a result, selection can be started in this generation, at the same time, keeping in mind hybrid effect was still effective in this generation. While the highest variation for the number of grains per spike is in the F_3 generation, the fact that the highest variation for the spike density and grain yield per hectare is in the F_4 generation shows that it is advisable to perform the selection for these characteristics in the F_3-F_4 generations.

Conclusions

It was concluded from the present research that populations showed highly significant variations for all the traits. Based on the results of this study, crosses of (Sana/Flamura 85), (Sana/Krasunia), (Krasunia/Pehlivan), (Pehlivan/Sana) and (Bezostaja1/Krasunia) were the best-performing populations for grain yield and spike traits indicating selected populations from these crosses can be used to improve these traits in breeding. Among the parents used in crosses, Sana, Pehlivan and Krasunia varieties showed high performance for spike characters indicating that it may be appropriate to use them as parents to improve the spike characteristics in subsequent breeding studies. Based on the statistical significance between generations for the traits seems that selection will be more efficient in generation for

the number of grains per spike and spikelets. Although it is statistically insignificant, the fact that the highest spike length and spike index values were obtained in the F_2 generation means that it may be appropriate to select in the very early generation for these traits. It was concluded that it would be more appropriate to make the selection for the remaining spike characters after the F_2 generation.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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Table 1. Cross combinations and their parents used as genetic material.

Crosses		Parents
1. Pehlivan/Flamura 85	11. Bezostaja1/Sana	21. Flamura 85
2. Flamura 85/Pehlivan	12. Sana/Bezostaja 1	22. Sana
3. Bezostaja 1/Flamura 85	13. Krasunia/Sana	23. Krasunia
4. Flamura 85/Bezostaja 1	14. Sana/Krasunia	24. Bezostaja 1
5. Krasunia/Flamura 85	15. Pehlivan/Krasunia	25. Pehlivan
6. Flamura 85/Krasunia	16. Krasunia/Pehlivan	
7. Sana/Flamura 85	17. Bezostaja1/Krasunia	
8. Flamura 85/Sana	18. Krasunia/Bezostaja 1	
9. Pehlivan/Sana	19. Pehlivan/Bezostaja 1	
10. Sana/Pehlivan	20. Bezostaja 1/Pehlivan	

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Table 2. The means of parents and populations used in the experiment for grain yield and spike characteristics.

Crosses and parents	Spike length (cm)	Number of grain per spike (no)	Number of grain per spikelet (no)	Grain weight per spike (g)	Spike density	Spike index (%)	Grain yield (kg ha^{-1})
1	8.66 d*	41.12 f	2.07 c	2.08 ab	2.05 c-g	72.07 a-d	5110 a-d
2	9.54 a-d	45.47 c-f	2.31 abc	2.13 ab	2.09 b-g	73.18 a-d	5059 a-e
3	9.12 bcd	47.16 a-f	2.28 abc	1.95 b	2.03 d-g	70.15 cd	3804 hı
4	10.10 a-d	47.10 a-f	2.38 abc	2.05 ab	2.02 efg	74.04 abc	4440 d-h
5	9.57 a-d	44.68 c-f	2.11 abc	2.04 ab	2.23 a-e	71.38 bcd	5306 ab
6	10.10 a-d	46.44 a-f	2.32 abc	2.28 ab	2.00 fg	75.01 ab	4730 b-g
7	9.41 a-d	52.64 abc	2.50 a	2.18 ab	2.29 ab	74.57 ab	5154 abc
8	9.91 a-d	49.98 a-d	2.44 abc	2.14 ab	2.11 b-f	73.43 a-d	5014 a-e
9	10.68 a	49.96 a-d	2.42 abc	2.46 a	2.02 efg	74.91 ab	4843 a-f
10	9.58 a-d	44.83 c-f	2.36 abc	2.09 ab	2.26 abc	72.68 a-d	4742 b-g
11	9.59 a-d	42.14 def	2.11 abc	1.89 b	2.21 a-e	70.02 cd	4239 f-ı
12	9.77 a-d	46.03 b-f	2.11 abc	2.21 ab	2.24 a-d	73.09 a-d	4147 ghı
13	9.82 a-d	46.84 a-f	2.25 abc	2.16 ab	2.21 a-e	74.16 abc	4888 a-f
14	10.14 a-d	52.89 abc	2.10 bc	2.24 ab	2.07 b-g	75.25 ab	5396 ab
15	10.28 abc	41.41 f	2.14 abc	1.97 b	1.93 fg	71.28 bcd	5100 a-d
16	10.92 a	54.96 a	2.44 abc	2.30 ab	2.11 b-f	73.53 a-d	4762 a-g
17	10.94 a	47.24 a-f	2.20 abc	2.16 ab	2.04 c-g	75.85 a	4007 hı
18	10.82 a	44.89 c-f	2.26 abc	2.15 ab	1.89 g	72.12 a-d	4123 ghı
19	9.83 a-d	41.08 f	2.07 c	2.29 ab	1.95 fg	71.47 bcd	4470 c-h
20	10.21 abc	41.74 e	2.44 abc	2.02 ab	2.25 abc	69.83 d	4381 e-h
PARENTS							
21	9.77 a-d	48.93 a-g	2.41 abc	2.01 b	2.08 b-g	73.74 a-d	4749 a-g
22	8.80 cd	54.12 ab	2.47 ab	2.08 ab	2.43 a	74.82 ab	5478 a
23	10.74 a	49.39 a-f	2.31 abc	2.21 ab	2.02 d-g	73.71 a-d	5071 a-e
24	10.38 ab	42.61 def	2.11 bc	1.92 b	2.06 b-g	70.13 cd	3592 ı
25	9.93 a-d	42.29 def	2.11 bc	2.06 ab	2.05 c-g	75.22 ab	5418 ab
MSE	*0.968*	*26.663*	*0.059*	*0.077*	*0.018*	*12.872*	*1897.925*
GENERATIONS							
F_2	10.41	49.17 a	2.48 a	2.19	1.94 b	74.26	4563 b
F_3	9.7	49.36 a	2.37 ab	2.15	2.11 a	72.97	4542 b
F_4	9.71	41.38 b	1.95 b	2.04	2.26 a	71.84	5059 a
MSE	*11.343*	*175.86*	*175.86*		*0.067*		*2200.253*

* The differences between the means for each trait denoted by the same letter are statistically insignificant.

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Assessment of Yield Performance and Stability of Winter Barley (*Hordeum vulgare* L.) Genotypes under Rainfed Conditions of Central Anatolia and Transition Regions

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ABSTRACT

Barley (*Hordeum vulgare* L.) is one of the most important agricultural crops in Türkiye. It is imperative to increase the grain yield of barley to meet the growing demand. One of the most important ways to do this is to develop high-yielding cultivars with good adaptation to varying environmental conditions. In breeding studies, the performance of candidate lines should be determined through multiple yield trials. In this study, eighteen advanced barley lines with six check cultivars were planted in nine locations under rainfed conditions of Central Anatolia and transitional regions in the 2019-2020 growing season. In addition to the yield performance of the genotypes, their yield stability in nine locations was also determined. According to the result of the study, the highest and lowest-yielding locations were Konya and Afyonkarahisar, respectively. This study revealed Sayım 40 had higher grain yield potential in Central Anatolia. Additionally, advanced line G23 was identified as the most promising feed barley genotype yielding approximately 3959 kg/ha across nine locations in the Central and transitional ecological zones of Anatolia.

Keywords: Barley, grain yield, multi-location trials

Introduction

Barley (*Hordeum vulgare* L.), one of the world's earliest crops, was cultivated in the Fertile Crescent 10500 years ago (Saisho and Purugganan, 2007). Today, barley is grown worldwide in both highly productive agricultural areas and harsh environments where cultivation is a challenge. In recent years, global barley grain production has reached approximately 150 million tons (FAOSTAT, 2023). Barley is traded worldwide and is of great economic importance for both feeding livestock and producing malting beverages (Newton et al., 2011).

Türkiye, one of the world's major barley-producing countries, produces about 8.5 million tons of barley grain on 3.2 million hectares of agricultural land (TSI 2023). In Türkiye, barley production is predominantly sustained in dryland areas, and the grain yield is highly impacted by the amount and distribution of annual rainfall throughout the growing season (Tokgöz 1997). In such areas, it is necessary to develop new varieties that are resistant to biotic and abiotic stress factors. Across diverse environmental conditions, conducting multi-location trials to assess the genotypic performances of the plant materials holds crucial importance for a breeding scheme (Lee et al., 2023).

The selection of high-yielding and stable genotypes is an unsubstituted strategy for developing new cultivars in rainfed conditions across various sub agro-ecologic zones. The ability of candidate genotypes to adapt to multi-environments increases their effectiveness (Becker and Leon, 1998). For selected traits, various statistical methods were applied to interpret the genotype-by-environment interactions

in diverse environments (Pour-Aboughadareh et al., 2022). The linear regression models are commonly used to calculate yield stability parameters (Sabaghnia et al., 2013).

In this study, eighteen advanced winter barley lines developed as part of the barley breeding studies of the Central Research Institute of Field Crops were observed in multi-location trials and compared with six check cultivars widely cultivated in the region. The yield performance of the candidate genotypes was analyzed based on the linear regression model across nine different locations. The study aimed to identify candidate lines with high and stable grain yield across Central Anatolia and the transitional regions.

Materials and Methods

Using six check cultivars, seven malting and eleven feed barley regional yield trial lines (MBRYT and FBRYT; hereafter referred to as G) developed by the Central Research Institute for Field Crops were tested as plant material in the study. All genotypes used in the study are given in Table 1.

The yield trials were conducted at nine locations (L) across Central Anatolia and the transition regions in the 2019-2020 growing season. Monthly precipitation amounts for the locations where the trials were carried out in the 2019-2020 growing season are given in Table 2. During the 2019-2020 growing season, the average precipitation varied from 208.8 to 413.0 mm throughout the locations of Ikizce (L1), Altınova (L2), Gözlü (L3), Malya (L4), Ulaş (L5), Sarkışla (L6), Konya (L7), Karapınar (L8) and Afyonkarahisar (L9). The total precipitation values in majority of the locations were lower than the long-term average (Table 2). The soil conditions at all locations were slightly alkaline and poor in organic matter while the levels of P_2O_5, K_2O, and $CaCO_3$ were relatively reasonable.

In all locations, field trials were arranged according to a randomized complete block design (RCBD) with four replicates. The experiments were conducted with a plot seeder during 15-30 October 2019. Genotypes were sown in plots (5 m long and 1.08 m wide) with a seed density of 500 seeds per m^2. As for fertilizer application, all the phosphorus (70 kg P_2O_5/ha) and half of the nitrogen (35 kg N/ha) were applied as di ammonium phosphate (DAP 18-46%) along with sowing. The remaining nitrogen (Ammonium nitrate, 35 kg N/ha) was applied following the tillering stage in spring 2019-20. Genotypes were harvested with a plot harvester when the grain moisture content was approximately 12%, and the grain yield values were converted to kg/ha.

Significance levels of differences between grain yields of genotypes and locations were determined using combined analysis of variance (ANOVA), and then genotypes were ranked using Student's t multiple comparison tests (LSD) (Montgomery 2013). Regression coefficient (b), coefficient of determination (R^2), deviation from regression (S^2d_i), and coefficient of variance (CV) were used as stability parameters. The stability parameters used here are a function of the deviations and slope from the regression of genotype yield on the environmental index introduced by Finlay and Wilkinson (1963), Eberhart and Russell (1966), Pinthus (1973) and the environmental coefficient of variance Francis and Kannenberg (1978). In addition, a Bi-Plot graph was created via Principal Component Analysis (PCA) to show the similarity of locations to each other and the specific adaptations of genotypes to environments (Yan and Tinker 2006). Combined analysis of variance and principal component analysis (PCA) were performed in the JMP 11 statistical package. Stability analysis was performed using the *avcıistatistik*® Excel add-in (Avcı, 2023) and the STABILITYSOFT, an online stability analysis platform (Pour-Aboughadareh et al., 2019).

Results and Discussion

During the 2019-2020 growing season, the combined analysis of variance results for grain yield indicates that the main effects of genotypes (G), locations (L), and the G by L interactions, were statistically significant at the $p<0.01$ level across nine locations (Table 3).

As the interactions between locations and genotypes were found to be statistically significant, the analyses of the variance of the genotypes were performed separately according to the locations in which the experiments were carried out. As a result of the variance analyses, the differences between the yield means of the genotypes in all locations were found to be statistically significant at the $p<0.01$ level. The grain yield values of the genotypes in nine locations and their overall mean yields are shown in Table 4. The grand mean yield of all locations was 3545 kg/da. Previous studies also reported similar yield results in this region (Akgun et al. 2012; Yüksel and Akcura 2012; Ergün et al. 2023). Among the locations, the highest yield was obtained from Konya (L7) with 5406 kg/ha, and the lowest yield was obtained from Afyonkarahisar (L9) with 2114 kg/ha. Konya was followed by Sarkısla with 4627 kg/ha and Ikizce with 4346 kg/ha (Table 4).

When the genotypes with the highest grain yield in the locations where the experiments were conducted, in Ikizce (L1), Gozlu (L3), Konya (L7), and Karapınar (L8),

were considered one by one, cv. Sayım 40 came first with yields of 5594, 5788, 6295 and 3269 kg/ha respectively. In Altınova (L2) and Malya (L4), cv. Larende was the first-ranked cultivar in these locations with yields of 3283 and 3534 kg/ha, respectively. In Ulaş (L5), cv. Asil (5241 kg/ha) and at Sarkısla (L6), numbered line G13 (5509 kg/ha) were the barley genotypes with the highest yields in these locations. In Afyonkarahisar (L9), which has the lowest average grain yield, cv. Tarm-92 was found as the highest-yielding genotype in this location with a grain yield of 2928 kg/ha. The cv. Sayım 40 ranked first among all genotypes with a grain yield of 4392 kg/ha, followed by cv. Larende with a grain yield level of 4076 kg/ha when considering the general mean data of the genotypes. Among the candidate lines, G23 was found to be the highest-yielding line (3959 kg/ha) in the same statistical group as these two registered cultivars. Cv. Burakbey was the fourth highest-yielding cultivar with a yield level of 3913 kg/ha after these genotypes (Table 4).

The data from the stability analyses carried out to evaluate the responses of the barley genotypes to different locations and to determine the most suitable areas are given in Table 5. When examining the (a) value, which is one of the determinants of the adaptability of genotypes for favorable or unfavorable locations. It was observed that the cv. Tarm 92 (1437.25) and the line G9 (1419.05) had the highest values. This result shows that these genotypes can adapt well to low-yielding locational conditions. On the other hand, G16, G4, G6, and G8 were the genotypes with negative and the lowest (a) values (-1466.29, -1197.10, -987.22 and -558.27 respectively). This indicates that these genotypes may be more suitable for favorable locations. The regression coefficient (b) is one of the most widely utilized indicators of yield stability (Akçura et al. 2005). If the b value of a genotype is closer to 1, this genotype is considered to have wide adaptability and good stability. Genotypes with a b-value less than 1 are well adapted to unfavorable locational conditions, while genotypes with a b-value greater than 1 are better adapted to high-yielding locational conditions (Finlay and Wilkinson 1963). Genotypes with yields close to the mean, b values around 1 and deviations from regression (S^2d_i) as close to zero as possible can be characterized as stable (Eberhart and Russel 1966). Among the genotypes in the study, the genotypes with b values closest to 1 were G11 and G17 with b values of 0.99 (Table 5 and Figure 1). In addition, G16, G14, cv. Asil, G2, G19 and cv. Tosunpaşa are the genotypes that can be classified as the most stable when their b values are considered. However, when genotypes above the average yield (3545 kg/ha) are considered, cv. Asil, cv. Tosunpaşa, G14, G19 and G11 are the genotypes with both stable and sufficiently high yields (Table 5 and Figure 1). According to the Bi-Plot stability graph generated according to regression coefficient (b) and average grain yields (kg/ha) of the genotypes (Figure 1), lines G23 and G6 increase their yield potential as locational conditions become more favorable. In addition, cv. Sayım 40, cv. Burakbey and the line G13 also showed acceptable levels of stability and it can be said that the yield potential of these genotypes is higher in favorable locations (Figure 1). On the other hand, Figure 1 reveals that the cultivars Tarm 92 and Larende have moderate stability when considering the b value and these genotypes can be classified as good adapted to unfavorable locational conditions.

The coefficient of determination (R^2) is another important parameter relating to stability and its higher value indicates that the genotype is more stable (Teich 1983). Among the genotypes in the study, the highest R^2 values were observed in lines G19 and G22 with 0.98, followed by G2 with 0.97 and G12 with 0.96. The lowest values were observed in lines G9 (0.45) and G18 (0.60). The R^2 values of lines G11 and G17, which had the closest b values to 1, were as high as 0.93 (Table 5). Another common method of assessing yield stability is to examine the deviation from regression. A deviation from regression (S^2d_i) is a measure of how much the yield of a particular genotype deviates from the yield predicted by the regression model under specific environmental conditions. The lower value of this parameter is interpreted as an indication that the yield of the genotype is close to the expected and more stable (Eberhart and Russell 1966; Teich 1983). Among the lines and cultivars in the study, the lowest S^2d_i values were found in lines G19, G22, G12, and G2. While cv. Tosunpaşa had the lowest S^2d_i value among the cultivars, the other cultivars generally had high values for this parameter. This value is relatively low in G11 and G17, which have the b value closest to 1 among the lines. The genotypes with the greatest deviation from the regression are the lines G18 and G9, which also have the lowest b values (Table 5). Francis and Kannenberg (1978) determined the stability of genotypes by evaluating the coefficient of variation (CV) and yield values together. In this concept, genotypes were divided into four groups according to low or high CV and yield values and it was suggested that genotypes with low CV and high yield could be defined as the most desirable group. Among the genotypes with the lowest CV values and yields above the overall mean were the cultivars Tarm 92, Larende, Sayım 40 and Asil and the lines G11 and G13 (Table 5).

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Bi-Plot analysis has become a useful statistical technique in plant breeding and agricultural studies (Yan and Tinker 2006). In the Bi-Plot generated from principal component analysis (PCA), the first two principal components (PC1 54.4% and PC2 24.6%) explained 79% of the total variation (Figure 2) in the yield of the genotypes across locations. PC1 shows a close relationship with the average yield values of the genotypes, while PC2 gives information about the stability (b value) of the genotypes. More stable genotypes are closer to the center of the PC2 axis. Ikizce (L1) and Konya (L7) were the most representative locations in PC1, while Sarkısla (L6) and Malya (L4) were the most representative locations in PC2. The distance between two locations is a function of their differences in genotype discrimination (Yan and Tinker 2006). According to the Bi-Plot graph, the experimental locations are divided into two main groups. While L3, L6 and L7 formed one group among themselves, L1, L2, L4, L5, L8 and L9 formed another main group and were the locations with the most similar results in the 2019-2020 growing season. Cv. Sayım 40 generally ranked the first in all locations. G23 seems to be a genotype better adapted to L7, L3 and L6 locations. On the other hand, G6 appears to be a genotype better adapted to higher-yielding locations (L3, L6 and L7). The cultivars Tarm 92, Asil, and Larende stood out, particularly in the L4, L5 and L9 locations. This shows that these varieties can perform well in unfavorable locations (Figure 2).

Conclusions

The differences among the grain yields of the barley genotypes used in this study and the locations as well as their interactions were statistically significant. Among all the genotypes, cv. Sayım 40 was the highest-yielding genotype, ranking first in four out of the nine locations across the Central Anatolia and Transitional Regions. It was followed by cv. Larende, line G23 and cv. Burakbey regarding high grain yield potential over the region. When the genotypes were considered in terms of grain yield and multiple stability parameters, the most stable and above-average yielding genotypes were identified as cv. Asil and cv. Tosunpaşa varieties and lines G23, G19, G13 and G11. Cv. Tarm 92 maintained its high performance in less favorable conditions. Overall, these results indicate that, cv. Sayım 40 highest yielding cultivar for Central Anatolia and Transitional Regions, while line G23 was the most promising barley line. The findings of this study suggest that line G23 can be evaluated for variety registration.

Conflict of interests

The authors declare that they have no conflict interests.

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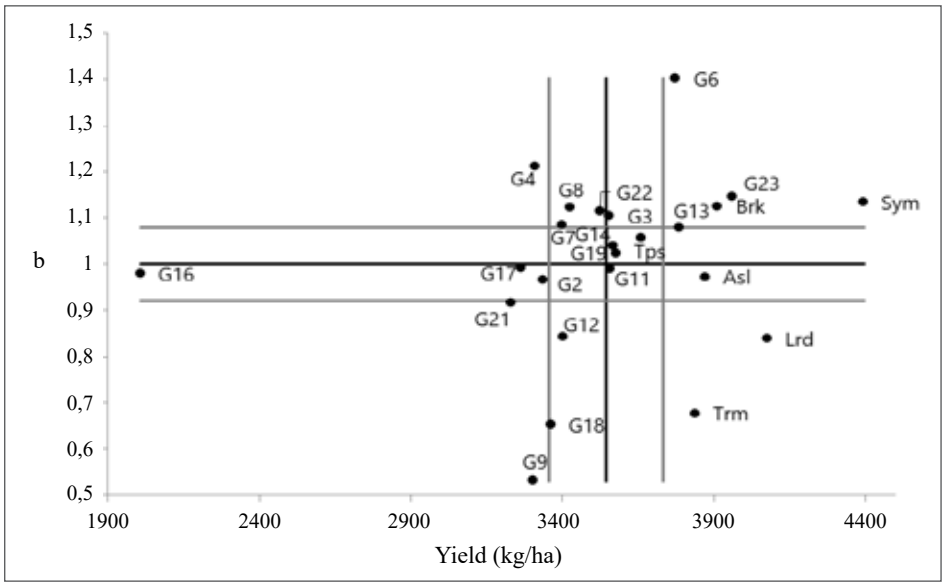

Figure 1. Two-way stability graph showing b value and yield averages of genotypes.

