Ekin Journal

July 2023, Volume 9, Issue 2

CROP BREEDING AND GENETICS

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Ekin Journal

International Journal of Crop Breeding and Genetics

Volume 9 Issue 2
9(2): 81-176, 2023
International biannual peer-reviewed journal

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International Journal of Crop Breeding and Genetics

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National Academy of Agricultural Sciences (NAAS) - ASOS Index
Eurasian Scientific Journal Index (ESJ) - Scientific Indexing Services (SIS)
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Volume 9 Issue 2
9(2):81-176, 2023
International biannual peer-reviewed journal
ISSN 2149-1275 • e-ISSN 2459-069X

Ekin Journal

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atölye KURUMSAL YAYINCILIK
Doğukan Karakaş +90 533 656 1366

Printing Office
KOZA Printing Industry
Saray Mah. 205 Cad. No.:4/2 Kahramankazan / Ankara / Türkiye • Phone: +90 312 385 9191

Printing Date
31.07.2023

ISSN Number
ISSN 2149-1275 • e-ISSN 2459-069X

Published By
bisab
Plant Breeders Union of Turkey
www.bisab.org.tr

Address Information
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Volume 9, Issue 2, July 2023

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EKIN

international biannual peer-reviewed crop breeding and genetics journal

Research Article

Ekin
Journal of Crop Breeding and Genetics

9(2):81-90, 2023

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Ekin International biannual peer-reviewed journal

Determination of Heat and Drought Tolerant Lines in Segregating Populations Produced by Interspecific Crosses in Eggplant

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Citation:
Cebeci E., Boyacı HF., Doğan Y., Toppino L., Rotino GL., 2023. Determination of Heat and Drought Tolerant Lines in Segregating Populations Produced by Interspecific Crosses in Eggplant. Ekin J. 9(2):81-90.

Received: 15.12.2022 ***Accepted: 10.03.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

ABSTRACT

Nowadays abiotic stresses originated by climate change are one of the main factors causing reductions to the agricultural production. Heat and drought are the most prominent abiotic stress factors affecting both eggplant production worldwide and food security. Although eggplant is known as more tolerant to these stresses compared to other vegetables and solanaceous crops, its quality and yield suffers from severe stress conditions. In this research, 256 F_2 plants developed from the interspecific cross between the wild relative of *Solanum insanum* L. and the pure line (BATEM-TDC47) from Batı Akdeniz Agricultural Research Institute, (BATEM) eggplant gene pool were used as plant materials. Seedlings at 3-4 true leaves stage bred in three-liter pots filled with a 1:1 mixture of peat and perlite were subjected to drought stress test to this end, a 75% deficit irrigation was applied to the plants, while control plants were irrigated with the required amount to recover the 100% of ETp as appropriate management strategy. The stress symptoms of plants were determined by morphological and chemical analyses. Plant heights were measured on the 25th day of the experiment and visual evaluation stress symptoms was observed according to the 0-5 scale. Morphological observations, MDA (malondialdehyde) and proline content of selected plants were performed to confirm their tolerance levels to heat and drought. Following the drought test, 100 F_2 lines, which were selected as drought tolerant, were transferred to the greenhouse for determination of heat tolerant individuals.

Keywords*:* Abiotic stress, drought, eggplant, heat, MDA, proline

[**] *This manuscript was oral-presented at 4th International Plant Breeding Congress, November 21-25, 2022, Antalya Türkiye.*

Introduction

Eggplant (*Solanum melongena* L.) is a crop species belonging to the family of Solanaceae but unlike the other solanaceous crops tomato, potato and pepper it has an old world origin as it is originated from both Southeast Asia and India (Barchi et al., 2022). It is widely grown in Southern and Southeast Asia, where great part of the world's population is located so and is included in the International Treaty on Plant Genetic Resources for Food and Agriculture's list of the most important 35 food crops substantial for global food security (Fowler et al., 2003). The most important factors limiting eggplant production are biotic and abiotic stresses (Rotino et al., 2014). For a sufficient yield and good quality of fruits, eggplant needs to be irrigated regularly, with an optimal temperature ranging between 22 and 30°C (Li et al., 2011).

Nowadays climate change is affecting the many parts of the world. Extreme weather events like floods after heavy rains, heat waves or low temperature shocks, increased soil salinity and prolonged water scarcity (drought) are the main results of the climate change, and are the cause of great losses in agricultural

production and fruit quality in recent years worldwide (Wakchaure et al., 2020). Due to climate change and global warming, crops started to encounter more and more frequently drought and heat stresses during their vegetation and harvesting period in arid as also in semiarid regions especially with Mediterranean climate (Fahad et al., 2017). Although results of previous studies suggested that eggplant has tolerance to the principal abiotic stresses (including drought, heat, salinity) compared to the other vegetables (Behboudian, 1977; Sarker et al., 2004; Díaz-Pérez and Eaton, 2015), it has been demonstrated in recent research that it suffers from extreme water stress deficit in terms of fruit quality and yield (Plazas et al., 2019; Wakchaure et al., 2020: Singh et al., 2021; Toppino et al., 2021). According to Karam et al., (2011) water deficiency application increasing from 20 to 40% of the optimal watering amount causes a yield decrease of nearly 60% with respect to the control; moreover, high temperatures above 38°C can seriously inhibit the growth of seedling, flower development, and eventually impact the fruit quality and yield of eggplant (Zong et al., 2018). Eggplant is among the top five vegetables constituting the diet of people living in drought-affected regions of the world (Wakchaure et al., 2020), as a consequence yield loss due to abiotic stresses could severely affect the daily diet and food supply chain in these regions. Adapting eggplant production to altered climatic conditions requires the development of tolerant cultivars. Crop wild relatives of eggplant display a wide genetic diversity and some of them possess tolerance traits against the principal biotic or abiotic stresses (Knapp et al., 2013; Fita et al., 2015; Plazas et al., 2019). Therefore, interspecific hybridization and introgression of useful traits from allied species into the eggplant genetic background may play a prominent role in increasing heat and drought tolerance of this crop species (Kouassi et al., 2016; Plazas et al., 2016). Evaluation of the response of segregating populations derived from interspecific hybridization to heat and drought stresses and the selection of best performing progenies would reveal as a useful tool for the development of breeding lines with improved tolerance to stress (Espanani et al., 2019).

In this study, an interspecific hybridization was performed between *Solanum melongena* L. and *Solanum insanum* L. and the response of the F_2 segregating population to heat and drought was assessed aimed at the selection of best performing lines and development of tolerant lines to stressed conditions.

Materials and Methods

In the present study, 256 individual F_2 seedlings derived from the selfing of the interspecific F_1 hybrid between the *S. melongena* L. BATEM-TDC47 pure line (sensitive parent) and the crop wild relative *S. insanum* L. (tolerant parent) were used as a plant material. The pure line "BATEM-TDC47" was developed in Bati Akdeniz Agricultural Research Institute under the project "Development of Qualified Genitors (Halfway Material) for Eggplant Breeding Programs and Seed Technology" (Project number: TAGEM/BBAD/10/A09/P01/12). The *S. insanum* L. (Coded as MM510 by INRAE) accession employed as donor of tolerance trait was provided from INRAE, France. In addition, 60 seedlings of the F_1 hybrid plus 60 seedlings of each parent line were tested for drought stress in a trial planned according to randomized block design with three replications to be compared with the F_2 individuals.

Seeds were sown in March 2021 and germinated in viols containing mixed peat moss and perlite (1:1) medium. Seedlings were equally watered with Hoagland solution (Hoagland and Arnon, 1950) until the 2nd-3rd true leaves stage and then they were transferred to the pots. Two seedlings were planted in each pot and they were normally watered with Hoagland solution until proper root development for two weeks. The application of the water deficit treatment started on April 30, 2021; the entire F_2 population and 10 plants for each of the three replications of F_1 and parent plants were subjected to stress by applying 75% deficit water compared to the control. To determine the water amount to be supplied in both control and stressed irrigation, all pots from control group were weighed daily and then the control group was watered up to full recover the ETp difference (the amount of weight lost each day due to evapotranspiration); a media performed on this value was considered as 100% control supply therefore deficit water supply was calculated as the 25% of the average control value and applied to the drought stressed group (Kıran et al., 2019).

On the 25th day of the drought application all plant heights were measured with a ruler and all F_2 plants were singularly evaluated according to the modified 0-5 visual scale already used by Banik et al., (2016) and Sseremba et al., (2018) where 0: No symptoms (control plants), 1: slow growth, 2: 25% yellowing and curling, 3: 26-50% yellowing and curling, dropping leaves, 4: 51-75% wilting and curling, drying, 5: more than 75% wilting and curling, dried plants. According to this scale, the 100 F_2 plants displaying "slow growth" (Scale = 1) were selected as "tolerant to drought" and transferred to the greenhouse for the heat tolerance study and further morphologic characterization.

Before the transfer, leaf samples were taken from each drought tolerant F_2 plant together with the F_1 and parents' both control and stressed plants to analyse the malondialdehyde (MDA) and proline leaf content. MDA was analysed according to Luts et al., (1996), while proline content was evaluated according to the Bates et al., (1973) method. Temperature and humidity were recorded during both the pot and greenhouse experiments and are presented in Figure 1 and 3.

Temperature and humidity ranged between 15-45°C and 23-65% respectively during the drought experiment in pots. While average temperature was measured as 36°C, average humidity was measured as 76%. Figure 2, shows general view from the compartment in which the experiment was established and the responses of F_2 population at the 25th day of drought application.

Selected drought tolerant plants were transferred to the greenhouse where their tolerance to heat was evaluated using the morphologic descriptors modified from Boyaci et al., (2015) and detailed in Table 1.

Greenhouse climatic conditions were also recorded and shown in Figure 3. In greenhouse, temperature ranged between 25-49 ^{o}C and the average temperature was recorded as 36 ^{o}C humidity ranged between, 13 - 99% and the average humidity was assessed as 76%. While the F_2 best performing plants were morphologically characterized in greenhouse for heat stress, they were also selfed to generate the F_3 generation. Characterized plants were self-pollinated by hand individually in greenhouse conditions to obtain F_3 progenies.

Results and Discussion

In terms of plant height, differences were observed between the drought-stressed groups of tolerant (*S. insanum* L.), sensitive (BATEM-TDC47) parents, and F_1 hybrid plants measured on 25th day of the drought experiment with respect to their corresponding control group (Table 2). The average plant height of the drought stressed group of *S. insanum* L. plants was 13.4 cm, while the average plant height of its control group was 17.6 cm. Drought stress accounted for 25.6% variation between the two groups. The average plant height of the sensitive genotype BATEM-TDC47 was 17.3 cm in the drought stressed group while 24.2 cm in the control group, with variation between the two groups of 28.5%. In F_1 plants, the mean values ranged 19.4 cm in the control group, while being 15.9 cm in the stressed group and the change rate was 18%. Simultaneously, wide variation was observed also among the F_2 population in terms of plant heights under drought effect.

Similarly, Semida et al., (2021) reported that water deficit in eggplant significantly affected and reduced plant height, stem diameter and number of leaves, as well as Fita et al., (2015) reported that plant height and fresh weight were the most distinctive morphological characters to determine drought tolerance in eggplant.

In the current study, morphologic damage level of the plants from F_2 population was observed on the 25th day of the drought experiment using a 0-5 scale. Hundred plants showed least damage due to drought stress and got scale value 1. Being drought tolerant according to Kiran et al (2015) these plants were transferred to green house for further evaluation under heat stress. Additionally, in terms of the scale evaluation, 150 F_2 showed symptoms as 25% yellowing and curling on plants and got "2" from the scale, being therefore noted as sensitive. Six among the 256 F_2 plants got "3" as a scale value as they showed symptoms 25%-50% yellowing and curling on plants and dropping leaves and were also noted as sensitive. In addition, studies on eggplant (Sseremba et al., 2018) potatoes (Banik et al., 2016), melon (Kusvuran, 2010), pea (Ajayi et al., 2018), and kiwifruit (Zhong et al., 2018) revealed that the 0-5 scale is an effective tool in determining to drought tolerance.

Leaf samples harvested from 50 among the 100 tolerant F_2 plants which also displayed desired morphological features were then analyzed for MDA and proline accumulations. MDA is the most frequently used biomarker of oxidative stress in plants, while proline is an amino acid which protects the plants from various stresses. An increase of proline levels under drought is related to a higher degree of adaptability to the plants to the stress; on the contrary an increase of the MDA values is linked to a higher sensitiveness. While the MDA values ranged between 9.87-15.97 nmol/g FW, proline content was determined between 2.40 – 16.70 µmol g^{-1} FW and Figure 4 showed the results of F_2 plants. MDA and proline values detected in Parents and F_1 plants' both under control and drought tested conditions are also presented in Table 3.

In this study, both MDA and proline amounts of F_1 and parents showed increases under the effect of drought with respect to their respective control plants (Table 3). However, as expected, the sensitive parent showed high MDA and low proline variation under drought stress with respect to the tolerant parent. Previously, Kıran et al., (2015) reported that there is an effective direct correlation between the scale value describing the level of visual symptoms and the levels of MDA, so that under drought stress conditions MDA levels display the lowest level of variation as the scale value decreases. Likewise previous studies conducted on beans (Kandemir et al., 2018) and tomatoes (Yekbun and Kabay, 2017), reported that MDA content increases

according to the degree of damage to the cell membrane in plants subjected to stress therefore, as the MDA content increases, the range scale increases as the drought tolerance of plant decreases.

In a study on tomatoes, it was determined that dehydrated plants accumulate osmolytes such as proline in their leaves and protect themselves from the stress accordingly (Noori et al., 2018). In another panel study aiming at determining the drought effect among different cultivar of rice, which is a semi-aquatic species requiring consistent irrigation prolonged during all season to grow, it was established that the higher levels of proline were accumulated in those cultivars which also displayed the higher tolerance to water deficiency (Lum et al., 2014).

A panel of 100 F_2 plants were selected as drought tolerant according to their overall good performance regarding plant height, symptoms scale value, MDA and proline content, and were therefore transferred to the greenhouse to be kept during the summer period, where their heat tolerance was assessed under normal irrigation conditions. For this purpose, the fruiting capacity of each plant was examined and the genotypes that could not set fruit under high temperature conditions were recorded as more sensitive to heat. Faiz et al., (2020) studied on heat tolerance of 4 local eggplant genotypes and they reported that, under high temperature (45°C) stress different eggplant genotypes performed physiologically and bio-chemically different.

The results of phenotypic characterization with regard of many qualitative traits are detailed in Table 4. Average plant height, fruit length, fruit width and fruit weight were measured as 106.4 cm, 14.0 cm, 6.8 cm and 55 g respectively.

Among the selected lines, differences were observed for all traits related to plant architecture, leaf prickles, leaf hairiness and fruit characteristics. The growth habit of the plants was neither upright nor widespread, the number of lobes in the leaf was average, and the plants were usually slightly spiny and hairy. Anthocyanin distribution in plant and leaves were also noted as mostly absent or low. The fruits were of various sizes, preferentially round but also oval or elongated (Table 5).

The morphologic parameters revealed a wide range of phenotypic combination in the F_2 population; Similarly, a wide segregation and variability, is usually expected in a segregant population obtained from a cross between cultivated eggplant and its wild relatives as reported in previous studies (Prohens et al., 2013; Frary et al., 2014; Boyaci, 2020). Interspecific hybridization is a strategy which extremely helps to transfer many useful features from wild species to cultivated eggplant, leading to increase the genetic diversity of the latter (Kouassi et al., 2016; Plazas et al., 2019). Meanwhile, due to the wide variability that usually is generated in a progeny when two different species are combined, before taking part in any breeding program the best performing progenies selected for a desired trait need to be submitted to a deep phenotypic characterization to better assess their combination ability and avoid selection of also negative traits (Gaufichon et al., 2010). With this study, the F_2 plants determined as heat and drought tolerant were also morphologically characterized with a sufficient number of descriptors created for eggplant, and only the progenies of the plants with the best traits combination will be considered for future employment in breeding programs to develop new eggplant breeding lines with improved adaptability to stress.

Conclusions

This research is aiming to develop heat and drought tolerant lines in eggplant. In this study, 100 F_2 lines selected as the most drought and heat tolerant were morphologically characterized, selfed and progressed to the F_3 generation. Drought tests and selfing of inbred lines in next generations will be continued to provide their durability and to be fixed at homozygous level, so that new eggplant hybrids with improved features as heat and drought tolerance will be developed in the future. In this respect, this study, which deals with the beginning of breeding studies for heat and drought stress, can be suggested as a model for breeding programs not only for eggplant but also for other species.

Acknowledgement

This study is funded by the Project C4C: Crops for Change, Tackling the Global Warming Effects on Crops and supported by the European Union FOSC ERA-NET program and TUBITAK (project no: 220N246) of Türkiye.

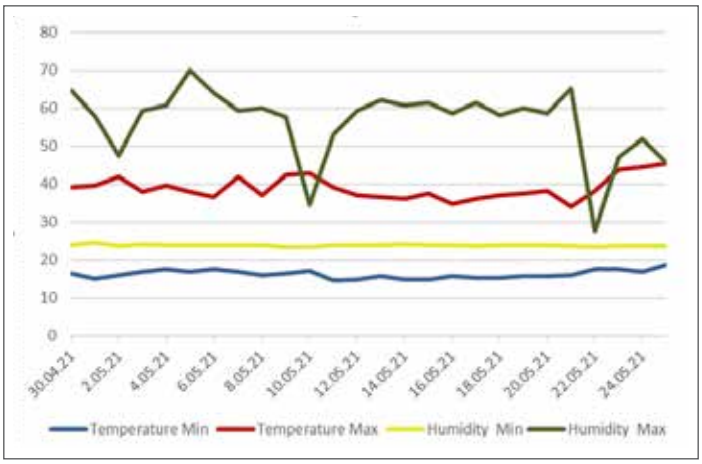

Figure 1. Temperature (°C) and humidity (%) recorded during the drought stress pot experiment.

Figure 2. A- General view from the compartment in which the drought experiment was established, B- The responses of F_2 population on the 25th day of drought application. (Original)

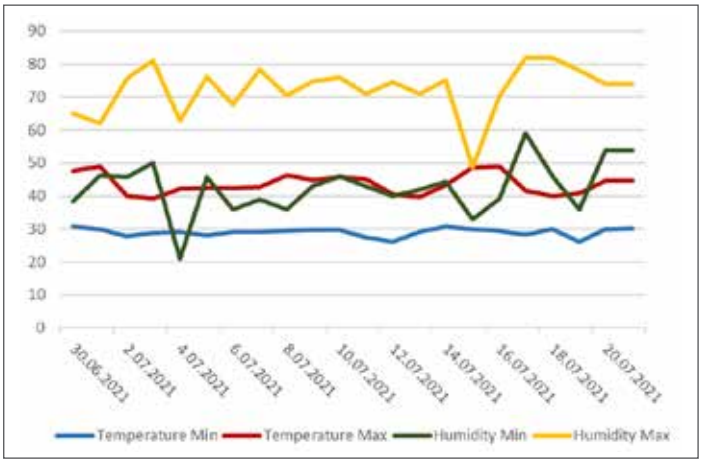

Figure 3. Temperature (°C) and humidity (%) recorded during the heat stress period.

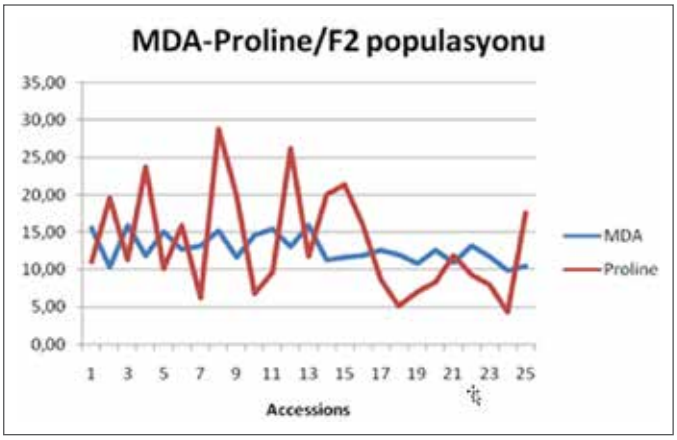

Figure 4. Graphic shows MDA (nmol/g FW) and proline (µmol g^{-1} FW) alteration in selected F_2 plants.

Table 1. The phenotypic descriptors and observation methods used in the study.

No	Descriptors	Explanation
1	Growth habit	1= very upright, 3= upright, 5= intermediate, 7= prostrate
2	Leaf blade lobs	1= very weak, 3= weak, 5= intermediate, 7=strong, 9= very strong
3	Anthocyanin distribution in plant	1=absent, 3= low, 5= intermediate, 7= high
4	Anthocyanin distribution in leaves	1=absent, 3= low, 5= intermediate, 7= high
5	Leaf prickliness	1= None, 3=Very few (1-2), 5= Few (3-5), 7= Intermediate (6-10), 9=Many (11-20), 11= Very many (>20)
6	Leaf hairiness	1=absent, 3= low, 5= intermediate, 7= high
7	Number of flowers/ inflorescence	number
8	Fruit load	1= very low, 3= low, 5= intermediate, 7=high, 9= very high
9	Leaf blade width	Measured in cm with ruler (Average of the best 3 leaves for each plant)
10	Leaf blade length	Measured in cm with ruler (average of the best 3 leaves for each plant.)
11	Total plant height	Measured in cm as the distance from the soil surface to the tip.
12	Varietal type	1=long, 3=oval, 5=round, 7=striped,
13	Predominant fruit colour	1=dark green, 3=green, 5=lilac, 7=dark lilac, 9=purple, 11=dark purple, 13=black,
14	Secondary fruit colour	1=dark green, 3=green, 5=lilac, 7=dark lilac, 9=purple, 11=dark purple, 13=black
15	Fruit glossiness	1=opaque, 3=intermediate, 5=bright peel colour
16	Fruit curvature	1=round, 3=no curvature, 5=slightly curved, 7=curved, 9=S shaped, 11= U shaped
17	Presence of grooves	1=absent, 3=few, 5=intermediate, 7=many
18	Calyx fruit coverage	1= less than 10%, 3=10-20%, 5=21-30%, 7=31-40%, 9=41-49%, 11=50% and more
19	Fruit firmness	1=spongy, 3=intermediate, 5= tight
20	Fruit weight	Measured in g (average of 2-3 fruits from each plant)
21	Fruit length	Measured in cm (average of 2-3 fruits from each plant)
22	Fruit maximum diameter	Measured in cm (average of 2-3 fruits from each plant)
23	Fruit length/breadth ratio	Calculated
24	Peduncle length	Measured in cm (average of 2-3 fruits from each plant)
25	Fruit calyx prickliness	1= none, 3= very few (1-2), 5= few (3-5), 7= intermediate (6-10), 9= many (11-20), 11= very many (>20)
26	Petiole length	Measured in cm
27	Fruit end button size	1=small, 3=intermediate, 5= large
28	Presence of chlorophyll on the pistil scar	1=absent, 3= present
29	Fruit color distribution	1=uniform, 3=mottled, 5=netted, 7=striped

Table 2. The effect of 75% water deficit on the plant height applied during the seedling period.

	S. insanum		TDC47		TDC47 × *S. insanum*		F_2**
	Control	**75% WD**	**Control**	**75% WD**	**Control**	**75% WD**	**75% WD**
Shortest plant height (cm)	12.0	10.0	18.5	14.0	15.0	11.0	10.0
Longest plant height (cm)	22.0	19.0	28.0	20.0	23.0	18.0	24.0
Average plant height (cm)	17.6	13.4	24.2	17.3	19.4	15.9	19.4
Standard deviation	3.1	2.7	3.1	2.2	2.8	1.9	3.1
% Variation	25.6%		28.5%		18.0%		-

WD: Water deficit **:max., min. and average values of 256 F_2 individual seedlings

Table 3. MDA (nmol/g FW) and proline (µmol g^{-1} FW) amounts showing different degrees of increases under drought compared to the control plants.

	S. insanum		TDC47		TDC47 × *S. insanum*	
	Control	**Application**	**Control**	**Application**	**Control**	**Application**
MDA (nmol/g FW)	11.1	12.5	7.5	9.0	14.1	15.6
% Variation	12.75%		19.20%		10.81%	
Proline (µmol g^{-1} FW)	17.6	13.4	24.2	17.3	19.4	15.9
% Variation	20%		3.7%		25%	

Table 4. Data of some phenotypic traits scored among the 100 F_2 plants determined as drought tolerant.

Trait	**Minimum**	**Maximum**	**Average**	**Standard Deviation**
Plant height (cm)	50.0	142.0	106.4	15.6
Leaf blade length (cm)	11.7	26.3	20.1	2.4
Leaf blade width (cm)	7.0	17.0	11.8	1.9
Petiole Length (cm)	3.0	15.5	7.3	1.5
Fruit length (cm)	3.0	14.0	8.4	1.7
Fruit width (mm)	28.0	68.0	48.9	6.8
Fruit weight (g)	26.0	140.0	55.0	20.9
Fruit length/breadth ratio (cm)	1.0	2.8	1.7	0.3
Peduncle length (cm)	3.0	15.5	7.3	1.1

Table 5. Some phenotypic characteristics of 100 lines selected as drought tolerant.

No	Descriptors	Results (*the numbers show how many plants are in which feature)
1	Growth habit	2- very upright, 42- upright, 50- intermediate, 7- prostrate
2	Leaf blade lobs	1-very weak, 12- weak, 67- intermediate, 20- strong
3	Anthocyanin distribution in plant	30- absent, 65- low 5- intermediate
4	Anthocyanin distribution in leaves	68- absent, 30- low. 2- intermediate
5	Leaf prickliness	37- none, 14- very few. 35- few, 14- intermediate
6	Leaf hairiness	2- absent, 51- low, 46- intermediate, 2- high
7	Number of flowers/inflorescence	2- 1/2, 78- 1/3, 20- 3/4
8	Fruit load	10- low, 56- intermediate, 24- high, 10- very high
9	Varietal type	12- long, 20- oval, 68- round
10	Predominant fruit color	15- dark green, 9- green, 19- lilac, 15- dark lilac, 22-purple, 20-dark purple
11	Secondary fruit color	3- dark green, 52- green, 6- lilac, 5- dark lilac, 18- purple, 16- dark purple
12	Fruit glossiness	30- opaque, 46- intermediate, 24- bright peel color
13	Fruit curvature	1- round, 66- no curvature, 33-slightly curvature
14	Presence of grooves	100- absent
15	Calyx fruit coverage	20- less than 10%, 55- 10-20%, 24-21-30%, 1-31-40%
16	Fruit calyx prickliness	36- none, 7-Very few (<3), 33-Few (~5), 24-Intermediate
17	Fruit firmness	2- spongy, 43- intermediate, 55- tight
18	Fruit end button size	25- small, 36-intermediate, 39- large
19	Presence of chlorophyll on the pistil scar	70- absent, 30- present
20	Fruit color distribution	52- uniform, 28- mottled, 1- netted, 19- striped

References

Ajayi AT, Gbadamosi AE and Olumekun VO, (2018). Screening for drought tolerance in cowpea (*Vigna unguiculata* L, Walp) at seedling stage under screen house condition. International Journal of BioSciences & Technology, 11(1), 1-10.

Banik P, Zeng W, Tai H, Bizimungu B and Tanino K, (2016). Effects of drought acclimation on drought stress resistance in potato (*Solanum tuberosum* L.) genotypes. Environmental and Experimental Botany, 126:76-89 http://dx,doi,org/10,1016/j,envexpbot, 2016,01,008

Barchi L, Portis E, Lanteri S, Alonso D, Diez MJ, Prohens J and Giuliano G, (2022). Worldwide population structure of eggplant identified by SPET genotyping over 3,400 accessions. Virtual Plant and Animal Genome XXIX Conference, Jan 2022, San Diego (CA), United States. Hal-03551609.

Bates LS, Waldren RP and Teare ID, (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39(1):205-207.

Behboudian MH, (1977). Responses of eggplant to drought. I. Plant water balance. Scientia Horticulturae, 7(4):303-310.

Boyaci HF, Topcu V, Tepe A, Yildirim IK, Oten M and Aktas A, (2015). Morphological and molecular characterization and relationships of Turkish local eggplant heirlooms. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 43(1):100-107.

Boyaci HF, (2020). Development of new valuable introgression lines from the interspecific cross in eggplant (*Solanum melongena* L.). Applied Ecology and Environmental Research, 18(1):1771-1781.

Diaz-Perez JC and Eaton TE, (2015). Eggplant (*Solanum melongena* L.) plant growth and fruit yield as affected by drip irrigation rate. HortScience, 50(11):1709-1714.

Espanani S, Majidi MM, Saeidi G and Alaei H, (2019). Physiological aspects of inter-specific gene introgression to improve drought tolerance in safflower. Euphytica, 215(10):1-18.

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S Nasim W, Adkins S, Saud S, Ihsan MZ, Alharby H, Wu C, Wang D and Huang J, (2017). Crop production under drought and heat stress: plant responses and management options, Frontiers in plant science, 8:1147.