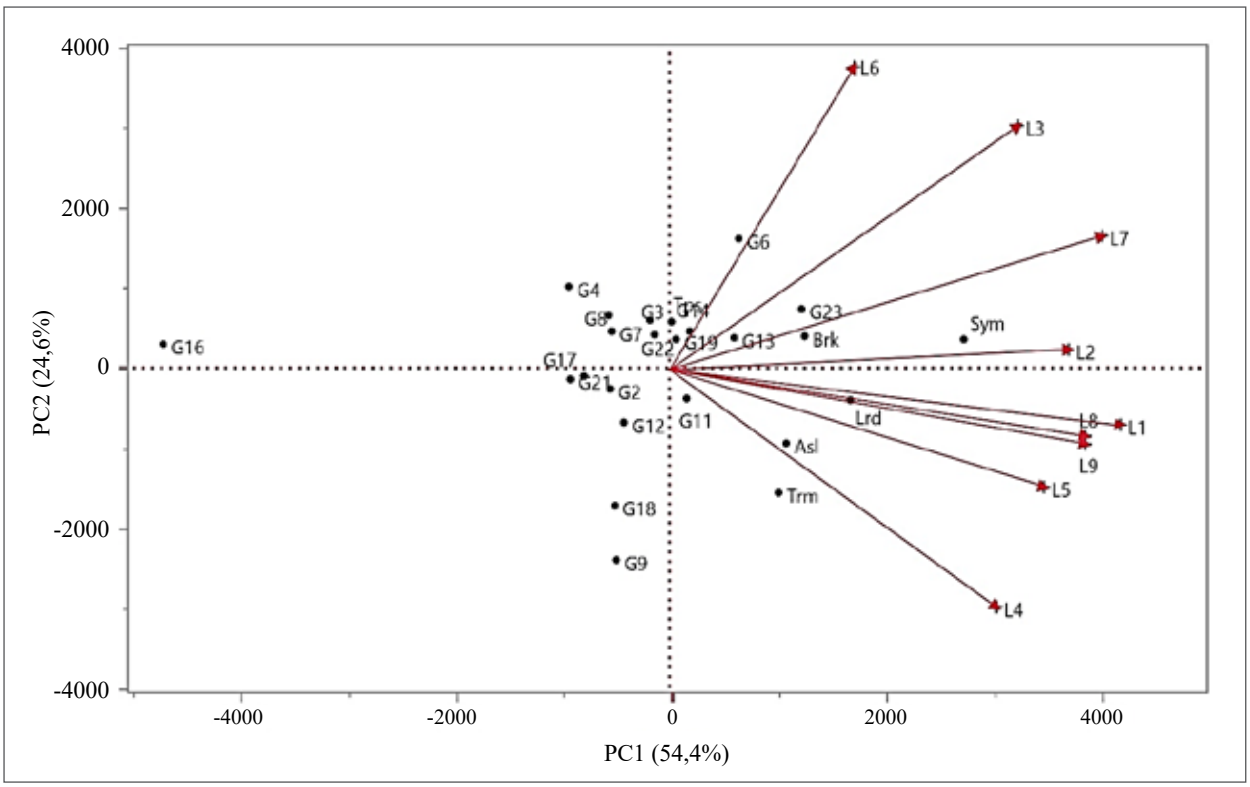

Figure 2. Bi-Plot displaying the result of Principal Component Analysis (PCA).

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Table 1. Barley genotypes in the multi-location yield trials.

Number	Identifier	Lines/Cultivars Name	Number	Identifier	Lines/Cultivars Name
1	Trm	Tarm 92 (Check)	13	G13	FBRYT -1-110
2	G2	MBRYT -1	14	G14	FBRYT -1-111
3	G3	MBRYT -4	15	Tps	Tosunpaşa (Check)
4	G4	MBRYT -5	16	G16	FBRYT -1-117
5	Brk	Burakbey (Check)	17	G17	FBRYT -1-122
6	G6	MBRYT -9	18	G18	FBRYT -2-201
7	G7	MBRYT -11	19	G19	FBRYT -2-202
8	G8	MBRYT -13	20	Asl	Asil (Check)
9	G9	MBRYT -23	21	G21	FBRYT -2-208
10	Sym	Sayım 40 (Check)	22	G22	FBRYT -2-221
11	G11	FBRYT -1-103	23	G23	FBRYT -2-222
12	G12	FBRYT -1-104	24	Lrd	Larende (Check)

MBRYT: Malting barley regional yield trial, FBRYT: Feed barley regional yield trial, G: Genotype

Table 2. Monthly and annual total precipitation (mm) data for the experimental locations.

Locations	Years	October	November	December	January	February	March	April	May	June	Total
Ikizce (L1)	19-20	23.4	31.8	50.8	28.6	38.7	13.8	28.6	47.8	27.0	290.5
	LT	22.7	29.1	37.7	36.3	34.0	35.7	40.2	46.9	35.7	318.3
Altınova (L2)	19-20	5.0	16.0	34.0	35.0	52.0	30.0	23.8	48.2	45.0	289.0
	LT	25.0	22.7	34.5	36.7	25.5	36.0	22.0	38.5	31.6	272.5
Gozlu (L3)	19-20	3.2	7.0	29.2	22.4	43.6	17.6	29.6	30.0	26.2	208.8
	LT	27.6	25.3	40.8	36.7	22.2	27.7	18.4	35.5	38.0	272.2
Malya (L4)	19-20	0.0	14.0	54.0	24.0	46.0	22.0	21.0	31.0	5.0	217.0
	LT	23.0	25.7	31.0	45.0	30.5	31.9	28.4	37.9	28.9	282.3
Ulas (L5)	19-20	23.0	13.0	16.3	42.3	57.0	60.3	14.7	32.1	38.9	297.6
	LT	37.9	36.1	23.8	34.8	29.0	38.2	37.7	54.7	47.1	339.3
Sarkısla (L6)	19-20	7.8	16.5	19.3	24.2	48.2	51.5	22.3	45.4	102.6	337.8
	LT	24.0	30.0	48.0	44.0	34.0	41.0	58.0	47.0	35.0	361.0
Konya (L7)	19-20	13.0	45.8	112.4	36.0	29.0	6.4	3.4	23.4	35.8	305.2
	LT	32.7	34.1	42.4	36.6	24.7	27.1	35.4	41.7	26.6	301.3
Karapınar (L8)	19-20	13.8	31.0	142.6	71.2	27.6	48.2	8.8	18.4	7.2	368.8
	LT	29.0	38.8	37.7	28.8	26.5	23.0	25.1	23.4	14.2	246.5
A.karahisar (L9)	19-20	11.1	11.7	64.2	63.3	57.8	42.2	18.2	83.5	60.6	412.6
	LT	35.3	33.2	46.7	44.9	39.7	45.0	45.1	54.5	42.0	386.4

19-20 : 2019-2020 growing period; LT : Long-term average (20 years)

Table 3. Result of combined analysis of variance.

Source	DF	Mean Square	F Ratio
Genotypes	23	69095	22.63*
Replications (Location)	27	31187	10.21*
Locations	8	1251293	409.78*
Genotype by Locations	184	8357	2.74*
Error	621	3053	-

*Statistically significant at p<0.01 level; DF: Degrees of Freedom

Table 4. Grain yield data of barley genotypes in nine locations (kg/ha).

Genotype	L1	L2	L3	L4	L5	L6	L7	L8	L9	Mean
Tarm-92	4776 ad*	2877 a	3524 ık	3472 ab	4495 ad	4054 bc	5433 ad	2980 ac	2928 a	3837 bf
G2	4302 bf	2670 a	3766 fj	1782 df	3894 be	4116 bc	5004 ad	2235 fg	2268 bg	3337 gı
G3	3714 df	3087 a	4278 dh	1846 df	4325 ae	5126 ab	5258 ad	2526 af	1835 gk	3555 eı
G4	3541 ef	2908 a	3973 fj	1163 fg	3711 ce	5140 ab	5358 ad	2286 eg	1695 ık	3308 hı
Burakbey	5383 ab	3162 a	4950 bd	2035 df	4079 be	4557 ab	5733 ac	2826 ae	2488 ad	3913 bd
G6	4652 ae	3202 a	5116 ac	1416 eg	3689 ce	5332 ab	6153 ab	2519 af	1878 fk	3773 bf
G7	3758 df	3013 a	4168 eı	1857 df	3667 ce	4647 ab	5359 ad	2709 af	1415 k	3399 gı
G8	3498 f	2957 a	4153 eı	1903 df	3959 be	5137 ab	5279 ad	2365 df	1578 jk	3425 gı
G9	4538 af	3025 a	2663 l	3080 ac	4048 be	2876 c	4639 ce	2900 ad	1955 ej	3303 hı
Sayım 40	5594 a	3044 a	5788 a	3078 ac	4820 ab	4914 ab	6295 a	3269 a	2730 ab	4392 a
G11	4638 af	2850 a	3781 fj	2137 cf	3884 be	4091 bc	5667 ad	2720 af	2255 cg	3558 eı
G12	4208 cf	2859 a	3506 ık	2536 bd	3880 be	4373 ab	4802 bd	2469 cf	1998 ej	3403 gı
G13	4730 ae	3033 a	4074 eı	2227 ce	4108 be	5509 a	5365 ad	2424 df	2603 ac	3786 bf
G14	4777 ad	2786 a	4282 dh	1902 df	3348 e	5331 ab	4809 bd	2439 cf	2518 ac	3577 dh
Tosunpaşa	3831 df	3092 a	4476 cf	2156 cf	4327 ae	4997 ab	5394 ad	2735 af	1935 ej	3660 cg
G16	2130 g	1368 b	2908 kl	600 g	2159 f	3963 bc	3298 e	933 h	705 l	2007 j
G17	4325 bf	3193 a	3716 gj	1772 df	3401 e	4112 bc	5016 ad	1810 g	2025 dj	3263 hı
G18	3850 df	2806 a	3316 jl	3490 ab	4614 ac	3973 bc	4279 de	2205 fg	1733 hk	3363 gı
G19	4513 af	2843 a	4391 cg	1970 df	3744 be	4803 ab	5103 ad	2541 af	2198 ch	3567 eh
Asil	5124 ad	2850 a	3621 hk	2621 bd	5241 a	4452 ab	5515 ad	3033 ab	2390 be	3872 be
G21	4381 bf	2584 a	3554 hk	1360 eg	3478 de	4206 ab	4642 ce	2543 af	2325 bf	3230 ı
G22	4288 bf	2831 a	4020 fj	1686 df	4062 be	4948 ab	5299 ad	2464 cf	2138 cı	3526 fı
G23	4596 af	3036 a	5231 ab	2686 bd	4161 ae	5312 ab	5768 ac	2588 af	2255 cg	3959 bc
Larende	5150 ac	3283 a	4783 be	3934 a	3744 be	5074 ab	5477 ad	2351 eg	2885 a	4076 ab
Mean	4346	2890	4085	2196	3951	4627	5406	2494	2114	3545
CV (%)	13.8	16.2	13.0	24.7	14.7	15.9	14.4	11.6	11.8	15.5
F Ratio	5.9*	2.4*	7.6 *	8.5*	4.1*	2.9*	2.7*	9.8*	15.8*	22.6*
LSD	1126	876	998	1019	1089	1377	1406	542	468	3365

* Means with the same letter are statistically in the same group, LSD: Least significant differences (0.01)

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Table 5. Stability parameters related to grain yield of barley genotypes.

Genotypes	Yield (kg/ha)	Rank	a	b	R^2	S^2d_i	CV
Trm	3837	6	1437.25	0.68	0.72	263489.64	23.71
G2	3337	19	-84.74	0.97	0.97	45356.09	33.55
G3	3555	13	-359.53	1.10	0.93	136287.86	36.77
G4	3308	20	-987.22	1.21	0.92	188294.07	43.57
Brk	3913	4	-76.37	1.13	0.92	156804.49	34.16
G6	3773	8	-1197.10	1.40	0.94	176146.70	43.67
G7	3399	17	-447.43	1.08	0.93	121939.64	37.69
G8	3425	15	-558.27	1.12	0.92	156308.19	38.97
G9	3303	21	1419.05	0.53	0.45	517384.75	27.42
Sym	4392	1	370.33	1.13	0.89	231094.87	31.21
G11	3558	12	47.93	0.99	0.93	107896.40	32.92
G12	3403	16	415.61	0.84	0.96	39578.52	28.79
G13	3786	7	-38.92	1.08	0.95	93821.38	33.40
G14	3577	10	-55.04	1.02	0.88	213071.95	34.85
Tps	3660	9	-85.08	1.06	0.94	101640.65	33.94
G16	2007	24	-1466.29	0.98	0.86	224602.96	59.94
G17	3263	22	-251.05	0.99	0.93	110247.76	35.96
G18	3363	18	1051.23	0.65	0.60	415525.98	28.48
G19	3567	11	-114.64	1.04	0.98	35621.92	33.60
Asl	3872	5	424.56	0.97	0.82	313015.75	31.70
G21	3230	23	-20.00	0.92	0.91	125194.24	33.99
G22	3526	14	-424.01	1.11	0.98	36817.00	36.43
G23	3959	3	-103.49	1.15	0.95	104718.52	33.91
Lrd	4076	2	1103.23	0.84	0.75	348306.45	27.11

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Morphological Characterization of Different *Ocimum* spp. Germplasm Lines under Semi-Arid Region of Haryana

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ABSTRACT

Knowledge about the genetic variation among the various plant traits enables the plant breeders to effectively utilize the available germplasm lines for the development of elite genotypes. The experimental material comprising of 40 germplasm lines of Tulsi (*Ocimum* spp.) received from National Bureau of Plant Genetic Resources, New Delhi were characterized for different morphological traits viz. leaf colour, leaf shape, leaf pubescence, stem colour, stem pubescence, petiole colour, flower colour, calyx colour, calyx pubescence, seed colour, plant height, inflorescence length and fresh herbage yield. The investigation was carried out in the Research Area of Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during *Kharif* 2022 and 2023. Among the various categories of different traits/descriptors, the maximum number of germplasm lines were observed for leaf lamina colour-green (25 germplasm lines), leaf shape-elliptical (40), stem colour-green (28), stem pubescence-pubescen t(5), petiole colour-green (36), flower colour-white(27), calyx colour-green (25), calyx pubescence-pubescent (13), seed colour-dull black (35). Plant height ranged from 70 cm (EC 388895) to 119 cm (IC 381185) and inflorescence length from 9.3 cm (IC 75730 and IC 381185) to 24 cm (IC 387838 and EC 469904). Maximum fresh herbage yield per plant was found for the genotype EC 388890 (1250 g) and minimum for IC 387838 (290 g). The study revealed that sufficient genetic variability was present among different germplasm lines. This genetic variability may further be exploited for crop improvement programme to develop improved varieties using appropriate breeding methodology.

Keywords: Tulsi germplasm, morphological characterization, genetic variability

Introduction

The genus *Ocimum* is represented by 66 species across the world. *Ocimum* is a versatile aromatic and most important genus of the family Lamiaceae due to its immense use in traditional system of medicine, perfumery and pharmaceutical industry (Simpson and Conner, 1986). All the aromatic plants belonging to the genus *Ocimum* are collectively called Basil. In India, so far about 9 species of *Ocimum* (*Ocimum tenuiflorum* L., *O. basilicum* L., *O. gratissimum* L., *O. kilimandscharicum* L., *O. micranthum* L., *O. campechianum* L., *O. americanum* L., *O. minimum* L. and *O. citriodorum* L.) have been reported of which the last three are exotic species (Balyan and Pushpangadan, 1988). The *O. basilicum* is known by various names such as 'Sweet Basil', 'Common Basil' or 'French Basil'. The *O. canum* species with a peculiar mint smell is known as 'Mint Basil'. The camphor containing species *O. kilimandscharicum* is commonly called 'Camphor Basil'. The species *O. canum* having borneol smell is known as 'Hoasy Basil' and the species *O. gratissimum* with high contents of eugenol is known as 'Spice Basil'. Hindus worship the plants of *O. sanctum* hence it is popularly known as 'Sacred

Basil' or 'Holy Basil'. The genus *Ocimum* exhibits a range of chromosome numbers, including various haploid chromosome numbers (12, 13, 16, 20, 24, 32, 36 and 38) in addition to the basic chromosome number. According to Carovic et al., (2010), the basic chromosome number for *Ocimum* species is x=12. Moreover, *O. basilicum* and *O. americanum* are known to be tetraploid (2n=4x=48) and hexaploid (2n=6x=72), respectively (Sobti and Pushpangadan, 1979).

The *Ocimum* species showcase remarkable morphological diversity owing to significant variations in their leaf size, shape, colour and pubescence, which have evolved over the centuries of cultivation. They are highly branched and can grow to a height ranging from 60-150 cm. Their stems and twigs are quadrangular in shape. The leaves are simple and have petioles and appear in a range of shapes from elliptical to ovate and have either entire or serrated margins. The leaves of these plants also bear sessile glands which secrete strongly scented volatile oils with aromatic flavours. The plants have small flowers that are white or purple in colour. These flowers are hermaphrodite and zygomorphic in nature. They are arranged in whorls on racemose inflorescence. The flowers have didynamous stamens and a style with bifid stigma. After the successful process of entomophilous pollination, the corolla naturally detaches and gives way to the development of four round seeds inside the bilabiate calyx. The seed's shape varies from elliptical to globose and becomes mucilaginous when wetted (Pushpangadan and Bradu, 1995).

Plants are the primary source of secondary metabolites and oils with therapeutic potential due to which people have relied on plant-based medicines for health care since the dawn of civilization. The therapeutic properties of *Ocimum* have been acknowledged since ancient times, not only in India but also in the ancient civilizations of China, West Asia, Europe and Africa. This recognition has elevated the status of *Ocimum* spp. to a highly valued medicinal and aromatic crop plants. Tulsi is a valuable natural source of various essential oils and fragrant compounds that hold great economic and medicinal significance. Its essential oils contain several notable chemicals including eugenol, methyl eugenol, thymol, linalool, methyl chavicol, camphor, citral, elimicin, sesquiterpene alcohols, linalyl acetate, geraniol, and methyl cinnamate (Khosla et al., 2000). The medicinal herb *O. basilicum* has generally been used to cure renal problems, warts, worms, cough, diarrhoea and headaches. Its oil is directly applied to the skin for treating acne and externally it can be used as an ointment for bug bites (Javanmardi et al., 2002). Its seeds are used in treatment of dysentery and chronic diarrhoea (Gangrade et al., 2000). Oil of *O. gratissimum* has been recommended for use in biological mosquito control because of its ability to repel insects. Leaves of *O. canum* are used to cure a variety of eye conditions, bronchitis as well as parasitic skin conditions (Naithani and Kakkar, 2002).

Materials and Methods

Experimental material: The study was carried out in the research area of the Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant breeding, CCS Haryana Agricultural University, Hisar in the *Kharif* season of the years 2022 and 2023. The experimental material was comprised of 40 genotypes of Tulsi (*Ocimum* spp.). Among these, 38 genotypes were received from ICAR- National Bureau of Plant Genetic Resources (NBPGR), New Delhi and 2 genotypes from the germplasm pool maintained at the Medicinal, Aromatic and Potential Crops Section, which were characterized for morphological traits. The species to which each of the studied genotypes belongs is presented in Table 1.

Field layout of experiment: To conduct the experiment, all the 40 genotypes of Tulsi were grown in the Research Area of the Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during *Kharif* 2022 and 2023. Hisar is located in semi-arid sub-tropical region at 29°-10°N latitude and 75°-46°E longitude with elevation of 215.52 m above mean sea level.

Nursery preparation and Transplanting: To raise the healthy plants, nursery was prepared by sowing the seeds of all the 40 different genotypes on the raised beds separately. Light irrigation was given to the young seedlings as per requirement for good growth. Twenty days old young healthy seedlings of all the 40 different genotypes were transplanted in the Randomized Block Design (RBD) with three replications. One seedling per hill was transplanted at 10 cm spacing between the plants in two rows each of 3 m length with a row to row distance of 30 cm. A light irrigation was applied immediately after transplanting. All the required cultural practices were followed to raise the good Tulsi crop.