Faiz H, Ayyub CM, Khan RW and Ahmad R, (2020). Morphological, physiological and biochemical responses of eggplant (*Solanum melongena* L.) seedling to heat stress. Pakistan Journal of Agricultural Sciences, 57(2):371-380

Fita A, Fioruci F, Plazas M, Rodriguez-Burruezo A and Prohens J, (2015). Drought tolerance among accessions of eggplant and related species. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca: Horticulture.72:461-462

Fowler C, Moore G and Hawtin GC, (2003). The international treaty on plant genetic resources for food and agriculture: A primer for the future harvest centres of the CGIAR, International Plant Genetic Resources Institute, Rome, Italy.

Frary A, Frary A, Daunay MC, Huvenaars K, Mank R and Doğanlar S, (2014). QTL hotspots in eggplant (*Solanum melongena*) detected with a high resolution map and CIM analysis. Euphytica, 197(2):211-228.

Gaufichon L, Prioul J and Bachelier B, (2010). What are the prospects for genetic improvement in drought-tolerant crops? Foundation Farm press 52 p.

Hoagland D and Arnon DI, (1950). The water culture method for growing plants without soil, California Agricultural Experiment Station, 347(2nd edit) 39 p., Newyork.

Karam F, Saliba R, Skaf S, Breidy J, Rouphael Y, and Balendonck J, (2011). Yield and water use of eggplants (*Solanum melongena* L,) under full and deficit irrigation regimes, Agricultural Water Management, 98(8):1307-1316.

Kandemir D, Balkaya A, Taşan M, Kobal Bekar N, Cemek B and Teksöz E, (2018). Nitelikli taze fasulye hatlarının kuraklığa dayanım düzeylerinin belirlenmesi ve kuraklık stresinde geliştirdikleri savunma mekanizmalarının incelenmesi, TÜBITAK TOVAG 116O881 No'lu Proje Raporu, 142s. (in Turkish)

Kıran S, Kuşvuran Ş, Özkay F and Ellialtıoğlu ŞŞ, (2015). Domates, patlıcan ve kavun genotiplerinin kuraklığa dayanım durumlarını belirlemeye yönelik olarak incelenen özellikler arasındaki ilişkiler. Nevşehir Bilim ve Teknoloji Dergisi, 4(2):9-25. (in Turkish)

Kıran S, Kuşvuran Ş. Özkay F and Ellialtioglu ŞŞ, (2019). Change of physiological and biochemical parameters under drought stress in salt-tolerant and salt-susceptible eggplant genotypes. Turkish Journal of Agriculture and Forestry 43(6):593-602.

Kouassi B, Prohens J, Gramazio P, Kouassi AB, Vilanova S, Galán-Ávila A and Plazas M, (2016). Development of backcross generations and new interspecific hybrid combinations for introgression breeding in eggplant (*Solanum melongena*), Scientia Horticulturae *213:*199-207, doi:10,1016/j,scienta,2016,10,039.

Knapp S, Vorontosova M and Prohens J, (2013). Wild relatives of eggplant (*Solanum melongena* L.:Solanaceae): New understanding of species names in a complex group, PLOS ONE 8:e57039.

Kuşvuran Ş, (2010). Kavunlarda kuraklık ve tuzluluğa toleransın fizyolojik mekanizmaları arasındaki bağlantılar, Çukurova Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı, Adana. 377 s. (in Turkish)

Li Y, Li Z, Luo S and Sun B, (2011). Effects of heat stress on gene expression in eggplant (*Solanum melongena* L.) seedlings, African Journal of Biotechnology, 10(79):18078-18084.

Lum MS, Hanafi MM, Rafii YM and Akmar ASN, (2014). Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice, J*,* Animal and Plant Sciences 24(5):1487-1493.

Lutts S, Kinet JM and Bouharmont J, (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance, Annals of Botany 78(3):389-398.

Noori M, Azar AM, Saidi M, Panahandeh J and Haghi DZ, (2018). Evaluation of water deficiency impacts on antioxidant enzymes activity and lipid peroxidation in some tomato (*Solanum lycopersicum* L.) lines, Indian Journal of Agricultural Research 52(3):228-235.

Plazas M, Rahma AF, Rodríguez-Burruezo A, Prohens J and Fita A, (2016). Screening for drought tolerance in eggplant relatives and interspecific hybrids, In Proceedings of XVIth Eucarpia Capsicum and Eggplant Working Group Meeting in memoriam Dr, Alain Palloix, 12-14 September 2016, Kecskemét, Hungary (pp, 306-310).

Plazas M, Nguyen HT, González-Orenga S, Fita A, Vicente O, Prohens J and Boscaiu M, (2019). Comparative analysis of the responses to water stress in eggplant (*Solanum melongena*) cultivars, Plant Physiology and Biochemistry, 143:72-82.

Prohens J, Whitaker BD, Plazas M, Vilanova S, Hurtado M, Blasco M, Gramazio P and Stommel JR, (2013). Genetic diversity in morphological characters and phenolic acids content resulting from an interspecific cross between eggplant, *Solanum melongena*, and its wild ancestor (*S. incanum*). Annals of Applied Biology, 162(2):242-257.

Rotino GL, Sala T and Toppino L, (2014). Eggplant in alien gene transfer in crop plants, Volume 2 (pp. 381-409). Springer, New York, NY.

Sarker BC, Hara M and Uemura M, (2004). Comparison of response of two C3 species to leaf water relation, proline synthesis, gas exchange and water use under periodic water stress, Journal of Plant Biology, 47(1):33-41.

Semida WM, Abdelkhalik A, Mohamed GF, Abd El-Mageed TA, Abd El-Mageed SA, Rady MM and Ali EF, (2021). Foliar application of zinc oxide nanoparticles promotes drought stress tolerance in eggplant (*Solanum melongena* L.). Plants, 10(2):421.

Singh M, Singh P, Singh S, Saini RK and Angadi SV, (2021). A global meta-analysis of yield and water productivity responses of vegetables to deficit irrigation. Scientific reports, 11(1):1-13.

Sseremba G, Tongoona P, Eleblu J, Danquah EY, and Kizito EB, (2018). Heritability of drought resistance in *Solanum aethiopicum* Shum group and combining ability of genotypes for drought tolerance and recovery, Scientia Horticulturae, 240:213-220.

Wakchaure GC, Minhas PS, Meena KK, Kumar S and Rane J, (2020). Effect of plant growth regulators and deficit irrigation on canopy traits, yield, water productivity and fruit quality of eggplant (*Solanum melongena* L,) grown in the water scarce environment, Journal of environmental management, 262:110320.

Yekbun A, Kabay T, (2017). Kuraklık stresinin yerli ve ticari domates çeşitlerinde bazı fizyolojik parametreler üzerine etkileri, Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi 22(2):86-96. (in Turkish)

Zhong YP, Li Z, Bai DF, Qi XJ, Chen JY, Wei CG and Fang JB, (2018). *In vitro* variation of drought tolerance in five *Actinidia* species, Journal of the American Society for Horticultural Science 143(3):226-234.

Research Article

Ekin
Journal of Crop Breeding and Genetics

9(2):91-97, 2023

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Genetic Variability among Winter Cereal Genotypes for Response to Protein Hydrolysate (PH) for Grain Yield and Its Attributes

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Citation:
Kumar P., Yadav P., Sharma PK., Singh M., Behl RK., 2023. Genetic Variability among Winter Cereal Genotypes for Response to Protein Hydrolysate (PH) for Grain Yield and Its Attributes. Ekin J. 9(2):91-97.

Received: 18.02.2023 ***Accepted: 20.04.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

ABSTRACT

A field experiment-based study was conducted using 19 different varieties of cereal crops i.e. eight of wheat (black wheat, WH1105, WH1142, HD2967, WH1124, DBW88, WH1025, WH1080), seven of triticale (TL3003, TL3002, TL3001, TL3004, TL2942, TL3005, TL2969 and four of barley (BH885, BH902, BH946, BH393) in research facility at agriculture farms of Jagan Nath University, Bahadurgarh, Jhajjar during Rabi 2020-21. The physiological and agronomical parameters were analyzed in three cereal crops wheat, triticale and barley using a waste human hair (amino acids hydrolysate developed by protein hydrolysis techniques). The test plot given the treatment of foliar spray having approximately 8% (v/v) nitrogen and 8-10% (w/v) carbon diluted 5ml/l with water after seed germination of 40 days followed by three sprays after interval of 25 days. The comparison of control and test showed increase in height of wheat (2.9±0.25%), triticale (3.5±0.45%), and barley (2.2±0.22%). The increase in spike length observed in wheat (2.01±0.6%), triticale (1.73±0.17%), and barley (2.9±0.27%). The overall production of plots increased as in wheat (11.9±0.86%), triticale (12.14±0.86%), and barley (12.8±0.29%) comparison to control plot. This analysis from this study concludes that the foliar application of protein hydrolysate shows significant results on the plant height, spike length and yield of crops. The accumulation of protein hydrolysate varies among cereal genotypes. This type of protein hydrolysate having short peptide and free amino acids are accumulated directly by plants and enhance the growth and maintained plants health. The application can be an alternative of chemical-based fertilizers and reduce the environment pollution.

Keywords: Amino acid, human hair "waste" hydrolysate, cereals, wheat, triticale, barley

Introduction

Wheat is a major crop in India and considered as second-largest producer of wheat in the world. As per the trends of last few seasons reduced production need to be focused to tackle the problem. In recent years the production is reported to be above 100 million tones, but not sufficient to feed ever growing population of India. Recent year 2021-2022 the estimated production was 105 million tones which was 5.7% less than the previous few years. The fall in wheat production has compelled the government of India to pose ban on export of the wheat. Besides, the government also failed in procurement of wheat due to issue related (MSP) Minimum Support Price (Vasudeva and Munjal, 2022).

The challenging environment and deteriorating soil health conditions are held responsible for the reduction in sustainable wheat crop production. The northern states Punjab, Haryana and Uttar Pradesh provide largest count of wheat in India. But the increased temperature in March caused the reduction in production of crops during last season. Even after the wheat season continues rise in temperature affect the overall health and production of different other crops like cotton deteriorating the fruit development. It's time to think about heat tolerance in wheat crops to understand the cause of reduced production. The onset of heat waves during March season may in crops plants.

Triticale is considered to be more nutritive than wheat with the ability to stand against the events of biotic and abiotic stress. In India triticale is grown in lower Himalayan regions. The triticale is developed by crossing between wheat and rye with advantages of both crops. Its acceptance at global level has made superior to wheat due to its quality of being tolerant to adverse conditions like drought and ability to flourish well in less fertile soil. It is also popular worldwide and grown in the European region. As wheat production is decreasing the adoption of triticale can resolve low production problems. Inputs of bio-based fertilizers can enhance the production as well as the nutritional values of the grains.

Barley crop is a major part of winter crops in India, and it is also considered as a major source of animal feedstock. The biotic and abiotic stresses are continuously decreasing production of barley. The use of amino acid-based protein hydrolysate has impacted physiology of plants through various biochemical pathways improvement (Mostafa et al., 2014). The amino acids based biostimulants improve soil health and enhance tolerance to abiotic and biotic stress (Calvo et al., 2014), as well as enhance maturation of leaves and roots (Popko et al., 2018). The plant-based protein hydrolysate biostimulants show enhancement in seed germination and productivity of many agronomic (Kumar et al., 2021) and horticultural crops (Colla et al., 2017).

In the present study, the protein hydrolysate formulated from human hair waste and experimented on 19 varieties of wheat, triticale and barley. Application of amino acids-based formulations showed an increase in plant growth, spike length and overall yield. Human hair waste is of no use in industries, and it is not degraded or broken down by microorganisms. This causes soil pollution and remains undegraded for so many years. Human hair is a rich source of amino acids, and these amino acids can directly support the growth of plants. The current scenario of fertilizers not supporting enough growth so it's time to change focus on alternative sophisticated methods that contribute directly to overall growth and health of plants. The formulation prepared by hydrolysis of human hair which converts complex protein into smaller peptides and free amino acids molecules. This formulation is applied as a biofertilizer in wheat, triticale, and barley varieties to analyze the impact on growth and health of crops. After harvesting and studying different parameters of plant growth, it was observed that this formulation has a significant effect on overall production of crops.

Materials and Methods

Investigations were undertaken using 19 different varieties of cereals crops: with eight varieties of bread wheat (black wheat, WH1105, WH1142, HD2967, WH1124, DBW88, WH1025, WH1080), seven varieties of winter triticale (TL3003, TL3002, TL3001, TL3004, TL2942, TL3005, TL2969 and four varieties of barley one is two-rowed BH885 and three are six-rowed BH902, BH946, BH393) at agriculture farms of Jagan Nath University, Bahadurgarh, Haryana. The study was carried out as an approved package of practice of wheat by the Department of Agriculture, Haryana. The agronomic and biological parameters were analyzed on wheat, triticale, and barley crops. The 19 varieties were sown in randomized complete block design in three replication and two plots for each genotype were assigned including one for control (no foliar spray) and one for treatment. In the control plot, all normal practice followed and in treatment with normal practice of foliar spray of protein hydrolysate formulation at the rate of 5 ml/1 ltr was carried out. Each plot was 2m x 2m in size accommodating 120 plants. The farm was situated at a height of 214 meters above mean sea level at 28.38°N, 76.45°E, and its average summer temperature was about 38°C, and its average winter temperature is around 12°C. In June, the temperature soars to 43°C. The soil was sandy loam alluvial. The experiment investigation varies crop wise on the basis of crops sowing and harvesting seasons because each crop have a different response to environment conditions.

According to ICAR, the Department of Agriculture in Haryana State adhered to the approved package of practices for growing wheat, and the agronomic and biological parameters of the cereal crops grown on a single acre were examined. One deep ploughing and a total of two to three harrowing's were used to prepare the ground. The seeds were sown at a rate of 100 kg per hectare in the month of November with row to row spacing 15 cm, plant to plant spacing 10 cm, and the sowing depth was approximately 4-5 cm. The fertilizer dosages were 150 kg of nitrogen, 80 kg of phosphorus, 60 kg of potash, and 12 kg of zinc sulfate per hectare. Applied 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.

After 21 days of sowing, the first irrigation was carried out, followed by additional irrigation as per the need of crop. At 27–35 day after sowing, Clodinafop Propargyl 15% WP (400 g/ha) was used to control weeds. Using two sprays as a tank mix at the first node and flag leaf stages: chlormequat chloride (Lihocin)

at 0.2% + tebuconazole (Folicur® 430 SC) at 0.1% of commercial product dose foliar spray was used as the primary treatment and control in a randomized plot design experiment.

In this experiment, the liquid formulation was obtained from human hair waste (amino acids) hydrolysate trademark "Plant Force Advance" from FloritechOrgano Industries, Nagpur, was evaluated for effectiveness on cereal plant growth sprayed after 25 days of seed germination. The test plots received a foliar spray of a liquid formulation with approximately 8% (v/v) nitrogen that was diluted to 1:200 with water. This was followed by three additional sprays, each after 30-day interval. Five randomly chosen plants were taken from the treatment and control groups at harvest time (in the month of mid-April) to record observations on the plant height, spike length, and overall yield parameters (total grains weight and yield of experiment plot). The Duncan multiple range test was used to statistically assess the results.

Result and Discussion

The height of a wheat plant is considered an important parameter, but it varies based on type of variety. The plant height is measured by normal ruler scale at fully grown stage (end of month March) with grains. The plant height was compared to control and significant amounts of increase were observed in plants treated with protein hydrolysate formulation. The comparison of control plot wheat (75.69 cm), triticale (83.97 cm), and barley (66.65 cm) and treated plot showed an increase in height wheat (2.9±0.25%), triticale (3.5±0.45%), and barley (2.2±0.22%) mentioned in Table 1. The increase in height proved that chlorophyll content also increased and by the help of biochemical energy the uptake of biostimulant formulation supports the growth. in various studies investigated the overall impact of protein hydrolysate in both controlled and open field condition showed the enhancement in root and shoot development, enhanced production and others crops productivity like tomato, passion fruit, pepper, papaya, and corn (Halpern et al., 2015; Colla et al., 2014, 2015, 2017; Nardi et al., 2016). Protein hydrolysate prepared from alfalfa and it's foliar spray on pepper had shown an increase in number of fruits as well as the amount of secondary metabolites (Ertani et al., 2014). In another study on lettuce, foliar application of plant-based protein hydrolysate demonstrated increase in salinity tolerance and concentration of nitrogen, phosphorus in leaves (Lucini et al., 2015). The amino acids (protein hydrolysate) formulation possesses amino acids with low molecular weight short peptides that are rich in a source of nitrogen and nitrogen is one of the major macro elements required for plant growth and metabolism (Subbarao et al., 2015). The availability of nitrogen from free amino acids as reduced source of nitrogen supports the overall growth and health of plants. Protein hydrolysate also possess hormone like activity that supports in seed germination to fruit maturation (Yadav et al., 2020)

The maximum increase of spike length was observed in wheat (2.01±0.6%), triticale (1.73±0.17%), and barley (2.9±0.27%) as compared to control plot wheat (8.91 cm), triticale (8.08 cm), and barley (6.75 cm) mentioned in Table -2. The spike length also varies depending on the variety of wheat, triticale and barley crops. In the experiment, an increase of spike length proved the uptake of biostimulants in the formation of fruits parts of plants. The increase in spike length of wheat determines the total yield of wheat crops. The significant amount of yield increased was noticed in wheat (11.9±0.86%), triticale (12.14±0.86%), and barley (12.8±0.29%) as compared to control plot in wheat (10.25 kg), triticale (10.39 kg), and barley (13.82 kg) mentioned in Table 3.

The human hair waste-based protein hydrolysate containsshort chain peptides and free amino acids that can be used as new range of biofertilizers. Eearlier also nitrogen is considered as the most important factor in biofertilizers that determines the growth of plants but recent trends the amino acid-based fertilizers are catching attention due to high uptake of amino acids by plant metabolic processes at molecular levels and amino acids also provide reduced nitrogen to plants (Teixeira et al., 2018).

Conclusions

To increase the production of any crop various techniques are available. In recent times genetic editing is in trend to increase production and nutritional value of crops but genetic modification is still not sure for the stability of crops, and it may cause threat to food security so, its required scientific evidence-based exploration of crop at molecular levels. The alternative of genetic editing biofertilizers is a very convenient source of enhancing production but due to disturbance in the environment like heat waves these biofertilizers do not provide effective results. The protein hydrolysate technique is now catching the attention due to their uptake of molecular level and solution to increasing the salon waste that naturally is not degraded in soil. Human hair is rich in amino acids and in this study, we got excellent results of bio stimulant foliar prepared from the human hair waste. The protein hydrolysate can be an alternative of biofertilizers and also maintains

overall plant health that leads to maturation of plants in abiotic and biotic stress. This study can conclude that bio stimulants based on protein hydrolysate technology are beneficial in terms of increase in growth, nutrient uptake and in minimization of chemical-based growth promoters. The application of protein hydrolysate in soil during cultivation and irrigation needs to be explored and studied the impact of soil health and microbiome of soil.

Acknowledgment

The authors are thankful to Er. Sonul D. Bodhane, FloritechOrgano Industries, Nagpur for supplying the Plant Force Advance® for field trails.

Table 1. Comparison of plant height in both control and test plots of three crops wheat, triticale, and barley.

			Plant Height (cm)	
Line No.	**Crops**	**Variety**	**Control**	**Treatment**
1	Wheat	Black Wheat	71.7±1.33	73.9±1.69
2		WH 1105	73.4±1.96	75.7±0.31
3		WH 1142	79.2±0.40	81.3±0.40
4		HD2967	77.3±1.40	79.9±0.76
5		WH 1124	74.5±1.41	76.8±1.13
6		DBW88	74.9±1.20	77.1±1.39
7		WH1025	75.2±0.90	77.8±1.30
8		WH1080	78.6±0.90	81.1±1.60
9	Triticale	TL3003	80.9±1.15	83.8±1.30
10		TL3002	80.9±0.50	83.9±0.94
11		TL3001	83.4±1.13	86.5±0.49
12		TL3004	80.5±0.52	83.5±0.98
13		TL2942	86.6±1.50	90.1±0.85
14		TL3005	86.4±0.41	89.9±0.75
15		TL2969	88.2±0.70	91.8±0.64
16	Barley	BH885	63.9±0.94	65.2±0.52
17		BH902	75.5±0.83	77.2±1.13
18		BH946	69.1±0.52	70.8±1.91
19		BH393	57.9±1.30	59.4±1.31

Table 2. Comparison of plant spike length in both control and test plots of three crops wheat, triticale, and barley.

Spike Length (cm)				
Line No.	**Crops**	**Variety**	**Control**	**Treatment**
1	Wheat	Black Wheat	6.4±0.28	7.9±0.91
2		WH 1105	6.8±0.49	9.6±0.44
3		WH 1142	8.4±0.61	9.6±0.46
4		HD2967	7.3±0.54	9.3±0.22
5		WH1124	6.2±0.31	8.2±0.45
6		DBW88	8.3±1.07	9.6±0.80
7		WH1025	7.2±1.21	9.7±0.15
8		WH1080	7.3±0.44	8.8±0.29
9	Triticale	TL3003	7.3±0.15	8.5±0.73
10		TL3002	6.6±0.09	8.1±0.32
11		TL3001	6.5±0.45	7.8±0.55
12		TL3004	7.1±0.55	8.8±0.68
13		TL2942	7.1±0.41	8.6±0.26
14		TL3005	6.6±0.55	8.2±0.37
15		TL2969	6.1±0.34	7.5±0.63
16	Barley	BH885	5.0±0.32	6.9±0.29
17		BH902	4.7±0.28	7.1±0.22
18		BH946	4.9±0.18	6.7±0.15
19		BH393	5.1±0.47	7.1±0.15

Table 3. Comparison of total yield in both control and test plots of three crops wheat, triticale, and barley.

			Plot Yield (kg)	
Line No.	**Crops**	**Variety**	**Control**	**Treatment**
1	Wheat	Black Wheat	17.57±0.46	20.27±0.77
2		WH1105	7.58±1.36	8.61±1.40
3		WH1142	10.21±2.86	11.73±2.06
4		HD2967	8.59±0.98	9.79±2.05
5		WH1124	11.17±2.69	12.57±1.49
6		DBW88	8.66±1.45	9.76±2.21
7		WH1025	5.67±2.35	6.37±2.04
8		WH1080	12.22±1.69	13.92±1.33
9	Triticale	TL3003	9.62±1.26	11.02±1.27
10		TL3002	13.37±0.96	15.37±1.59
11		TL3001	15.24±2.55	17.54±1.69
12		TL3004	7.55±1.77	9.65±2.39
13		TL2942	9.39±2.84	10.69±2.56
14		TL3005	8.33±1.37	9.43±1.69
15		TL2969	8.08±2.13	9.08±1.31
16	Barley	BH885	11.13±2.35	12.73±2.27
17		BH902	14.99±1.35	17.19±1.67
18		BH946	11.38±1.86	13.08±1.23
19		BH393	17.71±1.20	20.41±1.28

References

Calvo P, Nelson L, and Kloepper JW, (2014). Agricultural uses of plant biostimulants. Plant Soil 383:3-41. doi: 10.1007/s11104-014-2131-8.

Colla G, Rouphael Y, Canaguier R, Svecova E and Cardarelli M, (2014). Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. Front. Plant Sci. 5:448. doi: 10.3389/fpls.2014.00448.

Colla G, Nardi S, Cardarelli M, Ertani A, Lucini L and Canaguier R, (2015). Protein hydrolysate as biostimulants in horticulture. Sci. Horti. 96:28-38. Doi: 10.1016/j.scienta.2015.08.037

Colla G, Hoagland L, Ruzzi M, Cardarelli M, Bonini P, Canaguier R and Rouphael Y, (2017) Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. Front. Plant Sci. 8:2202. doi: 10.3389/fpls.2017.02202

Ertani A, Pizzeghello D, Francioso O, Sambo P, Sanchez-Cortes S and Nardi S, (2014). *Capsicum chinense* L. growth and nutraceutical properties are enhanced by biostimulants in a long-term period: chemical and metabolomic approaches. Front. Plant Sci. 5:1-12.

Halpern M, Bar-Tal A, Ofek M, Minz D, Muller T and Yermiyahu U, (2015). The use of biostimulants for enhancing nutrient uptake. Adv. Agron. 130:141-174. doi: 10.3389/fpls.2017.00597.

Kumar P, Behl J , Singh M and Behl R, (2021). Efficacy of protein hydrolysate (plant force advance) based formulation on cotton yield. Ekin Journal of Crop Breeding and Genetics, 7(1):43-47. https://dergipark.org.tr/en/pub/ekinjournal/issue/60162/872676

Lucini L, Rouphael Y, Cardarelli M, Canguier R, Kumar P and Colla G, (2015). The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. Sci. Hortic. 182:124-133.

Mostafa MA, Manal Mubarak NS, Khalil G and Mohamad H, (2014). Manufacturing amino acids bio fertilizers from agricultural wastes-Effect of synthetic organic fertilizers on the growth and yield of some forage crops as well as some soil properties J. Soil Sci. and Agric. Eng., Mansoura Univ., 5(2):279-294.

Nardi S, Pizzeghello D, Schiavon M and Ertani A, (2016). Plant biostimulants: Physiological responses induced by protein hydrolyzed based products and humic substances in plant metabolism. Sci. Agric. 73:18-23. doi: 10.1590/0103-9016-2015-0006.

Popko M, Michalak I, Wilk R, Gramza M, Chojnacka K and Górecki H, (2018). Effect of the new plant growth biostimulants based on amino acids on yield and grain quality of winter wheat. Molecules. 23(2):470. DOI: 10.3390/molecules23020470.

Subbarao SB, Aftab Hussain IS and Ganesh PT, (2015). Biostimulant activity of protein hydrolysate: Influence on plant growth and yield. J Plant Sci Res. 2(2):125.

Teixeira WF, Fagan EB, Soares LH,Soares JN, Reichardt K and Neto DD, (2018). Seed treatment and foliar application of amino acids improve variables of nitrogen metabolism and productivity in soybean Crop. Front. Plant Sci. 9:396.doi: 10.3389/fpls.2018.00396

Yadav S and Khosla B, (2020). Biostimulant effect of poultry feather hydrolysate on maize (*Zea mays* L.) seedlings. Annals of Biology 36 (3):508-512.

Vasudeva, V. (2022). Explained What are India's plans to avert a wheat crisis?

Research Article

Ekin
Journal of Crop Breeding and Genetics

9(2):98-105, 2023

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Comparison of Two and Six-rowed Barley (*Hordeum vulgare* L.) Genotypes under Rainfed Conditions for Yield, Quality and Biotic Stress Tolerance

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Citation:
Öztürk İ., Tülek B., Avcı R., Kahraman T., Tülek A., 2023. Comparison of Two and Six-rowed Barley (*Hordeum vulgare* L.) Genotypes under Rainfed Conditions for Yield, Quality and Biotic Stress Tolerance. Ekin J. 9(2):98-105.

Received: 11.10.2022 ***Accepted: 06.02.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

ABSTRACT

Barley is an essential crop in the Trakya region and it grows for feed and malting in the region. The experiments were carried out in the 2016-2017 growing cycle and composed of 36 two-rowed and 36 six-rowed barley genotypes in alpha-lattice blocks with three replications. The characters such as grain yield, net blotch, scald, plant height, days of heading, 1000-kernel weight (TKW), test weight (TW) and protein ratio and relationship among them were investigated in the study. The genotypes were screened for scald and net blotch under natural epidemic conditions. According to the results, there were significant differences among genotypes for the parameters investigated in the study. The means of grain yield for two-rowed and six-rowed genotypes were 8576 kg ha^{-1} and 8454 kg ha^{-1} respectively. In two-rowed genotypes mean 1000-kernel weight was 51.2 g, test weight 72.9 kg and protein ratio 11.6%. In six-rowed genotypes mean TKW was 37.0 g, TW 69.5 kg and protein ratio 10.8%. In two-rowed genotypes based on double-digit scores, a total of 11 barley genotypes highly tolerant to Net blotch (*Pyrenophora teres*) scored between 11 and 33. Net blotch effect on grain yield, 1000-kernel weight, test weight and protein ratio were slightly negative for two-rowed genotypes. Scald leaf disease negatively affected 1000-kernel weight (r=-0.391*) and test weight (r=-0.482**). Scald leaf disease also negatively affected grain yield. In the study, 5 genotypes from the 2-rowed experiments and 2 genotypes from a 6-rowed experiment were selected for breeding studies based on parameters investigated.

***Keywords*:** Barley, genotype, grain yield, quality parameter, biotic stress

Introduction

Barley is the main field crop in the Trakya region of Turkey. Environmental effects such as temperature, humidity and rainfall cause biotic and abiotic stress factors and constraint yield and quality in barley (Öztürk et al., 2018). Barley genotypes are classified as 2-row or 6-row according to the structure of the spike and has been used as animal feed, as a source of fermentable material for beer and certain distilled beverages, and as a compound for a variety of health foods (Marwat et al., 2012). Since two-row barley produces larger seeds with a higher test weight and seed weight than six-row barley, two-row barley is very likely to produce more useful quality forage than six-row barley (Reid et al., 2001). Understanding the potential grain yield of the 2 and 6 rows and the ways to get their yield can be helpful to the plant breeder. Grain filling, the final process associated with yield performance, is a very important determinant of grain yield in cereals products. In addition, abiotic stresses such as, drought and high temperature during the grain-filling phase of barley limit barley productivity Gouis, 1992; Przulj and Momcilovic, 2012).

According to the spike morphology, the two and six-rowed genotypes of barley usually differ in their end-use. Six-row barley is mainly used as feed due to its higher grain protein content and less uniform grain size and weight compared to two-row barley (Kandic et al., 2019; Zwirek et al., 2019; Lang et al., 2013). Two-row barley is used more often as a malting

material in brewing and produces higher malt extract than six-row barley (Gupta et al., 2010). The two-row barley genotypes generally had a higher absolute grain filling rate. Another benefit of two-row barley over six-row barley is the earliness. This is essential mechanism for the future climate scenario to avoid high temperatures and low rainfall during grain filling (Kandic et al., 2018). Grain yield and yield components in barley are complicated characters relying on a large number of genotypes, and environmental, agronomic and physiological characteristics. Based on the barley row type, there are various results concerning grain yield across stress environment conditions. Two-rowed barley genotypes generally had more 1000-kernel weight, test weight, protein ratio and grain uniformity than six-rowed genotypes under non-stress environment conditions. Another advantage of two-row barley over six-row barley is the earlier heading time. This is essential to avoid high temperatures and low precipitation during the grain-filling phase (Öztürk, 2019).

Due to changing environmental conditions, there are variations in yield, quality and leaf diseases depending on environmental factors in genotypes with 2 and 6 rows. In addition, biotic stress factors are also influential due to rainy and humidity conditions during the shooting and heading phase. Because of the favourable environmental factors such as precipitation and temperature, high yields can be obtained in barley in the region. However, the change between some years and locations may occur high infection of leaf disease and cause a decrease in yield. In addition, the low temperature of the booting and heading stages causes cold damage and sterility in the spike. For this reason, genotypic differences are also important for adapting to different environmental conditions in barley. The study aimed was to investigate and comparison of 2 and 6-rowed barley genotypes yield, quality and biotic stress factors such as scald and net blotch under rainfed conditions.

Materials and Methods

The study was carried out in the 2016-2017 growing season as two experiments that composed of 36 two-rowed and 36 six-rowed barley genotypes. Experiments were set up in alpha-lattice blocks design with three replications. Experiments were conducted in the Edirne location (latitude 41° 38′ 57″ N, longitude 26° 35′ 59″ E and altitude 41 m), Trakya region, Türkiye. The plot area was 6 m^{-2}, 6 meters long and 6 rows, spaced 0.17 meters apart. A seed rate of 500 seeds m^{-2} was used. In the study, grain yield (GY), plant height (PH), days of heading (DH), 1000-kernel weight (TKW), test weight (TW) and protein ratio (PRT) were investigated. Scald (*Rhynchosporium commune*) (RHY) and Net blotch (*Pyrenophora teres* f.sp. *teres*) (PYR) leaf diseases were screened under natural epidemic conditions at heading stages (Z75). Plots were naturally infected by *Pyrenophora teres* and *Rhynchosporium commune*. Disease assessments were made in Zadoks 75 growth stage of development (GS75) (Zadoks et al., 1974) using a 0-9 scale described by Saari and Prescott (1975) and Couture (1980).

Statistical Analyses

Data were analysed statistically for analysis of variance the method described by Gomez and Gomez (1984). The significance of differences among means was compared by using the Least Significant Difference (L.S.D. at a 5%). Pearson correlation coefficients were calculated between significant variables measured in this study and the results were plotted.

Cluster Analysis

Cluster analysis was performed on the barley genotypes using the seven measured parameters in clustering of the studied accessions (Chiu et al., 2001; Bacher et al., 2004). Hierarchical Cluster analysis with Ward's clustering method (Ward, 1963) based on Squared Euclidean Distances was performed to construct a cluster tree (Dendogram).

Temperature, monthly precipitation and mean humidity in 2016-2017 in the experimental area are given in Table 1. In the experimental area, the amount of precipitation was 417.2 mm less than a long year. The mean humidity was 71.2%. Rainfall in November and December was very low compared with the long year (Table 1).

Results and Discussion

According to the results, there were significant differences among genotypes for the parameters investigated in two-rowed genotypes. The mean grain yield in two-rowed genotypes was 8576 kg ha^{-1}. The highest grain yield was performed by G3 (9603 kg ha^{-1}) and followed by Yaba, G21 and G22. The minimum and maximum days of heading were 100 (G4) and 118 (G18 and G36). Plant height is an important component as it can cause lodging in rainy conditions and flat areas. In addition, tall varieties are preferred in arid regions. In the study, plant height varied from the shortest 85 cm (Yaba) to and tallest 109 cm (G25). In barley genotypes, TKW and TW vary according to genotype, environmental factors and cultural practices. Precipitation during the grain-filling period is the most important determining factor. In two-rowed genotypes mean 1000-kernel weight was 51.2 g and the test weight was 72.9 kg. Genotypes G17, G23 and G24 had the

highest 1000-kernel weight. The highest test weight was determined in G14, G12 and G13. The ratio of protein is much related to the amount and time of nitrogen fertilization. Nitrogen fertilization, especially in the pre-heading period, contributes to the increase in protein in the grain. In the study, the mean protein ratio was 11.6%. The highest protein ratio was determined in G8 and G12 (12.8%) and the lowest in G33 (10.5%).

Net blotch caused by *Pyrenophora teres* f. sp. teres and scald caused by *Rhynchosporium commune* are major foliar diseases of barley and often epidemics occur in the same region. In two-rowed genotypes based on double-digit scores, a total of 11 barley genotypes highly tolerant to Net blotch (*Prenophora teres*) scored between 11 and 33. A total of 10 genotypes were susceptible to Net blotch leaf disease. It has been determined that *Prenophora teres* leaf disease generally causes moderate and low epidemics in genotypes.

Scald (*Rhynchosporium commune*) is one of the important biotic stress factors in barley. Scald leaf disease negatively affected 1000-kernel weight (r=-0.391*) and test weight (r=-0.482**). Scald leaf disease also negatively affected grain yield. In the study, 5 genotypes from the 2-rowed experiments and 2 genotypes from a 6-rowed experiment were selected for breeding studies. In 2-rowed genotypes, protein ratio was positively associated with plant height and 1000-kernel weight. Genotypes with short plant height had higher yield potential. Net blotch (*Pyrenophora teres*) is one of the essential biotic stress factors associated with precipitation and humidity during plant growth stages. Correlation coefficients among tested characters in two-rowed genotypes were given in Table 3. Net blotch negatively slightly affected grain yield, TKW, TW and protein ratio. There was also a negative association between grain yield with plant height (r=-0.410*), days of heading, TKW, and protein ratio (Table 3).

Correlation coefficients among tested characters in six-rowed genotypes were given in Table 5. Scald (*Rhynchosporium commune*) leaf disease is one of the important biotic stress factors in barley and reduces grain yield and quality. Scald leaf disease negatively significantly affected and reduced 1000-kernel weight (r=-0.391*) and test weight (r=-0.482**). Scald leaf disease also negatively affected grain yield. In the study in six-rowed genotypes, grain yield was negatively and significantly associated with days of heading (r=-0.500**), TKW (r=-0.458**) and protein ratio (r=-0.554**). There was also a positive association between grain yield and test weight (r=0.369*), 1000-kernel weight and protein ratio (r=0.569*) (Table 5).

The mean grain yield in six-rowed genotypes was 8454 kg ha^{-1}. Genotype G22 had a higher yield (10086 kg ha^{-1}) and followed by G24 (9864 kg ha^{-1}), G12 (9774 kg ha^{-1}), and G21 (9652 kg ha^{-1}). In barley, 6-rowed genotypes are more sensitive to drought and heat stress due to the high number of grains per spike. For this reason, the grain weight of barley is affected the most by drought and high temperatures. In the research, while the earliest genotypes were G23, G22 and G24, the latest variety was Lord. Plant height with stem strength is an essential characteristic for lodging resistance. In the study, the shortest plant was 82 cm (G15) and the tallest was 118 cm (G9). In six-rowed barley genotypes, while the average TKW was 37.0 g, the lowest was 28.6 g (cv. Yaprak) and the maximum was 45.3 g (G26). The mean test weight was 69.5 kg. Genotype Lord had the highest TW (73.6 kg) and G3 and G3 had the lowest 64.1 kg. The protein ratio in barley varies depending on genotype, environment and agronomic practices such as nitrogen amount and time. The mean protein ratio was 10.8%. The higher protein ratio was established in G11 and followed by cultivar Martı. While 2 barley genotypes were highly tolerant to scald leaf disease in 6-row genotypes, 22 barley genotypes were found to be very sensitive. In six-rowed genotypes, scald negatively slightly affected grain yield, and significantly negatively affected 1000-kernel weight and protein ratio.

Cluster analysis

Genotypes were classified according to cluster analysis in terms of the traits examined. (Figure 1). Based on cluster analyses there was a significant difference classified of the 2 and 6- rowed genotypes. The first and second clusters included 36 accessions composed of 2 and 6-rowed barley genotypes. While the 2-row genotypes showed a different distribution according to the cluster analysis, most genotypes were in 1 subgroup. The clustering analyses in six-rowed genotypes were divided into seven subclusters (Figure 1 and 2). Genotypes G19 and G29 were the closest to each other, while G1 and G36 were the most distant genotypes in terms of the traits examined in the two-row genotypes. G15 and G18 were the closest to each other in terms of the investigated characteristics in the six-row genotypes, while G1 and G23 were the most different genotypes.

Conclusions

These results showed that the two-row genotypes had better performance under rainy conditions in terms of yield and some quality parameters. As expected, grain yield, 1000 grain weight and test weight of the two-row barley genotypes were higher than those of the six-row barley genotypes. Unexpectedly, the fact that

the protein ratio of the two-row genotypes was found to be higher than the six-row genotypes also means that they may be more suitable for use as feed. The fact that *Pyrenophora teres* disease for two-row genotypes and *Rhynchosporium secalis* leaf disease for 6-row genotypes were observed to cause more adverse effects with the effect of environmental factors confirms that the genotypic susceptibility factor is the determinant. These adverse effects were on grain yield, TKW, TW and protein ratio for two-row barley and grain yield for six-row barley. The 11 two-rowed barley genotypes and 2 six-rowed barley genotypes showed high tolerance to Net blotch (*Pyrenophora teres*) and scald leaf disease respectively. In the study, 5 2-rowed genotypes and 2 6-rowed genotypes were selected to use as a parent in breeding studies.

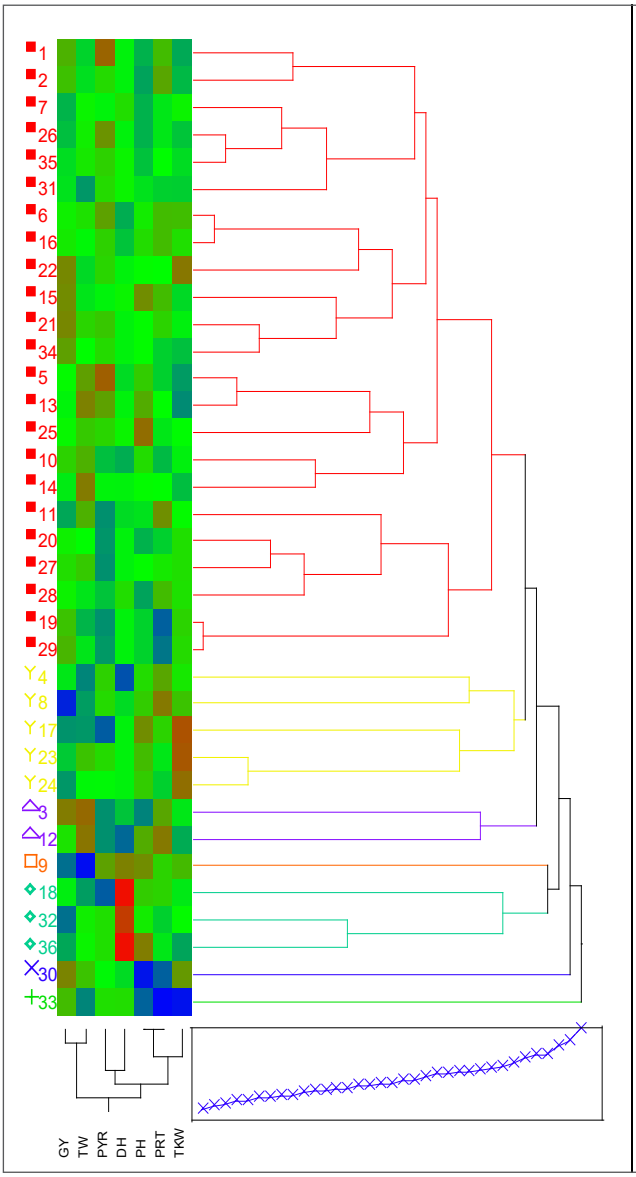

Figure 1. Dendrogram of 2-rowed 36 barley genotypes using the Hierarchical Ward's clustering method based on seven measured parameters.

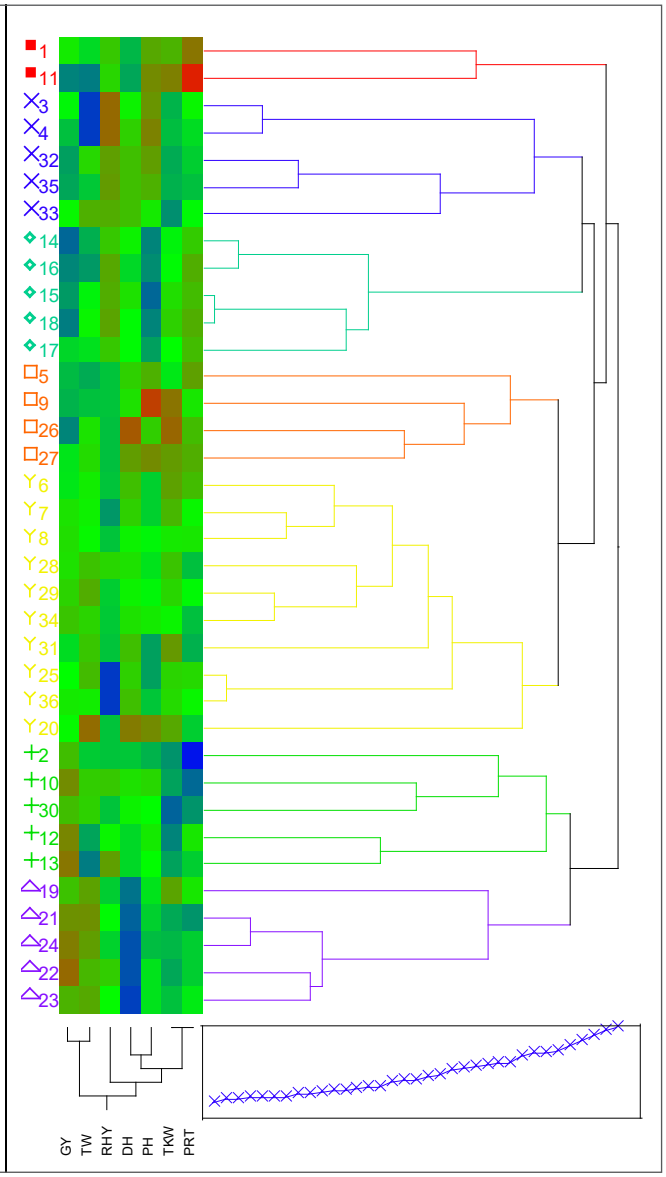

Figure 2. Dendrogram of 6-rowed 36 barley genotypes using the Hierarchical Ward's clustering method based on seven measured parameters.

Table 1. Rainfall, mean humidity and temperature in Edirne location in 2016-2017 growing year.

Months	Rainfall Long Year	Rainfall (mm)	Humidity (%)	Temperature (°C)		
				Min.	Max.	Mean
September 2016	34.0	9.2	57.5	5.0	33.8	20.8
October 2016	52.9	44.4	69.5	1.3	28.8	14.3
November 2016	72.4	3.2	72.9	-9.9	15.4	0.7
December 2016	61.7	3.2	72.9	-9.9	15.4	0.7
January 2017	48.1	67.8	83.7	-17.0	8.4	-1.9
February 2017	46.9	43.4	80.0	-8.4	20.6	5.3
March 2017	52.2	51.0	73.0	-1.9	25.5	10.2
April 2017	51.0	65.6	63.1	-1.6	28.6	12.5
May 2017	56.0	85.0	65.4	4.4	30.0	17.9
June 2017	41.5	44.4	74.4	12.9	40.0	21.2
Total/Mean	516.7	417.2	71.2	-17.0	40.0	10.2

Table 2. Mean grain yield quality and other parameters investigated in two-rowed barley genotypes in 2016-2017 cycles.

G No.	Genotypes	GY	PYR	DH	PH	TKW	TW	PRT
1	Sladoran (G1)	9175	78	107	95	47.5	72.2	11.9
2	G2	9059	53	107	94	48.2	72.4	12.0
3	G3	9603	22	105	92	50.2	75.4	12.0
4	G4	8391	55	100	102	52.2	70.9	12.0
5	Bolayır (G5)	8614	79	106	103	46.9	74.5	11.4
6	G6	8687	65	104	101	54.0	73.4	11.9
7	G7	7988	43	109	95	51.7	73.1	11.5
8	G8	6843	53	106	103	53.8	71.3	12.2
9	G9	7449	65	113	107	54.2	68.9	11.8
10	Harman (G10)	8924	32	104	102	50.6	74.2	11.3
11	G11	7889	22	106	98	51.4	74.2	12.1
12	G12	8789	22	101	105	47.6	75.2	12.2
13	G13	8491	65	107	105	46.2	75.0	11.6
14	G14	8426	43	107	100	48.4	75.1	11.6
15	Hasat (G15)	9479	43	108	107	49.6	72.5	11.9
16	G16	8740	55	105	102	52.6	72.8	11.9
17	G17	7729	11	107	107	58.7	71.2	11.8
18	G18	8446	11	118	103	50.3	71.3	11.8
19	G19	9041	22	107	97	53.1	71.7	10.9
20	Pınar (G20)	8688	23	107	95	52.6	72.9	11.4
21	G21	9517	57	107	100	50.6	73.6	11.8
22	G22	9508	54	107	100	57.1	72.3	11.6
23	G23	8162	53	107	104	58.5	73.9	11.5
24	G24	7760	44	107	103	57.5	72.8	11.4
25	G25	8633	54	108	109	51.1	73.8	11.5

Continuing table 2

G No.	Genotypes	GY	PYR	DH	PH	TKW	TW	PRT
26	G26	8077	68	107	95	48.7	73.2	11.5
27	G27	8843	22	107	100	52.6	73.8	11.7
28	G28	8677	33	109	94	52.5	72.5	11.9
29	G29	9148	24	107	97	52.8	72.5	11.0
30	Yaba (G30)	9535	44	106	85	55.6	73.9	10.9
31	G31	8353	53	108	98	49.1	71.2	11.4
32	G32	7457	52	116	101	51.4	73.2	11.4
33	G33	9096	52	109	90	40.9	70.9	10.5
34	G34	9316	53	107	100	48.5	72.9	11.4
35	G35	8313	55	108	96	49.7	73.3	11.6
36	G36	7886	52	118	108	47.3	73.1	11.5
	Mean	8576	45	108	100	51.2	72.9	11.6

GY: Grain yield (kg/ha^{-1}), PYR: Net blotch (0-99), DH: Days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%).

Table 3. The correlation coefficient among parameters in 2-rowed barley genotypes.

Parameters	GY	PYR	DH	PH	TKW	TW
PYR	-0.072					
DH	-0.319	-0.024				
PH	-0.410*	0.066	0.164			
TKW	-0.232	-0.264	-0.107	0.159		
TW	0.278	-0.066	-0.299	-0.066	-0.172	
PRT	-0.217	-0.013	-0.192	0.364	0.134	0.076

*: P<0.05, **: P<0.01; GY: Grain yield (kg/ha^{-1}), PYR: Net blotch (00-99), DH: Days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%).

Table 4. Mean grain yield quality and other parameters investigated in six-rowed barley genotypes in 2016-2017 cycles.

G No.	Genotypes	GY	RHY	DH	PH	TKW	TW	PRT
1	Martı (G1)	8669	78	104	107	41.1	68.5	11.8
2	G2	9161	53	105	90	31.2	68.1	9.2
3	G3	8371	99	109	109	33.0	64.1	10.9
4	G4	7768	99	111	111	33.5	64.1	10.6
5	G5	7717	53	111	106	35.8	67.2	11.5
6	G6	8200	53	112	93	42.1	70.0	11.3
7	G7	8752	43	111	93	40.9	69.9	10.9
8	G8	8807	53	109	97	38.2	69.7	11.0
9	G9	7634	53	110	118	44.5	67.8	11.0

Continuing table 4

G No.	Genotypes	GY	RHY	DH	PH	TKW	TW	PRT
10	Hazar (G10)	9701	78	110	102	32.0	70.9	9.8
11	G11	7107	75	103	110	43.9	65.9	12.4
12	G12	9774	68	106	100	30.4	67.0	11.0
13	G13	9944	87	106	98	32.0	65.9	10.5
14	G14	6798	78	109	85	36.4	67.3	11.2
15	G15	7361	84	110	82	38.8	69.2	11.3
16	G16	7136	85	106	86	36.8	66.7	11.4
17	G17	8010	78	108	88	37.0	68.7	11.3
18	G18	7043	86	108	85	39.5	69.8	11.4
19	G19	9110	55	100	95	41.9	72.1	11.0
20	Lord (G20)	8516	53	116	110	41.7	73.6	10.5
21	G21	9652	65	99	93	32.4	72.6	10.1
22	G22	10086	77	98	95	32.3	71.5	10.5
23	G23	9278	67	97	95	33.7	71.9	10.7
24	G24	9864	56	98	91	33.2	72.2	10.5
25	G25	8456	22	111	88	39.3	71.4	11.1
26	G26	7131	52	118	103	45.3	70.3	11.3
27	G27	8177	52	114	110	42.4	70.5	11.4
28	G28	8777	75	110	95	40.2	71.2	10.4
29	G29	8929	55	109	97	39.2	71.8	10.8
30	Yaprak (G30)	9156	53	109	98	28.6	70.8	10.1
31	G31	8073	53	112	88	42.5	71.1	10.3
32	G32	7416	87	112	108	32.5	70.6	10.5
33	G33	8531	84	112	100	31.0	71.7	10.8
34	G34	9079	54	110	100	37.6	70.7	10.4
35	G35	7489	88	112	106	32.8	68.0	10.4
36	G36	8673	22	112	92	39.0	70.0	10.9
	Mean	8454	66	108	98	37.0	69.5	10.8

*: P<0.05, **: P<0.01; GY: Grain yield (kg/ha^{-1}), RHY: Scald (00-99), DH: Days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%).

Table 5. The correlation coefficient among parameters in 6-rowed barley genotypes.

Parameters	GY	RHY	DH	PH	TKW	TW
RHY	-0.210					
DH	-0.500**	-0.160				
PH	-0.060	0.142	0.251			
TKW	-0.458**	-0.391*	0.333*	0.081		
TW	0.369*	-0.482**	-0.071	-0.252	0.127	
PRT	-0.554**	0.043	0.073	0.093	0.569**	-0.304

*: P<0.05, **: P<0.01; GY: Grain yield (kg/ha^{-1}), PYR: Net blotch (00-99), DH: Days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%).

References

Bacher J, Wenzig K and Vogler M, (2004). SPSS two-step cluster - a first evaluation. Univ. Erlangennürnb. 1:1-20

Chiu T, Fang D, Chen J, Wang Y and Jeris C, (2001). A robust and scalable clustering algorithm for mixed type attributes in a large database environment, in Proceedings of the Seventh ACM SIGKDD International Conference on Knowledge Discovery and Data Mining - KDD '01, (New York, NY: ACM Press), 263-268.

Couture L, (1980). Assessment of severity of foliage diseases of cereals in cooperative evaluation tests. Can. Plant Dis. Surv. 60:8-10.

Gomez KA and Gomez AA, (1984). Statistical procedures for agricultural research. 2nd Ed. John Willey and Sons, Inc. New York. 641.

Gouis Le J, (1992). A comparison between two and six-row winter barley genotypes for above-ground dry matter production and distribution. Agronomie, EDP Sciences, 12(2):163-171. <hal00885463>

Gupta M, Abu-Ghannam N and Gallaghar E, (2010). Barley for brewing. Characteristic changes during malting, brewing and applications of its by-products. Comprehensive Reviews in Food Science and Food Safety 9:318-328. https://doi.org/10.1111/j.1541-4337.2010.00112.x

Kandic V, Dodig D, Secanski M, Prodanovic S, Brankovic G and Titan P, (2019). Grain yield, agronomic traits, and protein content of two- and six-row barley genotypes under terminal drought conditions. Chilean Journal of Agricultural Research 79(4):648-657.

Kandic V, Dodig D, Zoric M, Nikolic A, Surlan Momirovic G, Kaitovic Z, Aleksic G and Duric N, (2018). Grain filling parameters of two-and six-rowed barley genotypes in terminal drought conditions. Italian Journal of Agrometeorology, 23(2):5-14.

Lang L, Rakszegi M and Bedo Z, (2013). Cereal production and its characteristics. Engineering aspects of cereal and cereal-based products. CRC Press, Boca Raton, Florida, USA.

Marwat SK, Hashimi M, Usman Khan K, Aslam Khan M, Shoaib M and Rehman F, (2012). Barley (*Hordeum vulgare* L.) a prophetic food mentioned in Ahadith and its ethnobotanical importance. American-Eurasian Journal of Agricultural and Environmental Sciences. 12:835-841.

Öztürk İ, (2019). Comparison of two and six-rowed barley (*Hordeum vulgare* L.) genotypes based on yield and agronomic characters. Agriculture & Food. 7:75-85.

Öztürk İ, Avcı R, Girgin VC, Kahraman T, Şili S, Kılıç TH, Vulchev D, Valcheva D and Goceva M, (2018). Effect of biomass at different growth stages on yield and agronomic characters in barley (*Hordeum vulgare* L.) cultivars. Agriculture & Food, 6:15-25. 2018

Przulj N and Momcilovic V, (2012). Spring barley performances in the Pannonian zone. Genetika. 44(3):499-512.

Reid JF, Choo TM, Ho KM, Martin RA and Konishi T, (2001). Comparisons of two-row and six-row barley for chemical composition using doubled-haploid lines. Crop Sci. 41:1737-1743.

Saari EE and Prescott JM, (1975). A scale for appraising the foliar intensity of wheat diseases. Plant. Dis. Rep. 59:377-380.

Ward JH, (1963). Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58:236-244.

Zadoks J, Chang T and Konzak C, (1974). A decimal code for the growth stages of cereals. Weed Research 14:415-421.

Zwirek M, Waugh R and McKim SM, (2019). Interaction between row type genes in barley controls meristem determinacy and reveals novel routes to improved grain. New Phytologist 221:1950-1965. https://doi.org/10.1111/nph.15548.

Research Article

Ekin
Journal of Crop Breeding and Genetics

9(2):106-118, 2023

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Determination of Promising Tulip Genotypes Belonging to Different *Tulipa* Species with Pedigree Selection Method

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Citation:
İzgi Y., Balkaya A., 2023. Determination of Promising Tulip Genotypes Belonging to Different *Tulipa* Species with Pedigree Selection Method. Ekin J. 9(2):106-118.

Received: 18.04.2023 ***Accepted: 20.05.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

ABSTRACT

Tulip (*Tulipa* spp.), belonging to the Liliaceae family, is a bulbous ornamental plant with approximately 280 natural species and 4000 varieties. The aim of this study was to select the most promising genotypes of different tulip (*Tulipa* spp.) species grown in Turkey with the objective of selection breeding and developing homogenous pure lines of these tulip genotypes (suitable for use as park and landscape plants, possessing traits like large flower, thick stem, long flower life, etc.). The study consisted of 74 tulip genotypes belonging to different *Tulipa* species. The weighted ranking method was used to select superior tulip genotypes with pedigree selection breeding. Local genotypes scored between 380 and 865 points according to the weighted ranking methods. In addition, tulip genotypes were grouped into classes based on the selection criteria, and the classes and distribution frequencies of tulip genotypes were identified. The top 10 most promising tulip genotypes were selected for evaluation in the variety breeding program. The first three highest-scoring genotypes were G2 (865 point), G3 (790 point), and G1 (785 point) of *T. agenensis* species, respectively.

***Keywords*:** Tulip, genetic resources, selection, frequency distribution, diversity, weighted ranking methods

Introduction

The breeding of high-quality new varieties in the ornamental plant sector is accomplished through the use of modern breeding techniques. The breeding studies in ornamental plants were first initiated by the private sector in the middle of the 19th century. The breeding programs were later carried out by institutions, universities, and research stations in ornamental plants for different purposes (Balkaya et al., 2021; Lal et al., 2022). The objectives of variety breeding studies can be summarized as resistance to biotic and abiotic stress conditions, gaining qualified fragrance characteristics, introducing new colors, morphological changes in plant and flower structure, differences in flowering time, longevity, and post-harvest performance (Horn and Peterson 2002; Gülbağ 2015; Balkaya et al., 2021).