Recording of observations: The observations for all the 40 genotypes were recorded at appropriate plant growth stage i.e. from flowering to reproductive stage of the crop for the following 10 different qualitative traits: Stem colour, Stem pubescence, Leaf shape, Leaf colour, Leaf pubescence, Petiole colour, Flower colour,

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Calyx colour, Calyx pubescence and Seed colour. All the morphological observations were recorded at flowering stage. Under natural field conditions, the data for the different traits were recorded on five randomly selected plants from each genotype in each replication.

Results

The 40 genotypes of Tulsi (*Ocimum* spp.) taken for the present investigation was characterized for 10 different qualitative traits mentioned as below:

Stem colour

Based on stem colour, five different groups *viz.* dark green, green, light green, purple, and light purple (Figure 1) were observed among 40 different genotypes of Tulsi and all the genotypes were categorized accordingly (Table 2, Figure11a). Four genotypes exhibited dark green stem colour, 28 genotypes green stem colour whereas light green stem colour appeared only in one genotype. Purple stem colour was observed in five genotypes and two genotypes displayed light purple stem colour.

Stem pubescence

On the basis of the presence or absence of the hairs on the stem, Tulsi genotypes were classified as sparse pubescent and non-pubescent (Figure 2). Five genotypes were observed to have sparse pubescence whereas the remaining 35 genotypes had no pubescence (Figure 11b).

Leaf shape

Based on the leaf shape, Tulsi genotypes may be characterized into three groups such as elliptical, sub-ovate and ovate. But, in the present investigation only elliptical leaf shape (Figure 3) was observed for all the 40 genotypes (Figure 11c). None of the genotypes showed sub-ovate and ovate leaf shape.

Leaf colour

All the 40 Tulsi genotypes were categorized into four different groups (dark green, green, light green and purple) based on the colour of their leaves (Figure 4). Dark green leaf colour was observed for 8 genotypes, green leaf colour for 25 genotypes, light green leaf colour for 6 genotypes whereas only one genotype exhibited purple leaf colour (Figure 11d).

Leaf pubescence

On the basis of the presence or absence of the hairs on the leaf surface, Tulsi genotypes can be classified as sparse pubescent, dense pubescent and non-pubescent (Figure 5). In the present investigation, only two genotypes were observed to have sparse pubescence. Dense pubescent was not observed in any of the genotype whereas 38 genotypes showed non-pubescent characteristic for leaf pubescence (Figure 11e)

Petiole colour

Fourty Tulsi genotypes were classified into three different groups based on their petiole colour namely green, purple and purple green (Figure 6). Thirty-six genotypes exhibited green petiole colour, one purple petiole colour whereas three genotypes displayed purple green petiole colour (Figure 11f).

Flower colour

On the basis of flower colour, Tulsi genotypes were classified into three categories which included white, light purple and purple (Figure 7). Among the 40 genotypes, 27 genotypes exhibited white flower colour, 8 genotypes light purple flower colour and purple flower colour expressed in the remaining 5 genotypes (Figure 11g).

Calyx colour

Five categories of calyx colour (Figure 8) *viz.* green (25 genotypes), light green (2 genotypes), purple (6 genotypes), light purple (6 genotypes) and dark purple (1 genotype) were observed for the 40 Tulsi genotypes studied for the present investigation (Figure 11h).

Calyx pubescence

Tulsi genotypes can be classified as sparse pubescent, dense pubescent and non-pubescent based on the presence or absence of the hairs on the calyx. In the present investigation, 13 genotypes were observed to have sparse pubescence, whereas 27 genotypes showed non-pubescent characteristic for calyx pubescence (Figure 9). None of the genotypes exhibited dense pubescence on calyx (Figure 11i).

Seed colour

Four categories of seed colour (Figure 10) *viz.* black (1 genotype), dull black (35 genotypes), brownish black (1 genotype) and brown (3 genotypes) were observed for the 40 Tulsi genotypes studied in the present investigation (Table 1, Figure 11j).

The maximum number of germplasm lines observed leaf lamina colour-green (25 germplasm lines), non pubescent leaf (38), leaf shape-elliptical (40), stem colour-green (28), stem pubescence-pubescent (5), calyx colour-green (25), calyx pubescence-pubescent (13), petiole colour-green (36), flower colour-white (27) and seed colour-dull black (35).

Discussion

Knowledge about the genetic variation of the various plant characters enables the plant breeders to effectively utilize the available germplasm lines for the development of elite genotypes (Gowda et al., 2019; Singh et al., 2020; Arya et al., 2024). Morphological and cytological studies have helped in resolving the identity issues in many genera (Paton and Putievsky, 1996).

The experimental material of the present investigation comprising of 40 genotypes of Tulsi (*Ocimum* spp.) were characterized for 10 qualitative traits *viz.* stem colour, stem pubescence, leaf shape, leaf colour, leaf pubescence, petiole colour, flower colour, calyx colour, calyx pubescence and seed colour.

On the basis of stem colour, all the 40 Tulsi genotypes were categorized into five groups: green (28 genotypes), purple (5), dark green (4), light purple (2) and light green stem colour (1). Based on presence or absence of minute hairs on the surface of the stem, two categories were formed: sparse pubescence (5 genotypes) and non-pubescent (35). Regarding leaf shape, elliptical leaf shape was observed in all the 40 genotypes of Tulsi. Based on leaf colour, all the Tulsi genotypes were classified into four group: green (25 genotypes), dark green (8), light green (6) and purple leaf colour (1). On the basis of leaf pubescence, two categories were observed: sparse pubescent (2 genotypes) and non-pubescent (38). Genotypes were classified into three groups on the basis of petiole colour: green (36 genotypes), purple green (3) and purple petiole colour (1). Based on flower colour, three categories were formed *viz.* white (27 genotypes), light purple (8) and purple (5). Based on the colour of the calyx, the genotypes were classified into five groups: green (25 genotypes), purple (6), light purple (6), light green (2) and dark purple calyx colour (1). On the basis of presence or absence of minute hairs on the calyx surface, two categories were observed such as non-pubescent (27) and sparsely pubescent (13). Genotypes were classified into four groups based on seed colour *viz.* dull black (35), black (3), brownish black (1) and brown seed colour (1). The characterization of Tulsi genotypes based on qualitative traits has also been reported by Kumar et al., (2012a), Chhaya et al., (2013), Nassar et al., (2013), Malav et al., (2015) and Kumar et al., (2019). Yaldiz, and Camlica, (2021) also studies the agro-morphological and phenotypic variability of sweet basil genotypes for breeding purposes. The morphological characterization in different medicinal plants is carried by different researchers for their proper identification and further utilization in breeding programme (Arya et al., 2024; Singh et al., 2024).

Conclusions

It may be concluded from present study that the sufficient morphological variability was present among different germplasm lines of Tulsi. The presence of genetic variation for different traits enables the plant breeders to utilize the germplasm lines EC 388890, IC 469938, IC 388785 and IC 328582 having maximum fresh herbage yield for the development of elite genotypes.

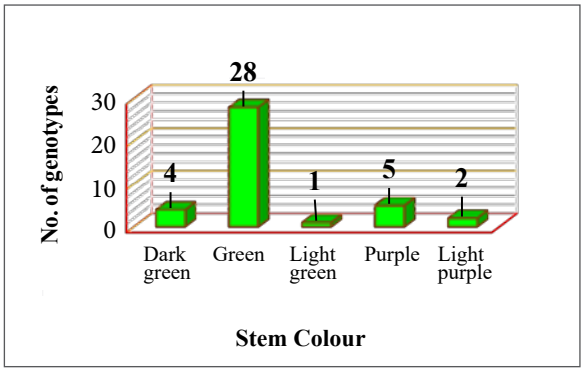

Figure 1. Classification of 40 Tulsi genotypes on the basis of stem colour.

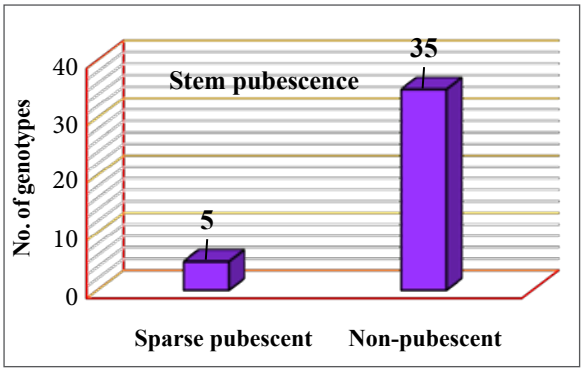

Figure 2. Classification of 40 Tulsi genotypes on the basis of stem pubescence.

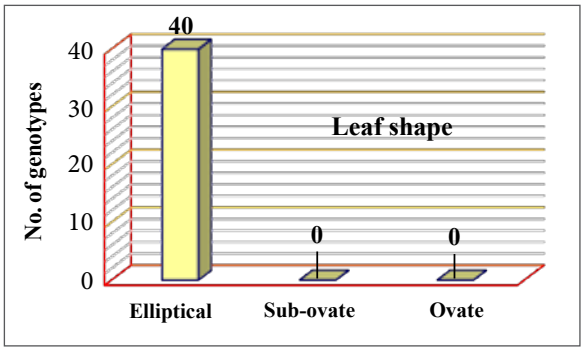

Figure 3. Classification of 40 Tulsi genotypes on the basis of leaf shape.

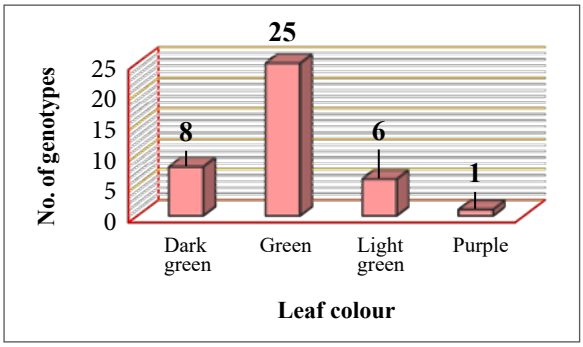

Figure 4. Classification of 40 Tulsi genotypes on the basis of leaf colour.

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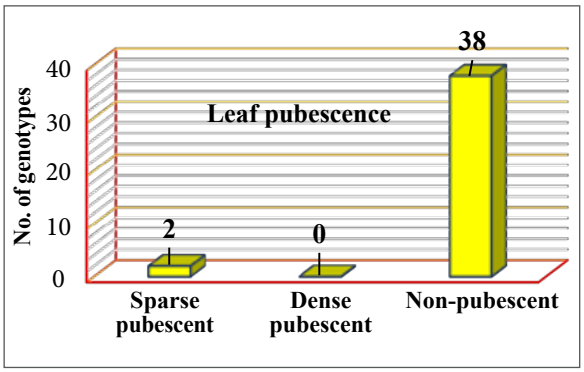


Figure 5. Classification of 40 Tulsi genotypes on the basis of leaf pubescence.

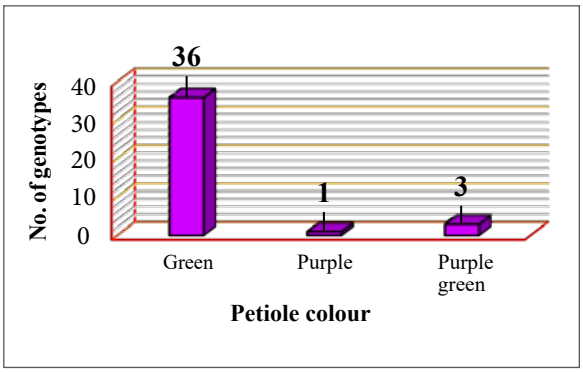


Figure 6. Classification of 40 Tulsi genotypes on the basis of petiole colour.

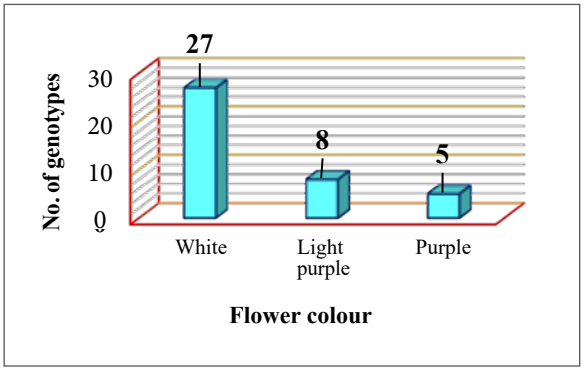


Figure 7. Classification of 40 Tulsi genotypes on the basis of flower colour.

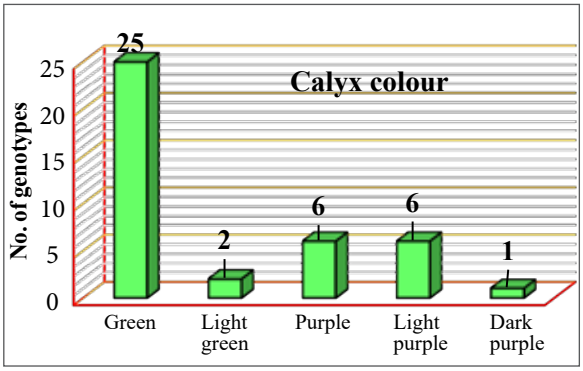


Figure 8. Classification of 40 Tulsi genotypes on the basis of calyx colour.

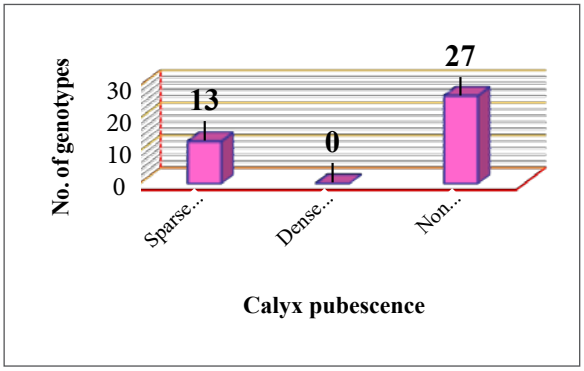


Figure 9. Classification of 40 Tulsi genotypes on the basis of calyx pubescence.

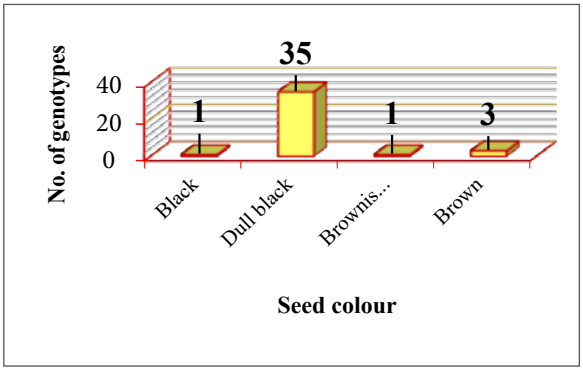
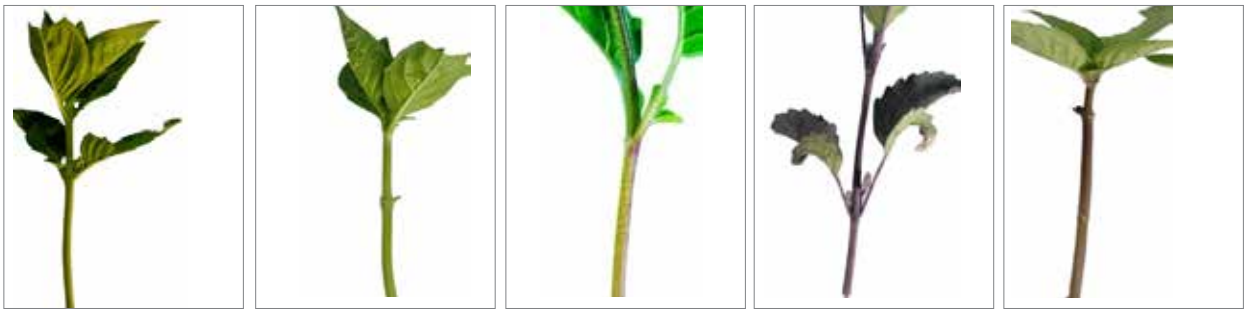


Figure 10. Classification of 40 Tulsi genotypes on the basis of seed colour.

a. Stem colours



Dark green | Green | Light green | Purple | Light purple

b. Stem pubescence

Sparse pubescent | Non-pubescent

c. Leaf shape

Elliptical leaf shape

d. Leaf colours

Dark green | Green | Light green | Purple

e. Leaf pubescence

Sparse pubescent | Non-pubescent

Figure 11. Figures represent morphological descriptors of various qualitative characters in *Ocimum* spp.

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Continuing Figure 11

f. Petiole colours

Green

Purple

Light purple

g. Flower colours

White with light green shade

White with light purple shade

White with purple shade

h. Calyx colours

Green

Light green

Light pink

Pink

Dark Pink

i. Calyx pubescence

j. Seed colours

Black

Dull black

Brownish black

Brown

Table 1. Species and source of 40 Tulsi genotypes used in present study.

Serial Number	Genotypes	Species	Source	Serial Number	Genotypes	Species	Source
1.	IC 44681	*Ocimum basilicum*	NBPGR	**21.**	EC 469904	*Ocimum basilicum*	NBPGR
2.	IC 387837	*Ocimum basilicum*	NBPGR	**22.**	EC 326771	*Ocimum basilicum*	NBPGR
3.	IC 369247	*Ocimum basilicum*	NBPGR	**23.**	EC 388893	*Ocimum basilicum*	NBPGR
4.	IC 387838	*Ocimum basilicum*	NBPGR	**24.**	EC 388887	*Ocimum basilicum*	NBPGR
5.	IC 388785	*Ocimum basilicum*	NBPGR	**25.**	EC 388895	*Ocimum basilicum*	NBPGR
6.	IC 469938	*Ocimum basilicum*	NBPGR	**26.**	EC 388896	*Ocimum basilicum*	NBPGR
7.	IC 326735	*Ocimum basilicum*	NBPGR	**27.**	EC 388782	*Ocimum basilicum*	NBPGR
8.	IC 312264	*Ocimum basilicum*	NBPGR	**28.**	EC 388737	*Ocimum basilicum*	NBPGR
9.	IC 110207	*Ocimum basilicum*	NBPGR	**29.**	EC 388889	*Ocimum basilicum*	NBPGR
10.	IC 338794	*Ocimum basilicum*	NBPGR	**30.**	EC 338772	*Ocimum basilicum*	NBPGR
11.	IC 336833	*Ocimum basilicum*	NBPGR	**31.**	EC 388788	*Ocimum basilicum*	NBPGR
12.	IC 201223	*Ocimum basilicum*	NBPGR	**32.**	EC 388890	*Ocimum basilicum*	NBPGR
13.	IC 328582	*Ocimum basilicum*	NBPGR	**33.**	NSV 38	*Ocimum basilicum*	NBPGR
14.	IC 338959	*Ocimum basilicum*	NBPGR	**34.**	RDV 45	*Ocimum basilicum*	NBPGR
15.	IC 333833	*Ocimum basilicum*	NBPGR	**35.**	Local 1	*Ocimum sanctum*	HAU
16.	IC 281185	*Ocimum basilicum*	NBPGR	**36.**	Local 2	*Ocimum tenuiflorum*	HAU
17.	IC 381552	*Ocimum basilicum*	NBPGR	**37.**	DOS 1	*Ocimum tenuiflorum*	DMAPR
18.	IC 436153	*Ocimum basilicum*	NBPGR	**38.**	IC 75730	*Ocimum basilicum*	NBPGR
19.	IC 381158	*Ocimum basilicum*	NBPGR	**39.**	IC 381185	*Ocimum basilicum*	NBPGR
20.	IC 326732	*Ocimum basilicum*	NBPGR	**40.**	EC 112548	*Ocimum basilicum*	NBPGR

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Table 2. Classification of 40 Tulsi genotypes on the basis of morphological characters.