Wild species of the natural flora, or plant species that are genetic resources used by growers, are disappearing over time due to genetic erosion. The extensive use of hybrid varieties, which have high productivity potential, has recently led to the extinction of many wild forms and local varieties in recent years. Sustainability in crop production can only be achieved through the conservation of wild species and local varieties (Akgün et al., 1998). Many of the traits that are directly affected by natural and artificial selection usually have quantitative variation. The studies on quantitative traits are of great importance for the economic use of germplasm resources. Therefore, agronomic traits and their genetic characteristics should be investigated concurrently while evaluating the genetic resources in breeding programs (Escribano et al., 1998).

The pre-breeding stage in plant breeding studies is to maintain heterogeneous and rich genetic diversity in the gene pools. Thus, qualified genetic materials are

constructed with genetic resources of heterogeneous structure having different characteristics from one another (Balkaya et al., 2021). The structure and genetic diversity of natural populations are affected by many factors, such as the diversity of habitat where the genotypes are located, plant fertilization biology, distribution of propagating materials (seeds and other vegetative plants), plant life cycle, population size, gene flow, and mutation rate (Ballesteros-Mejia et al., 2016). The wild forms and local cultivars are important genitors in transferring new traits to the cultivated crops. Identification of the variation present in populations is very important for the utilization of genetic resources in accordance with the targeted objectives in breeding studies (Tan, 2005). Understanding the structure and genetic diversity of the population is crucial for plant breeders to develop new varieties with agriculturally prominent and desirable traits by using valuable wild germplasm. The highest genetic diversities, especially in the traits related to flowering potential, flowering times, and flower structures have been obtained in the tulip breeding programs (İzgi Saraç et al., 2021).

Türkiye is a rich country with tulip genetic resources. This genetic diversity is very important, especially for the breeding of new commercial tulip varieties. Selection is one of the most important factors that may change the population structure in tulip breeding. The original gene frequency of a population is altered through selection methods and therefore, some genotypes are decreased or increased over time (Balkaya et al., 2011). The shortening of the breeding process with pre-selection is important in tulip breeding. The selection made in the early period when the bulbs do not have the ability to flower is the pre-selection. The aim of pre-selection is to make early selection in terms of bulb production, cut flower and disease resistance (İzgi Saraç et al., 2021). The breeders save labor, time, and space with a good pre-selection. Plant height, leaf and flower stalk strength, position and number of leaves, ratio between flower and leaf number, earliness, flower appearance, flower life and flower size are highly important in the selection of tulip genotypes. In addition, plant growth habit, stem thickness, and leaf appearance are other important traits (İzgi Saraç et al., 2010). The variety breeding studies in tulip species in Türkiye are insufficient compared to those in other plant species. The tulip varieties, namely Arda, Muş1071 and Kumru, were developed by population breeding within the scope of the project titled "Variety development in tulip (*Tulipa* spp.) and hyacinth (*Hyacinthus* spp.) species in Türkiye and introduction of new varieties to the ornamental sector" and these were registered as the first domestic tulip varieties in Türkiye (İzgi Saraç et al., 2021). However, these varieties with their existing characteristics cannot compete with the foreign F_1 varieties.

The purpose of this study was to select promising genotypes that are suitable for use in parks and landscapes, covering the area, having large flowers, thick stems, and long flower life, earliness and developing pure lines belonging to these tulip genotypes. Therefore, the selection breeding was carried out by the "Pedigree selection breeding" method in the available tulip gene pool.

Materials and Methods

The bulbs of 71 tulip genotypes, which were determined by Izgi Saraç et al., (2010) according to their adaptability and flower characteristics among the genetic sources of 114 tulip genotypes previously collected from the flora of Turkey, were used in the study (Table 1). The first local tulip varieties (Arda, Muş1071 and Kumru) developed by the Black Sea Agricultural Research Institute were also included as control (Table 1). The tulip bulbs were planted on January 15, 2018. Before planting, 30 bulbs of each tulip genotype were soaked in a 1% Captan + 0.1% Antracol® solution for 30 minutes to prevent fungal diseases. Then soil, peat, and perlite were mixed in a ratio of 1:1:1 and placed in plastic containers. Fifteen bulbs were planted in each plastic container (width, 37 cm; length, 56 cm, and height 24 cm dimensions) in the open field condition for each genotype. Standard fertilization and irrigation practices were applied for all genotypes.

In this study, pedigree selection method was used in tulip breeding. The flower and the other plant traits data were evaluated by the modified weighted ranked (WR) method ((İzgi Saraç et al., 2021). The WR method is a tool commonly used in statistical analyses. This method is known as "Tartılı derecelendirme" in Turkish and almost exclusively used in the studies with multivariate data generated in horticultural research (Balkaya and Yanmaz 2005; Balkaya and Ergün 2008; Çakır et al., 2019). Ten plants from each tulip genotype were examined for the selection criteria. The evaluations of the selection criteria are given below.

a. Plant stance: Classified as upright, semi-upright, and lateral.

b. Plant height (cm): The distance from the soil level to the tip of the tepals was measured using a tape measure during the full flowering period of the plant.

c. Stem thickness (mm): The thickness of the middle part of the stem was measured using a digital caliper.

d. Flower longevity (days): The flowering period

of the plant was determined in the field as the time difference (days) between the first flowering of the plant and the wilting of the flower petals.

e. Flower size (mm): Flower size was measured at the widest point of the flower with the help of a digital caliper when the plant was in the flowering stage.

f. Diameter of the main bulb (cm): The circumference of the bulb was measured using a tape measure.

g. Number of bulblet formed from the mother bulb (number): The number of bulblet formed from the mother bulb was counted.

Class values of selection criteria, Class Scores (CS) and Relative Scores (RS) were assigned to each tulip genotypes (Table 2). The total points of tulip genotypes were calculated by summing Class Scores (CS) and multiplied by Relative Scores (RS). At the end of this study, genotypes that were above the average score were selected as the superior tulip genotypes. In addition, the tulip genotypes were classified with respect to the detailed traits for the distribution frequencies (%).

Results and Discussion

The weighted ranking scores obtained by the selection of tulip genotypes are given in Table 3. The total scores calculated by multiplying the class and relative scores of tulip genotypes for each trait emphasized in the selection are also shown in Table 3. Accordingly, the scores of all the tulip genotypes ranged from 380 (G52) to 865 (G2) points. The three highest scoring genotypes were G2 (865 point), G3 (790 point) and G1 (785 point) which belong to *T. agenensis* species (Figure1), followed by G72 (750 point), G14 (745 point) and G18 (740 point), respectively (Table 3). The majority of the high-scoring tulip genotypes had the highest scores for all traits emphasized in the selection. The weighted ranking scores of 49 tulip genotypes were higher than the average score of all genotypes (513 points). Since the number of genotypes selected in the selection process was high, the top 10 most promising tulip genotypes with the highest scores were selected as a parental for the hybrid breeding program.

Selection technique is an important mechanism that modifies the structure of the original population in breeding studies. The gene frequency of the current population changes in accordance with the selection breeding aim, thus affecting the distribution of all genetic materials (Balkaya et al., 2011). Tulip genotypes were grouped into classes according to the weighted ranking method and the classes and distribution frequencies of tulip genotypes were determined in detail. The results of grouping demonstrated that 29 of the tulip genotypes were upright, 41 of them were medium, and 4 of them (G23, G27, G28, G43) had lateral plant habitus (Table 4). The tulip genotypes should be medium and erect in parks and landscaping and as potted plants, and the tulip genotypes should be erect in cut flowers according to consumer demands. The majority of the studied tulip genotypes had these characteristics.

The tulip (*Tulipa* spp.) genotypes displayed high variation and phenotypic diversity for plant height trait in this study. The plant height of 40.5% of the genotypes were grouped as moderately short, 25.7% as short, 25.7% as medium, 5.4% as tall, and 2.7% as very tall (Table 5). The plant height of more than 50% of the tulip genotypes evaluated in the study was between 10 cm and 25 cm. The plant height of *Tulipa mongolica* species in China was determined to range from 10 cm to 25 cm (Zhao, 2003). İzgi Saraç (2015) also reported that the plant height of 61 tulip genotypes varied between 10 cm and 25 cm. The results present study were similar with these findings.

The stem thickness in tulip genotypes is an important selection criterion that is directly correlated with the upright stance of the plant and its resistance to breakage ((İzgi Saraç et al., 2021). This trait is desired trait for the development of new varieties by the tulip breeders. More than half of the genotypes (52.5%) had a medium stem thickness and 39.2% had a thin stem thickness (Table 6). Moreover, the flowering life span of tulip (*Tulipa* spp.) genotypes indicated that 6.8% of the tulips were in the long, 47.3% in the medium, and 44.6% in the short flower longevity group (Table 7). Half of the tulip genotypes evaluated in the study had a medium flower longevity characteristic, which was considered significant. The flower longevity of tulip varieties varied between 6 and 22 days (İzgi Saraç et al., 2021). Breeders evaluate tulip plants with a long flowering period with respect to their vase longevity and select the genotypes with long vase longevity (Van der Meulen et al., 1997). In terms of flower size, 39.1% of the tulip genotypes had small flowers, 33.9% had medium flowers, 18.9% had very large flowers and 8.1% had large flowers (Table 8). The flower size is a considerably important trait of tulip plants because it enables them to visually stand out. The tulip genotypes in present study showed considerable phenotypic variation and the selected genotypes showed stand out with respect to their flower size.

The majority of the tulip genotypes (56.8 %) were in the medium bulb diameter group as targeted in the selection study (Table 9). Four tulip genotypes with the desired very large bulb diameter were identified. Two of

the very large genotypes were local tulip varieties. The size of 33.8% of the tulip genotypes was between 8 cm and 12 cm. Moreover, three tulip genotypes with small bulb diameters were also recorded (Table 9). According to the number of bulbs formed from the mother bulb of tulip genotypes, 55.4% of the genotypes were in the "low" group (between 3 and 4 pieces) and 18.9% were in the "very low" group (less than 2 pieces) (Table 10). Straathof et al., (1997) determined the annual increase in the diameter of the mother bulb and the number of bulbs in a pedigree selection test for the production of tulip bulbs. The researchers reported that a preliminary selection based on bulb production can be carried out by measuring the annual increase in the diameter of the mother bulb and the number of bulbs. This indicates that the number of bulbs is a very important criterion for selection breeding. The results revealed that the G1 and G2 genotypes of *T. agenensis*, which are in the 'very good' group, are the most promising tulip genotypes from the selection perspective.

Conclusions

The variety breeding studies in Türkiye, especially in tulip species among ornamental plants, are quite limited compared to other plant species. All the hybrid tulip varieties used in cultivation are imported from other countries. Therefore, the dependence on foreign countries for tulip bulbs is continuously increasing. Despite the favorable ecological conditions of Türkiye for bulbous plants, importing tulip bulbs is not an acceptable situation. However, private sector and/or public institutions, organizations, and universities do not have short- or long-term comprehensively breeding programs for the development of domestic hybrid tulip varieties. This study was carried out on tulip variety breeding. The pedigree selection method was used in selection breeding.

In this study, the tulip genotypes collected from different locations in Türkiye were distributed into frequency groups based on the selection criteria. Thus, tulip breeders will be able to easily select genotypes suitable for different breeding programs. The results of the study will contribute to the development of new hybrid tulip varieties, and evaluation of local tulip genotypes with different qualities in accordance with the objectives of the targeted variety breeding programs in the future.

Acknowledgements

This study is part of the PhD thesis of Yasemin İzgi. It was carried out with the support of OMÜ-BAP 1901.18.013 project. We gratefully acknowledge for the support of Agriculture Faculty of Ondokuz Mayıs University, and the Graduate School of Sciences Institute of Ondokuz Mayıs University.

Figure 1. The flower appearance of selected promising tulip genotypes in this study. (Original)

Table 1. Code, accession numbers and, scientific name, origins of the tulip (*Tulipa* spp.) genotypes.

Genotype Code	Accession No	Scientific Name	Origin
G1	248 05-04	*Tulipa agenensis* DC. (4 genotypes)	Amasya
G2	104 05-03		Amasya
G3	252 27-01		Gaziantep
G4	305 35-05		İzmir
G5	118 42-09	*Tulipa armena* Boiss. (30 genotypes)	Konya
G6	129 38-03		Kayseri
G7	124 66-02		Yozgat
G8	237 42-02		Konya
G9	125 38-01		Kayseri
G10	116 42-07		Konya
G11	121 01-02		Adana
G12	223 44-05		Malatya
G13	123 66-01		Yozgat
G14	401 09-01		Aydın
G15	239 44-02		Malatya
G16	245 26-03		Eskişehir
G17	402 60-01		Tokat
G18	316 04-02		Ağrı
G19	217 35-02		İzmir
G20	109 42-04		Konya
G21	250 21-02		Diyarbakır
G22	315 04-01		Ağrı
G23	243 26-04		Eskişehir
G24	119 70-01		Karaman
G25	236 69-01		Bayburt
G26	203 24-02		Erzincan
G27	127 38-04		Kayseri
G28	128 38-05		Kayseri
G29	202 46-01		Kahramanmaraş
G30	103 05-05		Amasya
G31	211 46-01		Kahramanmaraş
G32	107 42-07		Konya
G33	225 58-01		Sivas
G34	103 05-02		Amasya
G35	218 48-04	*Tulipa saxatilis* Sieber (1 genotype)	Muğla

Continuing table 1

Genotype Code	Accession No	Scientific Name	Origin
G36	115 42-06	*Tulipa pulchella* (Regel) Baker (4 genotypes)	Konya
G37	110 68-01		Aksaray
G38	117 42-08		Konya
G39	310 10-04		Balıkesir
G40	220 65-10	*Tulipa humilis* Herb. (1 genotype)	Van
G41	230 10-02	*Tulipa sylvestris* L. (2 genotypes)	Balıkesir
G42	235 11-01		Bilecik
G43	228 06-03	*Tulipa sintenisii* Baker (3 genotypes)	Ankara
G44	304 27-01		Gaziantep
G45	313 49-01		Muş
G46	311 65-01	*Tulipa julia* C. Koch (8 genotypes)	Van
G47	301 63-01		Şanlıurfa
G48	212 65-03		Van
G49	129 38-06		Kayseri
G50	319 08-02		Artvin
G51	209 62-01		Tunceli
G52	221 65-04		Van
G53	301 63-01		Şanlıurfa
G54	240 59-01	*Tulipa undilatifolia* B. (1 genotype)	Tekirdağ
G55	101 07-05	*Tulipa orphanidea* Boiss.ex Heldr (12 genotypes)	Antalya
G56	224 34-01		İstanbul
G57	306 35-06		İzmir
G58	216 45-01		Manisa
G59	242 45-02		Manisa
G60	232 22-01		Edirne
G61	102 48-01		Muğla
G62	229 59-02		Tekirdağ
G63	233 43-01		Kütahya
G64	241 35-04		İzmir
G65	309 10-03		Balıkesir
G66	204 17-01		Çanakkale
G67	303 63-03	*Tulipa aleppensis* Boiss.ex Regel (3 genotypes)	Şanlıurfa
G68	251 21-03		Diyarbakır
G69	222 23-01		Elazığ
G70	317 05-07	*Tulipa praecox* Ten (1 genotype)	Amasya
G71	401 09-01	*Tulipa clusiana* DC. (1 genotype)	Aydın

Table 2. Weighted ranking criteria used in the pedigree selection of local tulip genotypes.

Selection Criteria	Classes	Class Score (CS)	Relative Scores (RS)
Plant Stance	Upright	10	15
	Medium	7	
	Lateral	3	
Plant Height (cm)	Short (2.9-9.0)	1	15
	Medium short (9.0-15.1)	2	
	Medium (15.1-21.2)	3	
	Medium tall (21.2-27.3)	4	
	Tall (27.3-33.4)	5	
Stem Thickness (mm)	Thick (3.84-5.10)	10	15
	Medium (2.57-3.84)	7	
	Thin (1.3-2.57)	3	
Flower Lifespan (day)	Long (20 >)	10	15
	Medium (14-20)	8	
	Short (8-14)	5	
	Very short (8 <)	2	
Size of Flower (mm)	Very big (39.4-47.6)	10	15
	Big (31.2-39.4)	8	
	Medium (23.0-31.2)	5	
	Small (14.8-23.0)	3	
Main Bulb Diameter (cm)	Very large (12 >)	10	10
	Large (8-12)	7	
	Medium (4-7)	5	
	Small (3 <)	3	
Number of Bulbs Formed From The Main Bulb (pcs.)	High 5>	10	15
	Medium 4-5	8	
	Low 3-4	5	
	Very low 2<	3	

Table 3. Relative score x class scores and total scores of tulip genotypes in terms of each trait.

Genotype	A	B	C	D	E	F	G	Total
G1	150	60	150	75	150	50	150	785
G2	150	75	150	120	150	70	150	865
G3	150	45	150	150	150	70	75	790
G4	105	30	105	75	75	50	45	485
G5	105	30	105	150	120	70	75	655
G6	105	15	105	75	75	70	75	520
G7	105	15	105	75	75	70	75	520
G8	105	15	45	150	75	70	75	535
G9	105	30	105	120	75	70	75	580
G10	105	15	105	120	150	70	75	640
G11	105	15	105	120	75	70	75	565
G12	105	30	105	75	45	50	120	530
G13	105	30	105	120	150	70	75	655
G14	150	60	105	120	120	70	120	745
G15	105	30	45	150	75	50	75	530
G16	105	15	105	120	45	50	45	485
G17	150	30	45	75	45	50	75	470
G18	150	45	105	120	150	50	120	740
G19	105	30	150	120	150	90	75	720
G20	105	15	45	150	75	30	120	540
G21	150	45	105	120	45	50	75	590
G22	150	15	45	30	45	30	120	435
G23	45	15	105	120	45	50	45	425
G24	105	30	105	120	45	50	75	530
G25	105	30	45	120	45	50	45	440
G26	105	30	105	120	150	70	75	655
G27	45	60	45	150	120	50	75	545
G28	45	30	105	150	150	50	45	575
G29	105	30	105	75	45	50	75	485
G30	150	45	105	120	45	50	75	590
G31	150	30	105	75	45	50	120	575
G32	105	45	105	120	150	70	75	670
G33	105	15	45	75	45	50	75	410
G34	150	30	105	75	150	50	45	605

Continuing table 3

Genotype	A	B	C	D	E	F	G	Total
G35	150	15	105	75	75	50	120	590
G36	105	15	45	30	45	90	75	405
G37	150	30	45	150	150	50	120	695
G38	105	15	45	75	75	50	75	440
G39	105	30	45	75	45	50	75	425
G40	105	30	45	75	45	50	75	425
G41	105	45	105	75	45	50	75	500
G42	105	15	45	75	45	50	75	410
G43	45	30	105	75	75	30	45	405
G44	105	45	105	150	150	70	75	700
G45	105	45	105	75	45	70	120	565
G46	105	30	45	120	45	50	75	470
G47	105	45	45	120	45	50	75	485
G48	105	15	45	75	45	50	75	410
G49	105	15	45	120	75	70	75	505
G50	150	15	45	75	75	50	120	530
G51	105	30	45	75	75	50	75	455
G52	105	15	45	75	45	50	45	380
G53	150	30	105	75	120	50	75	605
G54	105	45	105	120	75	70	75	595
G55	150	30	105	150	120	70	75	700
G56	150	45	45	120	45	70	120	595
G57	150	45	105	120	75	50	75	620
G58	150	45	105	120	75	70	75	640
G59	150	30	45	150	45	50	75	545
G60	150	45	105	75	75	50	75	575
G61	150	45	150	120	75	70	75	685
G62	150	45	105	120	75	50	75	620
G63	105	30	45	75	45	50	75	425
G64	150	30	45	120	75	50	120	590
G65	105	15	45	75	45	50	75	410
G66	105	30	45	120	75	30	75	480
G67	105	30	105	120	45	50	45	500
G68	150	30	105	75	75	70	75	580

Continuing table 3

Genotype	A	B	C	D	E	F	G	Total
G69	105	30	105	75	75	50	75	515
G70	150	45	45	75	75	50	75	515
G71	150	45	45	120	75	70	75	580
G72	150	45	150	120	75	90	120	750
G73	150	60	105	120	75	90	120	720
G74	150	75	45	120	75	50	12	527

*A: Plant habitus, B: Plant height (cm), C: Stem thickness (mm), D: Lifespan of flower (day), E: Diameter of flower (mm), F: Diameter of bulb (mm), G: Number of bulblet (pcs.)

Table 4. Distribution frequencies of local tulip genotypes according to the plant habitus.

Plant Habitus	Genotype No	Distribution Frequencies (%)
Upright	G1, G2, G3, G14, G18, G21, G22, G35, G30, G31, G34, G37, G48, G50, G55, G56, G57, G58, G59, G60, G61, G62, G64, G68, G70, G71, Kumru, Muş1071, Arda	39.2
Moderate	G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G15, G16, G17, G19, G20, G24, G25, G26, G29, G32, G33, G36, G38, G39, G40, G41, G42, G44, G45, G46, G47, G49, G51, G52, G53, G54, G63, G65, G66, G67, G69	55.4
Lateral	G23, G27, G28, G43	5.4

Table 5. Distribution frequencies of local tulip genotypes in terms of plant height.

Plant Height (cm)	Genotype No	Distribution Frequencies (%)
Short (2.9-9.0 cm)	G6, G7, G8, G10, G11, G16, G20, G22, G23 G33, G35, G36, G38, G42, G48, G49, G50 G52, G65	25.7
Medium Short (9.1-15.1 cm)	G4, G5, G9, G12, G13, G15, G17 G19, G24, G25, G26, G28, G29, G31, G34, G37, G39, G40, G43, G46, G51, G53, G55, G59, G63, G64, G66, G67, G68, G69	40.5
Medium (15.2-21.2 cm)	G3, G18, G21, G30, G32, G41, G44, G45, G47 G54, G56, G57, G58, G60, G61, G62, G70, G71, G72	25.7
Tall (21.3-27.3 cm)	G1, G14, G27, G73	5.4
Very Tall (27.4-33.4 cm)	G2, G74	2.7

Table 6. Distribution frequencies of the local tulip genotypes in terms of stem thickness.

Stem Thickness (mm)	Genotype No	Distribution Frequencies (%)
Thick (3.85-5.10 mm)	G1, G2, G19, G22, G61, Kumru	8,1
Medium (2.58-3.84 mm)	G3, G5, G7, G8, G9, G10, G11, G12, G13, G16, G18, G20, G21, G23, G24, G26, G35, G28, G29, G30, G31, G32, G34, G41, G43, G44, G45, G47, G49, G54, G55, G57, G58, G60, G62, G67, G68, G69, Muş1071	52.5
Thin (1.3-2.57 mm)	G4, G6, G14, G15, G17, G25, G27, G33, G36, G37, G38, G39, G40, G42, G46, G48, G50, G51, G52, G53, G56, G59, G63, G64, G65, G66, G70, G14, Arda	39,2

Table 7. Distribution and frequencies of local tulip genotypes in terms of flower longevity.

Flower Longevity	Genotype No	Distribution Frequencies (%)
Long (20 > days)	G27, G28, G44, G55, G59	6.8
Medium (14-20 days)	G2, G3, G5, G7, G8, G9, G10, G13, G14, G15, G18, G19, G20, G21, G24, G26, G32, G34, G37, G46, G53, G54, G56, G57, G58, G61, G62, G64, G66, G67, G68, G71, Kumru, Muş1071, Arda	47.3
Short (8-14 days)	G1, G4, G6, G11, G12, G16, G17, G23, G25, G35, G29, G30, G31, G33, G36, G38, G39, G40, G41, G42, G43, G45, G47, G48, G49, G50, G51, G52, G60, G63, G65, G69, G70	44.6
Very Short (8 <days)	G22	1.3

Table 8. Distribution and frequencies of tulip genotypes in terms of flower size.

Flower Size	Genotype No	Distribution Frequencies (%)
Very Large (39.5-47.6 mm)	G1, G2, G3, G8, G10, G18, G20, G26, G28, G30, G32, G37, G40, G44	18.9
Large (31.3-39.4 mm)	G5, G11, G14, G27, G47, G55	8.1
Medium (23.1-31.2 mm)	G6, G9, G15, G35, G36, G38, G43, G49, G50, G51, G54, G57, G58, G60, G61, G62, G64, G66, G68, G69, G70, G71, Kumru, MuŞ1071, Arda	33.9
Small (14.8-23.0 mm)	G4, G7, G12, G13, G16, G17, G19, G21, G22, G23, G24, G25, G29, G31, G33, G34, G39, G41, G42, G45, G46, G48, G52, G53, G56, G59, G63, G65, G67	39.1

Table 9. Distribution frequencies of tulip genotypes according to main bulb diameter values.

Bulb Diameter (cm)	Genotype No	Distribution Frequencies (%)
Very Big (12 > cm)	G19, G36, Kumru, Muş1071	5.4
Big (8-12 cm)	G2, G5, G6, G7, G8, G9, G10, G11, G13, G14, G17, G26, G35, G27, G28, G32, G44, G45, G49, G54, G56, G58, G61, G68, G71	33.8
Medium (4-7 cm)	G1, G3, G4, G12, G15, G16, G18, G21, G23, G24, G25, G29, G30, G31, G33, G34, G37, G38, G39, G40, G41, G42, G43, G46, G47, G48, G50, G51, G52, G53, G55, G57, G59, G60, G62, G63, G64, G65, G67, G69, G70, Arda	56.8
Small (3 < cm)	G20, G22, G66	4.0

Table 10. Distribution frequencies of local tulip genotypes according to the number of bulblets from the main bulb.

The Number of Bulbs from The Main Bulb	Genotype No	Distribution Frequencies (%)
Very Good (5> number)	G1, G2	2.7
Good (4-5 number)	G12, G14, G18, G20, G22, G35, G29, G31, G37, G45, G50, G56, G64, G71, Kumru, Muş1071, Arda	23.0
Low (3-4 number)	G3, G5, G6, G8, G9, G10, G11, G13, G15, G17, G19, G21, G24, G26, G27, G32, G33, G34, G36, G39, G40, G41, G42, G44, G46, G47, G48, G49, G51, G53, G55, G57, G58, G59, G60, G61, G62, G63, G65, G66, G70	55.4
Very Low (2< number)	G4, G7, G16, G23, G25, G28, G30, G38, G43, G52, G54, G67, G68, G69	18.9

References

Akgün İ, Tosun M and Sağsöz S, (1998). Bitkisel gen kaynaklarının önemi ve Erzurum'un bitkisel gen kaynakları yönünden değerlendirilmesi. Doğu Anadolu Tarım Kongresi, 363-372. (in Turkish)

Balkaya A and Yanmaz R, (2005). Promising kale (*Brassica oleracea* var. *acephala*) populations from Black Sea region, Turkey. New Zealand Journal of Crop and Horticultural Science, 33(1):1-7

Balkaya A and Ergün A, (2008). Diversity and use of pinto bean (*Phaseolus vulgaris*) populations from Samsun, Turkey. New Zealand Journal of Crop and Horticultural Science 36(3):189–197

Balkaya A, Kurtar ES, Yanmaz R and Özbakır M, (2011). Karadeniz Bölgesi kestane kabağı (*Cucurbita maxima*) populasyonlarından seleksiyon ıslahı yoluyla geliştirilen çeşit adayları. Türkiye IV. Tohumculuk Kongresi, Bildiriler Kitabı-1:17-22. (in Turkish)

Balkaya A, İzgi Saraç Y and Tütüncü M, (2021). Melezleme ıslahı, süs bitkileri ıslahı, Klasik ve biyoteknolojik yöntemler, Editörler: Mendi YY, Kazaz S, Gece Kitaplığı. pp:107-144. (in Turkish)

Ballesteros-Mejia L, Lima NE, Lima-Ribeiro MS and Collevatti RG, (2016). Pollination mode and mating system explain patterns in genetic differentiation in neotropical plants. PLoS One, 11(7):e0158660

Çakır Z, Balkaya A, Kandemir D and Sarıbaş Ş, (2019). Determination of superior Turkish eggplant (*Solanum melongena* L.) genotypes by pedigree selection method. Ekin Journal of Crop Breeding and Genetics, 5(1):56-67

Escribano MR, Santalla M, Casquero PA, and Ron AM, (1998). Patterns of genetic diversity in landraces of common bean populations from Northwestern Spain. Euphytica, 93:71-81

Gülbağ F, (2015). Türkiye'de süs bitkileri ıslah çalışmaları. Türkiye Tohumcular Birliği Dergisi, 14:12-15. (in Turkish)

Horn PJ and Peterson CL, (2002). Chromatin higher order folding wrapping up transcription. Science, 297(5588):1824 -1827

İzgi Saraç Y, Arslan D, Altun B, et al., (2010). Cultivation of *Tulipa* spp. and *Hyacinthus* spp. in Turkish Flora. IX International Symposium on Flower Bulbs and Herbaceous Perennials, 28 March-01 April, Antalya

İzgi Saraç Y, (2015). Türkiye florasından toplanan lale (*Tulipa* spp.) genotiplerinin UPOV kriterlerine göre karakterizasyonları. Yüksek Lisans Tezi, Gaziosmanpaşa Ünversitesi, Fen Bilimleri Enstitüsü, Bahçe Bitkileri Ana Bilim Dalı, pp:95. (in Turkish)

İzgi Saraç Y, Balkaya A and Deligöz İ, (2021). Lale, Süs bitkileri ıslahı, türler, Editörler: Mendi YY, Kazaz S, Gece Kitaplığı. pp:375-414. (in Turkish)

Lal M, Baruah J, Begum T, Darnei RL, Nyitan J, (2022). Identification of novel five variants of caladium species through multi-environmental evaluation. Ekin Journal of Crop Breeding and Genetics, 8(2):146-151.