Characters: Stem colour	No. of Genotypes	Genotypes
Dark green	4	IC 369247, IC 381552, EC 388887, EC 388895
Green	28	IC 44681, IC 387837, IC 387838, IC 388785, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, EC 469904, EC 326771, EC 388896, EC 388782, EC 388737, EC 388889, EC 338772, IC 436153, IC 381158, IC 326732, RDV 45, NSV 38, Local 1, Local 2
Light green	1	IC 336833
Purple	5	EC 388788, EC 388890, IC 75730, IC 381185, DOS 1
Light purple	2	EC 388893, EC 112548
Stem pubescence		
Sparse pubescent	5	IC 388785 , EC 469904, IC 75730, IC 381185, DOS 1
Non-pubescent	35	IC 44681, IC 387837, IC 369247, IC 387838, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 381552, EC 326771, EC 388893, EC 388887, EC 388895,EC 388896, EC 388782, EC 388737, EC 388889, EC 338772, EC 388788, EC 388890, IC 436153, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, Local 1, Local 2
Leaf shape		
Elliptical	40	IC 44681, IC 387837, IC 369247, IC 387838, IC 388785, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 381552, IC 436153, IC 381158, IC 326732, EC 469904, EC 326771, EC 388893, EC 388887, EC 388895, EC 388896, EC 388782, EC 388737, EC 388889, EC 338772, EC 388788, EC 388890, RDV 45, NSV 38, Local 1, Local 2, DOS 1, IC 75730, IC 381185, EC 112548
Sub-ovate	0	-None-
Ovate	0	-None-
Leaf colour		
Dark green	8	IC 369247, IC 381552, EC 388893, EC 388887, EC 388895, IC 75730, Local 1, DOS 1
Green	25	IC 44681, IC 387837, IC 387838, IC 388785, IC 326735, IC 312264, IC 110207, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, EC 469904, EC 326771, EC 388896, EC 388782, EC 388737, EC 388889, EC 388788, EC 388890, IC 381158, IC 326732, NSV 38, EC 112548
Light green	6	IC 469938, IC 338794, EC 338772, IC 436153, RDV 45, Local 2
Purple	1	IC 381185
Leaf pubescence		
Sparse pubescent	2	IC 388785, IC 381185
Dense pubescent	0	-None-
Non-pubescent	38	IC 44681, IC 387837, IC 369247, IC 387838, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 381552, EC 469904, EC 326771, EC 388893, EC 388887, EC 388895,EC 388896, EC 388782, EC 388737, EC 388889, EC 338772, EC 388788, EC 388890, IC 436153, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, IC 75730, DOS 1, Local 1, Local 2

Continuing Table 2

Characters: Stem colour	No. of Genotypes	Genotypes
Petiole colour		
Green	36	IC 44681, IC 387837, IC 369247, IC 387838, IC 388785, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 336833, IC 201223,IC 328582, IC 338959, IC 333833, IC 281185, IC 381552, EC 469904, EC 326771, EC 388887, EC 388895, EC 388896, EC 388782, EC 388737, EC 388889, EC 338772, EC 388788, EC 388890, IC 436153, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, Local 1, Local 2
Purple	1	IC 381185
Purple green	3	EC 388893, IC 75730, DOS 1
Flower colour		
White	27	IC 44681, IC 387837, IC 387838, IC 388785, IC 312264, IC 110207, IC 338794, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, EC 469904, EC 326771, EC 388887, EC 388895, EC 388782, EC 388737, EC 388889, EC 338772, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, Local 1, Local 2
Light purple	8	IC 369247, IC 469938, IC 326735, IC 381552, EC 388896, EC 388788, EC 388890, IC 436153
Purple	5	IC 336833, EC 388893, IC 75730, IC 381185, DOS 1
Calyx colour		
Green	25	IC 387838, IC 388785, IC 312264, IC 110207, IC 338794, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, EC 469904, EC 326771, EC 388887, EC 388895, EC 388782, EC 388737, EC 388889, EC 338772, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, Local 1, Local 2
Light green	2	IC 44681, IC 387837
Purple	6	EC 388893, EC 388788, EC 388890, IC 436153, IC 75730, DOS 1
Light purple	6	IC 369247, IC 469938, IC 326735, IC 336833, IC 381552, EC 388896
Dark purple	1	IC 381185
Calyx Pubescence		
Sparse pubescent	13	IC 44681, IC 369247, IC 388785, IC 469938, IC 312264, IC 338794, IC 381552, IC 75730, IC 381185, EC 469904, EC 388893, EC 338772, DOS 1
Dense pubescent	0	-None-
Non-pubescent	27	IC 387837, IC 387838, IC 326735, IC 110207, IC 336833, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 436153, IC 381158, IC 326732, EC 326771, EC 388887, EC 388895, EC 388896, EC 388782, EC 388737, EC 388889, EC 388788, EC 388890, RDV 45, NSV 38, EC 112548, Local 1, Local 2
Seed colour		
Black	1	IC 44681
Dull black	35	IC 387837, IC 369247, IC 387838, IC 388785, IC 469938, IC 326735, IC 312264, IC 110207, IC 338794, IC 201223, IC 328582, IC 338959, IC 333833, IC 281185, IC 381552, EC 469904, EC 326771, EC 388893, EC 388887, EC 388895, EC 388896, EC 388782, EC 388737, EC 388889, EC 338772, EC 388788, EC 388890, IC 436153, IC 381158, IC 326732, RDV 45, NSV 38, EC 112548, Local 1, DOS 1
Brownish black	1	IC 336833
Brown	3	IC 75730, IC 381185, Local 2

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Determination of Resistance Status of Qualified Tomato Genotypes to *Meloidogyne incognita*, *Tomato spotted wilt virus*, *Tomato mosaic virus*, Verticillium Wilt

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the most cultivated vegetables both in Türkiye and in the world. Türkiye is the 4th tomato producing country after China, India and the USA. According to consumer demands, tomato varieties such as pink, beef, bunch, cocktail are grown. The main goals in breeding are high yield, quality and resistance to stress factors, and molecular marker-assisted selection is a fast and reliable method, especially in determining the presence or absence of resistance genes to biotic stress factors. In this study, the resistance genes determined for 339 advanced tomato lines, which have the potential to become parent lines due to their agro morphological characteristics and disease resistance against *Meloidogyne incognita* (Root-knot nematode), *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV) and Verticillium wilt in the plant material. 235 were homozygous resistant to root-knot nematode, 172 homozygous resistant to Verticillium wilt, 201 homozygous resistant to TSWV and 211 homozygous resistant to ToMV were determined. It has been seen that breeding programs can be created with the results obtained.

Keywords: *Meloidogyne incognita*, Tomatoes, ToMV, TSWV, Verticillium wilt

Introduction

In Türkiye, tomato is an economically important crop and it faces many diseases and pests in terms of cultivation. These diseases and pests cause a loss of yield and quality in tomato production. To increase tomato production and obtain quality products, it is of very important to protect against diseases and pests in tomato cultivation. It is possible to gain an initial resistance advantage by selecting tomato varieties that are resistant to diseases and pests. This increases the plant's natural ability to cope with diseases and makes them less susceptible to pests. Breeding programs are carried out using genetic resources to increase resistance to various diseases and pests. As a result of these studies, more than 50 disease and pest-resistance genes have been identified in tomato and these genes are used in the development of commercial varieties (Bai and Lindhout, 2007).

Root-knot nematodes (*Meloidogyne* spp.) are an important pest that can cause serious economic losses in agricultural production. Root-knot nematodes are one of the species of plant parasitic nematodes that live in the soil. These nematodes colonize the roots of plants and inhibit the growth of the plant while feeding. Root-knot nematodes disrupting the plant's uptake of nutrient and water by forming tumor-shaped nodules on the roots of the plant. This situation negatively affects the development of the plant and causes weakening of the roots, yellowing, wilting and in general loss of yield and quality of the plant (Agrios, 2005). Root-knot

nematodes can reduce vegetable production by 50% to 80% (Siddiqi, 2000). The *Mi-1.2* molecular marker is linked to the *Mi-23* gene of *S. lycopersicum* is known to be a marker used to determine nematode resistance in plants, *Mi-23* marker of *S. lycopersicum* about 450 bp is amplified in susceptible plants of (mi/mi). In addition, it is stated that a 400 bp fragment is seen in resistant phenotypes (Mi/Mi) and both fragments are seen in heterozygous plants (Mi/mi) (Pérez-Almeida et al., 2016). *Meloidogyne incognita* resistance to the breed is provided by the *Mi* gene. This gene family includes subtypes such as *Mi-1.1*, *Mi-1.2* and *Mi-1.3* (Milligan et al., 1998). These genes are important genetic resources used in the development of varieties resistant to root-knot nematodes in tomato cultivation.

TSWV (*Tomato spotted wilt virus*) is a viral disease that is common in the tomato plant that causes severe economic losses. TSWV infection can cause crop losses of up to 60% or even 100% in tomato growing areas. (Roselló et al., 1996). Symptoms due to TSWV infection in genotype during development period in which the plant was in when the infection occurred (Moriones et al., 1998; Chaisuekul et al., 2003) may vary depending on the virus isolate and environmental conditions (Kaminska, 1993; Mitidieri et al., 2000). These factors can affect the symptoms and severity of the disease and determine how TSWV infection will appear in tomato plants. There are genes for resistance to TSWV in the tomato plant. These resistance genes make plants more resistant to TSWV infection in *Solanum peruvianum.* The *Sw-5* gene found in the genome is a dominant resistance gene to TSWV. This gene has been reported as an effective source of resistance to TSWV. The *Sw-5* gene may be effective against various isolates of TSWV and is considered a non-race-specific resistance gene. That is, it can be generally effective against different TSWV isolates (Stevens et al., 1991).

Tomato mosaic virus (ToMV) is a virus that causes viral disease in tomato plants. Symptoms associated with ToMV infection can vary among plants and cultivars, but generally include stunting, curling of leaves, mosaic patterns and mottling, necrosis, and textural yellowing (Ullah et al., 2017). *S. lycopersicum* resistance to ToMV (*Tomato mosaic virus*) in cultivated tomato plants is usually *S. habrochites,* it is provided by genes transferred from the wild species (Lee et al., 2015). Genes known as *Tm-1*, *Tm-2* and *Tm-2*^{2} play an important role in conferring resistance to ToMV in tomatoes. These genes limit or inhibit the infection of the virus in tomato by genetic resistance mechanisms. The *Tm-1* gene is known to be a resistance gene transferred from *S. habrochites* to *S. lycopersicum.* This gene has been used in the development of tomato varieties that are resistant to ToMV. If the *Tm-2* gene of *S. habrochites* is transferred, it has been reported that there is another resistance gene that has been passed down from some populations. The *Tm-2* gene works by a mechanism of action that limits the spread of ToMV. Finally, the *Tm-2*^{2} gene has been described as a genetic variant that has been introduced as a supplement to the *Tm-2* gene to confer stronger resistance to ToMV (Weber et al., 2004; Labate et al., 2007).

Verticillium wilt rarely causes death in the plant, but there is a important loss of yield due to the drying of all the lower leaves (Pegg and Brady, 2002). Resistance to Verticillium wilt disease is known to be a genetically controlled trait in some plant species. On the tomato plant *Verticillium dahliae* Kleb resistance to race 1 is controlled by a single dominant gene (*Ve*) located on the short arm of chromosome 9 of the plant. This resistance gene makes the plant resistant to Verticillium wilt disease (Simko et al., 2004).

In this study, it was aimed to determine the resistance levels of qualified tomato pure lines at different stages against *Meloidogyne incognita* (root-knot nematode), Verticillium wilt (Ve), *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV) by molecular methods and to show the usability of these materials in breeding programs.

Materials and Methods

In the study carried out in cooperation with the public-private sector, 339 tomato lines selected from the gene accession of Selko R&D company constituted the plant material of the study. In the study, the resistance levels of lines to root-knot nematode, *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV), Verticillium wilt were determined using DNA markers. 2 markers were used for the selection of root-knot nematode hardiness status (Table 1). The REX-1 and *Mi23* markers successfully distinguish between nematode-resistant and susceptible genotypes (Bhavana et al., 2019). The presence of resistant *Tm* genes was identified for the first time using sequence-characterized amplified region (SCAR) and allele-specific (AS1) markers for the *Tm-1* and *Tm-2* genes, respectively (Ashwini and Nagaraju, 2022). Several PCR-based markers related to the resistance gene have been reported (Kawchuk et al., 1994; Kawchuk et al., 1998; Kawchuk et al., 2001; Acciarri et al., 2007; Park et al., 2008). SCAR and SNP markers have been reported to be related to this durability (Kawchuk et al., 1998; Kawchuk et al., 2001). Markers developed to accurately and quickly screen for resistance to *Tomato spotted wilt virus* (TSWV) disease are designed to

identify different allele combinations of the *Sw-5* gene (Stevens et al., 1995; Chagué et al., 1996; Folkertsma et al., 1999; Śmiech et al., 2000).

DNA isolation was performed in tissues taken from healthy leaf samples for molecular characterization. The work on the effective fragmentation of samples for DNA extraction and the release of DNA from cells was carried out in the Qiagen Tissue Lyzer II device. The SCAR and CAPS marker portions of DNA samples obtained from tomato leaves are indicated (Table 1). PCR mix Liu et al. (1995) it has been prepared according to the protocol. SCAR and CAPS marker ranges were visualized by the Qiaxcel Fragment Analyzer (Qiagen Sample & Assay Technologies) capillary electrophoresis system and agarose gel electrophoresis.

Results and Discussion

To study the different genes that are reported to confer resistance to different diseases and pests, each (Maurya et al., 2023), primer pair specifically designed for the gene was used. Marker fragments were imaged with the capillary electrophoresis system, an analytical method in which DNA and other molecules move in an electric field. Out of all *Meloidogyne incognita* (Root-Knot Nematode) genotypes 235 homozygous (RR) and 79 heterozygous (Rr) genotypes were identified. Maurya et al. (2023) scan at the *Mi23* locus using the *Mi1.2* SCAR marker revealed that 5 plants carried a resistance allele in the homozygous state, and 6 plants reported no resistance alleles. Bhavana et al. (2019) REX-1 and *Mi23* markers successfully differentiated between nematode-resistant and susceptible genotypes. In addition, *Mi23* did not require restriction enzyme analysis and separated homozygous/heterozygous resistance sources.

211 homozygous (RR) and 76 heterozygous (Rr) genotypes were obtained against *Tomato mosaic virus* (ToMV). In the greenhouse study conducted by Ashwini and Nagaraju (2022) on ToMV, a total of 35 tomatoes were screened to determine the source of resistance by mechanical inoculation, and 6 were determined as hardy, 11 as moderately resistant, 12 as moderately susceptible, and 6 as susceptible.

172 homozygous (RR) and 78 heterozygous (Rr) genotypes were identified against Verticillium wilt. In their study, Kiymaci et al. (2023) reported that out of 70 tomatoes, 45 RR (homozygous resistant), 15 Rr (heterozygous) and 10 RR (susceptible) were found against Verticillium wilt. 201 homozygous (RR) and 75 heterozygous (Rr) genotypes were obtained against *Tomato spotted wilt virus* (TSWV). Pinar et al. (2013) tested 92 F_2 tomato populations with the *Sw-5.2* SCAR marker and 26 of them reported that they obtained a durability band at 550 bp and reported that 23 of them obtained a sensitivity band at 500 bp. Table 2 shows the resistance levels of *Meloidogyne incognita*, *Tomato mosaic virus*, Verticillium wilt, *Tomato spotted wilt virus* against diseases and pests.

Conclusions

In tomato cultivation, the presence of genotypes with disease and pest-resistance genes is of very important. These genotypes make plants resistant to diseases and make them less susceptible to pests. Diseases and pests can lead to a loss of yield and a decrease in quality in tomato production. Therefore, the development of genotypes with resistance genes is of great importance for tomato cultivation. Breeding studies are conducted to identify genotypes with resistance genes, to transfer these genes to more resistant genotypes, and eventually to produce more resistant tomato varieties. When the results of the study were examined, 235 lines resistant to Root-Knot-Nematode, 172 lines resistant to Verticillium wilt, 201 lines resistant to TSWV and 211 lines resistant to ToMV were determined among 339 genotypes. As a result, 339 tomato genotypes with known agro morphological structures at different levels of stability were determined to be homozygous and heterozygous for 4 different pathogen species.

In accordance with the data obtained, a valuable gene accession has been created with genes resistant to disease factors. It will allow us to obtain genotypes that are advantageous in terms of disease resistance in future tomato breeding studies. In this direction, the development of more resistant varieties for tomato varieties will contribute to the adoption of a more sustainable approach to diseases and pests.

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Table 1. Primers used to determine the level of resistance of genotypes to specified diseases.

Pathogen Name	Marker Name	Gene	Primer Forward	Primer Reverse	Reference
Meloidogyne incognita	SCAR	*MI-REX*	TCGGAGCCTTGGTCTG AATT	GCCAGAGATGATTCGTGA GA	(Garcia et al., 2007)
	SCAR	*Mi23*	TGGAAAAATGTTGAAT TTCTTTTG	GCATACTATATGGCTTGTT TACCC	
Tomato mosaic virus (ToMV)	SCAR	*Tm-2*	CACCTTTCCCTCTCCAA	CACCTTTCCCCTAAAGC	(Sobir et al., 2000)
Verticillium wilt	SCAR	*V2LeO3*	CAAACATAGCTGGAAG AATC	TAGGAGGAAAAGAATTGG	(Acciarri et al., 2007)
Tomato spotted wilt virus (TSWV)	SCAR	*Sw-5–2*	AATTAGGTTCTTGAAGC CCATCT	TTCCGCATCAGCCAATAG TGT	(Dianese et al., 2010)

Marker amplicon sizes: *MI-REX,* Durable: tapes digested with TaqI of 570 and 160 bp, Susceptible: remains uncleared (750 bp); *Mi23*, Rugged: 380 bp, Susceptible: 420 bp; *Tm-2*, Durable: 574 bp, Precision: 534 bp; *V2LeO3,* Resistant: HincII digested 428 and 601 bp fragments, Susceptible: remains uncleared (1029 bp); *Sw-5-2,* Resistive: 574 bp, Susceptible: 464 bp; *TY-1*, Resistant: TaqI-digested 500, 300 and 160 bp bands, Susceptible: TaqI-digested 500, 300 and 200 bp bands; *TY-2*, Resistive: 120 bp, Susceptible: 213 bp; *TY-3,* Resistant: 630 bp, Susceptible: 320 bp. The markers found in the relevant genes are: *MI-REX, Mi23, TY-2, Sw-5-2, I3* and *V2LeO3*

Table 2. Resistance status of *Meloidogyne incognita*, *Tomato spotted wilt virus*, *Tomato mosaic virus*, Verticillium Wilt.

Disease and Pest	Homozygote (RR)	Heterozygote (Rr)
Meloidogyne incognita	235 genotypes	79 genotypes
Tomato mosaic virus (ToMV)	211 genotypes	76 genotypes
Verticillium wilt	172 genotypes	78 genotypes
Tomato spotted wilt virus (TSWV)	201 genotypes	75 genotypes

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Performance of Different Okra Varieties for Fruit Yield and Resistance Against Yellow Vein Mosaic Virus (YVMV) and Okra Enation Leaf Curl Virus (OELCV) under Climate Change

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ABSTRACT

A field experiment was carried out to evaluate the performance of 12 new entries of okra during 2019 and 2020 at the Research Farm of Vegetable Science, CCS HAU, Hisar. In the present evaluation, during 2019, out of 12 tested entries of okra, 19/OKYVRES-5 recorded the maximum fruit yield (116.4 q/ha) with no incidence of YVMV (0.0%) and f OELCV (0.0%), whereas the minimum yield (77.2 q/ha) with higher percentage of YVMV (13.4%) and OELCV (22.1%) was found in 19/OKYVRES-7. Likewise, during 2020, twelve entries of Okra were again tested, the maximum fruit yield (113.3 q/ha) with minimum incidence of YVMV (0.2%) and no incidence of OELCV (0.0%) was recorded in 9/OKYVRES-5, whereas the minimum yield (72.4 q/ha) with maximum percentage of YVMV (19.6%) and OELCV (25.4%) was found in 19/OKYVRES-7. It may concluded that the newly developed variety 19/OKYVRES-5 of okra has average fruit weight 114.85 q/ha and also reflected resistant against YVMV and OELCV, therefore, may recommended for commercial cultivation after testing it over the locations and years.

Keywords: Okra, cultivars, climate change, growth, fruit yield, YVMV, OELCV

Introduction

Today, the entire world is worried about the impact of climate change on plants including the vegetable crops. In the last 200 years, due to industrial revolution the climatic factor is changing very fast that's why some of crops are facing problem of adaption in changed climate. (Arya et al., 2014). The effect of climate on vegetables production is associated to the variabilities in local climatic factors rather than in international climate patterns. The average temperature of earth crust has increased by 1 degree F in just over the last century (Arya et al., 2020). Consequently, researchers consider any assessment has to be individually considering each location. Therefore, to face the challenges of climate change, concerted efforts are needed to evaluate, the released varieties of vegetable crops against the stressed environment.

Okra (*Abelmoschus esculentus* L. Moench) is one of the most important export oriented vegetable crop of India (Desh Raj et al., 2013 and 2014). It ranks first in vegetable production with 6095 thousand tonnes (Anonymous, 2018a). In India, it is commonly grown in the states of Maharashtra, Gujarat, Karnataka, TN, Punjab, Haryana, UP, Bihar, WB and Odisha (Swarup, 2006). In Haryana, it is grown for its tender immature green fruits which are cooked in curry as well as in soups. The roots and stem are used for clearing cane juice during preparation of jaggary. Its dry seeds contain 13-22% edible oil and 20-24% protein. Its seed cake is also used as animal feed. The dry fruit

shell and stem contain crude fibers which are used in manufacture of paper and cardboard. Okra has certain medicinal values like curing ulcers and relief from haemorrhoids. It is beneficial to people suffering from leucorrhoea and general weakness. The high iodine content in its fruits is considered useful to control goiter (Thamburaj and Singh, 2018). It is very important source of protein, carbohydrates, vitamin A, Vitamin C, Ca, K, Mg, and several other mineral elements, which generally found missing in our daily food. In addition to dietary benefits, okra is also serves a good medicine in case of peptic ulcer problems as it is one of the cheap source of plasma replacement in our body fluid system (Makinde et al., 2022).

For profitable commercial production of any vegetable crop, the high yielding and disease resistant variety is the first basic requirement of vegetable growing farmers (Majoka et al., 2021; Oo et al., 2022). Therefore, to achieve the maximum yield production for a variety identify the specific sowing duration under a set of specific climatic conditions. The assessment of available vegetable crop varieties under different environments provides valuable information to the scientist for further improvement in yield (Vu et al., 2017; Oo et al., 2023). In India, okra cultivation is influenced by several abiotic and biotic agents and significant fruit yield reduction has been noticed (Sanwal et al., 2016). These constraints further increase with increasing cropping intensity of very few HYV. Under this critical situation, fruit yield and quality can be improved by addressing the key factors responsible for yield losses, i.e. viral diseases (Sanwal et al., 2014). Among the viruses affecting the okra, YVMV and OELCV causes big losses in okra cultivation. Therefore, in the present investigation, twelve newly developed entries were evaluated for yield and its contributing triats and against YVMV and OELCV diseases.

Materials and Methods

A field experiment was planned to evaluate the performance of twelve okra new entries during seasons of 2019 and 2020 at the Research Farm of Vegetable Science, CCS Haryana Agricultural University, Hisar, India in RBD with three replications. The experimental location in Hisar is situated at the latitude of 29º 10' N, the longitude of 75º46' E and at an altitude of 215.2 m above mean sea level on South-Western edge of the Rajasthan state and at a remoteness of about 175 kilometers in West direction, New Delhi. This region is characterized as semi-arid climate along with hot and dry winds during summer and dry severe cold in winters which are common features of this region. The temperature in this area exhibit wide range from 44-48ºC in summer season and as low as up to freezing point accompanied with chill frost in the winter season (Majoka et al., 2021). Highest rainfall in this area is received during the months of July to September with showers in the month of January to late spring. In the present experiment, each entry was accommodated in plot size of 3.0 m x 2.7 m with spacing 60 cm x 45 cm and all the good cultural practices were followed to raise the okra crop (Anonymous, 2023). The observations were recorded on five competitive plants in each treatment for fruit length (cm), average fruit weight (g), fruit yield per plant (kg), YVMV (%) and OELCV (%). The data was subjected to the RBD statistical analysis as per procedure suggested by Panse and Sukhatme, 1985.

Results and Discussion

The analysis of variance (ANOVA) for number of fruits per plant, fruit length, average fruit weight (g), fruit yield (q/ha), YVMV (%) and OELCV (%) reflected the significant differences among the entries of okra under the present investigation.

Number of fruits per plant

The results of present investigation are presented in Table 1. Out of 12 tested entries of Okra, during 2019, maximum no. of fruits per plant 24.03 was recorded by 19/OKYVRES-5 which was at par with 19/OKYVRES-9 (20.62) and followed by 19/OKYVRES-13 (19.29), 19/OKYVRES-11 (16.91), 19/OKYVRES-2 (15.43). During 2020, maximum no. of fruits per plant 21.10 was recorded in 19/OKYVRES-5 followed by 19/OKYVRES-9 (17.45), 19/OKYVRES-13 (16.60), 19/OKYVRES-11 (13.80), 19/OKYVRES-2 (13.41). Based on the average of both the years, maximum no. of fruits per plant 22.57 was recorded in 19/OKYVRES-5 followed by 19/OKYVRES-9 (19.04), 19/OKYVRES-13 (17.95), 19/OKYVRES-11 (15.36) and 19/OKYVRES-2 (14.42). Similar findings were also reported by Batra and Singh. 2000; Rashid et al., 2002 and Malshe et al., 2016. While working on cowpea, Vu et al. (2019) also revealed the lot of variations in fruit yield and reported some promising genotypes.

Fruit length (cm)

The data presented in Table 1 revealed that out of 12 tested entries of Okra, during 2019, maximum fruit length 11.30 cm was recorded by 19/OKYVRES-8 which was at par with 19/OKYVRES-5 (11.00 cm) and followed by 19/OKYVRES-13 (10.40 cm), 19/OKYVRES-2 (10.00 cm), 19/OKYVRES-6 (9.80 cm). During 2020, maximum fruit length 11.10 cm was recorded in 19/OKYVRES-8 which was at par with

19/OKYVRES-5 (10.70 cm), 19/OKYVRES-13 (10.60 cm) and followed by 19/OKYVRES-2 (9.80 cm), 19/OKYVRES-7 (9.50 cm) and 19/OKYVRES-9 (9.50 cm). Based on the average of both the years, maximum fruit length 11.20 cm was recorded in 19/OKYVRES-8 followed by 19/OKYVRES-5 (10.80 cm), 19/OKYVRES-13 (10.55 cm), 19/OKYVRES-2 (9.90 cm), and 19/OKYVRES-6 (9.45 cm). Similar findings were also reported by Batra and Singh. 2000; Rashid et al., 2002 and Malshe et al., 2016.

Average fruit weight (g)

The data on average fruit weight of okra presented in Table 2 shows that during 2019, maximum average fruit weight 12.40 g was recorded in 19/OKYVRES-5 which was at par with 19/OKYVRES-7 (12.10 g) and followed by 19/OKYVRES-13 (11.10 g), 19/OKYVRES-1(10.90 g) and 19/OKYVRES-2 (10.70 g). During 2020, maximum average fruit weight 11.80 g was recorded in 19/OKYVRES-5 which was at par with 19/OKYVRES-13 (11.50 g), 19/OKYVRES-7 (11.40 g), 19/OKYVRES-2 (10.70 g) and 19/OKYVRES-9 (10.70 g). Based on the average of both the years, maximum fruit length 12.10 g was recorded in 19/OKYVRES-5 followed by 19/OKYVRES-7 (11.75 g), 19/OKYVRES-13 (11.30 g), and 19/OKYVRES-2 (10.70 g). Above finding were supported by Abdul et al., 2004 and Malshe et al., 2016.

Fruit yield (q/ha)

Data on fruit yield of okra is depicted in Table 2 for the year 2019 and 2020. Out of 12 tested entries of Okra, during 2019, maximum average fruit yield 116.40 q/ha was recorded in 19/OKYVRES-5 followed by 19/OKYVRES-9 (108.00 q/ha), 19/OKYVRES-13 (107.20 q/ha), 19/OKYVRES-2 (102.20 q/ha) and 19/OKYVRES-11(101.20 q/ha). During 2020, maximum average fruit yield 113.30 q/ha was recorded in 19/OKYVRES-5 followed by 19/OKYVRES-9 (106.10 q/ha), 19/OKYVRES-13 (104.10 q/ha), 19/OKYVRES-11 (103.00 q/ha) and 19/OKYVRES-2 (100.30 q/ha). Based on the average of both the years, maximum average fruit yield 114.85 q/ha was recorded in 19/OKYVRES-5 followed by 19/OKYVRES-9 (107.05 q/ha), 19/OKYVRES-13 (105.65 q/ha), 19/OKYVRES-11 (102.10 q/ha) and 19/OKYVRES-2 (101.25 q/ha). Similar findings were also reported by Abdul et al., 2004 and Malshe et al., 2016. Singh et al., (2024) evaluated 10 hybrids of okra and reported that the hybrid Amanat was found to be best in term of fruit yield production.

YVMV (%)

The information in disease incidence is presented in Table 3 for year 2019 and 2020. During 2019, no incidence of YVMV was noticed in 19/OKYVRES-5 and very low incidence of YVMV was observed in 19/OKYVRES-13 (1.20%), 19/OKYVRES-2 (2.30%), 19/OKYVRES-11 (2.50%) and 19/OKYVRES-9 (3.20%). During 2020, lowest incidence (1.20%) of YVMV was observed in19/OKYVRES-5 followed by 19/OKYVRES-13 (2.70%), 19/OKYVRES-11 (3.40%), 19/OKYVRES-10 (5.90%) and 19/OKYVRES-2 (6.10%). Based on the average of both the years, lowest incidence (0.60%) of YVMV was observed in 19/OKYVRES-5 followed by 19/OKYVRES-13 (1.95%), 19/OKYVRES-11 (2.95%), 19/OKYVRES-2 (4.20%) and 19/OKYVRES-10 (5.35%). Above finding were supported by Singh et al., 2002; Zulfequar and Patil, 2004; Sanwal et al., 2014 and Malshe et al., 2016.

OELCV (%)

Out of 12 tested entries of Okra, during 2019, no incidence of OELCV was noticed in 19/OKYVRES-2, 19/OKYVRES-5, 19/OKYVRES-6, 19/OKYVRES-9, 19/OKYVRES-10 and 19/OKYVRES-13. In addition to this, low incidence (2.70%) of YVMV was observed in 19/OKYVRES-3 followed by 19/OKYVRES-11 (2.90%), and 19/OKYVRES-1 (3.50%). During 2020, zero incidence of OELCV was noticed in 19/OKYVRES-2, 19/OKYVRES-5, 19/OKYVRES-6, 19/OKYVRES-9, 19/OKYVRES-10 and 19/OKYVRES-13. In addition to this, low incidence 3.10% was seen in 19/OKYVRES-12 followed by 19/OKYVRES-3 (3.30%) and 19/OKYVRES-11 (3.40%). Based on the average of both the years, zero incidence of OELCV was noticed in 19/OKYVRES-2, 19/OKYVRES-5, 19/OKYVRES-6, 19/OKYVRES-9, 19/OKYVRES-10 and 19/OKYVRES-13. In addition to this, low incidence (3.0%) of YVMV was observed in 19/OKYVRES-3 followed by 19/OKYVRES-11 (3.15%), and 19/OKYVRES-1 (3.85%). Above finding were supported by Singh et al., 2002; Zulfequar and Patil, 2004; Sanwal et al., 2014.

Out of 12 tested entries of Okra, during 2019, the maximum fruit yield (116.40 q/ha) with no incidence of YVMV and OELCV was recorded in 19/OKYVRES-5, whereas the minimum yield (77.2 q/ha) with higher percentage of YVMV (24.7%) and OELCV (5.2%) was found in 19/OKYVRES-8. Likewise, during 2020, Twelve entries of Okra were tested, the maximum fruit yield (113.3 q/ha) with minimum incidence of YVMV (1.2%) and no incidence of OELCV was recorded in 19/OKYVRES-5, whereas the minimum yield (72.4 q/ha) with maximum percentage of YVMV (27.4%) and OELCV (6.8%) was found in 19/OKYVRES-8. However, maximum incidence of OELCV (22.1 and 25.4% resp.) was recorded in 19/OKYVRES-7 during both the years. Singh et al., (2024) evaluated 10 hybrids were Anmol, Aryushi, Super Sneha, Sonali-99,

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Sharmili, Julie, Amanat, Karishma, Nandini-7080, Best Green-11 Prayagraj agro climatic conditions reported that the hybrid Amanat was found to be best in term of vegetative parameters, quality parameters, yield.

In India, Okra fruit yield loss is directly associated with viral disease, probably due to favorable tropical warm climatic conditions for the survival of whitefly vector. In addition to this, the overlapping and mixed cropping pattern favors the growth of whitefly vector. Therefore to overcome the yield losses in okra, development of new resistant varieties is the eco-friendly, economical and practical way (Sanwal et al., 2014 and 2016). Therefore, development of new variety 19/OKYVRES-5 with maximum average fruit yield 114.85 q/ha accompanied with resistance against YVMV and OELCV may recommended for commercial cultivation after testing it over the locations and years. The disease incidence of YVMV and OELCV was found negatively correlated with yield during both the seasons (Fig. 3a-d). As the diseased plant photosynthetic capacity is decreased which ultimately leads to reduction in yield. During second year, rainfall was less and almost season was dry, which fevers the growth of white flies - a vector responsible for transmission of viral diseases. Only the resistance genotype e.g. 19/OKYVRES-5 performed better results. Therefore, it can be utilized to develop the resistant genotypes of okra for commercial cultivation in era of climate change.

The increase in okra yield, under Hisar climatic conditions can be credited to several environmental factors and the characteristics of the okra cultivars themselves. Firstly, Hisar climatic is characterized by warm temperatures, low rains, and fertile soils that is optimum for okra growth and yield. The newly developed varieties may have improved resistance against disease, tolerance to climatic up and downs, and leads to healthier crop growth and increased yield.

Conclusion

It may concluded that the newly developed variety 19/OKYVRES-5 of okra has average fruit yield 114.85 q/ha and also resistant against YVMV and OELCV diseases, therefore, may recommended for commercial cultivation after testing it over the locations and years. The disease incidence of YVMV and OELCV was found negatively correlated with yield. Therefore, there is an urgent to develop the resistant genotypes of okra for commercial cultivation in era of climate change.

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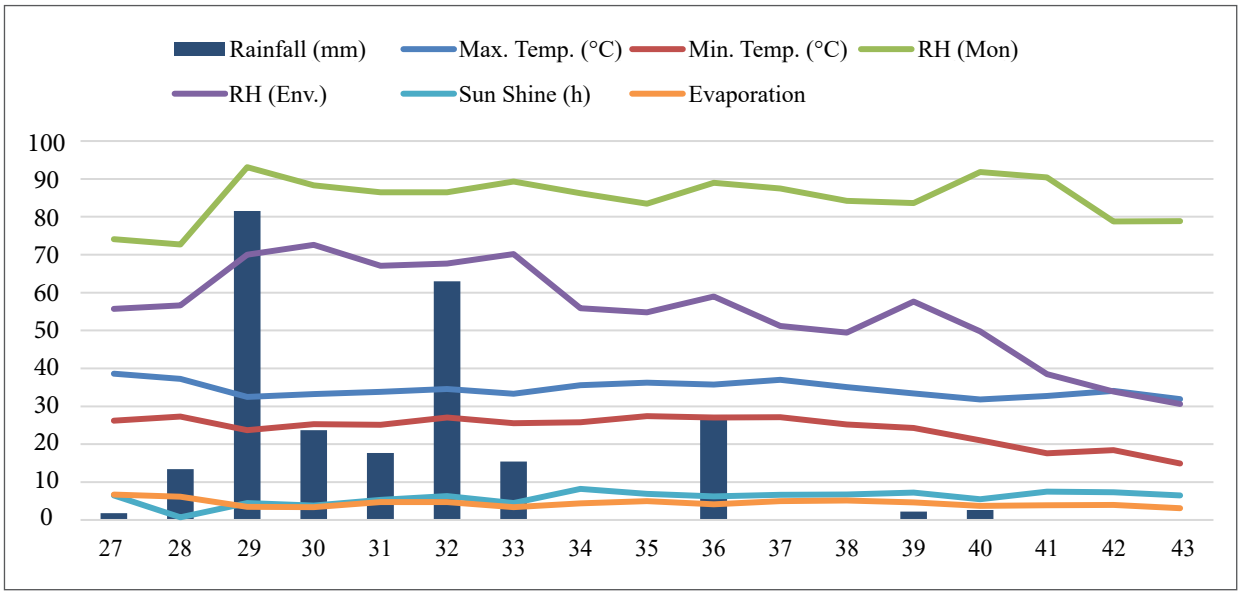

Figure 1. Weather parameters recorded during season 2019.

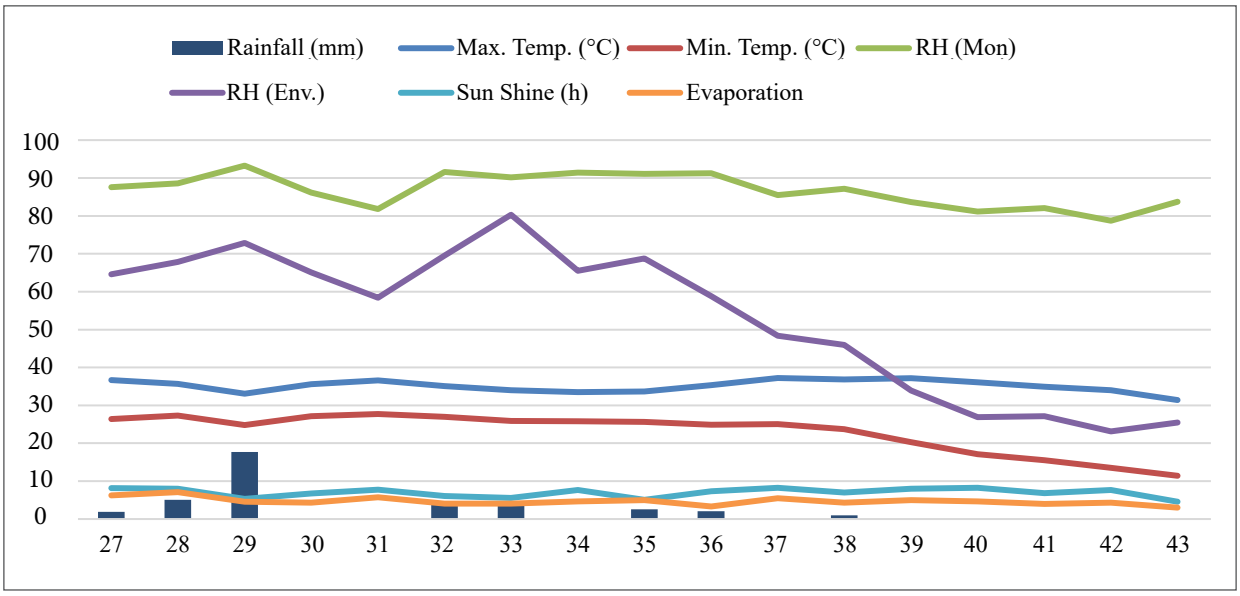

Figure 2. Weather parameters recorded during season 2020.

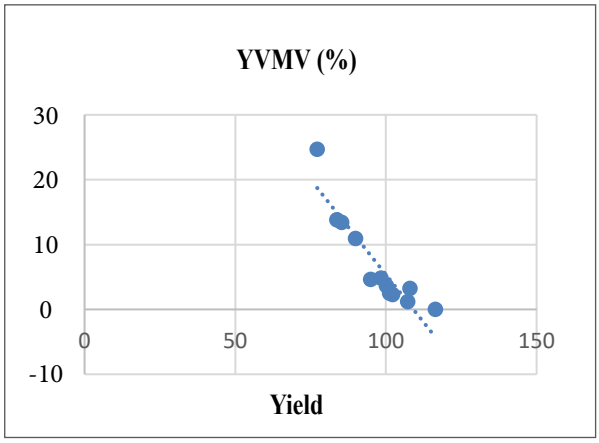

Figure 3a. Correlation between yield and YVMV (%) during 2019.

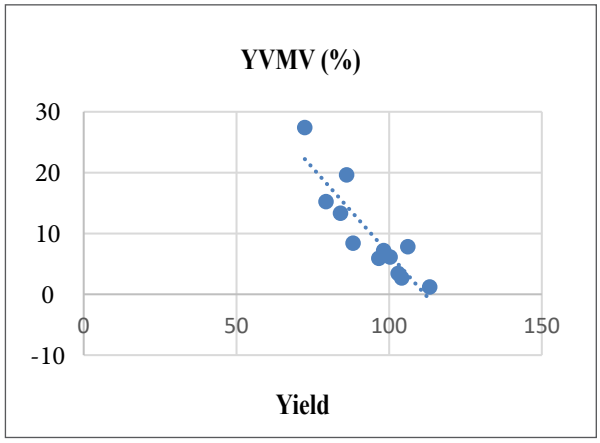

Figure 3b. Correlation between yield and YVMV (%) during 2020.

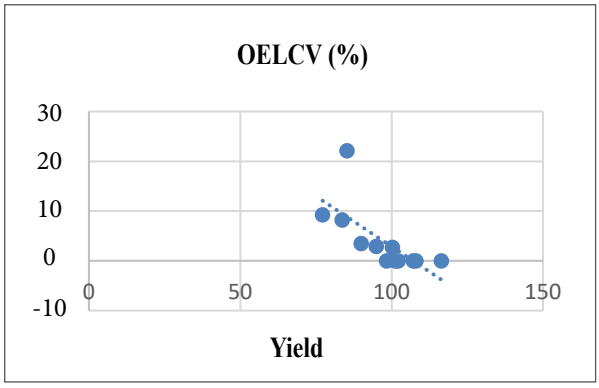

Figure 3c. Correlation between yield and OELCV (%) during 2019.

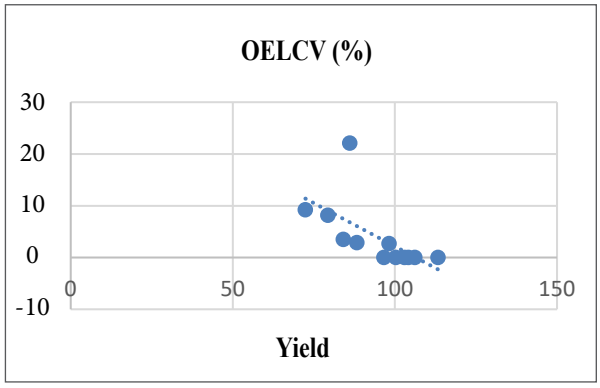

Figure 3d. Correlation between yield and OELCV (%) during 2020

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Table 1. Performance of okra entries for no. of fruits per plant and fruit length (cm) at Hisar.