Straathof TP, Eikelboom W, Van Tuyl JM and Peters D, (1997). Screening for Tulip breaking virus resistance in seedling populations of *Tulipa* L., Acta Horticulturae, 432:391-395

Tan Ş, (2005). Bitki ıslahında istatistik ve genetik metotlar. Ege Tarımsal Araştırma Enstitüsü Müdürlüğü, Menemen/İzmir, Yayın No.: 121:129-145. (in Turkish)

Van der Meulen JJM, Van Oeveren JC and Van Tuyl JM, (1997). Breeding as a tool for improving postharvest quality characters of lily and tulip flowers. Acta Hortic. 430: 569-575

Zhao YZ, (2003). A new species of the genus *Tulipa* (Liliaceae) from China. Novon, 13(2): 277-278

Research Article

Ekin
Journal of Crop Breeding and Genetics

9(2):119-125, 2023

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Variability for Phenological Traits and Fruit Yield Attributes in Bael (*Aegle marmelos* Correa) Cultivars under Semi-Arid Conditions

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Citation:
Kumar M., Sharma S., Kumar M., Nibhoria A., Yadav BS., 2023. Variability for Phenological Traits and Fruit Yield Attributes in Bael (*Aegle marmelos* Correa) Cultivars under Semi-Arid Conditions. Ekin J. 9(2):119-125.

Received: 10.05.2023 ***Accepted: 18.06.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

ABSTRACT

The study on evaluation of bael cultivars for phenology, fruit set, fruit drop, fruit shape and fruit yield was conducted at Experimental Orchard, CCS HAU, Regional Research Station, Bawal (Rewari). Among phenological parameters, leaf bud burst was recorded earliest (30 April) in NB 16, while it was late (12 May) in NB 5. The initiation of flowering was recorded earliest (19 June) in NB 5, while it was late (12 July) in NB 17. Time taken for expansion of leaf in different genotypes ranged from 67 days (NB 5) to 85 days (NB 16). Flowering to fruit setting took less time (45 days) in CISHB 2, however, it took more time (63 days) in NB 9. Time taken from fruit setting to fruit maturity was observed least (224 days) in NB 16. It was at par with NB 17 (229 days) and Pant Aparna (229 days), while maximum time taken from fruit setting to fruit maturity (242 days) was recorded in NB 5. Time taken to complete leaf fall was recorded minimum (336 days) in CISHB 1. However, maximum time taken to complete leaf fall (345 days) was recorded in NB 5. The range of fruit set per cent varied from 11.48 % (Pant Sujata) to 18.35 % (NB 17). Fruit drop among different cultivars ranged from 88.33 to 92.67 %. It was lowest in NB 5 and highest in Pant Aparna. Fruit apex and base of cultivars varied as shallow, flattened and depressed, whereas shape varied as round, ovate, globose and elliptical.

Keywords: Bael (*Aegle marmelos*), fruit crack, fruit drop, fruit shape, germplasm, phenology, yield

Introduction

Bael (*Aegle marmelos* Correa) is one of the important underutilized medicinal, indigenous fruit crops of India. It can adapt a wide range of habitat therefore spread over different parts of the country. Plant shows enormous variability with respect to qualitative as well as quantitative characters. Apart from the tree morphological characters, wide variability exists in size and shape, bearing habit, flesh colour and texture of fruit (Misra et al., 2000). The wider genetic variability is priority of breeders to develop new variety(s) with better quality and higher production. The erosion of plant genetic resources results in a severe threat to the world's food security. In recent statistics the area under bael is 8320 ha and production 82260 MT (Anonymous, 2021).

Normally, the fruits are considered mature after litter fall at yellowish-green stage. Therefore, fruits are harvested after litter fall and kept for one week so that it loses green tint. A ripe bael fruit has great demand in market for therapeutic use. Beverages prepared from fruit pulp during summer give smothering and cooling effect. Besides, it is a good source of vitamins, minerals, alkaloids and steroids (Kumar et al., 2013). The bael fruit juice is useful to release the stomach problems and also provide cooling effect to brain (Arya et al., 2021).

Recently, few land races have been developed for commercial cultivation from NDUA&T, Faizabad; GBPUAT, Pantnagar; CISH, Lucknow; Regional Station, IARI, Samastipur, but their adaptability under different arid and semi-arid conditions has not been studied adequately. In order to identify distinct characters of various bael cultivars, the morphological characters are equally important. Bael gene pool with enormous variability with respect to qualitative as well as quantitative characters is spread over different parts of the country (Nagar et al., 2018). Identification of suitable genotypes for arid and semi-arid region is demand of time for better production, productivity and quality of the fruits. The evaluation of different cultivars will help the orchardist in selection of appropriate cultivar(s) of this neglected crop for large scale adoption. Unproductive land races of this region are being grown by orchardist due to their hardy nature. It holds promise for nutritional security and also helpful in curing various ailments.

Materials and Methods

The experiment on evaluation of bael cultivars *viz.,* NB 5, NB 9, NB 16, NB 17 collected from NDUA&T, Faizabad; Pant Aparna, Pant Sujata collected from GBPUAT, Pantnagar; and CISHB 1, CISHB 2 collected from CISH, Lucknow was carried out at Experimental Orchard, CCS HAU, RRS, Bawal. The experiment was laid out in a randomized block design. Twelve years old, uniformly grown trees were selected randomly and maintained under uniform conditions of orchard management practices during the study period, where all the agronomic practices were carried out as per recommended package of practices. This location has a typical semi-arid climate with hot and dry summer and extremely cold winter. The mean monthly maximum temperature during summer months (June, July) ranged from 44 to 47ºC, while minimum temperatures as low as freezing point during winter months (December, January). About 65-70 per cent of total rainfall is received during July to September.

Different cultivars were observed for variability in dates of appearance of leaf. It was observed with naked eyes and the average of dates of leaf bud burst or sprout was calculated. Leaf expansion period was measured by counting the days from date of leaf bud bursts to the date of leaf becomes fully mature. Unpleasant smell produced from bruised leaf was considered as indication of mature leaf. Days taken to leaf fall were calculated as period from date of leaf bud burst to the date of complete leaf fall and its average was calculated.

The branches of plants were tagged in different directions to observe the date of initiation of flowering. Date of 50% flower opening on each tagged branch was considered as date of initiation of flowering and their average was considered as a date of initiation of flowering. The average of time taken to open 50 per cent flowers on each tagged branch to 50% fruit setting was taken as days to fruit setting. The period from 50 per cent fruit setting on each tagged branch to the date of complete leaf fall was considered as a time taken (days) from fruit setting to fruit maturity.

The fruit set per cent was calculated as fruits developed out of total number of flowers on tagged branches and average fruit set was calculated. Fruit drop was calculated as fruits dropped out of total fruits set. The fruits dropped from fruit setting to the harvesting were added at weekly interval. Splitting of fruit bark or formation of cracks on the outer surface of the fruit were counted in the fruit crack. The cracked fruits were also calculated by counting cracked fruits from fruit set to harvesting of fruits out of total fruit set on the plant. Some fruits dropped due to cracking were counted in cracked as well as dropped fruits. The fruit yield per plant was calculated by adding the weight of fruits harvested in each picking. Fruit shape *viz.,* fruit apex, fruit base and fruit shape of different bael cultivars were observed by matching with the standards figures available in the descriptors of bael crop.

In order to evaluate comparative performance of the various treatments, the data were analyzed by the techniques of analysis of variance described by Fisher (1958). The statistical method described by Panse and Sukatme (1967) was followed for analysis and interpretation of the experimental results. The test of significance was worked out at 5 per cent level of the significance and results were compared by critical difference (CD).

Results and Discussion

Phenological parameters: The data collected from various genotypes of bael planted at CCS HAU, RRS, Bawal during 2007 under semi-arid region of Haryana revealed that the genotypes varied in different life cycle events (Table 1). It was observed from the data, leaf bud burst was recorded earliest (30 April) in NB 16, followed by CISHB 1 (4 May) and Pant Aparna (5 May), however, it was recorded late (12 May) in NB 5. The initiation of flowering was recorded earliest (19 June) in NB 5; followed by NB 9 and CISHB 1, whereas it was observed late (12 July) in NB 17. The variation in duration of flowering might be due to variability in genetic make-up of the particular germplasm. Mazumdar et al., (2006) observed flowering in bael during May and June.

Time taken for expansion of leaf in different cultivars ranged from 67 days to 85 days. Minimum time taken for expansion of leaf (67 days) was observed in NB 5, it was at par with Pant Sujata (73 days), however, highest leaf expansion period was observed (85 days) in NB 16, which was at par with NB 17 (83 days) and Pant Aparna (80 days). Minimum time taken from flowering to fruit setting (45 days) was recorded in CISHB 2, which was at par with NB 17, however, the highest time taken from flowering to fruit setting (63 days) was observed in NB 9, which was at par with NB 5. The variability in time taken from flowering to fruit setting may be due to variation in flowering behavior of the germplasm. The variation in different characters of germplasm may vary with genotypes and agro-climatic conditions also (Singh et al., 2011). The variation in these parameters shows the performance of these cultivars under semi-arid conditions. The reason of the variation may be due to genetic make-up, adaption of germplasm, variability in agro-climatic conditions. More or less similar results have been obtained with respect to variation in phenology of various bael genotypes (Singh et al., 2006). Flower bud emergence, flowering duration, time of anthesis, dehiscence of anther, stigma receptivity and pollen viability vary with variety and locality (Srivastava and Singh, 2000). Generally, flower bud emergence takes place in the month of April and flowering in full bloom stage appears in the month of May under hot semi-arid ecosystem of western India (Singh et al., 2008). Among different bael genotypes *viz*., CISH Bael-1, CISH Bael-2, NB 5, NB 7, NB 9, Pant Aparna, Pant Sujata, Pant Urvashi, Pant Siwani, Dhara Road and PB 1 under Gujarat conditions the earliest flower bud emergence was observed in CISHB 2 (30 April), whereas it was delayed in CISHB 1 and PB 1 (6 May), however, the flower bud emergence continued till last week of June. Similar findings have also been reported in other fruit crops like jamun (Singh and Singh, 2005).

Time taken from fruit setting to maturity was least (224 days) in NB 16, it was at par with NB 17 as well as Pant Aparna (229 days), while maximum time taken from fruit setting to fruit maturity (242 days) was recorded in NB 5. Time taken to complete leaf fall ranged from 336 days to 345 days (Table 1). Maximum time taken to complete leaf fall (345 days) was observed in NB 5, however, minimum time taken to complete leaf fall (336 days) was recorded in CISHB 1 and it was statistically at par with NB 16 and Pant Aparna. The maturity of the fruits may vary with the ripening behavior of the germplasm.

Fruit set, drop, cracks and yield: There was significant variation in fruit set per cent among different genotypes (Table 2). The range of fruit set per cent was varied from 11.48 per cent to 18.35 per cent (Table 2). The highest fruit set percent (18.35 %) was recorded in NB 17, it was statistically at par with CISHB 1 (17.58 %), while lower fruit set per cent (11.48 %) was observed in Pant Sujata. This variation in fruit set per cent among various germplasm of bael might be due to their inherent characters. Sometimes fruit set per cent may vary due to agronomic practices and local environmental conditions. However, Uniyal and Misra (2013) reported maximum fruit set in Pant Aparna and minimum fruit set in Pant Shivani.

This study showed a significant variation in fruit drop per cent among different cultivars. It ranged from 80.33 per cent 92.67 per cent. Least fruit drop (80.33%) was observed in NB 5. Fruit drop per cent was significantly higher (92.67%) in Pant Aparna, these values were statistically at par with NB 17 (90.33%), CISHB 1 (91.0%), NB 16 (91.67%) and Pant Sujata (90.33%). However, Uniyal and Misra (2013) reported the maximum fruit drop in Pant Sujata, followed by Pant Uravshi, while minimum fruit drop was observed in Pant Shivani and they reported that fruit drop may be due to embryo abortion. Dropping of fruits due to embryo abortion after fertilization was reported in Litchi by Ray et al., (2002). One of the reasons might be due to deficiency of nutrient especially Ca, Zn, B and K (Choi et al., 2020). The fruit drop may be due to competition among fruit lets for carbohydrates, water, nutrients, hormones and other metabolites (Uniyal and Misra, 2013). Fruit cracking in bael germplasm is also one of the reasons for fruit drop.

Fruit cracking in all the genotypes showed significant variation. Fruit cracking per cent was significantly higher (40.67%) in NB 16 and lowest (5.67%) in NB 5 (Table 2). In young plants, fruit cracking may occur due to boron deficiency but in fully grown tree, it may be due to dry conditions or soil moisture imbalances (Choi et al., 2020). Dhaker et al., (2013) reported that the fruit cracking reduced to 2.14 per cent with spraying of 0.6 per cent of borax. They also reported that the boron is helpful in improving the appropriate growth of bael tree and it is constituent of cell membrane and essential for cell division, which reduces disorders like cracking in fruits. Uniyal and Misra (2013) reported maximum fruit cracking in Pant Sujata, followed by Pant Urvashi, while minimum in Pant Aparna. Wani et al., (2015) reported that fruit cracking in different pomegranate cultivars varying from 6.31% to 31.40% under Kashmir valley conditions. Mean fruit yield was observed highest (63.2 kg/ plant) in NB 9 among different cultivars during the study period, which was at par with NB 5 and NB 17.

However, mean fruit yield was observed lowest (39.8 kg/ plant) in Pant Sujata, it might be due to less fruit set percent and more fruit drop. Variation in qualitative attributes of different germplasm at different locations might be due to adaptability to varied agroclimatic conditions, root distribution pattern of the crop and genetic make-up of the germplasm (Nagar et al., 2017). Increase in yield might be due to the specific climatic requirement of the variety and the genetic makeup of the cultivar (Kumar et al., 2021).

Fruit shape: Fruit shape of different bael genotypes exhibit wide range of genetic variability. The results pertaining to variation in fruit shape such as fruit apex, fruit base (shallow, flattened and depressed), fruit shape (round, ovate, globose and elliptical) in respect of various cultivars are presented in Figure 1. The fruit apex was observed as shallow in NB 5, CISHB 1, CISHB 2, NB 16; flattened in NB 9 and depressed in NB 17, Pant Aparna and Pant Sujata. The fruit base of cultivars NB 5, CISHB 2 and NB 16 was observed depressed, while in rest of the cultivars it was observed as shallow. There was a great variation in fruit shape of different cultivars. The shapes of different cultivars were observed as ovate (NB 9), globose (NB 17) and elliptical (CISHB 2), whereas, all other cultivars were round in shape. Sharma and Dubey (2013) also observed fruit shape as spherical, oblong, cylindrical, pear-shaped and flat in different cultivars. Variation in fruit shape of different bael cultivars was also observed in Uttar Pradesh and Bihar (Jauhari et al., 1969).

Correlations: Fruit yield is main parameter in all kind of studies in horticultural crops and it relay in several yield contributing traits. The results on fruit yield correlation with its contributing traits are presented in figures 2-4. In the present study, bael fruit yield showed significant positive correlation with fruit setting (%). Above findings were supported by Dahal et al., 2015. But fruit yield was found negatively correlated with fruit drop (%) and fruit creaking (%).

Conclusions

NB 9 and NB 5 cultivars of the bael performed best in terms of different parameters such as fruit drop, fruit yield, fruits cracking as compared to other cultivars. These cultivars can be recommended for commercial cultivation or social forestry in semiarid zone of Haryana.

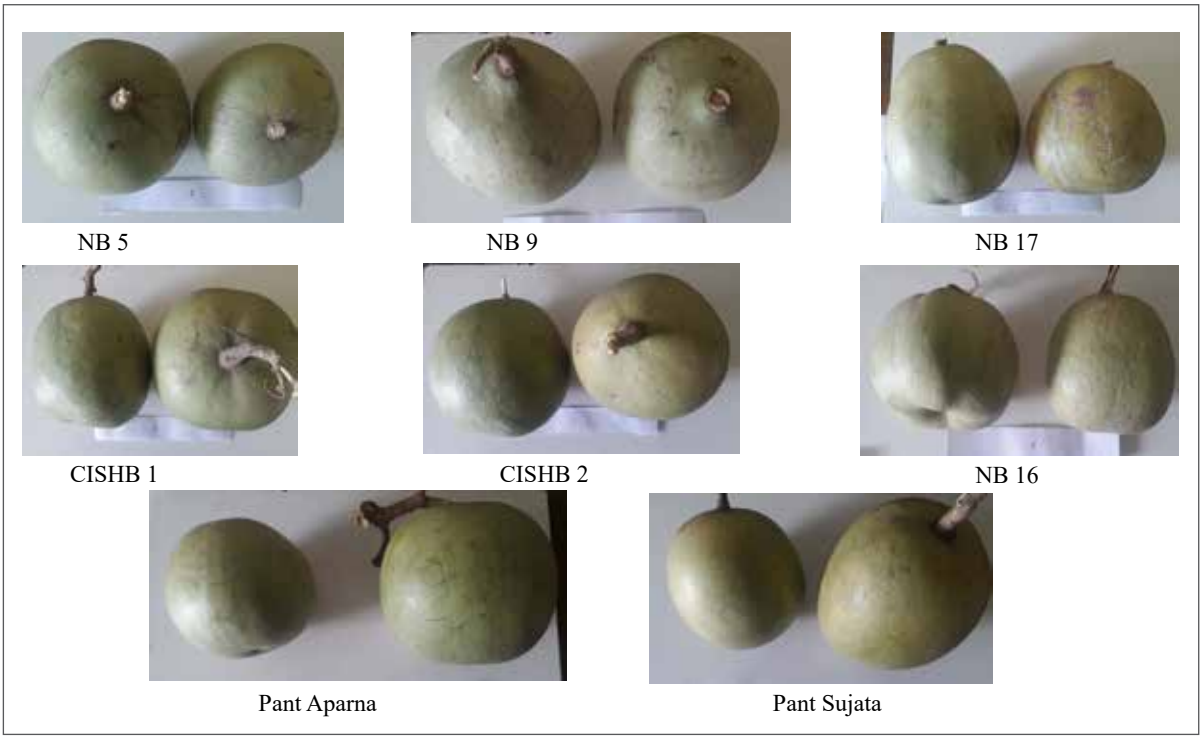

Figure 1. Fruit shape of different bael genotypes. (Original)

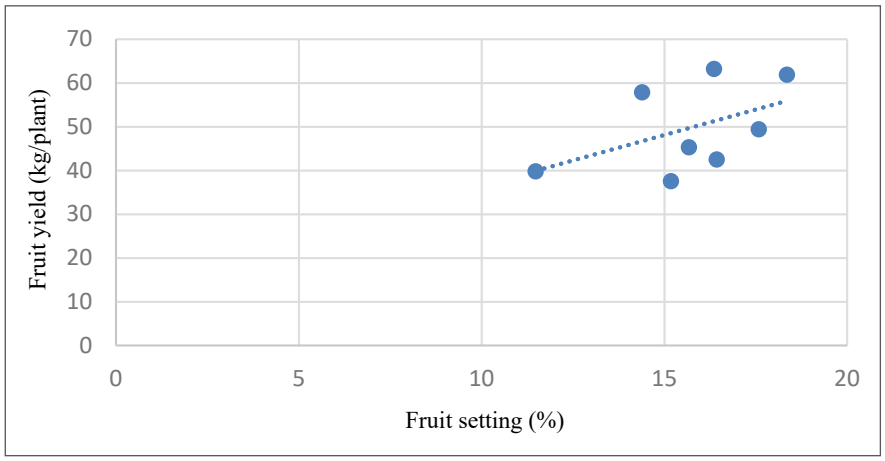

Figure 2. Fruit yield positive association with fruit setting (%).

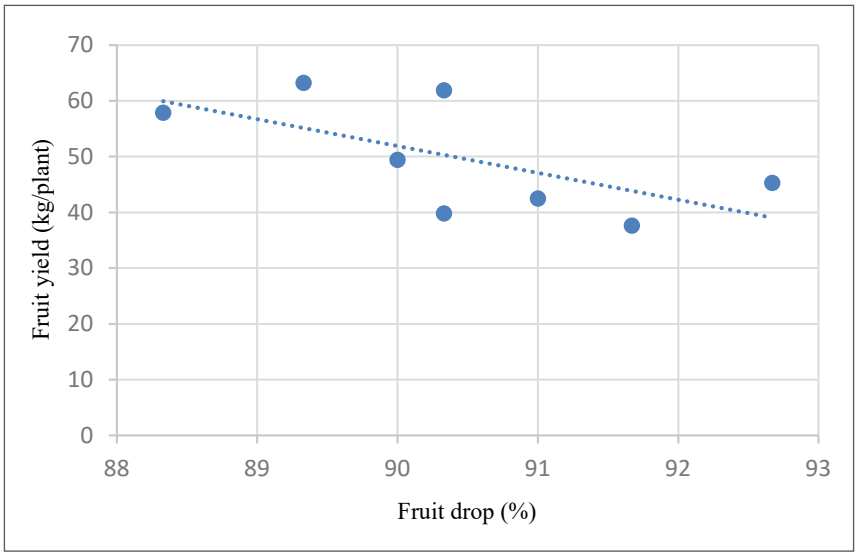

Figure 3. Fruit yield negative association with fruit drop (%).

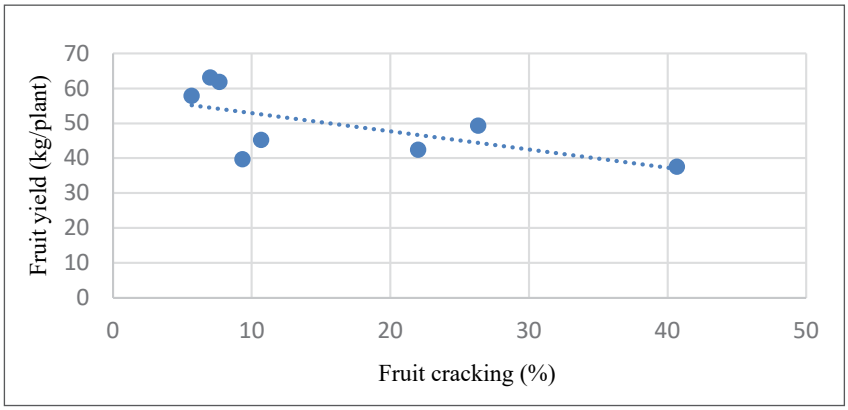

Figure 4. Fruit yield negative association with fruit cracking (%).

Table 1. Phenological parameters of bael cultivars under semi-arid conditions of Haryana.

Cultivars	Date of Leaf Bud Burst	Date of Initiation of Flowering	Leaf Expansion Period (days)	Time Taken From Flowering to Fruit Setting (days)	Time Taken From Fruit Setting to Fruit Maturity (days)	Time Taken to Complete Leaf Fall (days)
NB 5	12 May	19 June	67	60	242	345
NB 9	9 May	22 June	76	63	235	342
NB 17	7 May	12 July	83	47	229	342
CISHB 1	4 May	25 June	75	54	231	336
CISHB 2	6 May	5 July	75	45	235	341
NB 16	30 April	27 June	85	57	224	340
Pant Aparna	5 May	27 June	80	58	229	339
Pant Sujata	10 May	29 June	73	54	237	341
Range	30 April to 12 May	19 June to 12 July	67-85	45-63	224-242	336-345
CD at 5%			6	3	6	4

Table 2. Fruiting and yield character of bael cultivars under semi-arid conditions of Haryana.

Cultivars	Fruit Set (%)	Fruit Drop (%)	Fruit Cracking (%)	2019 (kg/ plant)	2020 (kg/ plant)	2021 (kg/ plant)	Mean (kg/ plant)
NB 5	14.39	88.33	5.67 (13.02) *	55.2	59.9	58.6	57.9
NB 9	16.36	89.33	7.00 (15.37) *	60.1	65.1	64.5	63.2
NB 17	18.35	90.33	7.66 (16.12) *	56.7	65.4	63.6	61.9
CISHB 1	17.58	90.00	26.33 (30.89) *	48.0	51.8	48.3	49.4
CISHB 2	16.43	91.00	22.00 (27.98) *	39.5	44.8	43.1	42.5
NB 16	15.18	91.67	40.67 (39.63) *	37.2	38.1	37.5	37.6
Pant Aparna	15.67	92.67	10.67 (19.10) *	42.2	46.1	47.5	45.3
Pant Sujata	11.48	90.33	9.33 (17.83) *	36.1	40.7	42.6	39.8
Range	11.48-18.35	88.33-92.67	5.67-40.67	36.1-60.1	38.1-65.4	37.5-64.5	39.8-63.2
CD at 5%	1.32	2.46	1.65	3.7	4.7	4.6	4.5

*Values in parenthesis are angular transformed

Table 3. Fruit shape of bael cultivars under semi-arid conditions of Haryana.

Cultivars	Fruit Apex	Fruit Base	Fruit Shape
NB 5	Shallow	Depressed	Round
NB 9	Flattened	Shallow	Ovate
NB 17	Depressed	Shallow	Globose
CISHB 1	Shallow	Shallow	Round
CISHB 2	Shallow	Depressed	Elliptical
NB 16	Shallow	Depressed	Round
Pant Aparna	Depressed	Shallow	Round
Pant Sujata	Depressed	Shallow	Round

References

Anonymous, (2021). India statistics 2020-21. Ministry of Agriculture & Farmers Welfare, Govt of India (www.indiastat.com).

Anonymous, (2023). Package and practice of horticultural crops. CCS HAU, Hisar.

Arya RK, Pawan Kumar, Dahiya GS, Sutaliya JM, Ravi Kumar and Chhabra AK, (2021). Medicinal garden at a glance (Publication No. CCSHAU/PUB#21-053), MAP Section, CCS HAU, Hisar.

Choi JH, Lee B, Gu M, Lee UY, Kim MS, Jung SK and Choi HS, (2020). Course of fruit cracking in 'Whansan' pears. Hortic. Environ. Biotechnol. 61:51-59.

Dahal KC, Sharma MD and Sapkota R, (2015). Fruit set, fruit retention and yield of chilli genotypes under hot and dry condition of western Terai of Nepal. J. Inst. Agric. Anim. Sci. 33-34:229.

Dhaker M, Soni, AK, Yadav PK, Chandra A and Kumar H, (2013). Response of different levels of farm yard manure and born on growth and yield of bael (*Aegle marmelos* Correa). Asian J. Hort., 8(2):767-771.

Fisher RA, (1958). Statistical methods for research workers, Ed. 10th Oliver and Boyd. Edinburg, London. p. 224.

Jauhari OS, Singh RD and Awashi RK, (1969). Survey of some important varieties of bael (*Aegle marmelos* Correa). Punjab Hort. J.,9:48-53.

Kumar M, Singh S and Yadav VK, (2013). Arid fruits: post-harvest handling and processing (Chapter published in: Emerging Science and technology for food, agriculture and environment ed by Kumar S, Yadav PK and Kumar S) p 435-448.

Kumar M, Arya R, Kumar Manoj, Gaur RK and Sharma S, (2021). Evaluation of aonla varieties under semi-arid conditions of Haryana. Ekin, J. Crop Breed. Genetics.,7(2):139-144.

Mazumdar R, Bhattacharya S, Mazumdar A, Pattnaik AK, Tiwary PM and Chaudhary S, (2006). Antidiarrhoeal evaluation of *Aegle marmelos* (Correa) Linn. root extract. Phytotherapy Research, 20:82-84.

Misra KK, Singh R and Jaiswal HR, (2000). Performance of bael (*Aegle marmelos* Correa) genotypes under foot hills region of Uttar Pradesh. Indian J. Agric.Sci.,70:682-683.

Nagar S, Kumar M, Kumatkar RB, Sharma JR and Singh S, (2017). Evaluation of bael (*Aegle marmelos* Corr.) germplasms for seed and qualitative characters under semi-arid conditions of Haryana, Int. J. Pure App. Biosci., 5 (3):436-442.

Nagar S, Kumar M, Sharma JR, Baloda S and Godara RK, (2018). Bael germplasm evaluation for leaf and fruits pulp variability under south Haryana conditions, Int. J. Pure App. Biosci., 6 (1):959-964.

Panse VG and Sukatme PV, (1967). Statistical methods for agricultural workers. Indian Council of Agricultural Research, New Delhi. p.155.

Ray PK, Rani R and Singh SK, (2002). Sex ratio and fruit set pattern in 10 litchi cultivars. Progress. Hortic., 34:17-21.

Sharma N and Dubey W, (2013). History and Taxonomy of *Aegle marmelos*: A Review. Int. J. Pure App. Biosci., 1(6): 7-13.

Singh AK, Singh S, Apparao VV, Mesh Ram DT, Bagle BG and Dhandar DG, (2006). Studies on floral biology of bael (*Aegle marmelos* Correa) under semi-arid ecosystem. In proceeding: National Symposium on Production, Utilization and Export of Underutilized Fruits with Commercial Potentialities, 22-24 November 2006, B.C.KV. p-31.