Serial Number	Entries	No. of Fruits/plant			Fruit Length (cm)		
		2019	2020	Mean	2019	2020	Mean
1	19/OKYVRES-1	14.12	10.32	12.22	9.60	9.10	9.35
2	19/OKYVRES-2	15.43	13.41	14.42	10.00	9.80	9.90
3	19/OKYVRES-3	14.61	11.24	12.93	8.80	9.30	9.05
4	19/OKYVRES-5	24.03	21.10	22.57	11.00	10.70	10.80
5	19/OKYVRES-6	13.89	10.14	12.01	9.80	9.10	9.45
6	19/OKYVRES-7	8.85	8.10	8.48	8.40	9.50	8.95
7	19/OKYVRES-8	11.86	10.50	11.18	11.30	11.10	11.20
8	19/OKYVRES-9	20.62	17.45	19.04	9.00	9.50	9.25
9	19/OKYVRES-10	14.35	10.92	12.63	7.90	7.70	7.80
10	19/OKYVRES-11	16.91	13.80	15.36	8.50	9.00	8.75
11	19/OKYVRES-12	12.96	13.10	13.03	7.70	9.10	8.40
12	19/OKYVRES-13	19.29	16.60	17.95	10.40	10.60	10.55
	Mean	**15.58**	**13.06**	**14.32**	**9.37**	**9.54**	**9.45**
	Range	8.85 - 24.03	8.1 - 21.10	8.48 -22.57	7.70 - 11.30	7.70 - 11.10	7.80 - 11.20
	CD (5%)	2.75	2.29	2.52	0.60	1.00	0.80

Table 2. Performance of okra entries for average fruit weight (g) and fruit yield (q/ha) at Hisar.

Serial Number	Entries	Average fruit weight (g)			Fruit yield (q/ha)		
		2019	2020	Mean	2019	2020	Mean
1	19/OKYVRES-1	10.90	9.60	10.25	89.90	84.10	87.00
2	19/OKYVRES-2	10.70	10.70	10.70	102.20	100.30	101.25
3	19/OKYVRES-3	10.10	10.10	10.10	100.20	98.20	99.20
4	19/OKYVRES-5	12.40	11.80	12.10	116.40	113.30	114.85
5	19/OKYVRES-6	10.30	9.80	10.05	94.90	88.20	91.55
6	19/OKYVRES-7	12.10	11.40	11.75	85.30	86.10	85.70
7	19/OKYVRES-8	10.00	10.50	10.25	77.20	72.40	74.80
8	19/OKYVRES-9	10.50	10.70	10.60	108.00	106.10	107.05
9	19/OKYVRES-10	8.90	8.50	8.70	98.30	96.60	97.45
10	19/OKYVRES-11	9.90	10.10	10.00	101.20	103.00	102.10
11	19/OKYVRES-12	9.60	9.60	9.60	83.70	79.30	81.50
12	19/OKYVRES-13	11.10	11.50	11.30	107.20	104.10	105.65
	Mean	**10.54**	**10.36**	**10.45**	**97.04**	**94.31**	**95.68**
	Range	8.90- 12.4	8.50- 11.8	8.70-12.10	77.2- 116.4	72.4- 110.3	74.8- 113.35
	CD (5%)	1.20	1.60	1.40	7.30	6.70	7.00

Table 3. Performance of okra entries for YVMV (%) and OELCV (%) at Hisar.

Serial Number	Entries	YVMV (%)			OELCV (%)		
		2019	2020	Mean	2019	2020	Mean
1	19/OKYVRES-1	10.90	13.30	12.10	3.50	4.20	3.85
2	19/OKYVRES-2	2.30	6.10	4.20	0.00	0.00	0.00
3	19/OKYVRES-3	3.60	7.20	5.40	2.70	3.30	3.00
4	19/OKYVRES-5	0.00	1.20	0.60	0.00	0.00	0.00
5	19/OKYVRES-6	4.60	8.40	6.50	2.90	3.40	3.15
6	19/OKYVRES-7	13.40	19.60	16.50	22.10	25.40	23.75
7	19/OKYVRES-8	24.70	27.40	26.05	9.20	10.80	10.00
8	19/OKYVRES-9	3.20	7.80	5.50	0.00	0.00	0.00
9	19/OKYVRES-10	4.80	5.90	5.35	0.00	0.00	0.00
10	19/OKYVRES-11	2.50	3.40	2.95	0.00	0.00	0.00
11	19/OKYVRES-12	13.80	15.20	14.50	8.20	3.10	5.65
12	19/OKYVRES-13	1.20	2.70	1.95	0.00	0.00	0.00
	Mean	**7.08**	**9.85**	**8.47**	**4.05**	**4.18**	**4.12**
	Range	0.00 -24.70	1.20 -27.40	1.95 -26.05	0.00 -22.10	0.00 -25.40	0.00 -23.75

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Assessment Through Multilocation Trial for Unique Germplasm of *Hibiscus sabdariffa* L. with Special Reference to Flower and Fruit Colour

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ABSTRACT

Hibiscus sabdariffa L. commonly referred to as "Roselle" and "red sorrel" is a globally known annual herbaceous species of Malvaceae family holds utmost significance owing to its medicinal properties namely antioxidant, antimicrobial, and also for its proven utility as a natural colorant. The dark-red colored anthocyanin rich calyx has multi-purpose utilities including food, beverages, and medicines. The plant species shows its significant and promising potential in avenues of natural red colorants particularly water-soluble ones. Highly adaptive to varying soil conditions and humidity, the North Eastern states of India serve as a perfect cultivation house for this exquisite plant. In the present study 32 germplasm were planted for subsequent year *Kharif* 2021 and *Kharif* 2022 in randomized block design with three replicates. From the two years' evaluation 5 identified lines were subjected to multi-locational trials (MLT) along with one check variety named as local variety comprising of four different locations of NE India during *Kharif* 2023. Diverse climatic conditions of this region are responsible for the formation of new genotypes with varying chemical compositions. For this purpose, the MLT was performed at four different locations of North East India to confirm their stability performance. The main objective was to carry out a morphological and qualitative data analysis of *H. sabdariffa*. Plant height, colour of flower, colour of fruit, number of fruits per plant, average fruit weight and stem colour were evaluated. The qualitative and quantitative results demonstrate the identified lines were superior to that of the local (check) variety.

Keywords: Calyx, fruit size, flower colour, multilocation trial, roselle

Introduction

Hibiscus sabdariffa L. of the Malvaceae family is a herb commonly named as "red sorrel" or "roselle" in English (Betiku and Adepoju, 2013; Mahadevan and Kamboj, 2009). It is commonly known as Jamaica in Mexico and Spain, Bissap in Senegal, Karkade in Egypt, Wonjo in the Gambia, Congo in France, Zobo in Nigeria, Saudi Arabia and Sudan (Cisse et al., 2009; Mckay et al., 2009; Ramirez-Rodrigues et al., 2012). In India it is also known as Lal ambari, Gongura, Lal Mista, Patwa, Chukar in Bengali, Pulichchaikerai in Malayalam; Yerra Gogu in Telegu, Tengamora and Chutkiar in Assam (Gautam, 2004). *H. sabdariffa*, a perennial plant, is one of the most trafficked commercial and medicinal plants worldwide (Amlashi et al., 2020). *Hibiscus* genus includes more than 300 species of annual, perennial herbs, shrubs and trees (Wang et al., 2012). The *Hibiscus* species possesses various medicinal properties, among all the species one of them is *Hibiscus sabdariffa* (Orwa et al., 2014). It is distributed in subtropical and tropical regions of the world, popular for high fiber content and is used to make cloth and rope, cultivated in the late December to February (Ali et al., 2014).

In warmer countries like India, Malaysia, Indonesia, Sudan, Mexico, Saudi Arabia, Egypt, Philippines, Thailand and Vietnam *H. sabdariffa* is distributed (Wisetmuen et al., 2008). The main producing areas of Roselle are China, Mexico, Sudan, Thailand, Egypt (Carvajal-Zarrabal et al., 2012), native to tropical Africa and cultivated in Nigeria, tropical

America and West Indies (Inikpi et al., 2014). *H. sabdariffa* has red stems that can grow up to 3.5 meters tall, with deep-penetrating taproots. Its alternate leaves, which range from dark green to red, have long petioles measuring 3 to 5 inches (7.5-12.5 cm) in length. The flowers, known as Roselle, form in axillary or terminal racemes and feature white petals with a reddish center at the base of persistent columns, often used as food (Ali et al., 2014). The seeds are kidney-shaped, brown, and covered with minute hairs. The leaves grow alternately, have reddish veins, and are edible. At tender age, the fruits are found to be green and when it matures, it has five valves and each valve possess 3-4 seeds (Ali et al., 2014). Around the world, in different countries for many years this plant has been used as culinary and therapeutic resources (Ubani et al., 2010). Most commonly used in food industry as flavoring agents in puddings, cakes, as hot or cold beverages. In some parts of Africa, people ground the roselle seeds and add to meals (Duke and Du Cellier, 1993) whereas the calyx and leaves are served as vegetables (Mungole and Chaturvedi, 2011).

The red calyx is used to prepare ice creams, jams, jellies whereas, the leaves juice are used to improve the health and immune system (Idris et al., 2012). In traditional medicine, during summer the drink of *H. sabdariffa* provide relief by increasing blood flow to the skin surface (Mungole and Chaturvedi, 2011). The leaves in combination with ginger are used to suppressing high blood pressure (Ali et al., 2005). The ripe calyx is boiled in water can cure bilious attacks and ulcers, the flower is used as tonic, tea and proper kidney functioning. The leaves on heating can be applied in the feet for avoiding cracks and on boils (Ali et al., 2005). *H. sabdariffa* flower extract is caffeine free and helps in digestion and effective for inflammatory skin disorders (Wisetmuen et al., 2008).

Roselle is mostly known for the nutritional, medicinal purposes (Kolawole et al., 2014) and has various pharmacological properties like antioxidant (Mahadevan and Kamboj, 2009), antiviral (Idris et al., 2012), anticancer (Ubani et al., 2010), antimicrobial (Wisetmuen et al., 2008), hepatoprotective (Ologundudu et al., 2009), diuretic (Sandeep et al., 2010), immunomodulation, antiobesity (Carvajal-Zarrabal et al., 2012), hypocholesterolemic (Ali et al., 2005), antifungal (Ali et al., 2011), antipyretic (Bako et al., 2009), antianaemic (Khaghani et al., 2011), renoprotective (Kolawole et al., 2014), hypotensive, and antiurolithiatic (Kristen et al., 2014). Almost all parts of the plant contain bioactive compounds and inorganic minerals. The seeds are rich in carbohydrates, cellulose, fibers, and starch. Both the calyx and seeds are good sources of minerals such as potassium, magnesium, zinc, nickel, manganese, iron, phosphorus, calcium, sodium, aluminum, etc. (Rao, 1996).

Roselle seeds are a good source of edible oil (Ahmed and Hudson, 1979). The seeds on a dry weight basis are found to contain approximately 15% highly unsaturated triglycerides and small amounts of other lipid components (Nyam et al., 2009). In roselle seed oil, oleic and lenoleic acid are the major unsaturated fatty acids. High content of linoleic acid may provide a good source of essential fatty acids. Another major constituent found to be rich in the oil is alpha-tocopherol (Nyam et al., 2009). The seed also contain phytosterol compound such as desmethylsterol that has the ability to reduce dietary cholesterol absorption (Jones et al., 2000). The extract of *H. sabdariffa* possesses secondary metabolites such as tannins, saponnins, glycosides, phenols, and flavonoids. Due to the presence of flavonoid, anthocyanin, and polyphenol the flower acts as antioxidant (Camelo-Méndez, 2013). Two major anthocyanins are present in the plant namely cyanidin-3-sambubioside and delphinidin-3-sambubiside, which gives deep red pigment of the calyx and is the main contributors of antioxidant activity (Ali et al., 2005; Mungole and Chaturvedi, 2011; Camelo-Méndez, 2013). The compounds found in the calyx are galactose, galacturonic acid, gallic acid, gossypetin, hibiscetin, hibiscin, myricetin, protocatechuic acid, quercetin, rhamnose, and sabdaritrin (Zhang and Wang, 2007).

H. sabdariffa essential oil contains 17 compounds, constituting 99.8% of the total oil. In oil the compounds present are sesquiterpenes hydrocarbons, oxidized sesquiterpenes, diterpenes, aliphatic compounds, phenylpropanes and fatty acids. The main components of essential oils are the linoleic acid, hexadecanoic acid, and fatty acids. Minor components include tetradecanoic, methyl ester, heptadecanoic acid, methyl hexadecanoic acid, isophytol, and methyl linoleate (Inikpi et al., 2014). The calyx oil of *H. sabdariffa* is also rich in geraniol, menthol and undecalactone. Sterols include β-sitosterol, campesterol, avenasterol, cholesterol and clerosterol (Mungole and Chaturvedi, 2011). Roselle holds significant traditional and commercial value in both the food and household industries. Its diverse applications extend to the cosmetic, poultry, herbal, medical, and food industries, contributing to its constant demand. This suggests a promising future for *H. sabdariffa* in pharmaceutical and other sectors in the upcoming generations. Therefore, the present study emphasizes on the identification of elite germplasm of *H. sabdariffa* for the greater benefit of mankind.

Materials and methods

In the current study, a total of 32 germplasm were planted during *Kharif* 2021 in randomized blocks design (RBD) with three replications. The trial was replicated during the consecutive *Kharif* 2022. The plantation was done during May and harvested in the month of November for each of the year. The line to line spacing of 60 cm and plant to plant spacing of 45 cm was maintained. The germplasm was screened for the plant height (cm), colour of flower, fruit, number of fruits per plant, average fruits weight (g), and stem colour during *Kharif* 2021 and *Kharif* 2022. After two years evaluation, five unique lines were identified namely Jor Lab HS- 6, Jor Lab HS- 9, Jor Lab HS- 16, Jor Lab HS- 19, Jor Lab HS- 22. All identified lines along with one check variety named as local were planted in RBD with three replicated at four different locations of NE India (Jorhat, Bokakhat in Assam and Runne, Modai in Arunachal Pradesh) during *Kharif* 2023. All the morphological and quality data was reported as per standard protocol. For the colour data recording the Royal Horticultural Society (RHS) colour chart was taken as the standard reference.

Results and Discussion

Among the thirty-two germplasm, five elite lines of *H. sabdariffa* were identified namely, Jor Lab HS-6, Jor Lab HS- 9, Jor Lab HS- 16, Jor Lab HS- 19, Jor Lab HS- 22. The identified germplasm was subjected to morphological and qualitative data studies in MLT along with one local variety during *Kharif* 2023. During the study period the range of variation observed for plant height, flower colour, fruit colour, number of fruits per plant, fruit weight, and stem colour were recorded (Table 1). The colour of flower, fruit and stem were identified by the help of the Royal Horticultural Society (RHS) colour chart.

In Jor Lab HS-6, plant height ranged from 145-155 cm, flower colour was deep purplish pink, and fruit colour was vivid red, number of fruits per plant was found to be 55-59, fruits weight ranged from 4-6 g, stem colour was moderate purplish red. While, Jor Lab HS-9 exhibited 166-177 cm plant height, light purplish pink flower colour, vivid red fruit colour, 32-35 number of fruits per plant, 4-5 g was the range for fruit weight and stem colour was deep purplish red. Jor Lab HS-16 showed 183-190 cm range for plant height, deep purplish pink flower colour, deep purplish red fruit colour, range of 90-93 for number of fruits per plant, 6-7 g for fruit weight and deep purplish red stem colour. Meanwhile, Jor Lab HS-19 showed 187-193 cm range of plant height, pale yellowish pink flower colour, vivid red fruit colour, number of fruits per plant ranged from 74-77, 5-6 g for fruit weight and moderate purplish red stem colour. Jor Lab HS-42 exhibited plant height in the range of 210-220 cm, deep purplish pink flower colour, deep purplish red fruit colour, 103-108 number of fruits per plant, 7-8 g was the range for fruit weight and moderate purplish red stem colour respectively. Compared to the identified germplasm, the local variety showed 115-123 cm range for plant height, light purplish pink flower colour, moderate red fruit colour, number of fruits per plant ranged from 20-25, 3-4 g for fruit weight and grayish purple stem colour (Table 1).

Based on the MLT data for the average data for identified germplasm and local variety were tabulated (Table 2). From the data it was found that the germplasm Jor Lab HS-42 exhibited the highest average plant height of 218 cm, followed by Jor Lab HS-19 (192 cm), Jor Lab HS-16 (189 cm), Jor Lab HS-9 (175 cm) and Jor Lab HS-6 (153 cm). The local variety had a comparable average plant height (184 cm) similar to the identified germplasm. A similar trend was observed for the traits average fruit weight and average number of fruits per plant, where the average value of the local variety was below than that of the identified five germplasm. For the trait average fruit weight, the highest value was observed in Jor Lab HS-42 (7 g), followed by Jor Lab HS-16 (6.5 g), Jor Lab HS-19 (5.9 g), Jor Lab HS-6 (5.2 g) and Jor Lab HS-9 (4.1 g) which was higher than the local variety (3.6 g). Similarly, for the trait number of fruits per plant the highest value was observed in Jor Lab HS-42 (105) followed by Jor Lab HS-16 (91), Jor Lab HS-19 (75), Jor Lab HS-6 (58) and Jor Lab HS-9 (35) which was again higher than the local variety (30).

Considering the colour aspect, variation was observed for the traits flower colour, fruit colour and stem colour where RHS N66-D indicate deep purplish pink colour, RHS 65-B indicate light purplish pink colour, RHS-27-D indicate pale yellowish pink colour, RHS 62-C indicate light purplish pink colour, RHS 46-B indicate vivid red, RHS 59-B indicate deep purplish red, RHS N45-D indicate moderate red, RHS 184-C indicate moderate purplish red, RHS 71-A indicate deep purplish red and RHS N77-A indicate grayish purple. The colour of the flower for Jor Lab HS-6, Jor Lab HS-16, Jor Lab HS-42 was deep purplish pink (RHS N66-D), for Jor Lab HS-9 the colour was light purplish pink (RHS 65-B) and for Jor Lab HS-19 the colour was pale yellowish pink (RHS-27-D) compared to light purplish pink (RHS 62-C) for the local variety (Fig 1). Meanwhile the colour of the fruit for Jor Lab HS-6, Jor Lab HS-9 and Jor Lab HS-19 was vivid red (RHS 46-B), and for Jor Lab HS-16 and Jor Lab HS-42

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the colour was deep purplish red (RHS 59-B) compared to moderate red (RHS N45-D) for the local variety (Fig 2). The stem colour for Jor Lab HS-6, Jor Lab HS-19, Jor Lab HS-42 was moderate purplish red (RHS 184-C), for Jor Lab HS-9 and Jor Lab HS-16 was deep purplish red (RHS 71-A) compared to grayish purple (RHS N77-A) for the local variety (Fig 3). Therefore, in this study, it revealed that all five germplasm (Jor Lab HS-6, Jor Lab HS-9, Jor Lab HS-16, Jor Lab HS-19 and Jor Lab HS-42) were found to be better than the check variety (local). Among the five selected elite germplasm Jor Lab HS-42 was the best performing.

A study conducted by Ilodibia et al. (2019) identified two varieties of *H. sabdariffa*: The red variety and the green variety. Morphological studies show no significant differences in their habits, and structure, but differ mainly in size and colour. Larger leaf area (94.25±0.310 cm^2) and petiole length (6.50±0.620 cm) was observed in the red variety as compared to the green variety. The leaf area and petiole length of the green variety are 53.95±0.400 cm^2 and 3.60±0.332 cm respectively. In a survey undertaken by Daudu et al. (2015), sixty Roselle accessions were collected showed that 41.7% of them were green in colour, and 31.7% had red calyxes. Among the accessions, 20% had deep red calyxes, while only 6.7% exhibited red and pink calyxes. Thus it can be said that aside from its medicinal properties, it is extensively utilized in food and beverage preparation. Another report by Sanders et al. (2020) revealed that based on a study on four genotypes of *H. sabdariffa* (African Green, Indian Red, Indian Variegated, and Thai Red) in New Jersey, the highest dry leaf weight was recorded in African Green Roselle (81.89 g), followed by Indian Variegated (79.94 g), Indian Red (74.23 g) and Thai Red (55.70 g) genotypes. Another report by Richardson and Arlotta, (2021) studied the yield of selected seven genotypes of *H. sabdariffa* near Washington, DC planted in three production systems (green roof, field row, high tunnel). From the study, it was found that in the field row the production of leaves was highest. According to Stevels (1990), Roselle plants containing anthocyanin pigments demonstrate greater resilience and tolerance to harsh environments compared to green varieties. From the present study, it can be clearly stated that the identified germplasm of *H. sabdariffa* holds promise for higher yield and further breeding program.

Conclusions

The present study revealed some important and unique morphological characteristics as well as excellent yield characteristics of some selected Roselle accessions. Considering the present result all the five germplasm (Jor Lab HS-6, Jor Lab HS-9, Jor Lab HS-16, Jor Lab HS-19 and Jor Lab HS-42) performed better than the local check variety. Moreover, considering the distinct morphological and colour variation of the selected germplasm all the germplasm are unique with the germplasm Jor Lab HS-42 performing with the highest yield across all the locations. Moreover, considering the different locations, Runne of Arunachal Pradesh was found to be best performing. Roselle has been reported to be used as a flavoring and coloring agent in food and beverage industry. It appears to be a good and promising source of water-soluble natural red dyes for use in the food and pharmaceutical industries. The overall data can be used as a useful tool in Roselle breeding to increase Roselle yield and improve accurate taxonomic characterization and plant species identification with great economic potential. This, in turn, can foster entrepreneurial opportunities and contribute to the upliftment of the socio-economic status of farming communities.

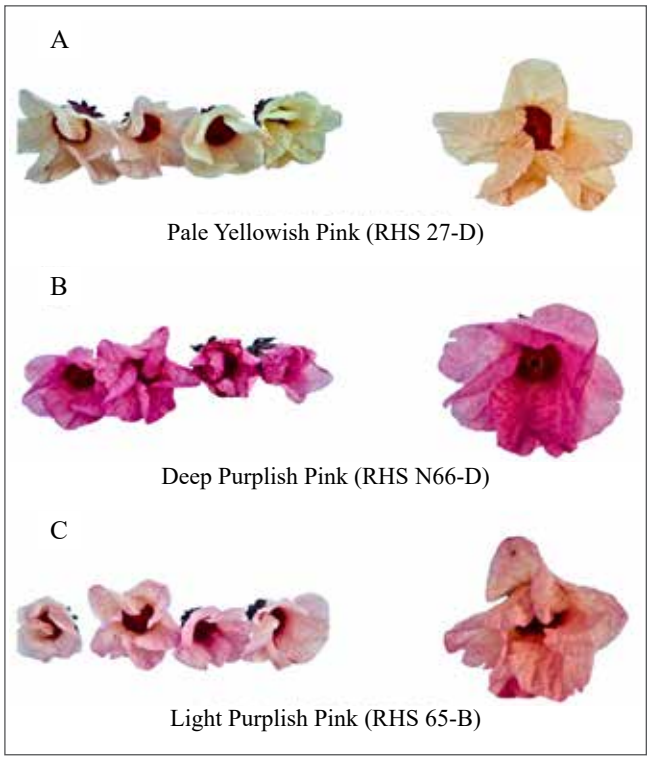

Figure 1. Different varieties of *Hibiscus sabdariffa* flower. (A) Jor Lab HS-19, (B) Jor Lab HS-6, Jor Lab HS-16, Jor Lab HS-42, (C) Jor Lab HS-9.

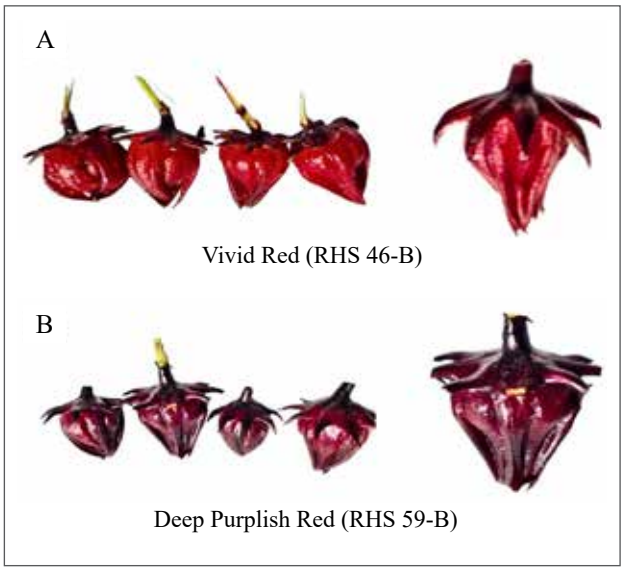

Figure 2. Different varieties of *Hibiscus sabdariffa* fruit (A) Jor Lab HS-6, Jor Lab HS-9, Jor Lab HS-19, (B) Jor Lab HS-16, Jor Lab HS-42.

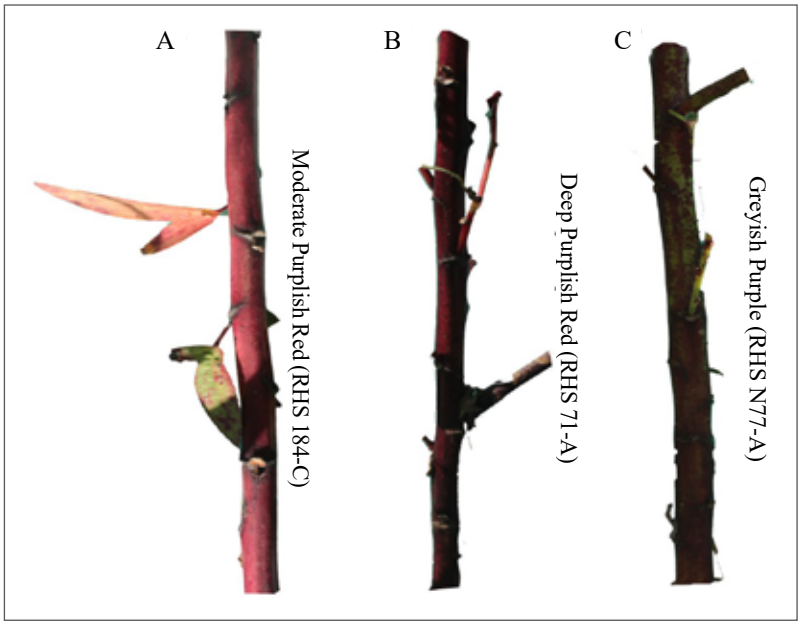

Figure 3. Different varieties of *Hibiscus sabdariffa* stem (A) Jor Lab HS-6, Jor Lab HS-19, Jor Lab HS-42, (B) Jor Lab HS-9, Jor Lab HS-16, (C) Check variety (Local)

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Table 1. Range of traits taken for study among the germplasm of *Hibiscus sabdariffa* during *Kharif* 2021 and 2022.

Traits	Jor Lab HS-6	Jor Lab HS-9	Jor Lab HS-16	Jor Lab HS-19	Jor Lab HS-42	Check variety (Local)
Plant height (cm)	145-155	166-177	183-190	187-193	210-220	115-123
Colour of flower	Deep purplish pink	Light purplish pink	Deep purplish pink	Pale yellowish pink	Deep purplish pink	Light purplish pink
Colour of fruit	Vivid red	Vivid red	Deep purplish red	Vivid red	Deep purplish red	Moderate red
No. of fruits per plant	55-59	32-35	90-93	74-77	103-108	20-25
Fruits weight (g)	4-6	4-5	6-7	5-6	7-8	3-4
Stem colour	Moderate purplish red	Deep purplish red	Deep purplish red	Moderate purplish red	Moderate purplish red	Greyish purple

Table 2. Average morphological and qualitative data of identified germplasm along with the Check variety (Local) of *Hibiscus sabdariffa* during multilocational trial.

Pedigree	Plant height (cm)	No. of fruits per plant	Average fruits weight (g)	Colour of flower	Colour of fruit	Stem colour
Jor Lab HS-6	153	58	5.2	Deep purplish pink (RHS N66-D)	Vivid Red (RHS 46-B)	Moderate purplish red (RHS 184-C)
Jor Lab HS-9	175	35	4.1	Light purplish pink (RHS 65-B)	Vivid Red (RHS 46-B	Deep purplish red (RHS 71-A)
Jor Lab HS-16	189	91	6.5	Deep purplish pink (RHS N66-D)	Deep purplish red (RHS 59-B)	Deep purplish red (RHS 71-A)
Jor Lab HS-19	192	75	5.9	Pale yellowish pink (RHS-27-D)	Vivid Red (RHS 46-B)	Moderate purplish red (RHS 184-C)
Jor Lab HS-42	218	105	7	Deep purplish pink (RHS N66-D)	Deep purplish red (RHS 59-B)	Moderate purplish red (RHS 184-C)
Check variety (Local)	184	30	3.6	Light purplish pink (RHS 62-C)	Moderate red (RHS N45-D)	Grayish purple (RHS N77-A)
SD	19.49	27.53	1.25			
SE	4.59	6.49	0.29			
CV	10.52	21.93	13.25			

*SD= standard deviation, SE= standard error, CV= coefficient of variance

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Relationships Between Seeding Densities and Selection Parameters in Bread Wheat (*Triticum aestivum* L.) Genotypes under Rainfed Conditions

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ABSTRACT

Agronomic approaches are important for high yield in changing environmental conditions such as climate and soil structure. Drought and heat stress due to climate change require the determination of tolerant genotypes using different seeding densities in bread wheat. The experiment was carried out in the Trakya Agricultural Research Institute experimental field in the 2015-2016 and 2016-2017 growing cycles. In the research, 8 bread wheat genotypes and three different seed densities of 400, 500 and 600 grains per square meter were used. The study was conducted in the randomized complete blocks design. Data on, the number of spikes and grains per spike, the number of spikes per square meter, grain yield, peduncle length and spike length, flag leaf area and normalized difference vegetation index were examined. In the study; year, genotype and year×genotype interaction were significant. The mean grain yield was 6197 kg ha^{-1}. Genotype G6 produced a higher grain yield with 7730 kg ha^{-1}. The results of the research showed that there was no significant difference between the seed densities for grain yield. However, it was observed that using 500 seeds per square meter had a higher grain yield (6280 kg ha^{-1}). The maximum peduncle length (31.04 cm) and spike length (8.94 cm) were determined in the application using 400 seeds per square meter. The use of 400 seeds per square meter produced the maximum spikelet number per spike (17.90). According to seeding density, the maximum number of spikelet's (535.6) and flag leaf area (24.51 cm^2) were obtained when 500 grains per square meter were used.

Keywords: Wheat genotypes, seeding density, yield, yield component, physiological parameters

Introduction

The number of plants or ears per unit area according to different environmental conditions and soil structure is the most significant factor determining the yield of bread wheat. Tillering potential and environmental conditions should be considered for wheat's planting density. Genotypes with low tillering potential show a higher effect on yield and spike weight due to the increase in seed density. The number of grains per ear reveals the lowest genotype effect and is strongly affected by the planting rate (Valerio et al., 2013). Plant rate in a square meter is one of the main factors determining the crop's ability to capture resources. Wheat production is important because it is under the control of the farmer in the planting system (Satorre, 1999). Optimum plant densities vary significantly between regions, climates, soil types, planting times and cultivars. Because varieties are genetically different in their yield components, varieties require to be examined over a broad range of seeding rates to establish optimum seeding rates (Wiersma, 2002). The planting date is highly affected by the optimum planting percentage, mainly controlled by climatic conditions and crop rotation requirements. Latitude and longitude, as well as the temperature of the production area, also affect the growing time (Gooding and Davies, 1997). It is important to use the most appropriate planting density to achieve the highest production efficiency. If an excessive seed rate is used, as the plant population will increase, there will be competition for water, nutrients and sunlight

in the plant, and as a result, productivity and quality will decrease. If a lower seed rate is used, the yield will be lower as the plant number per unit area will decline (Attarde and Khuspe, 1989). However, this compensatory mechanism may vary depending on bread wheat genotypes (Dahlke et al., 1993; Lloveras et al., 2004). It is known that the optimum seed rate may increase with the influence of environmental factors (Arduini et al., 2006; Gooding et al., 2002). Nevertheless, increasing planting frequency does not always increase yield because as seed density increases, competition between plants also increases (Park et al., 2003). Appropriate planting rates help to ease the conflict between individuals and populations by establishing a reasonable population structure. It is also useful to coordinate the development of spike number, spike grain number and grain weight of winter wheat (Wang et al., 2011). The seeding densities influence the wheat population, which further influences the use of soil water and nutrients and ultimately affects wheat growth and yield formation (Kühling et al., 2017). Wheat yield rises with the increase in seeding densities under the low planting densities and rises at a certain threshold value, after which it might not increase and may even cause a decrease in grain yield (Bhatta et al., 2017).

Determining the optimum seed density in varieties is very essential for high-yield potential. Seed frequency to be used in sowing may vary according to dry and productive conditions. Different soil structures also such as sandy or loamy can also be effective in seed density. Soil moisture and temperature after planting are also important factors in tillering. The genetic factor is the most important factor in determining seed density, as it requires the use of lower seed density in varieties with high tillering ability. Depending on the vernalisation needs, some cultivars may have early and fast tillering, while in some cultivars it may be late. This is especially important in early drought and provides an advantage to early and fast-growing varieties. In the research, the impacts of various planting density treatments on grain yield and yield components in some wheat genotypes under rainfed conditions and the relationships between the parameters were examined.

Materials and Methods

The experiments were completed during the period of the 2015-2016 and 2016-2017 growing cycle's breeding field area in Edirne locations, in the northwest of Türkiye. The study site is located in the Trakya region lowland, the experiment site is 41° 38′ 55″ N, 26° 36′ 06″ E and the altitude is 40 m. In the research, 8 bread wheat genotypes (Saban, Köprü, Yüksel, BBVD7-2014, BBVD24-2014, BBVD3-2015, BBVD12-2016, BBVD17-2016) and 3 different seed density (400, 500 and 600 kernel per square meter) applications were examined under rainfed conditions. The study was a split plot randomized complete blocks design with four replications. In the experiment plot area was 6 m^2, with 6 rows and 0.17 cm spaced rows. The following field characters were evaluated; a number of spikes per square meter (SNM), the number of spikelet per spike (SNS) and the number of kernels per spike (KNS) were counted after harvesting for each genotype. Grain yield (kg ha^{-1}) was determined in a 6 m^{-2} plot area. Spike length (SL) and peduncle length (PL) were determined by scaling ten randomly selected plants in each subplot at physiological maturity for tested genotypes. At the heading stage (Z55) normalized difference vegetative index (NDVI) was measured defined by Reynolds et al., 2001, Reynolds et al., 2012 and Pask et al., 2012. During the heading period, the width and length of 10 flag leaves were measured and multiplied by 0.68 to determine the average flag leaf area (Fowler and Rasmusson, 1969). Normalized difference vegetative indices (NDVI) was scaled at the heading phase of the genotypes (Gutierrez-Rodriguez et al, 2004). The Zadoks Decimal Code (Z) defined plant growth stages (Zadoks et. al., 1974).

Statistical Analyses

Data were analysed statistically using L.S.D (Least Significant Difference at 5%) to test the significant difference between means (Gomez and Gomez, 1984; Steel and Torrie, 1980). Correlation coefficient analysis for the relationship between parameters was performed according to the Dewey and Lu (1959) method.

Climate Data

Total precipitation and temperature in the studied field area in the 2015-2016 and 2016-2017 cycles are given in Table 1. In the experimental area, monthly total precipitation in the 2015-2016 cycles was higher and in 2016-2017 lower than the long year. Total rainfall in November and December in both cycles was much lower than in a long year. Average temperature was very low in 2016-2017 and higher than the average in 2015-2016 cycles.

Results and Discussion

Plant density per unit area is an important factor affecting the many components in wheat genotypes. Plant ratio per unit area is one of the essential factors and it varies according to climatic conditions, soil type, planting time and genotype effect. The results of variance analysis indicate considerable variations ($p<0.01$) among genotypes (G), among years (Y) and their interaction (Y×G) for all parameters examined.

The interaction of seeding densities (treatment) was non-significant statistically in all data investigated except for the flag leaf area. In the flag leaf area there was a significant difference in treatment, Y×T and G×T interactions. The grain number per spike, which is one of the important factors determining yield, is affected by drought and temperature during grain filling, sowing frequency also has an important effect. The interaction of G×T and Y×G×T was statistically significant according to grain number per spike (Tables 2 and 3).

The appropriate seeding rate in wheat genotypes may differ based on climate effect, soil type and genetic structure. Grain yield in genotypes differed from the highest 7730 kg ha^{-1} in G6 to the lowest 4994 kg ha^{-1} in G1 (Table 5). In the experiment, the average yield was 6197 kg ha^{-1}. According to genotypes, the maximum yield was produced by genotypes G6 and G5. The data showed that spike length and peduncle length differed significantly by the seeding rate of the genotypes. Based on seeding rates, the longest peduncle (33.37 cm) was measured in genotype G7 followed by G4. Genotypes G7 and G4 exhibited the longest spike length with 9.52 cm and 9.50 cm, respectively. Different seeding densities significantly affected the spikelet number per spike, genotype G2 produced a maximum spikelet number in spike (19.28) closely followed by G7 with an 18.71 spikelet number (Table 4).

Kernel number per spike varied among genotypes. While the highest kernel number was determined in G4 (44.78) the lowest grains were established in G1 (33.91). The spike number in a square meter may vary depending on the sowing depth, plant ratio and genotype. In the study, the spike number in a square meter varied among genotypes, the minimum was 455.6 in genotype G7 and the highest was 592.9 in genotype G3. Flag leaf is an important plant organ that contributes to productivity through photosynthesis. The maximum flag leaf area was scaled in genotype G1 (26.20 cm^2) and followed by G7 (25.88 cm^2), while the minimum (17.68 cm^2) flag leaf area was recorded in genotype G3. The mean normalized difference vegetative index (NDVI) of wheat genotypes was 0.59; genotype G1 had a higher NDVI and G2 had the lowest NDVI (Table 4).

In the study, no significant difference was found in yield according to seed density. The use of only three different seed densities can be considered one of the factors. Nevertheless, the using of 500 seeds in a square meter yielded higher yield potential in genotypes. There were also no statistically differences among seeding rates for other parameters tested. Using only one lower and upper ratio of the recommended seed density caused the application to be insignificant. In the study, longer spike (8.94 cm) and peduncle length (31.04 cm) were obtained when 400 seeds were used per square meter. As expected in the study, a higher number of spikelets was determined at sparse seed density. The highest number of spikelet was 17.90 when 400 seeds per square meter were used (Table 5). Peduncle length, spike length, spikelet number per plant and kernel number per plant decreased along with the seeding rate. Normalised difference vegetative index increased with increasing seeding rate.

The number of grains per ear is among the essential yield components. The highest number of grains per spike was determined at 400 and 500-grain frequencies. The maximum spikelet number per square meter (535.6) was scored when 500 seeds were used per square meter. The results showed that seeding rate significantly influenced the flag leaf area of wheat genotypes. The largest flag leaf area (24.51 cm^2) was determined in the application using 500 seeds per square meter. In the experiments, NDVI increased with increasing seed frequency (Table 5). The fact that the application of seeding density is not important has shown that these studies should carry on under various environmental conditions and various seeding densities. It is possible to interpret that the expected result cannot be obtained in some parameters according to the seed frequency, the genotypes are similar and the research area has a similar environment. Therefore, similar studies should use different genotypes and be conducted in different environments.

Comparisons among the parameters examined in the study and different relationships were found (Table 6). In the study, all parameters except normalised difference vegetative index were found to have a positive relation with yield. A positive association was found between grain yield with the number of spikes in a square meter (p=0.809), the number of spikelet in a spike (p=0.592), the number of grains in a spike (p=0.907), the length of the peduncle (p=0.618) and flag leaf area (r=0.966*). Positively relation was found to be with flag leaf area and spikelet number in spike, kernel number in spike (r=0.766), spikelet number per square meter (r=0.934) and peduncle length. Correlation coefficients for wheat genotypes showed that a negative association between normalised difference vegetative index and grain yield (p=-0.659), peduncle length (r=-0.998**), spike length (r=-0.903), spikelet number per spike (r=-0.996**), kernel number per spike (r=-0.915). The results showed that the largest flag leaf area significantly influenced the yield components such as kernel number per spike, spikelet

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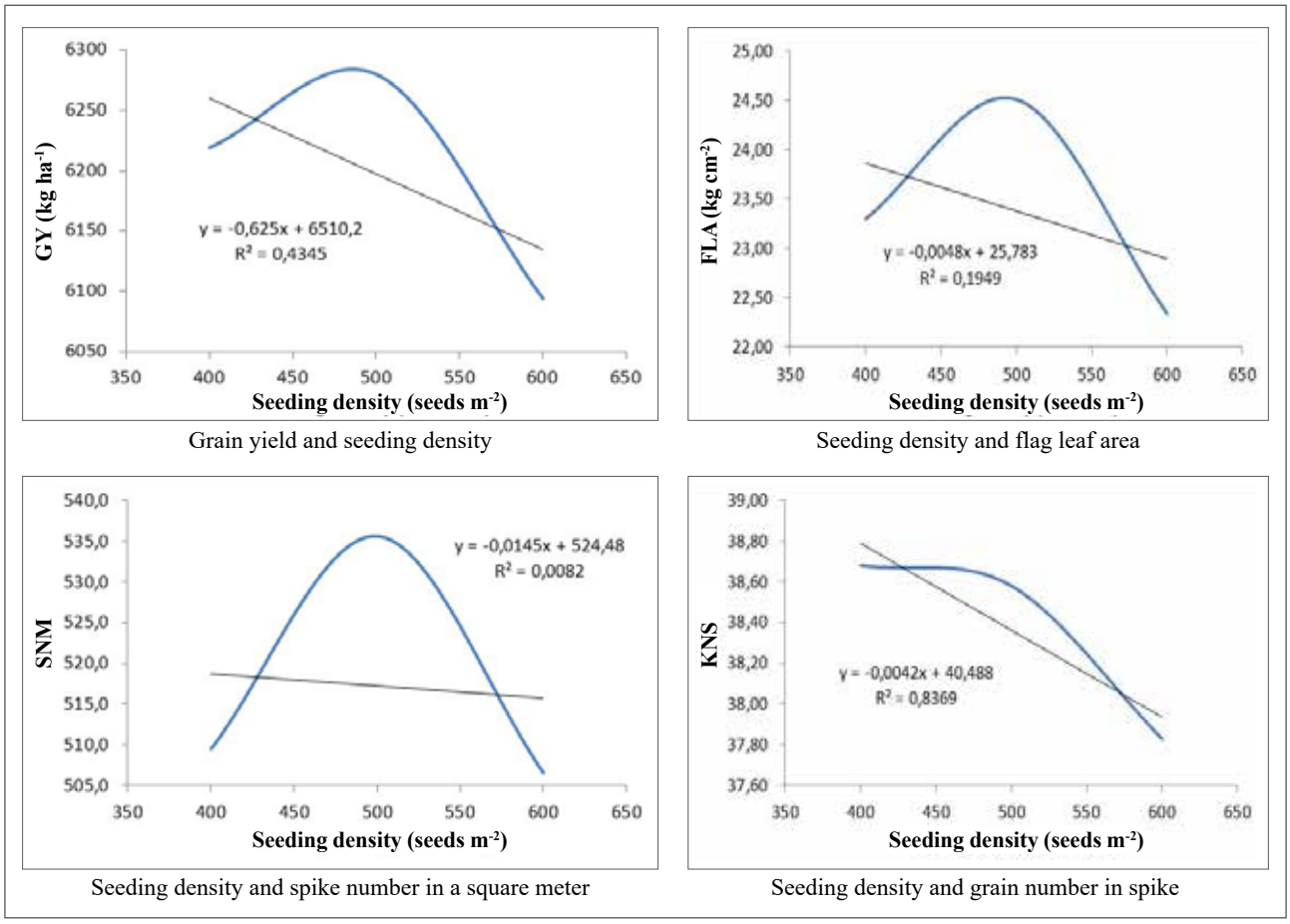
number per spike and spike number per square meter of winter wheat.