Singh AK, Singh S, Joshi HK, Bagle BG and More TA, (2008). Evaluation of bael genotypes for growth behavior and floral traits under semi-arid ecosystem of western India. Hort. J., 21(30): 140-142.

Singh AK, Singh S, Singh RS, Bagle, BG and Sharma BD, (2011). The bael fruit for dryland. Technical Bulletin No 38 published from Central Horticultural Experiment Station, Godhra (Gujrat) Regional Station of Central Institute for Arid Horticulture, Bikaner.

Singh S and Singh AK, (2005). Genetic variability in jamun (*Syzygium cumini*) in Gujrat, Progress. Hortic., 37:44-48.

Srivastava KK and Singh HK, (2000). Floral biology of bael (*Aegle marmelos*) cultivars. Indian J. Agric. Sci., 70(11):797-798.

Uniyal S and Misra KK, (2013). Study on the kinetics and causes of fruit drop in bael (*Aegle marmelos* Correa). Ann. Agri. Bio. Res., 18(2):214-218.

Wani IA, Sheikh FA and Bhat MY, (2015). Genetic diversity among indigenous pomegranate (*Punica granatum*) accessions collected from Kashmir valley. Int. J. Current Sci. Res., 1(4): 53-66.

Research Article

Ekin
Journal of Crop Breeding and Genetics

9(2):126-140, 2023

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

In Vitro and *In Vivo* Screening of Rice Genotypes for Yield Attributes and Proximate Analysis in Relation to Salinity Tolerance

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Citation:
Bisht SS., Behl RK., Kishor C., Arya RK., 2023. *In Vitro* and *In Vivo* Screening of Rice Genotypes for Yield Attributes and Proximate Analysis in Relation to Salinity Tolerance. Ekin J. 9(2):126-140.

Received: 25.04.2023 ***Accepted: 12.06.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

ABSTRACT

An experiment on *in vivo* and *in vitro* screening of rice germplasm for salinity tolerance was carried out to screen rice genotypes. The results revealed that all the genotypes were affected by the increasing salinity levels but there were genotypic differences also. Among morphological characters almost all the characters showed reduction with increasing salinity levels except proline content, sodium content and chloride content. Sodium and Potassium content increased tremendously with increasing salinity levels where as there was significant reduction in K/Na ratio. On the basis of this the genotype CSR 23, CSR 36, HKR 47 and F_1s viz. CSR 36 x HKR 126, CSR 36 x IR 64, CSR 36 x HKR 127 and HKR 126 x IR 64 expressed to be salt tolerant whereas CSR 23 x CSR 36, IR 64 x HKR 127 and HKR 47 x HKR 127 as sensitive to salinity. The salinity tolerant genotypes may be used in rice breeding to develop elite genotypes.

***Keywords*:** Rice, salinity, tolerance, *in vitro*, *in vivo,* screening

Introduction

Rice is a most important cereal crop in India and it contributes about 45% to the cereal production, 41% of the total food grain production. It is cultivated round the year in one or the other parts of the country, in diverse ecologies spread. Now a days, salinity is a serious environmental constraint to crop production in many parts of the world (Krishanamurthy et al., 2022). It is especially prevalent in irrigated agriculture and in marginal lands, associated with poor drainage or high water tables. Around 30% of the world's rice cultivation land is affected by soil salinity (Hopmans et al., 2021). The development of crops/varieties with improved salt tolerance is proposed as part of the solution to some of these problems (Sajid et al., 2017).

High salt stress disrupts homeostasis in water potential and ion distribution. This disruption of homeostasis occurs at both the cellular and whole plant levels. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest and even death. Increased salt tolerance is need for rice crop grown in salt affected areas and those at risk of salinization. This requires new genetic sources of salt tolerance and more efficient techniques for identifying salt tolerant germplasm, so that new genes for tolerance can be introduced into crop cultivars (Singh et al., 2021). Conventional techniques of screening rice germplasm for tolerance to soil salinity include growing of plants for long period of times to measure biomass or yield. However, this is subjected to genotype-environment interactions and environment effects to unknown extent. This sometimes jeopardizes whole selection effort. The other approach is based on physiological traits, accumulation of osmolytes for osmotic adjustment by way of amino acids, sugars *etc.* and sodium exclusion trait. While many physiological

traits have been tried but sodium exclusion trait has been accepted as one reliable trait for screening crop germplasm for salt tolerance (Ahmadizadeh et al., 2016; Singh et al., 2021).

Most of the cultivated rice varieties are susceptible to salinity but rice germplasm do have source for salt tolerance character traditional land races/ varieties such as Pokkali, Dasal, Getu *etc*. have sufficient salt tolerance level and thus can be involved in breeding programme (De Leon et al., 2015). At CSSRI, Karnal, a series of salt tolerant rice varieties (*e.g*. CSR 10, CSR 11, CSR 13, CSR 19, CSR 26, CSR 30) have been developed through traditional breeding methods. However the selection under field conditions (*in vivo*) requires longer time and efforts but selection done under real conditions can stand the test of time. Keeping above facts in view the present study was carried out.

Materials and Methods

The present investigation was carried out for *in vivo* and *in vitro* screening of rice genotypes. The experiment was conducted in the screening house complex, Regional Research Station, CCS HAU, Uchani, Karnal. The soil was air dried, ground and passed through the rough 2 mm sieve before filling the pots. Polyethylene lined earthen pots were filled in with five kg air dried soil. The soil was added in lots to maintain uniform bulk density though out the pot. In this study, 10 genotypes *viz*., IR 64, HKR 46, HKR 47, HKR 120, HKR 126, HKR 127, CSR 13, CSR 23, CSR27, CSR36 were screened under four levels of salinity (0(control), 2dS/m, 4 dS/m and 6 dS/m) in three replications. To test the effect of different levels of salinity, ten genotypes of rice were sown. A population of five plants per pot was maintained after germination and allowed to grow up to maturity. All recommended package of practices were followed.

Creation of salinity levels

Amount of salts required for creating different salinities on soil saturation basis as follows:-

Desired Ece (dS/m)	TDS (me/l)	Amount of salts (me/l)				
		Na	Ca	Mg	Cl : SO_4	
2	25	12.5	3.12	9.38	17.5	7.5
4	50	25.0	6.25	18.75	35.0	15.0
6	72	36.0	9.00	27.00	50.4	21.6

Development of salinity levels in the soil: Varying levels of salinity *viz*., control, 2, 4 and 6 dSm^{-1} were created by saturating the respective number of pots with distilled water (control) and artificially prepared saline waters of 2, 4 and 6 dSm^{-1} electrical conductivity, respectively. The pots were kept covered with polyethylene sheet for one week to attain equilibrium. Thereafter, the pots were uncovered and allowed to approach the moisture level suitable for rice sowing. The surface soil was remixed thoroughly before sowing. The pots were irrigated with deionised/ distilled water on as and when required basis in order to maintain the constant level of salinity in the pots. The pots were also protected from rain water so as not to allow any interference due to rain water.

Observations: The crop was harvested at maturity. The observations on four morphological characters were recorded namely plant height (cm), number of panicle/plant, 1000- grain weight (g) and Seed yield/plant. The crop matured at different times due to the treatment effects and nature of genotypes. After harvesting, the plant samples were washed in tap water and then with distilled water, dried at 65±2°C in a forced air oven to a constant weight, the grains were then separated out. The straw and grain yields were recorded before grinding the sample. The samples were stored in sealed polyethylene bags for further analysis.

Chlorophyll (mg/g fresh weight): Chlorophyll (a & b) were extracted as per standard procedure of Hiscox and Israestam (1979). Method: 80 mg of washed and fine chopped leaf tissue was placed in a test tube containing 7 ml of DMSO (Di Methyl Sulphoxide). The chlorophyll was extracted without grinding by incubating at 65°C for one hour. The extracted liquid was transferred to a graduated cylinder and volume made up to 10 ml with DMSO and O.D. was recorded using spectrophotometer at 645 and 663 nm. When stored at 0-4°C for 24 hours there was no effect on the absorbance. Chlorophyll content was calculated following the standard equation as follows:

Chl a (mg/g): 11.63 X A663 – 2.39 X A645

Chl b (mg/g): 20.11 X A645 – 5.18 X A663

Plant Analysis: After the harvest, the plant samples were washed first with tap water and then by distilled water, dried at 65±2°C to a constant weight, ground and analysed for different constituents. One gram of the ground plant material (straw) was digested in 4:1 HNO_3: $HClO_4$ mixture. The material was heated for 90 minutes at 160°C and finally for 30 minutes at 220°C. After cooling the digest was made 50 ml with distilled water. Then this end product was filtered into plastic bottle of 100 ml and such digest were further used for analysis of K, Na, Ca, Mg and SO_4 content.

Chemical Parameters: Proline content (Mg/g dry weight) was estimated by the standard procedure of Bates et al., (1973). Potassium and Sodium content (ppm) was determined by flame photometer. Calcium content (ppm) was measured by Versenate titration method using Calcon indicator (Hesse, 1971). Magnesium content (ppm) was estimated by subtracting the Ca content from the Ca^{+} Mg content, obtained by Versenate titration method as outlined in USDA hand book-60 (Richards, 1954). Chloride content (%) was determined by chloride specific ion selective electrode (Orion) using 0.5 M HNO_3 and 0.5 KNO_3 as supporting electrolyte according to procedure of Chhabra et al., (1976). Sulphate content (ppm) was determined colorimetrically by turbidity method at 440 nm wave length. K^{+}/Na^{+} ratio was calculated by dividing potassium content with sodium content. Ca^{++}/Mg^{++} ratio was calculated by dividing Calcium content with Magnesium content. The statistical parameters were calculated as per Completely Randomized Design experimental analysis (Sheoran et al., 1998).

Results

The present investigation was resolved into experiments dealing with *in vivo* and *in vitro* screening of rice genotypes. The salient features of results are described here under:

Plant height (cm): Plant height decreased with increasing salinity levels in all the genotypes (table 1). The overall genotypic mean decreased from 70.73 to 57.13 cm with an increase from control to 6 dSm^{-1}. It means that increasing salinity stress led to dwarfing. The least affected genotypes were HKR 47 X HKR 127, followed by CSR 23 and CSR 23 X HKR 47 at 6 dSm^{-1} of salinity stress.

Number of panicle/plant: Increasing salinity levels exhibited adverse effects on the number of panicle/plant as evident from table 1. The maximum number of panicle/plant at 6 dSm^{-1} salinity level was exhibited by IR 64, followed by CSR 36 X HKR 126, CSR 23, and CSR 36 X IR 64. The maximum decrease in number of panicle/plant was observed in CSR 36 X IR 64 followed by CSR 13, HKR 46 and HKR 126 X HKR 127 at 6 dSm^{-1} salinity level.

1000-grain weight (g): The 1000-grain weight at 6 dSm^{-1} was reduced for every genotype (table 2). Genotypes HKR 127 and CSR 36 X HKR 127 had lowest per cent reduction whereas maximum reduction was found in genotype HKR 120 X IR 64 in 1000 grain weight at 6 dSm^{-1} compared to the control.

Seed yield/plant (g): The salinity level of 2 dSm^{-1} acted as a stimulus for seed yield/plant for the genotype CSR 36 X HKR 127. While at salinity level 6 dSm^{-1}, the seed yield/plant decreased drastically in all genotypes (table 2). Lowest per cent decrease was noticed in CSR 23 (32.23%), followed by HKR 47 (39.07%) and CSR 36 (39.62%) whereas, maximum per cent decrease was noticed in the genotype CSR 23 X CSR 36 (90.14%) followed by CSR 36 X HKR 47 (89.09%), IR 64 X HKR 127 (88.60%) and HKR 47 X HKR 127 (86.75) at 6 dSm^{-1}.

Chlorophyll 'a' (mg/g, fresh weight): All stresses *i.e.* 2 dSm^{-1}, 4 dSm^{-1} and 6 dSm^{-1} proved as stimulus for increased chlorophyll 'a' content in genotype HKR 127 whereas, salinity level 2 dSm^{-1} and 4 dSm^{-1} proved as stimulus for genotype CSR 23 X HKR 127 and CSR 36 X HKR 126 as shown in table 3. The maximum decrease in chlorophyll 'a' content was found in genotype CSR 27 at 6 dSm^{-1} salinity level.

Chlorophyll 'b' (mg/g, fresh weight): Chlorophyll 'b' of all the genotypes reduced significantly with increasing salinity levels. It was evident from the data in table 3 that minimum reduction percentage in chlorophyll 'b' content was found in the genotype HKR 47 followed by HKR 46 and IR 64 whereas maximum reduction percentage was observed in the genotypes HKR 120, followed by CSR 36 X HKR 126 and CSR 23 X HKR 127.

Proline content (mg/g): Proline, a stress indicator, increased with increasing salinity levels in every genotype but with a varying magnitude due to genotypic differences (Table 4). Comparative evaluation of proline content at 6 dSm^{-1} with that at control, revealed the highest proline content in HKR 47 (6.99 mg/g), followed by CSR 13 (6.868 mg/g), CSR 36 (6.857 mg/g) and CSR 27 (6.591 mg/g). On the other hand, the minimum proline content was observed in genotype IR 64 X HKR 47 (5.898 mg/g), followed by CSR 36 X HKR 126 (5.971 mg/g) and CSR 23 X HKR 126 (6.041) at 6 dSm^{-1} salinity level.

Potassium content (ppm): Potassium content of rice genotypes decreased drastically with increasing salinity levels, that with genotypic differences too. But all the F_1's except genotype CSR 36 X HKR 47 showed reverse trend with increase in salinity levels (table 4). The maximum increase in potassium content was found in the genotype CSR 23 X CSR 36, followed by CSR 36 X HKR 127 and CSR 23 X HKR 126 whereas, maximum decrease was observed in the genotype in genotype CSR 36 X HKR 47 followed HKR 46, HKR 120 and HKR 126.

Sodium content (ppm): A significantly increase in sodium content was observed with increasing salinity levels (Table 5). At a salinity level of 6 dSm^{-1}, the

lowest sodium content was observed in the genotype HKR 47 X HKR 127 (4133.35 ppm), followed by HKR 47 (4400.88 ppm), HKR 126 X HKR 127 (7566.76 ppm) and CSR 23 X CSR 36 (4600.85 ppm) whereas the highest sodium content was observed in the genotype HKR 126 (7766.67 ppm), followed by IR 64 (7634.21 ppm), HKR 127 (7400.86 ppm) and CSR 27 (7234.20 ppm).

Calcium content (%): With the increasing salinity levels there was a linear decrease in the calcium content in rice straw. The highest decrease was noticed in HKR 46 i.e. from 2.159% (at control) to 1.044% (at 6 dSm^{-1}), followed by CSR 36 from 2.087% (at control) to 1.058% (at 6 dSm^{-1}) and HKR 126 from 2.394% (at control) to 1.249% (at 6 dSm^{-1}). The minimum decrease in calcium content was noticed in CSR 23 from 1.769% at control to 1.466% at 6 dSm^{-1} (table 5). Maximum calcium content in rice at 6 dSm^{-1} was observed in the genotypes HKR 126 X HKR 127 (1.837%), followed by HKR 126 X IR 64 (1.732%) and CSR 36 X HKR 126 (1.710%).

Magnesium content (%): A linear decrease in the magnesium content was observed with increasing salinity levels in each genotype (table 6). Highest decrease in magnesium content was observed in CSR 23 *i.e.* from 1.806% at control to 0.466% at 6 dSm^{-1}, followed by CSR 23 X CSR 36 from 1.415% at control to 0.386% at 6 dSm^{-1}, CSR 23 X IR 64 from 1.737% at control to 0.482% at 6 dSm^{-1} and CSR 36 X HKR 126 from 1.142% at control to 0.324% at 6 dSm^{-1} whereas, the minimum decrease was found in the genotypes CSR 13, followed by CSR 27 and HKR 126.

Chloride content (%): The chloride content in rice increased in a linear fashion with increasing salinity levels (table 6). The highest increase was observed in HKR 126 from 2.034% at control to 6.185% at 6 dSm^{-1}, about three times increase, followed by CSR 13 from 2.679% at control to 7.323% at 6 dSm^{-1}, CSR 23 X CSR 36 from 2.757% at control to 7.375% at 6 dSm^{-1}, CSR 46 from 2.439% at control to 6.510% at 6 dSm^{-1} and CSR 27 from 2.493% at control to 6.609% at 6 dSm^{-1}. The highest chloride content in rice at 6 dSm^{-1} was recorded in CSR 23 X CSR 36 (7.375%), followed by CSR 23 X HKR 126 (7.324%), CSR 13 (7.323%) and CSR 36 (7.020%).

Sulphate content (ppm): The sulphate content decreased substantially at all salinity levels i.e. 2 dSm^{-1}, 4 dSm^{-1} and 6 dSm^{-1} compared to control in all genotypes (table 7). But in case HKR 127 at 2 dSm^{-1} sulphate content increased. Maximum decrease in sulphate content from control to 6 dSm^{-1} was observed in the genotypes HKR 126 (26433.33 ppm to 17801.61 ppm), followed by HKR 46 (26566.67 ppm to 15266.75 ppm), CSR 36 X HKR 47 (25233.48 ppm to 14637.51 ppm) and CSR 36 X HKR 126 (24833.37 ppm to 14533.83 ppm). On the other hand the minimum decrease in sulphate content from control to 6 dSm^{-1} was observed in the genotype HKR 127 (21233.82 ppm to 19133.67 ppm).

K^+/Na^+ ratio: Every genotype exhibited a significant decrease in K^+/Na^+ ratio with increasing salinity levels (table 8). At salinity level 4 dSm^{-1} the maximum K^+/Na^+ ratio was observed in CSR 23 (4.12), followed by CSR 23 X CSR 36 (3.99), CSR 23 X HKR 126 (3.93), HKR 47 X HKR 127 (3.92) and HKR 47 (3.91). When compared at salinity level of 6 dSm^{-1}, the maximum K^+/Na^+ ratio was observed in the genotypes CSR 23 X HKR 126 (3.54), followed by HKR 126 X HKR 127 (3.51), CSR 23 X CSR 36 (3.48), IR 64 X HKR 127 (3.43) and CSR 23 X IR 64 (3.30).

Ca^{++}/Mg^{++} ratio: It is evident from data in table 8 that the Ca^{++}/Mg^{++} ration increased at all the salinity levels i.e. 2 dSm^{-1}, 4 dSm^{-1} and 6 dSm^{-1} as compared to control in all genotypes but the magnitude of increase varied from genotype to genotype. At 6 dSm^{-1} salinity level maximum increase was noticed in CSR 23 (0.980 to 3.163), followed by CSR 23 X IR 64 and CSR 23 X CSR 36.

Discussion

Salinity limits rice yield or prevent rice planting over large land areas around the world. Investigation on the effects of salinity on plant growth and productivity have been conducted to enhance the salt tolerance in rice. Genetic improvement of crop plants depends upon availability of requisite genetic variability in germplasm. In genus *Oryza*, traditional landraces/varieties such as Pokkali, Dasal, Getu *etc*. are well adapted to saline conditions and can be used as donor of salt tolerance trait in rice breeding programs. Some progress in developing improved varieties has been made through conventional breeding method by introgression of salt tolerance genes/traits from salt tolerant germplasm to cultivated rice varieties. In spite of considerable efforts, only a few salt-tolerant cultivars have been released. CSR 10 is one of the salt tolerant variety developed from crosses between CSR 1 (Damodar, salt tolerant landrace) and Jaya (high yielding indica) by Central Soil Salinity Research Institute, Karnal, India.

In the present investigation, a screen house experiment was conducted for the screening of 10 rice genotypes and their 15 F_1 hybrids for their salt tolerance at

varying levels of salinity (Control, 2, 4 and 6 dSm^{-1}). The genotypes were grown up to maturity. The grain and straw yields were recorded and also analyzed for different chemical/biochemical parameters. The yield and yield attributes of all the genotypes were affected differentially by salinity levels. Minimum reduction in leaf area over control was observed in HKR 47. The decrease in leaf area might be due to poor development of meristematic tissue due to stresses caused by increasing levels of salinity. Similar results were also reported by Bhatt et al., (2020).

The increasing levels of soil salinity also resulted in decreased plant height, number of panicle/plant, number of seeds/panicle, 1000-grain weight, seed yield and dry aerial biomass. This was observed in all the genotypes and the magnitude of reduction varied between cultivars. The number of seeds/panicle decreased in IR 64, from 85.17 to 22.27 seeds/panicle with an increase in salinity level from control to 6 dSm^{-1}, respectively. Similarly seed yield/plant of CSR 13 decreased from 18.10 to 2.47 gram per plant with increasing salinity, from control to 6 dSm^{-1}, respectively. Seed yield per plant reduction in F_1's was high as compared to their parents. Except genotypes CSR 36 x HKR 126 and CSR 36 x HKR 127 all F_1's had more than 50% reduction in seed yield per plant. Whereas in parents only HKR 127 and IR 64 had more than 50% reductions in seed yield. Similar findings were also reported by Krishanamurthy et al., (2022).

The adverse effect of increasing salinity levels on the yield and yield traits of almost all the genotypes of rice may be attributed to the adverse effect of soluble salts on nutrient and water absorption by roots, probably due to high osmotic pressure/potential of soil solution than that of root cell sap. This is the most important single factor which influences the growth of crops grown in saline environments. Further the poor growth of genotypes of rice in saline environments may also be ascribed due to its inhibitory effect on cell division and its enlargement in plants growing points. Reduced growth of shoot caused by excess soluble salts may be due to their adverse effect on tissues (Singh et al., 2021). Consequently, stunted growth of plants was observed.

All stresses *i.e.* 2 dSm^{-1}, 4 dSm^{-1} and 6 dSm^{-1} proved as stimulus for increased chlorophyll 'a' content in genotype HKR 127 whereas, salinity level 2 dSm^{-1} and 4 dSm^{-1} proved as stimulus for genotype CSR 23 X HKR 127 and CSR 36 X HKR 126. The maximum decrease in chlorophyll 'a' content was found in genotype CSR 27 at 6 dSm^{-1} salinity level. Chlorophyll 'b' of all the genotypes reduced significantly with increasing salinity levels. The minimum reduction percentage in chlorophyll 'b' content was found in the genotype HKR 47 followed by HKR 46 and IR 64 whereas, maximum reduction percentage was observed in the genotypes HKR 120, followed by CSR 36 X HKR 126 and CSR 23 X HKR 127. The decrease in the chlorophyll content due to increasing salt stress might have affected the photosynthetic activity in plants resulting in drastic reduction in grain and dry aerial biomass yield. Plants have evolved diverse strategies of acclimation and avoidance to cope with adverse environmental conditions; various solutes accumulate under stress conditions to protect the plant from damage. Out of these, proline is only one which has been shown to protect plants against singlet oxygen and free radical induced damages. It is thought to play a role as a singlet oxygen quencher and scavenger of OH radicals. Increase in proline accumulation under salt-stress as witnessed in present study was also observed by various investigators (Bhatt et al., 2020). Comparative evaluation of proline content at 6 dSm^{-1} with that at control, revealed the highest proline content in HKR 47 (6.99 mg/g). On the other hand, the minimum proline content was observed in genotype IR 64 X HKR 47 (5.898 mg/g) at 6 dSm^{-1} salinity level. Elevated proline content with enriched salt stress tolerance has been described by Bhatt et al., (2020).

Na exclusion or uptake reduction and increased absorption of K to maintain a good Na: K balance in the straw has been associated with salinity tolerance in rice. The potassium content decreased drastically with increasing salinity levels of almost all rice genotypes. But all the F_1's except genotype CSR 36 X HKR 47 showed reverse trend with increase with salinity levels. Potassium is well known for its role in stress tolerance in plants. The maximum increase in potassium content was found in the genotype CSR 23 X CSR 36, followed by CSR 36 X HKR 127 and CSR 23 X HKR 126 whereas, maximum decrease was observed in the genotype CSR 36 X HKR 47 followed HKR 46, HKR 120 and HKR 126. The accumulation of K in salt tolerant genotypes also influences the K/Na ratio of straw. The ranking according to Na, K absorption alone is not a reliable parameter for salinity tolerance reactions. However, the classification of susceptible and tolerant based on field laboratory and greenhouse tests is clearly related to the Na:K concentration. Thus Na: K ratio, which is the balance between Na and K in straw, could be a valid criterion in measuring salinity tolerance in rice.

Calcium content decreased with the increasing salinity levels in rice straw. The highest decrease was noticed in HKR 46 *i.e.* from 2.159% (at control)

to 1.044% (at 6 dSm^{-1}). The minimum decrease in calcium content was noticed in CSR 23 from 1.769% at control to 1.466% at 6 dSm^{-1}. Maximum calcium content in rice at 6 dSm^{-1} was observed in the genotypes HKR 126 X HKR 127 (1.837%). A linear decrease in the magnesium content was observed with increasing salinity levels in each genotype. Highest decrease in magnesium content was observed in CSR 23 i.e. from 1.806% at control to 0.466% at 6 dSm^{-1} whereas, the minimum decrease was found in the genotypes CSR 13, followed by CSR 27 and HKR 126. In F_1's highest decrease in magnesium content was observed in CSR 23 X CSR 36 from 1.415% at control to 0.386% at 6 dSm^{-1} followed by CSR 23 X IR 64 from 1.737% at control to 0.482% at 6 dSm^{-1} and CSR 36 X HKR 126 from 1.142% at control to 0.324% at 6 dSm^{-1}.

The sulphate content decreased substantially with the increasing salinity levels in all genotypes. But in case HKR 127 sulphate content increased at 2 dSm^{-1}. Maximum decrease in sulphate content from control to 6 dSm^{-1} was observed in the genotypes HKR 126 (26433.33 ppm to 17801.61 ppm). The higher accumulation of sulphate content in HKR 127, CSR 23, CSR 36, IR 64 and HKR 126 might have alleviated the toxic effect of Cl on plants. The chloride content in rice increased in a linear fashion with increasing salinity levels. The highest increase was observed in HKR 126 from 2.034% at control to 6.185% at 6 dSm^{-1}, about three times increase. The highest chloride content in rice at 6 dSm^{-1} was recorded in CSR 23 X CSR 36 (7.375%). Bhatt et al., (2020) studied the salinity tolerance mechanism in rice and also reported tolerant land races.

Conclusions

It may be concluded that the parents CSR 23, CSR 36, HKR 47 and crosses CSR 36 x HKR 126, CSR 36 x IR 64, CSR 36 x HKR 127 and HKR 126 x IR 64 figured to offer promise as they depicted less reduction in the yield and involved divergent parents. Proximate studies postulate them as salt shock protein mediated salinity stress tolerant genotypes which may be used as potent parents in crosses for further rice improvement through recombination breeding.

Table 1. Performance of rice genotypes at different salinity levels for Plant height (cm).

Genotypes	Plant Height (cm)					Number of Panicle/Plant				
	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean
IR 64	70.50	60.33	60.27	58.00	**62.28**	9.33	8.50	8.50	8.50	**3.37**
HKR 46	56.67	54.67	54.50	49.77	**53.90**	7.00	6.33	5.50	4.00	**3.37**
HKR 47	72.00	65.77	65.00	63.67	**66.61**	10.50	8.50	8.00	7.00	**3.43**
HKR 120	82.50	73.00	69.33	54.50	**69.83**	7.17	6.00	5.00	4.50	**3.44**
HKR 126	71.50	66.00	59.50	51.00	**62.00**	7.50	6.67	5.83	5.33	**3.51**
HKR 127	73.50	70.83	66.17	64.00	**68.63**	7.50	7.17	6.50	6.00	**3.56**
CSR 13	63.00	55.33	49.50	45.33	**53.29**	11.50	9.83	6.50	6.50	**3.55**
CSR 23	77.00	76.77	73.50	70.50	**74.44**	9.00	7.50	7.50	7.50	**3.56**
CSR 27	71.50	67.33	66.67	62.00	**66.88**	7.00	7.17	6.83	5.50	**3.58**
CSR 36	59.00	58.00	56.83	52.83	**56.67**	9.50	6.50	6.50	6.17	**3.62**
CSR 23 X CSR 36	75.33	65.83	65.00	60.50	**66.67**	9.33	8.83	8.00	6.67	**3.65**
CSR 23 X HKR 126	74.57	64.00	60.50	60.00	**64.77**	7.67	7.17	4.83	4.27	**3.57**
CSR 23 X IR 64	74.70	62.57	59.17	58.00	**63.61**	7.67	7.10	7.00	6.70	**3.60**
CSR 23 X HKR 47	74.57	67.50	65.27	63.30	**67.66**	8.00	7.50	7.00	6.50	**3.58**
CSR 23 X HKR 127	74.90	71.67	69.23	67.93	**70.93**	8.00	7.67	7.50	7.17	**3.56**
CSR 36 X HKR 126	72.00	70.33	69.17	50.50	**65.50**	9.50	7.50	6.00	8.33	**3.54**
CSR 36 X IR 64	83.00	60.50	55.00	49.67	**62.04**	14.83	12.00	8.33	7.50	**3.52**
CSR 36 X HKR 47	73.17	65.00	61.50	59.50	**64.79**	8.00	7.50	6.50	6.00	**3.47**
CSR 36 X HKR 127	65.27	61.00	60.00	58.50	**61.19**	8.00	8.00	7.67	6.50	**3.53**
HKR 126 X IR 64	66.47	62.67	58.73	57.30	**61.29**	7.60	7.50	7.33	7.17	**3.54**
HKR 126 X HKR 47	66.40	64.23	59.13	58.00	**61.94**	7.00	6.83	6.67	6.50	**3.53**
HKR 126 X HKR 127	67.00	67.00	64.67	56.50	**63.79**	7.50	7.00	6.50	4.50	**3.53**
IR 64 X HKR 47	69.43	66.90	50.83	47.63	**58.70**	8.17	7.83	7.50	7.17	**3.53**
IR 64 X HKR 127	70.60	67.33	50.87	49.47	**59.57**	7.67	7.50	7.33	7.20	**3.53**
HKR 47 X HKR 127	63.67	63.17	62.00	59.87	**62.18**	7.50	6.50	6.33	5.87	**3.53**
Mean	**70.73**	**65.11**	**61.29**	**57.13**		**3.52**	**3.54**	**3.53**	**3.53**	
			CD	**SE (d)**	**SE (M)**			**CD**	**SE (d)**	**SE (M)**
Salinity Levels			0.56	0.28	0.20			0.17	0.08	0.06
Genotypes			1.40	0.71	0.50			0.42	0.21	0.15
Salinity Levels x Genotypes			2.79	1.41	1.00			0.83	0.42	0.30

Table 2. Performance of rice genotypes at different salinity levels for number of panicle/plant.