In the study, pairwise relations between seeding rate and the parameters tested were examined. Peduncle length, spike length, spikelet number per plant and kernel number per plant decreased along with the seeding rate. Normalised difference vegetative index increased with increasing seeding rate (Figure 1).

Conclusions

Adjustment of seeding densities is one of the main crop management procedures that most impact grain yield components. In the study, genotypes, years and the interaction between them were statistically significant. The use of 500 seeds per square meter had a higher grain yield in genotypes. The highest yielding potential was performed by genotypes G6 and G5. The data showed that peduncle length and spike length differed significantly by the seeding rate of the genotypes. The longest peduncle length was observed in G7 followed by G4. Genotypes G7 and G4 exhibited the longest spike length. Seeding densities greatly influenced the spikelet number in spike and genotype G2 produced the maximum spikelet number. The spike number in a square meter differed based on genotypes and seeding densities used. Genotype G1 had the maximum flag leaf area. In the research, the longest peduncle and spike with the highest spikelet number per spike were determined in the application of 400 seeds in a square meter. The highest spikelet number per spike and flag leaf area was obtained using 500 kernels per square meter. It would be useful to conduct studies using different seed densities to determine the adaptation of bread wheat genotypes to changing environmental conditions. Drought and heat stress due to climate change increases its effect on grains produced under rainy conditions. Morphological, agro-physiological and biochemical responses occur in plants against drought and heat stress. In drought conditions, the number of plants per unit area that share water in the soil is the most important factor in drought tolerance. Therefore, it is necessary to determine tolerant genotypes using different planting densities in drought stress conditions.



Grain yield and seeding density

Seeding density and flag leaf area

Seeding density and spike number in a square meter

Seeding density and grain number in spike

Figure 1. Association with yield and yield component with curves for the regression analysis at different seeding density levels.

Table 1. Total rainfall and mean temperature in 2015-2016 and 2016-2017 cycles and long year.

Months	Long year		2015-2016		2016-2017	
	Rainfall (mm)	Mean temp. (°C)	Rainfall (mm)	Mean temp. (°C)	Rainfall (mm)	Mean temp. (°C)
October	52.9	14.1	52.6	15.6	44.4	14.3
November	72.4	8.5	26.2	13.5	3.2	0.7
December	61.7	4.2	0.3	5.5	3.2	0.7
January	48.1	2.8	114.8	2.8	67.8	-1.9
February	46.9	4.2	91.4	9.2	43.4	5.3
March	52.2	7.6	54.8	10.2	51.0	10.2
April	51.0	12.8	116.1	15.5	65.6	12.5
May	56.0	17.9	81.4	17.4	85.0	17.9
June	41.5	22.3	10.2	23.9	44.4	21.2
Total/Mean	482.7	10.5	547.8	12.6	408.0	8.9

Table 2. Analysis of variance for genotypes, year and its interaction for yield and yield component.

Source of variation	DF	GY		PL		SL		FLA	
		MS	F Ratio	MS	F Ratio	MS	F Ratio	MS	F Ratio
Year (Y)	1	87739.4**	29.41	422.45**	133.92	15.67**	39.70	293.96**	31.01
Genotype (G)	7	101187.1**	33.92	54.27**	17.20	5.81**	14.72	149.39**	15.76
Y×G	7	61894.4**	20.75	65.60**	20.79	4.76**	12.06	85.63**	9.03
Treatment (T)	2	4310.5	1.44	0.13	0.04	0.83	2.11	56.50**	5.96
Y×T	2	5087.1	1.71	7.14	2.26	0.05	0.13	42.05*	4.44
G×T	14	4212.8	1.41	2.9	0.92	0.27	0.67	7.09**	0.75
Y×G×T	14	3572.4	1.20	2.11	0.67	0.48	1.21	9.66	1.02
Error	92			3.15		0.39		9.48	
C. Total	143								

* and ** indicate significances, at p<0.05 and p<0.01, respectively. ns: non-significant. GY: Grain yield (kg ha^{-1}), PL: Peduncle length (cm), SL: Spike length (cm), FLA: Flag leaf area (cm^2)

Table 3. Analysis of variance for genotypes, year and its interaction for yield components.

Source of variation	DF	SNS		KNS		SNM		NDVI	
		MS	F Ratio	MS	F Ratio	MS	F Ratio	MS	F Ratio
Year (Y)	1	35.57**	10.64	704.06**	23.23	1136356.0**	141.17	0.1320**	18.54
Genotype (G)	7	14.45**	4.32	254.56**	8.40	29169.0**	3.62	0.0353**	4.95
Y×G	7	11.75**	3.52	175.73**	5.80	24190.4**	3.01	0.0176*	2.47
Treatment (T)	2	1.95	0.58	10.31	0.34	12238.8	1.52	0.0053	0.74
Y×T	2	2.27	0.68	14.34	0.47	4864.4	0.60	0.0316*	4.44
G×T	14	2.33	0.69	71.30**	2.35	4357.3	0.54	0.0029	0.41
Y×G×T	14	4.24	1.27	77.74**	2.56	8962.7	1.11	0.0051	0.72
Error	92	3.34		30.304		8049.7		0.0071	
C. Total	143								

*, ** Significance at respectively 5% and 1% level probability, ns: non-significant. SNS: Number of spikelet in square meter, KNS: Number of grain in spike, SNM: Number of spike in a square meter, NDVI: Normalized differences vegetative index.

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Table 4. The average data of genotypes based on yield and agronomic parameters and standard deviation.

Genotype	GY	PL	SL	SNS	KNS	SNM	FLA	NDVI
G1	4994±209^{d}	28.17±0.7^{e}	8.56±0.2^{cd}	17.09±1.2^{c}	33.91±.2^{d}	517.1±38^{b}	26.20±1.2^{a}	0.67±0.05^{a}
G2	6291±103^{bc}	31.78±0.3^{bc}	8.31±0.1^{de}	19.28±5.5^{a}	38.55±1.0^{bc}	520.9±31^{b}	24.08±0.5^{bc}	0.52±0.02^{d}
G3	5945±441^{c}	29.85±0.4^{d}	8.40±0.2^{de}	17.61±1.8^{bc}	38.16±0.7^{bc}	592.9±11^{a}	17.68±1.2^{e}	0.58±0.01^{bc}
G4	6056±82^{bc}	32.91±0.7^{ab}	9.50±0.2^{a}	17.89±3.1^{bc}	44.78±0.6^{a}	487.6±28^{bc}	24.93±1.3^{abc}	0.59±0.01^{bc}
G5	6321±133^{b}	30.73±0.7^{cd}	9.20±0.1^{ab}	16.94±2.0^{c}	35.65±0.4^{cd}	521.6±35^{b}	23.43±1.1^{c}	0.62±0.03^{ab}
G6	7730±378^{a}	31.36±1.0^{c}	8.01±0.2^{e}	16.76±2.1^{c}	34.19±0.4^{d}	542.0±24^{ab}	20.60±1.4^{d}	0.55±0.03^{cd}
G7	6029±301^{bc}	33.37±0.4^{a}	9.52±0.5^{a}	18.71±3.2^{ab}	40.14±0.8^{b}	455.6±31^{c}	25.88±2.8^{ab}	0.61±0.01^{b}
G8	6218±236^{bc}	29.69±0.7^{d}	8.83±0.2^{bc}	17.22±4.7^{c}	41.53±0.3^{ab}	500.2±32^{bc}	24.27±1.2^{abc}	0.59±0.01^{bc}
Mean	6197	30.98	8.79	17.69	38.36	517.2	23.38	0.59
LSD $_{(0.05)}$	36.05	1.17	8.79	1.21	3.62	59.2	2.02	0.05
CV (%)	8.8	5.7	7.1	10.3	14.3	17.3	13.1	13.5

GY: Grain yield (kg ha^{-1}), PL: Peduncle length (cm), SL: Spike length (cm), SNS: Number of spikelet in square meter, KNS: Number of grain in spike, SNM: Number of spike in a square meter, FLA: Flag leaf area (cm^{2}), NDVI: Normalized differences vegetative index.

Table 5. Mean performance of genotypes yield and yield component based on treatment

Seed Rate	GY	PL	SL	SNS	KNS	SNM	FLA	NDVI
400	6219^{a}	31.04^{a}	8.94^{a}	17.90^{a}	38.68^{a}	509.5^{a}	23.30^{ab}	0.58^{a}
500	6280^{a}	30.98^{a}	8.73^{a}	17.67^{a}	38.58^{a}	535.6^{a}	24.51^{a}	0.59^{a}
600	6094^{a}	30.93^{a}	8.71^{a}	17.50^{a}	37.83^{a}	506.6^{a}	22.34^{b}	0.60^{a}
Mean	6197	30.98	8.79	17.69	38.36	517.2	23.38	0.59
LSD $_{(0.05)}$	22.05	0.71	0.24	1.04	2.22	36.25	1.23	0.03

GY: Grain yield (kg ha^{-1}), PL: Peduncle length (cm), SL: Spike length (cm), SNS: Number of spikelet in square meter, KNS: Number of grain in spike, SNM: Number of spike in a square meter, FLA: Flag leaf area (cm^{2}), NDVI: Normalized differences vegetative index.

Table 6. Correlation coefficients between grain yield and tested parameters for treatment

Parameter	GY	PL	SL	SNS	KNS	SNM	FLA
PL	0.618						
SL	0.271	0.924					
SNS	0.592	0.999**	0.936				
KNS	0.907	0.892	0.652	0.877			
SNM	0.809	0.039	-0.347	0.005	0.485		
FLA	0.966*	0.394	0.012	0.362	0.766	0.934	
NDVI	-0.659	-0.998**	-0.903	-0.996**	-0.915	-0.091	-0.441

Significant at **: p<0.01, *: p<0.05, GY: Grain yield (kg ha^{-1}), PL: Peduncle length (cm), SL: Spike length (cm), SNS: Number of spikelet in square meter, KNS: Number of grain in spike, SNM: Number of spike in a square meter, FLA: Flag leaf area (cm^{2}), NDVI: Normalized differences vegetative index.

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Registration of "Kürşad" Bread Wheat (*Triticum aestivum* L.) Variety

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Kürşad is a hard-red facultative wheat (*Triticum aestivum* L.) cultivar registered in 2020 by the Central Research Institute for Field Crops. The cultivar's pedigree is KS82142/SERI//SOYER02 and YE15269-0E. The crossing was carried out in the 2002-03 growing season and segregating populations were carried out in a modified bulk method. The yield trials for the line began in the 2010-11 growing seasons with the preliminary yield trials followed by yield trials and regional yield trials which were carried out between 2011 to 2016. Application for registration of the line ANK-35 was submitted to the Seed Registration and Certification Institute in 2016, in 2018 the line was registered and named Kürşad.

Kürşad is of medium to tall height (90-95 cm) and is late maturing. The spikes of the cultivar are white, awned, and medium-length (Figure 1). It is characterized by high grain yield, high grain quality, and resistance to lodging and yellow rust. The variety has a high tillering capacity as well as high water and fertilizer use efficiency. Kürşad is adapted to Central Anatolia Regions that receive low to intermediate rainfall (300-350 mm average annual precipitation). The average yield of the cultivar varied from 3500-4000 kg ha^{-1} in arid and 4500-6000 kg ha^{-1} in semi-arid areas of Central Anatolia. Bread-making quality values for Kürşad variety are: thousand kernel weight: 27.2-36.8 g, hectoliter weight: 69.9-78.9 kg/hl, protein content: 15.3%-16.0%, Zeleny sedimentation: 68-72 ml, baking strength (W): 301-456, water absorption: 58.8-60.1%, flour yield: 62.5-68%, wet gluten: 32.1-33.1%, dry gluten: 11.3-11.8%, and the gluten index: 97.6-99.2%.

The cultivar is recommended for arid, semi-arid, and irrigated areas of the Central Anatolian Region and the Transitional Zones. The appropriate sowing rate is 180-200 kg ha^{-1} and the suggested sowing dates are between October 15-November 15.

Figure 1. Spike and grain of the Kürşad variety. (Original)

References and Notes

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Registration of "Çavuş" Bread Wheat (*Triticum aestivum* L.) Variety

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Citation:
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Çavuş is a hard-red winter wheat (*Triticum aestivum* L.) cultivar registered in 2019 by Central Research Institute for Field Crops. Çavuş is of medium height and it is adapted to Central Anatolia Regions which receive low to intermediate rainfall (300-350 mm average annual precipitation). The spikes of the cultivar are white, awnless, and medium length (Figure 1). It is characterized by high grain yield, high grain quality, and resistance to lodging and yellow rust. The variety is mid-early maturing and has a high tillering capacity as well as high water and fertilizer use efficiency. The pedigree of Çavuş is KNYAGHNA/BEZOSTAYA and YA:739-0A-0A-0A-0A-0A-8A. The crossing took place in 2005 and yield trials began in the 2012-13 growing season. Application for registration of Çavuş bread wheat variety was submitted to the Seed Registration and Certification Institute in 2016. The average yield of the cultivar varied from 3000-4500 kg ha^{-1} in arid and 4500-6500 kg ha^{-1} in semi-arid areas of Central Anatolia. It was the top cultivar in terms of bread-making quality values among all the genotypes and checks during the registration process. Bread-making quality values for Çavuş variety are: thousand kernel weight: 31.2-39.5 g, hectoliter weight: 75.8-79.6 kg/hl, protein content: 14.0-16.8%, Zeleny sedimentation: 47-68 ml, baking strength (W): 300-538, water absorption: 59.7-62.0%, flour yield: 59.6-70.5%, wet gluten: 35.3-39.7%, dry gluten: 12.2-13.3%, and the gluten index: 93.6-94.2 %. Çavuş has the best quality characteristics among the bread wheat cultivars grown in Central Anatolia. The cultivar is recommended for arid, semi-arid, and irrigated areas of the Central Anatolian Region and the Transitional Zones. The appropriate sowing rate is 180-200 kg ha^{-1} and suggested sowing dates are between October 15-November 15.

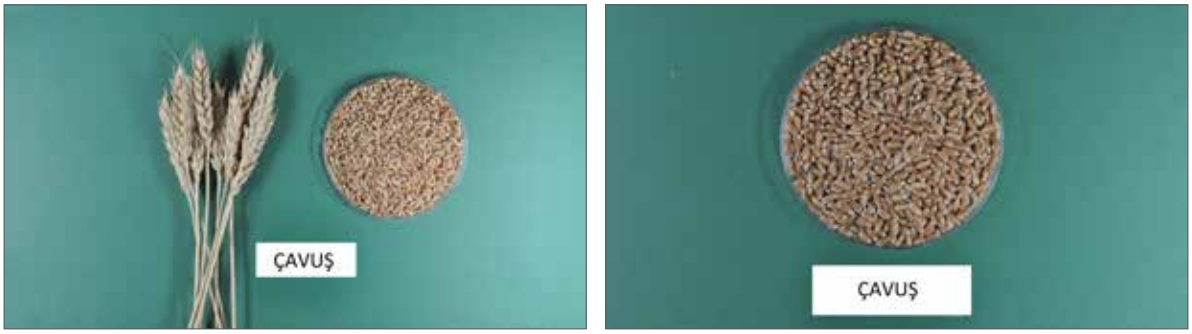

Figure 1. Spike and grain of the Çavuş variety. (Original)

References and Notes

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Registration of "TB Yaman" Bread Wheat (*Triticum aestivum* L.) Variety

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TB Yaman is a winter bread wheat (*Triticum aestivum* L.) variety developed by Trakya Agricultural Research Institute (TARI) and registered in 2023. TB Yaman is developed through pedigree method by crossing Albana/7/Sau41/Sad1/5/Agri"S"/093-44/3/Kkk/ltd/Lov29/4/FKong15//Bow/Pwn/6/1518-4-38K with TE7223-0T-0T-0T-5T-0T. The crossing was made in 2010 and the yield test began in the 2018-2019 growing year.

The spike of the TB Yaman cultivar is medium-long, white, smooth, with awn and medium compact (Figure 1). The flag leaf is medium dark-green and with medium glaucousity. Grain is oval, hard and red colour. Yaman is a tall cultivar, similar to Değirmen. Plant height is between 90 and 105 cm depending on the growing conditions. It is medium-early and as it has good adaptation ability, it has been grown throughout the Trakya-Marmara region and some other transitional-zone parts of Türkiye. It gives high yield both on fertile and less fertile soils. It has resistance to winterkilling and is tolerant to drought conditions. TB Yaman is highly tolerant to stripe rust (*Puccinia striiformis* f. sp. *tritici*) and leaf rust (*Puccinia triticina*). It has tolerant to septoria leaf disease and powdery mildew *Blumeria graminis* f. sp. *tritici* (syn. *Erysiphe graminis*).

Its yield potential is high, however, a high yield can be obtained if environmental conditions are favorable and good agronomic practices followed. The highest grain yield obtained was 10851 kg ha^{-1} in a variety testing experiment (Edirne location in the 2020-2021 cycle). The mean yield of the variety testing experiment was 7138 kg ha^{-1} in Trakya growing conditions. The suggested planting rate is between 500-550 seeds/m^2.

Its grain quality is good. The mean values of some bread-making qualities of the variety tested during 2022 and 2023 are; test weight 78.3 kg hl, thousand kernel weight 31.3 g, protein content 13.5-16.1%, sedimentation (Zel) 69 ml, gluten index 99.4%, gluten value 32.7%, alveograph energy value (W) 309 and flour yield 70.9%. The highest quality values in the 2020-2021 growing seasons application of the variety were; thousand kernel weight 45.0 g, test weight 83.0 kg, protein content 12.7%, gluten value 36.7%, gluten index 93.0% and sedimentation (Zel) 68 ml.

Pre-basic and basic seeds of the TB Yaman cultivar have been produced by Trakya Agricultural Research Institute (TARI) and Trakya Birlik Seeds Company. Certified seed of the TB Yaman are produced by both private companies and state farms.

Figure 1. Spike and grain of the TB Yaman cultivar. (Original)

References and Notes

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About the Journal

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In Turkey, wheat was produced 10 million tons in 1923 (Gokgol 1939).

This result was in agreement with result of Sahin and Yildirim (2004).

Similar effect has been widely studied prior to this study (Eser 1991; Bagci et al. 1995; Uzun and Yol 2013).

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Journal article:

Toker C (1998). Adaptation of kabuli chickpeas (*Cicer arietinum* L.) to the low and high lands in the West Mediterranean region of Turkey. Turk J Field Crop 3:10-15.

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Article by Digital Object Identifier (DOI) number:

Yasar M, Ceylan FO, Ikten C and Toker C (2013). Comparison of expressivity and penetrance of the double podding trait and yield components based on reciprocal crosses of kabuli and desi chickpeas (*Cicer arietinum* L.). Euphyticadoi:10.1007/s001090000086

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Yasar M (2012). Penetrance and expressivity of double podding characteristic in chickpea (*Cicer arietinum* L.). Dissertation, Akdeniz University, Antalya.

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Abbreviations

Abbreviations should be defined at first mention and used consistently.

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