Genotypes	1000-Grain Weight (gr)					Seed Yield/Plant (gr)				
	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean
IR 64	47.00	45.00	42.33	35.00	**42.33**	17.67	13.67	9.70	7.90	**12.24**
HKR 46	47.00	46.00	43.67	41.00	**44.42**	20.33	15.60	15.00	7.53	**14.62**
HKR 47	39.00	36.00	33.67	31.67	**35.09**	21.50	21.27	16.47	13.10	**18.09**
HKR 120	43.00	41.67	31.67	30.67	**36.75**	19.63	18.50	10.83	10.47	**14.86**
HKR 126	45.00	40.33	37.00	35.33	**39.42**	20.43	20.23	13.00	11.47	**16.28**
HKR 127	39.33	38.33	36.00	35.67	**37.33**	22.67	19.77	14.97	9.13	**16.64**
CSR 13	36.00	36.00	31.00	28.67	**32.92**	18.10	9.80	3.63	2.47	**8.50**
CSR 23	44.67	42.33	39.33	36.67	**40.75**	16.63	15.43	12.43	11.27	**13.94**
CSR 27	45.33	43.33	38.00	36.00	**40.67**	21.53	15.33	13.73	7.17	**14.44**
CSR 36	43.00	43.00	38.33	35.33	**39.92**	13.53	11.53	10.83	8.17	**11.02**
CSR 23 X CSR 36	42.67	42.33	37.67	35.00	**39.42**	24.33	14.37	10.07	2.40	**12.79**
CSR 23 X HKR 126	45.00	43.00	38.00	36.00	**40.50**	14.33	13.90	13.27	4.23	**11.43**
CSR 23 X IR 64	44.00	41.67	37.00	35.00	**39.42**	16.47	16.47	12.43	3.30	**12.17**
CSR 23 X HKR 47	40.67	39.00	36.00	33.67	**37.34**	19.33	19.03	17.20	5.40	**15.24**
CSR 23 X HKR 127	40.00	38.67	36.67	32.67	**37.00**	14.87	14.50	11.77	2.13	**10.82**
CSR 36 X HKR 126	44.00	41.33	37.67	34.67	**39.42**	19.00	14.97	11.70	11.33	**14.25**
CSR 36 X IR 64	43.00	40.00	37.00	35.33	**38.83**	18.87	12.77	9.70	8.77	**12.53**
CSR 36 X HKR 47	47.33	44.33	37.67	35.33	**41.17**	16.50	13.50	6.60	1.80	**9.60**
CSR 36 X HKR 127	38.00	36.33	36.67	34.33	**36.33**	10.90	11.17	6.67	6.47	**8.80**
HKR 126 X IR 64	47.00	45.67	40.00	36.67	**42.34**	15.27	15.10	12.87	6.57	**12.45**
HKR 126 X HKR 47	41.00	38.67	35.67	32.67	**37.00**	19.00	18.87	16.97	6.10	**15.24**
HKR 126 X HKR 127	47.00	45.00	42.00	37.00	**42.75**	15.50	10.03	2.77	2.23	**7.63**
IR 64 X HKR 47	44.67	41.00	37.00	34.00	**39.17**	18.67	18.43	16.03	7.40	**15.13**
IR 64 X HKR 127	42.00	40.33	38.00	34.67	**38.75**	15.53	3.67	3.30	1.77	**6.07**
HKR 47 X HKR 127	41.33	39.00	37.00	33.33	**37.67**	12.60	2.73	2.30	1.67	**4.83**
Mean	**43.08**	**41.13**	**37.40**	**34.65**		**17.73**	**14.43**	**10.97**	**6.41**	
			CD	**SE (d)**	**SE (M)**			**CD**	**SE (d)**	**SE (M)**
Salinity Levels			0.47	0.24	0.17			0.40	0.20	0.14
Genotypes			1.18	0.60	0.42			0.99	0.50	0.35
Salinity Levels x Genotypes			2.35	1.19	0.84			1.98	1.00	0.71

Table 3. Performance of rice genotypes at different salinity levels for Chlorophyll 'a' & 'b' (mg/g fresh wt.).

Genotypes	Chlorophyll 'a' (mg/g fresh wt.)					Chlorophyll 'b' (mg/g fresh wt.)				
	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean
IR 64	3.487	3.347	3.146	2.651	**3.158**	0.763	0.716	0.706	0.688	**0.718**
HKR 46	2.999	2.816	2.807	2.235	**2.714**	0.867	0.820	0.801	0.781	**0.817**
HKR 47	3.924	3.872	3.612	3.234	**3.661**	0.855	0.827	0.819	0.796	**0.824**
HKR 120	2.345	2.277	2.146	1.768	**2.134**	0.751	0.716	0.694	0.523	**0.671**
HKR 126	2.939	2.822	2.748	2.478	**2.747**	0.812	0.789	0.700	0.684	**0.746**
HKR 127	2.740	2.974	2.879	2.754	**2.837**	0.674	0.650	0.585	0.572	**0.620**
CSR 13	3.034	2.867	2.654	2.426	**2.745**	0.829	0.799	0.773	0.734	**0.784**
CSR 23	2.869	2.796	2.651	2.051	**2.592**	0.870	0.800	0.785	0.765	**0.805**
CSR 27	2.468	2.058	1.824	1.383	**1.933**	0.768	0.728	0.684	0.620	**0.700**
CSR 36	2.675	2.414	2.301	1.808	**2.300**	0.828	0.793	0.766	0.696	**0.771**
CSR 23 X CSR 36	2.598	2.314	2.259	1.780	**2.238**	0.850	0.793	0.780	0.712	**0.784**
CSR 23 X HKR 126	2.058	1.996	1.944	1.529	**1.882**	0.669	0.603	0.568	0.562	**0.601**
CSR 23 X IR 64	2.099	1.829	1.892	1.691	**1.878**	0.700	0.627	0.610	0.551	**0.622**
CSR 23 X HKR 47	1.719	1.363	1.266	1.184	**1.383**	0.568	0.498	0.482	0.451	**0.500**
CSR 23 X HKR 127	1.206	1.264	1.382	0.998	**1.213**	0.392	0.384	0.355	0.299	**0.358**
CSR 36 X HKR 126	1.650	1.768	1.811	1.202	**1.608**	0.524	0.483	0.443	0.367	**0.454**
CSR 36 X IR 64	2.366	2.263	2.037	1.934	**2.150**	0.726	0.683	0.630	0.597	**0.659**
CSR 36 X HKR 47	2.622	2.303	2.375	2.307	**2.402**	0.803	0.773	0.747	0.695	**0.755**
CSR 36 X HKR 127	2.026	2.028	1.837	1.622	**1.878**	0.635	0.606	0.579	0.520	**0.585**
HKR 126 X IR 64	2.496	2.339	2.154	2.062	**2.263**	0.701	0.691	0.665	0.617	**0.669**
HKR 126 X HKR 47	2.834	2.313	2.445	2.108	**2.425**	0.749	0.697	0.678	0.629	**0.688**
HKR 126 X HKR 127	2.035	2.004	1.767	1.565	**1.843**	0.562	0.526	0.488	0.427	**0.501**
IR 64 X HKR 47	2.553	2.124	2.055	1.960	**2.173**	0.688	0.634	0.611	0.559	**0.623**
IR 64 X HKR 127	2.016	1.949	1.826	1.686	**1.869**	0.622	0.600	0.579	0.524	**0.581**
HKR 47 X HKR 127	2.826	2.617	2.503	2.112	**2.515**	0.789	0.724	0.681	0.645	**0.710**
Mean	**2.503**	**2.349**	**2.253**	**1.941**		**0.720**	**0.678**	**0.648**	**0.601**	
			CD	**SE (d)**	**SE (M)**			**CD**	**SE (d)**	**SE (M)**
Salinity Levels			0.043	0.022	0.015			0.004	0.002	0.001
Genotypes			0.108	0.055	0.039			0.010	0.005	0.004
Salinity Levels x Genotypes			0.215	0.109	0.077			0.020	0.010	0.007

Table 4. Performance of rice genotypes at different salinity levels for Proline content (mg/g dry wt.) and Potassium Content (ppm).

Genotypes	Proline Content (mg/g dry wt.)					Potassium Content (ppm)				
	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean	Control	2 dSm^{-1}	4dSm^{-1}	6 dSm^{-1}	Mean
IR 64	4.960	5.745	5.930	6.317	**5.738**	14934.15	15535.08	13367.19	12202.63	**14009.76**
HKR 46	5.323	5.967	6.058	6.270	**5.905**	15836.94	15367.86	12668.09	12402.08	**14068.74**
HKR 47	6.414	6.253	6.631	6.919	**6.554**	14803.21	13900.86	13100.85	12702.22	**13626.79**
HKR 120	5.264	5.893	6.049	6.230	**5.859**	15269.50	14368.75	13434.01	12102.18	**13793.61**
HKR 126	4.900	5.583	5.774	6.112	**5.592**	15469.21	14266.67	13833.33	12366.67	**13983.97**
HKR 127	5.209	5.780	5.890	6.169	**5.762**	14767.96	13441.74	13534.56	12901.48	**13661.44**
CSR 13	5.568	5.185	6.645	6.868	**6.067**	15538.30	13967.45	13540.71	12967.55	**14003.50**
CSR 23	5.427	6.340	6.360	6.574	**6.175**	15668.94	15033.33	14268.33	12800.11	**14442.68**
CSR 27	5.330	5.951	6.537	6.591	**6.102**	15203.12	14473.72	13633.41	12833.33	**14035.90**
CSR 36	5.701	6.275	6.822	6.857	**6.414**	14635.74	14234.42	13567.09	12908.08	**13836.33**
CSR 23 X CSR 36	5.584	6.148	6.463	6.524	**6.180**	15000.42	15200.00	15569.86	16000.02	**15442.58**
CSR 23 X HKR 126	5.226	5.734	5.894	6.041	**5.724**	15801.71	16134.04	16400.86	16633.33	**16242.49**
CSR 23 X IR 64	5.139	5.703	5.985	6.115	**5.736**	14801.11	15066.67	15300.53	15466.67	**15158.75**
CSR 23 X HKR 47	5.628	6.049	6.329	6.460	**6.117**	15467.86	15653.08	15933.45	16002.86	**15764.31**
CSR 23 X HKR 127	5.401	5.769	5.945	6.149	**5.816**	14966.67	15133.75	15302.12	15433.33	**15208.97**
CSR 36 X HKR 126	5.400	5.712	5.960	5.971	**5.761**	15034.20	15200.90	15434.07	15702.22	**15342.85**
CSR 36 X IR 64	5.309	5.822	6.044	6.307	**5.871**	14202.22	14500.01	14666.90	14833.58	**14550.68**
CSR 36 X HKR 47	5.730	6.080	6.247	6.360	**6.104**	14803.28	15055.12	15133.56	10710.04	**13925.50**
CSR 36 X HKR 127	5.446	5.858	5.992	6.046	**5.836**	14400.85	14866.67	15100.42	15337.51	**14926.36**
HKR 126 X IR 64	5.262	5.623	6.075	6.194	**5.789**	15100.85	15400.22	15533.89	15721.12	**15439.02**
HKR 126 X HKR 47	5.294	5.800	6.136	6.212	**5.861**	15602.39	15834.19	16033.85	16200.29	**15917.68**
HKR 126 X HKR127	5.075	5.684	5.901	6.062	**5.681**	15302.85	15633.33	15802.11	16001.39	**15684.92**
IR 64 X HKR 47	5.389	5.774	5.835	5.898	**5.724**	14901.09	15101.09	15333.34	15368.84	**15176.09**
IR 64 X HKR 127	5.024	5.771	5.934	6.042	**5.693**	14536.10	14801.40	14935.71	15100.02	**14843.31**
HKR 47 X HKR 127	5.331	5.906	5.815	6.136	**5.797**	15068.08	15201.20	15435.53	15701.62	**15351.61**
Mean	**5.373**	**5.856**	**6.130**	**6.297**		**15084.67**	**14934.86**	**14674.55**	**14255.97**	

	CD	SE (d)	SE (M)	CD	SE (d)	SE (M)
Salinity Levels	0.061	0.031	0.022	268.79	136.3	96.38
Genotypes	0.153	0.078	0.055	671.96	340.74	240.94
Salinity Levels x Genotypes	0.306	0.155	0.110	13343.93	681.48	481.88

Table 5. Performance of rice genotypes at different salinity levels for Sodium content (ppm).

Genotypes	Sodium Content (ppm)					Calcium Content (%)				
	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean
IR 64	2533.74	3368.42	5822.89	7634.21	**4839.82**	1.917	1.707	1.587	1.134	**1.586**
HKR 46	2368.75	3066.67	4000.00	5133.44	**3642.22**	2.159	1.985	1.764	1.044	**1.738**
HKR 47	2069.60	2908.45	3368.76	4400.88	**3186.92**	1.913	1.782	1.374	1.178	**1.562**
HKR 120	2400.00	3433.33	4000.41	5634.83	**3867.14**	2.029	1.861	1.462	1.115	**1.617**
HKR 126	3202.85	3802.55	4101.54	7766.67	**4718.40**	2.394	1.865	1.479	1.249	**1.747**
HKR 127	2066.67	3033.33	4300.00	7400.86	**4200.22**	2.180	1.905	1.496	1.389	**1.743**
CSR 13	2001.20	3012.08	3600.00	6100.00	**3678.32**	2.109	1.831	1.566	1.343	**1.712**
CSR 23	2342.18	2633.33	3468.42	6067.09	**3627.76**	1.769	1.744	1.625	1.466	**1.651**
CSR 27	2666.67	3369.60	3966.68	7234.20	**4309.29**	1.844	1.797	1.610	1.224	**1.619**
CSR 36	3068.41	3500.00	3666.67	6400.00	**4158.77**	2.087	1.897	1.752	1.058	**1.699**
CSR 23 X CSR 36	2766.67	3200.00	3902.75	4600.85	**3617.57**	1.899	1.828	1.615	1.424	**1.692**
CSR 23 X HKR 126	2902.99	3466.74	4166.95	4700.00	**3809.17**	2.137	1.767	1.755	1.510	**1.792**
CSR 23 X IR 64	2467.19	3004.26	4236.09	4700.86	**3602.10**	1.792	1.732	1.631	1.460	**1.654**
CSR 23 X HKR 47	2500.00	3200.00	4366.67	5067.20	**3783.47**	1.798	1.675	1.647	1.447	**1.642**
CSR 23 X HKR 127	2200.78	2933.33	4134.86	5000.00	**3567.24**	1.945	1.783	1.735	1.284	**1.687**
CSR 36 X HKR 126	3402.22	3834.20	4201.90	5402.98	**4210.33**	2.219	2.044	1.926	1.710	**1.975**
CSR 36 X IR 64	3066.67	3400.00	4402.08	4902.98	**3942.93**	1.937	1.730	1.622	1.278	**1.642**
CSR 36 X HKR 47	2834.83	3834.01	4600.00	5300.15	**4142.25**	1.913	1.782	1.671	1.421	**1.697**
CSR 36 X HKR 127	2737.53	3336.08	4369.10	4933.33	**3844.01**	2.059	1.872	1.778	1.583	**1.823**
HKR 126 X IR 64	3066.67	4000.00	4900.42	5401.42	**4342.13**	2.160	2.030	1.915	1.732	**1.959**
HKR 126 X HKR 47	3167.40	4007.20	5166.67	5908.78	**4562.51**	2.121	1.938	1.820	1.610	**1.872**
HKR 126 X HKR 127	2768.06	3200.00	4069.42	4566.76	**3651.06**	2.293	2.185	2.000	1.837	**2.079**
IR 64 X HKR 47	2600.00	3700.52	5267.86	5633.33	**4300.43**	1.831	1.691	1.627	1.198	**1.587**
IR 64 X HKR 127	2369.55	3000.00	4067.45	4402.75	**3459.94**	2.044	1.855	1.750	1.316	**1.741**
HKR 47 X HKR 127	2302.92	3072.21	3934.79	4133.35	**3360.82**	2.044	1.843	1.815	1.444	**1.787**
Mean	**2634.94**	**3332.65**	**4243.30**	**5537.08**		**2.024**	**1.845**	**1.681**	**1.378**	
			CD	SE (d)	SE (M)			CD	SE (d)	SE (M)
Salinity Levels			57.40	29.11	20.58			0.020	0.010	0.007
Genotypes			143.51	72.77	51.46			0.050	0.025	0.018
Salinity Levels x Genotypes			287.03	145.54	102.92			0.099	0.050	0.036

Table 6. Performance of rice genotypes at different salinity levels for Magnesium content (%).

Genotypes	Magnesium Content (%)					Chloride Content (%)				
	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean
IR 64	1.627	0.743	0.609	0.539	**0.880**	2.373	3.499	4.592	5.955	**4.105**
HKR 46	1.555	0.800	0.568	0.520	**0.861**	2.439	4.090	4.900	6.510	**4.485**
HKR 47	1.336	0.766	0.631	0.513	**0.812**	2.728	4.008	5.232	6.880	**4.712**
HKR 120	1.149	0.551	0.627	0.447	**0.694**	2.419	3.835	4.692	6.185	**4.283**
HKR 126	1.049	0.630	0.502	0.481	**0.666**	2.034	2.897	4.662	6.042	**3.909**
HKR 127	1.079	0.497	0.459	0.416	**0.613**	2.599	3.474	4.725	6.410	**4.302**
CSR 13	1.260	0.774	0.750	0.625	**0.852**	2.679	4.342	5.533	7.323	**4.969**
CSR 23	1.806	0.715	0.757	0.466	**0.936**	2.762	4.515	5.431	6.463	**4.793**
CSR 27	1.275	0.612	0.676	0.585	**0.787**	2.493	3.816	4.888	6.609	**4.452**
CSR 36	1.135	0.787	0.587	0.431	**0.735**	2.785	3.871	4.910	7.020	**4.647**
CSR 23 X CSR 36	1.415	0.793	0.655	0.386	**0.812**	2.757	4.284	5.407	7.375	**4.956**
CSR 23 X HKR 126	1.430	0.803	0.672	0.433	**0.835**	2.837	4.361	5.487	7.324	**5.002**
CSR 23 X IR 64	1.737	0.847	0.653	0.482	**0.930**	2.599	3.431	4.301	5.750	**4.020**
CSR 23 X HKR 47	1.622	0.818	0.627	0.511	**0.895**	2.782	3.796	4.753	6.219	**4.388**
CSR 23 X HKR 127	1.423	0.783	0.585	0.429	**0.805**	2.679	3.817	4.769	6.662	**4.482**
CSR 36 X HKR 126	1.142	0.641	0.456	0.324	**0.641**	2.365	3.511	4.584	5.908	**4.092**
CSR 36 X IR 64	1.237	0.712	0.565	0.437	**0.738**	2.558	3.489	4.526	5.829	**4.101**
CSR 36 X HKR 47	1.142	0.616	0.507	0.352	**0.654**	2.735	4.206	4.951	6.898	**4.698**
CSR 36 X HKR 127	1.136	0.580	0.416	0.404	**0.634**	2.551	3.888	4.746	6.428	**4.403**
HKR 126 X IR 64	1.312	0.763	0.525	0.432	**0.758**	2.363	3.297	4.627	6.012	**4.075**
HKR 126 X HKR 47	1.164	0.529	0.428	0.415	**0.634**	2.352	3.517	4.535	6.122	**4.132**
HKR 126 X HKR 127	1.147	0.497	0.443	0.398	**0.621**	2.271	3.068	4.049	5.831	**3.805**
IR 64 X HKR 47	1.437	0.753	0.578	0.533	**0.825**	2.554	3.521	4.555	5.907	**4.134**
IR 64 X HKR 127	1.326	0.660	0.470	0.403	**0.715**	2.480	3.392	4.451	5.667	**3.998**
HKR 47 X HKR 127	1.232	0.530	0.469	0.376	**0.652**	2.579	3.935	4.905	5.801	**4.305**
Mean	**1.327**	**0.688**	**0.569**	**0.454**		**2.551**	**3.754**	**4.808**	**6.365**	
			CD	**SE (d)**	**SE (M)**			**CD**	**SE (d)**	**SE (M)**
Salinity Levels			0.013	0.007	0.005			0.060	0.030	0.021
Genotypes			0.033	0.017	0.012			0.149	0.076	0.054
Salinity Levels x Genotypes			0.066	0.033	0.024			0.298	0.151	0.107

Table 7. Performance of rice genotypes at different salinity levels for Sulphate content (ppm).

Genotypes	Salinity Levels				Mean
	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	
IR 64	24234.23	21035.83	19633.75	17871.24	**20693.76**
HKR 46	26566.67	22100.42	17000.53	15266.75	**20233.59**
HKR 47	25433.38	20033.85	17500.07	17166.67	**20033.49**
HKR 120	26433.33	22100.00	17701.73	15036.26	**20317.83**
HKR 126	23600.09	21101.65	18733.33	17801.61	**20309.17**
HKR 127	21233.82	21500.88	20333.42	19133.67	**20550.45**
CSR 13	21435.53	19233.34	17201.53	15733.60	**18401.00**
CSR 23	27066.67	23501.39	18300.86	18600.42	**21867.34**
CSR 27	23867.52	21769.65	19600.00	16834.73	**20517.98**
CSR 36	25333.33	23200.08	22100.53	18402.38	**22259.08**
CSR 23 X CSR 36	25888.79	23100.00	17500.09	16534.44	**20755.83**
CSR 23 X HKR 126	25100.86	22602.47	16466.68	15468.25	**19909.57**
CSR 23 X IR 64	25433.33	22833.33	16633.42	15168.19	**20017.07**
CSR 23 X HKR 47	25801.53	23100.00	17600.42	16967.41	**20867.34**
CSR 23 X HKR 127	24802.89	21903.12	17202.94	14700.49	**19652.36**
CSR 36 X HKR 126	24833.37	21735.73	16968.06	14533.83	**19517.75**
CSR 36 X IR 64	25100.00	22333.33	17433.33	14969.50	**19959.04**
CSR 36 X HKR 47	25233.48	22635.21	17566.75	14637.51	**20018.24**
CSR 36 X HKR 127	23900.00	19966.67	18900.22	15067.49	**19458.60**
HKR 126 X IR 64	24400.89	21400.53	19066.67	15767.61	**20158.93**
HKR 126 X HKR 47	24500.00	21336.29	19369.24	16100.50	**20326.51**
HKR 126 X HKR 127	23000.00	19568.29	17901.11	14868.27	**18834.42**
IR 64 X HKR 47	24900.56	21400.00	17969.52	14801.39	**19767.87**
IR 64 X HKR 127	23200.03	20401.39	17534.20	14668.06	**18950.92**
HKR 47 X HKR 127	23200.09	20221.20	17400.35	15038.02	**18964.92**
Mean	**24580.02**	**21604.59**	**18144.75**	**16045.53**	

	CD	SE (d)	SE (M)
Salinity Levels	107.34	54.43	38.49
Genotypes	268.35	136.08	96.22
Salinity Levels x Genotypes	536.71	272.16	192.44

Table 8. Performance of rice genotypes at different salinity levels for K^+/Na^+ ratio and Ca^{++}/Mg^{++} ratio.

Genotypes	K^+/Na^+ Ratio					Ca^{++}/Mg^{++} Ratio				
	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Mean
IR 64	5.966	4.626	2.298	1.599	**3.622**	1.179	2.301	2.607	2.117	**2.051**
HKR 46	6.775	5.019	3.167	2.417	**4.345**	1.389	2.483	3.107	2.017	**2.249**
HKR 47	7.283	4.786	3.911	2.890	**4.718**	1.433	2.337	2.177	2.310	**2.064**
HKR 120	6.489	4.190	3.368	2.149	**4.049**	1.772	3.391	2.345	2.497	**2.501**
HKR 126	4.838	3.755	3.379	1.593	**3.391**	2.287	2.966	2.954	2.597	**2.701**
HKR 127	7.169	4.439	3.159	1.744	**4.128**	2.026	3.835	3.266	3.343	**3.118**
CSR 13	7.785	4.638	3.765	2.127	**4.579**	1.676	2.373	2.095	2.150	**2.074**
CSR 23	6.716	5.726	4.120	2.111	**4.668**	0.980	2.443	2.158	3.163	**2.186**
CSR 27	5.716	4.301	3.449	1.775	**3.810**	1.447	2.963	2.384	2.093	**2.222**
CSR 36	4.779	4.070	3.714	2.019	**3.646**	1.844	2.414	2.984	2.470	**2.428**
CSR 23 X CSR 36	5.432	4.753	3.991	3.485	**4.415**	1.342	2.305	2.468	3.693	**2.452**
CSR 23 X HKR 126	5.449	4.655	3.938	3.540	**4.396**	1.494	2.200	2.613	3.491	**2.450**
CSR 23 X IR 64	6.016	5.019	3.617	3.296	**4.487**	1.032	2.047	2.496	3.028	**2.151**
CSR 23 X HKR 47	6.193	4.894	3.651	3.160	**4.475**	1.108	2.048	2.626	2.829	**2.153**
CSR 23 X HKR 127	6.811	5.161	3.704	3.087	**4.691**	1.367	2.279	2.965	2.994	**2.401**
CSR 36 X HKR 126	4.422	3.977	3.675	2.907	**3.745**	1.951	3.193	4.234	5.291	**3.667**
CSR 36 X IR 64	4.652	4.267	3.333	3.026	**3.820**	1.565	2.430	2.871	2.937	**2.451**
CSR 36 X HKR 47	5.231	3.930	3.291	2.024	**3.619**	1.678	2.894	3.492	4.046	**3.028**
CSR 36 X HKR 127	5.287	4.461	3.456	3.112	**4.079**	1.815	3.226	4.290	3.918	**3.312**
HKR 126 X IR 64	4.933	3.852	3.171	2.913	**3.717**	1.646	2.661	3.650	4.020	**2.994**
HKR 126 X HKR 47	4.933	3.957	3.104	2.742	**3.684**	1.825	3.665	4.256	3.877	**3.406**
HKR 126 X HKR 127	5.547	4.888	3.890	3.509	**4.459**	2.019	4.398	4.514	4.615	**3.887**
IR 64 X HKR 47	5.736	4.082	2.912	2.731	**3.865**	1.275	2.248	2.817	2.248	**2.147**
IR 64 X HKR 127	6.150	4.937	3.674	3.431	**4.548**	1.542	2.815	3.731	3.264	**2.838**
HKR 47 X HKR 127	6.552	4.955	3.928	3.799	**4.809**	1.662	3.479	3.873	3.849	**3.216**
Mean	**5.874**	**4.534**	**3.507**	**2.687**		**1.574**	**2.786**	**3.089**	**3.154**	
			CD	**SE (d)**	**SE (M)**			**CD**	**SE (d)**	**SE (M)**
Salinity Levels			0.107	0.054	0.038			0.059	0.030	0.021
Genotypes			0.266	0.135	0.095			0.147	0.075	0.053
Salinity Levels x Genotypes			0.532	0.270	0.191			0.295	0.150	0.106

References

Ahmadizadeh M, Vispo NA, Calapit-Palao CD, Pangaan I D, Vina, CD and Singh RK, (2016). Reproductive stage salinity tolerance in rice: a complex trait to phenotype. Indian J Plant Physiol 12:528-536.

Bates LS, Waldren RP and Teare LD, (1973). Rapid determination of free proline of water-stress studies. Plant and Soil. 39:205-207.

Bhatt T, Sharma A, Puri S, and Minhas AP, (2020). Salt tolerance mechanisms and approaches: future scope of halotolerant genes and rice landraces. Rice Sci. 27(5):368-383. https://doi.org/10.1016/j.rsci.2020.03.002.

Chhabra R, Ringoet A and Lamberts D, (1976). Kinetics and interaction of chloride and phosphate absorption by intact tomato plants (*Lycopersicon esculentum* Mill) from a dilute nutrients solution. Zeitschriftfur Pflanze Physiologie. 78:253-261.

De Leon TB, Linscombe S, Gregorio G, and Subudhi PK, (2015). Genetic variation in Southern USA rice genotypes for seedling salinity tolerance. Front Plant Sci. 6:374. https://doi.org/10.3389/fpls.2015.00374.

Hesse PR, (1971). A text book of soil chemical analysis. John Murvay, London.

Hiscox JD, and Israestam GM, (1979). A method for extraction of chlorophyll from leaf tissue without maturation. *Can. J.* 57: 1332-1334.

Hopmans JW, Qureshi AS, Kisekka I, Munns R, Grattan SR, Rengasamy P, Ben-Gal A, Assouline S, Javaux M, Minhas PS, Raats PAC, Skaggs TH, Wang G, De Jong Q, van Lier H, Jiao RS, Lavado N, Lazarovitch BL and Taleisnik E, (2021). Critical knowledge gaps and research priorities in global soil salinity. Adv Agron. https://doi.org/10.1016/bs.agron.2021.03.001.

Krishanamurthy SL, Lokeshkumar BM, Rathor S, Warraich AS, Yadav S, Gautam RK, Singh RK and Sharma PC, (2022). Development of salt-tolerant rice varieties to enhancing productivity in salt-affected environments. Environ. Sci. Proc. 16(1):30. https://doi.org/10.3390/environsciproc2022016030.

Richards LA, (1954). Diagnosis acid improvement of saline-alkali soils. USDA Hand Book No. 60.

Sajid H, Jun-Hua Z, Chu Z, Lian-Feng Z, Xiao-Chuang C, Sheng-Miao Y, James AB, Ji-Jie H and Qian-Yu J, (2017). Effects of salt stress on rice growth, development characteristics, and the regulating ways: A review. J Integr. Agric. 16:2357-2374. https://doi.org/10.1016/S2095-3119 (16)61608-8.

Sheoran OP, Tonk DS, Kaushik LS, Hasija R C and Pannu RS, (1998). Statistical software package for agricultural research workers. Recent Advances in Information Theory, Statistics & Computer Applications by D SHooda and R C Hasija CCS HAU, Hisar (139-143).

Singh RK, Kota S and Flowers TJ, (2021) Salt tolerance in rice: seedling and reproductive stage QTL mapping come of age. Theor Appl Genet 134, 3495-3533. https://doi.org/10.1007/s00122-021-03890-3.

Research Article

Ekin
Journal of Crop Breeding and Genetics

9(2):141-149, 2023

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Accelerated Pepper Breeding Using Molecular Markers and Doubled Haploidy Technique

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Citation:
Nar M., Albezirgan HN., Demirkaya B., Fidan H., Aktaş H., Ellialtıoğlu ŞŞ., 2023. Accelerated Pepper Breeding Using Molecular Markers and Doubled Haploidy Technique. Ekin J. 9(2):141-149.

Received: 14.12.2022 ***Accepted: 13.02.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

ABSTRACT

Hatay pepper is preferred in terms of quality characteristics e.g. hot, fresh and suitable for dried consumption, but it does not contain any disease resistance genes. Within the scope, we established the study is aiming to develop resistant hybrid varieties to TSWV is causing high yield and quality losses in pepper production areas. For this purpose, studies are carried out to obtain inbreed resistant lines in a short time, a Tsw gene found in *Capsicum chinense*, which provides resistance to TSWV, was combined with Hatay pepper lines by crossbreeding. First of all, 20 of the Hatay pepper lines from the gene pool were selected according to weighted grading criteria, then the number was reduced to 4. In *C. chinense* lines, molecular screening was performed with SCAC568 primers and individuals with Tsw gene were determined from donor parents and crosses. After then, backcrosses to Hatay peppers were conducted and BC_1F_1 was generated. Their seed samples were sown from them and brought until the first true leaves during the transition from seed to seedling stage. Meanwhile, molecular analyses were applied to find resistant individuals with Tsw gene. The homozygous and heterozygous plants were planted in a greenhouse and used in anther culture study. Whole study takes 22 months from the initiation of hybridization to the emergence of androgenic embryos and acclimatization to external conditions and development of DH seeds. Here, these findings are presented as a case study in biotechnology and the combined techniques are an indispensable part of accelerated breeding processes.

Keywords: Anther culture, *Capsicum* spp., Tsw gene, Hatay pepper, molecular marker

[**] *This article was presented at fourth International Plant Breeding Congress, November 21-25, 2022, Antalya Türkiye.*

Introduction

Annual pepper production in the world is around 37 million tons (FAOSTAT 2020). Pepper, which is in the same family as tomato and eggplant, ranks second after tomato in terms of production value among Solanaceae vegetables in our country and has a total production amount of 2.6 million tons in an area of 792,617 ha (Anonymous 2020). Pepper is one of the vegetables with a wide distribution of types. Blocky bell peppers, Cubanella, Long sweet/hot, Poblano types of peppers are widely grown in our country. Pepper originated in South America and entered Anatolia in the 16th century and is cultivated. Anatolia, which has different ecologies and is on migration routes, has had a very rich diversity in terms of genetic resources in pepper over the years. One of them is our local variety, which is grown in and around Hatay, also known as Hatay pepper or Samandağ pepper, which is preferred in its smooth pointed form,

and which is also consumed as fresh and dried chili peppers. However, crop losses have been experienced in recent years due to Tomato Spotted Wilt Virus (TSWV), which is one of the leading viral diseases that negatively affect yield and quality in open pepper cultivation. TSWV; causes diseases and losses in important agricultural products such as tomatoes, peppers, eggplant, lettuce, beans, artichokes, celery and tobacco (Şevik 2014). It is estimated that the TSWV causes more than 1 billion dollars of damage to agricultural products every year (Griep et al., 2000). TSWV viral infection causes yield losses of 30-100% (German et al., 1992). The use of resistant cultivars against the disease factor has become mandatory. Resistance is not available in the current native cultivars. Commercial foreign hybrid varieties have this resistance. Although the producer does not want to give up traditional pepper varieties, he necessarily turns to foreign varieties from which he can buy products. If resistance to TSWV factor is not transferred to the native material, it does not seem possible to maintain our genetic resources and existing native varieties in the market. It takes many years to develop varieties with traditional breeding methods, and although the cost is high, its effectiveness remains low. DH technologies especially anther culture in pepper has advantages to short breeding time. On the other hand, complex genotypes are fixed by completely homozygous plants obtained through dihaploidization of haploids (Heberle-Bors 1985, Vagera 1990). Haploids and diploids can be successfully applied to study pepper resistance to viruses (Dumas De Vaulx et al., 1982, Pochard et al., 1983) and transmission of resistance to *Phytophthora capsici* (Abak et al., 1982). It has been determined that the conditions found to be successful in pepper with sufficient androgenesis frequency in previous studies also gave positive results in the Hatay pepper population in the preliminary trials (Nar et al., 2022). The inclusion of folded haploid techniques and molecular marker technologies in breeding programs is a basic need in today's conditions. In this study, the determination of the TSWV resistance gene (Tsw) with a molecular marker and its transfer to selected Hatay pepper breeding lines constituted the first stage of the study. The development of haploid pepper pure lines folded by the androgenesis technique, which is one of the genotypes with resistance in the first backcross generation, is the subject that is aimed to be done in the second stage.

Materials and Methods

In this study, which is aimed at breeding Hatay pepper varieties resistant to TSWV; Two basic biotechnological methods were used to accelerate the classical breeding methods: a. Determining the presence of disease resistance gene with a molecular marker, b. Obtaining homozygous pure lines using the folded haploid technique by androgenesis. To create a pepper gene pool, foreign hybrid cultivars, genitors, and local populations were collected from all over our country, some of which were interbred and some of them were inbred up to the F_4-F_7 stage.

In the first stage, morphological characterization (according to the selected UPOV criteria) and observation of agronomic traits were performed during a growing period in 20 local Hatay pepper breeding lines from the gene pool. Some features selected from the UPOV criteria were examined (UPOV 2021). For example; plant features: Maturation, height; (1 very small-5 large), aspect; (1 spreading-5 upright), leaf length diameter ratio (1<, 1, >1), leaf color (1 light green-5 dark green), fruit set (1 weak -5 good); and fruit features: Length (cm), diameter (cm), Cross-sectional shape, immature fruit color, ripe fruit color, flesh thickness (1 thin-5 thick), stance position (1 up - 5 down), number of lobes, flavor (1 bitter - 5 sweet), Stem gap (absent / present), tip shape, curling at the tip (absent/present), wrinkling (absent/present). Hatay pepper local populations have different fruit shapes (Öntürk and Çürük, 2019). 20 lines used in this study have more or less different characteristics from each other. Following the recording of the observations obtained from this stage, the selection was made with the weighted grading method to determine the 4 lines from the gene pool of AG Seed Co., that are preferred to market primarily (Sönmez et al., 2015).

Determination of the main (repetitive) parent lines by weighted grading method: In 20 local Hatay pepper breeding lines selected Weighed Grading Criteria (Table 1) such as plant structure, plant vigor, yield per plant, leaf cover, bitterness, fruit color and brightness were determined from Hatay type pepper populations, which is one of our local gene resources, and 4 different pepper lines with the highest scores were selected accordingly.

Used plant material and its preparation for speed breeding technologies

Sowing seeds from selected Hatay pepper and TSWV resistant lines in the nursery (greenhouse). Main parent (Hatay pepper lines) and paternal parent (lines carrying the resistance gene) plants and seeds were planted in seedling trays filled with a mixture of peat perlite (3:1) and in a fully controlled seedling greenhouse. They are grown until they have 5 true leaves.

Greenhouses are arranged with materials suitable for growing plants and ventilation system in a way

that will not cause disease and insect damage. To grow the plants in an environment free from diseases and thrips, aphid whitefly pests, applications such as 40 mesh insect netting, yellow and blue sticky traps, chemical control etc. were applied to the greenhouse ventilation openings. When the seedlings reach the stage with 4-5 true leaves, they were transferred to 1000 m^2 polyethylene covered greenhouses with 50x50 row spacing.

When the plants of 4 lines selected from Hatay peppers and 3-4 paternal parents that would give the resistance gene reached the flowering stage, crossing procedure was performed. Pollen was first collected from fully opened paternal flowers into tubes, petri dishes or other suitable material with the help of a vibrator. Then, the fully developed but not opened buds in the main parent, and the anthers that have not yet burst, were selected and the petals and anthers were removed. The stigmas of the emasculated buds were pollinated by dipping into previously collected pollen, and the process was completed by attaching a label to the flower stem. Seeds from the fruits developed as a result of hybridization were harvested during the red maturity period, dried and replanted. In the second half of 2021, at this stage in the autumn, backcrossing was done to Hatay pepper types and their seeds were taken in a healthy way.

In crosses made in 4 different Hatay pepper lines, pollen taken from 4 paternal parents was mixed and used in the pollination process with the mixed pollen technique. This method has a positive effect on obtaining hybrids with high adaptability and seed set rate. When the fruits formed were reddened, the seeds were removed, cleaned and dried. The seedlings were obtained from the seeds and they planted in AG Seed Co.'s greenhouses on August 16, 2021. Hybridizations were started in the bud development period. Thus, backcrossings were started in F_1 plants and each line was crossed with its parent and BC_1F_1 A, B, C and D seeds were reached during the harvest period of the fruits. Seeds of these fruits were used as donor plants in anther culture studies in the 2022 spring period.

Molecular marker resistance tests

Our lines developed from Chile peppers were tested for the presence of the Tsw gene. At this stage, 4 different pepper materials were selected as the parental plants. The pollen of these parental parents was mixed and used in crosses with 4 Hatay pepper lines. The lines formed as a result of hybridizations were tested with the help of molecular markers in the molecular genetics laboratory established within the AG Seed Co. During the seedling period, 100 mg of leaves were taken from the true leaf samples of the pepper plant, and the CTAB protocol (Doyle and Doyle 1987) was applied and DNA was isolated (Figure 1). DNA samples were run on agarose gel and after quality control and concentration equalization, Moury et al., (2000) PCR reaction was carried out using the CAPS marker (SCAC568). The protocol used as follows: SCAC568 primers specific to the Tsw gene were used forward (5'GTGCCAGAGGAGGATTTAT 3') and reverse (5'GCGAGGTGACACTGATACT 3'). The PCR reaction is completed to a final volume of 50 µl with EcoTaq 2x PCR Master Mix 25 µl, Forward primer 10 µM 2 µl, Reverse primer 10 µM 2 µl, genomic DNA 10 pg-500 µg and ddH_2O. Cycle conditions 98 ºC for 1 minute; 94 ºC for 30 seconds, 57 ºC for 30 seconds, 72 ºC for 30 seconds 36 cycles and final elongation at 72 ºC for 1 minute. The mixture prepared as 10 µl of the obtained PCR products, 1 µl of Thermo Scientific FastDigest XbaI enzyme, 2 µl of buffer and 17 µl of ddH_2O was cut at 37 ºC for 5 minutes and incubated at 65 ºC for 15 minutes. Laboratory processes were completed with gel readings (Nar et al., 2022).

The PCR result of Scac 568 primer is given in Figure 2 and the gel image after cutting with XBAI enzyme is given in Figure 3.

Androgenesis studies

In anther cultures, buds in the morphological development stage must be collected, subjected to surface sterilization in the laboratory and then cultured. It has been determined in previous studies that the buds in the suitable microspore period in pepper are the period when the petals and sepals reach the same level or pass 1-2 mm (Çömlekçioğlu and Ellialtıoğlu 2018). The blue-purple color transformation at the tips of the anthers is the key feature.

The protocol, which includes combinations of MS nutrient medium, activated charcoal, $AgNO_3$, NAA and BAP in pepper, gives very successful results (Alremi et al., 2014). Using the same method with some modifications is also recommended by Keleş et al., (2015). They used MS medium containing 4 mg/L NAA, 1.0 mg/L BAP, 0.25% activated charcoal, 30 g/L sucrose, 7 g/L agar and 15 mg/L silver nitrate was used as nutrient medium. In this study, process flow in anther cultures Bat et al., (2020) according to the method described (Figure 4). After the prepared nutrient media were adjusted to pH 5.8, they were sterilized in an autoclave at 121 ºC for 20 minutes. The media removed from the autoclave was poured into petri dishes with a diameter of 60 mm in a sterile cabinet in equal amounts and left to solidify.

The cultures were incubated in a growth cabinet at a temperature of 25±1 ºC, with 16 hours of light and 8 hours of darkness (Vural et al., 2019). After the anthers

were cultured, embryo emergence was observed in the 30-70 days of the incubation period in the climate chamber and the emergence was determined. A few days after the embryos were seen, they were first arranged in contact with the same environment, then germinated in hormone-free MS medium.

Plantlets that formed fringe roots and 4-6 true leaves were planted in mini pots filled with autoclaved ½ perlite + ½ peat mixture, giving life water. Developed plants were transferred to the greenhouse.

Results and Discussion

Identification of suitable lines from the gene pool

Weighed grading has been used for many years as a statistical and consistent selection method used in the selection of starting material suitable for breeding purposes from the gene pool or in highlighting the candidate variety among the variants obtained after the breeding program (Sönmez et al., 2015). It was also used effectively in the selection of breeding material for our study. Figure 5 shows the 4 Hatay pepper genotypes selected to be used as replicates in the study.

To transfer the Tsw gene to the sensitive Hatay peppers, the chile peppers at the F_1-F_3 stage found in our gene pool were tested with the molecular marker technique, and the genitors whose Tsw gene presence was confirmed and similar to the structure of the Hatay type pepper were selected.

Crossbreeding, generation advancement

Crosses were made with 4 selected Hatay-type pepper lines and Tsw-resistant chile peppers with mixed pollen technique. Harvesting and seed extraction of fruits that are at the stage of red ripening in the greenhouse was performed (Figure 6). Backcrossing was carried out on the plants grown from the seeds obtained. Backcrossings in the autumn season were made towards Hatay pepper types and their seeds were taken in a healthy way. Obtained individuals were subjected to molecular marker testing and it was examined whether they contain the Tsw gene. Those containing the resistance gene were used as donor plants for androgenesis studies.

After the backcrossing, molecular marker tests were carried out in BC_1F_1 generation plants of 4 different Hatay lines using SCAC568 primers specific to the Tsw gene. In this context, the lines whose seeds were taken as a result of crosses in the previous season were grown and samples were taken from the real leaves while they were still in the seedling stage, and PCR tests were carried out after total nucleic acid isolation. Approximately 376 plants were tested during the season (Table 2). As a result, individuals with homozygous or heterozygous resistance were separated from individuals with homozygous recessive disease susceptibility characteristics in both alleles. Plants found to contain the resistance gene were transferred to the donor plant growing greenhouse to be used in the process of obtaining DH-lines by androgenesis method, and anther culture was made from them at the stage of flower bud formation.

From androgenesis studies, haploid embryo formation was obtained successfully (Figure 7). The process of obtaining haploid plants, chromosome doubling and obtaining DH seeds are completed. DH seeds harvested and stored at the cool conditions (+7 °C) in the paper bags.

In Table 3, anther planting was made in the spring of 2022 and the embryo and plant numbers taken from them are given. Embryo emergence is continuing. Embryo formation frequencies close to each other were obtained from anthers taken from backcross donor plants in lines A, B, C and D, which have genetically similar characteristics. An average embryo formation frequency of 2.05% was obtained. This number within the embryo formation frequency range compatible with many previous studies (Çömlekçioğlu and Ellialtıoğlu 2018; Atasoy et al., 2021). With the increase in the performance of the working system and personal over time in the laboratory, it can be predicted that this ratio will also improve somewhat. However, since the frequency of embryo formation is a genetic feature, it is thought that the average success will still be in the same range.

Conclusions

This article has been prepared from a presentation made to provide an overview of the phases carried out in a project that lasted 3 years, and to share the outputs. For this reason, it is designed to explain the flow of the breeding program rather than giving the details and procedure of a single study. The study mainly consisted of the following stages and was performed successfully:

a. To identify 4 prominent genotypes from the Hatay pepper gene pool;

b. Identifying parental parents with the Tsw resistance gene from the chile pepper gene pool using molecular markers;

c. Crossing and backcrossing;

d. Performing anther cultures from BC_1 F_1 plants and obtaining Tsw resistant DH lines.

In order to develop hybrid varieties in pepper, obtaining parent lines requires 6 generations of selfing, and breeding takes a long time. Pepper androgenesis, which was first initiated in the 1980s in Turkey, can be used successfully (Çömlekçioğlu and Ellialtıoğlu 2018). Until recently, almost all of the F_1 hybrid

varieties grown in our country were imported from abroad. In recent years, domestic hybrid varieties have started to take place in the market in some of the vegetable species, thanks to the establishment of their R&D enterprises by local seed companies, projects and incentives received with from government facilities. With the subject of trade in pepper and pepper products, an economic activity of approximately 1 billion dollars occurs in Turkey. In 2016, around 40,000 k of open-pollinated and 350 k of hybrid pepper seeds were produced, and some of the hybrid pepper seeds, all of which were purchased from abroad, could be met by domestic companies. The number of hybrids is less because Turkish consumer habits have peculiar characteristics in pepper, these demands cannot be met with varieties produced abroad, and most of the varieties grown in the open are local populations. Significant developments are expected in the seed sector in Turkey as well. Firms need to constantly increase their competitiveness.

Since tissue culture and MAS technological infrastructure will contribute to product development in new areas, it will bring important innovations in the breeding process and shorten the development period of the variety. Obtaining pure lines in a short time, dihaploidization technique and anther culture can be practically integrated into breeding studies. When MAS technology is combined with selection and backcrossing programs, both faster and more reliable and easy results are obtained.

With the use of both techniques, it is possible to complete a 10-12 year breeding program in a short period of 3 years. Short-term breeding efforts and the rapid introduction of new varieties will generally improve the seed potential of Turkey. Thanks to the transfer of TSWV resistance trait with MAS, which is one of the accelerator breeding techniques, and the acquisition of DH plants through androgenesis, qualified genotypes were obtained in about 2 years. With this ongoing study, inbreed lines in which the TSWV disease resistance gene is transferred to Hatay-type peppers, which is one of the genetic resources of Turkey, are successfully obtained. This material will be used as a parent in hybrid cultivar breeding and thus disease resistant local pepper varieties will be developed. In this way, a chance to compete in the seed sector can be provided.

Acknowledgements

This project was supported by TÜBİTAK TEYDEB 1507 SME R&D Startup Support Program. (Project No: 7210289). The authors thank TÜBİTAK for their contributions.

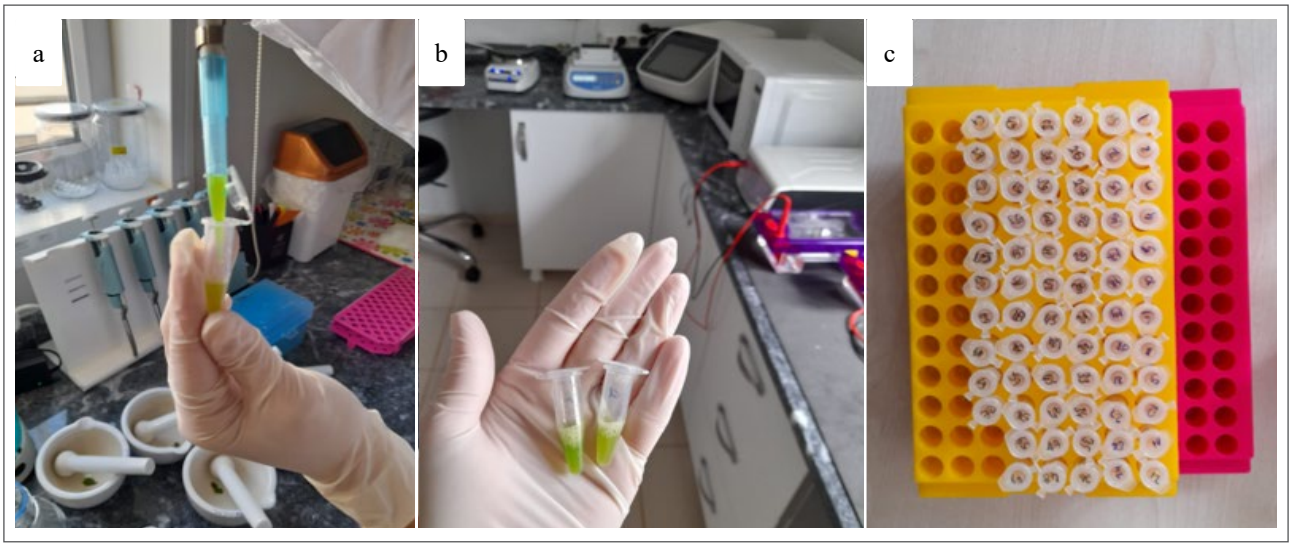

Figure 1. Examples of DNA isolation steps. a. Crushing the leaf samples with CTAB solution in a mortar, b. Transferring the crushed samples to 1.5 microtubes, c. Arrangement of the crushed samples. (Original)

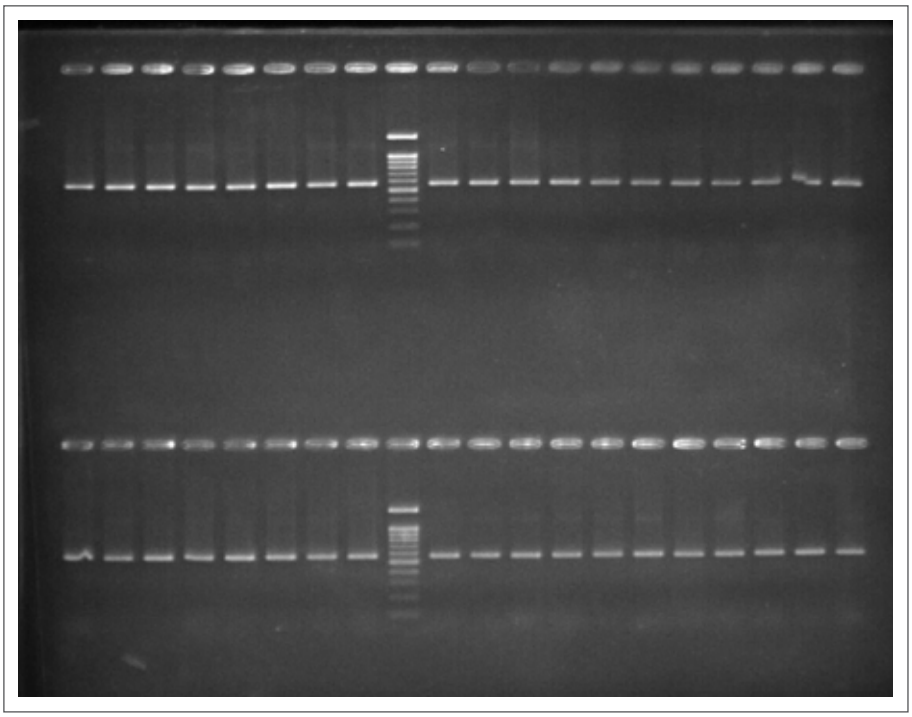

Figure 2. The PCR result of SCAC 568 primer.

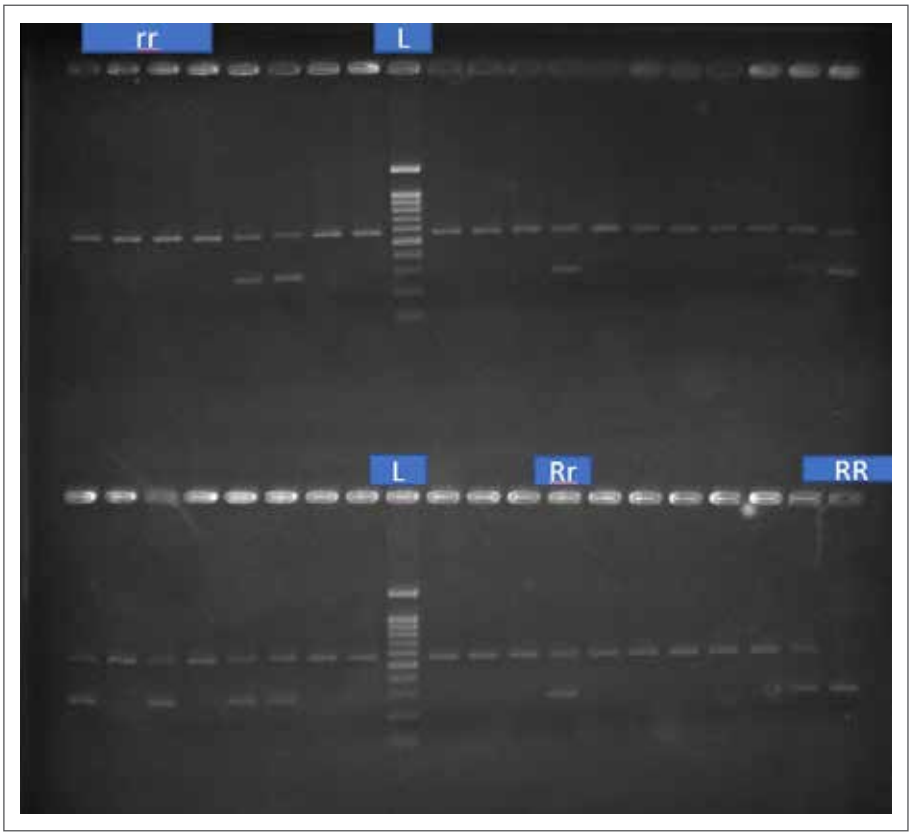

Figure 3. The gel image after cutting with Xbal enzyme (280bp:RR, 280bp, 568bp:Rr, 568bp: rr, L: 100bp ladder)

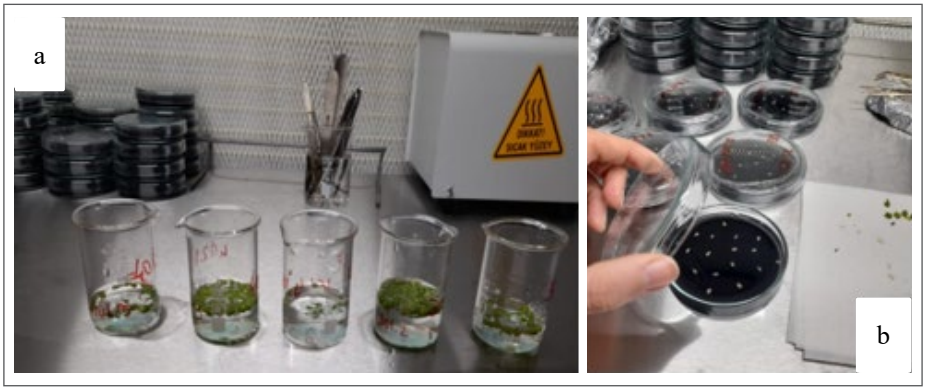

Figure 4. Anther culture stages. a. Disinfection of buds in 15% sodium hypochlorite for 12 minutes, b. Culturing of anthers in nutrient media. (Original)

Figure 5. Plant habitus and fruit appearance of selected Hatay type peppers (a-b Hatay 1, c-d Hatay 2, d-e Hatay 3, f-g Hatay 4). (Original)

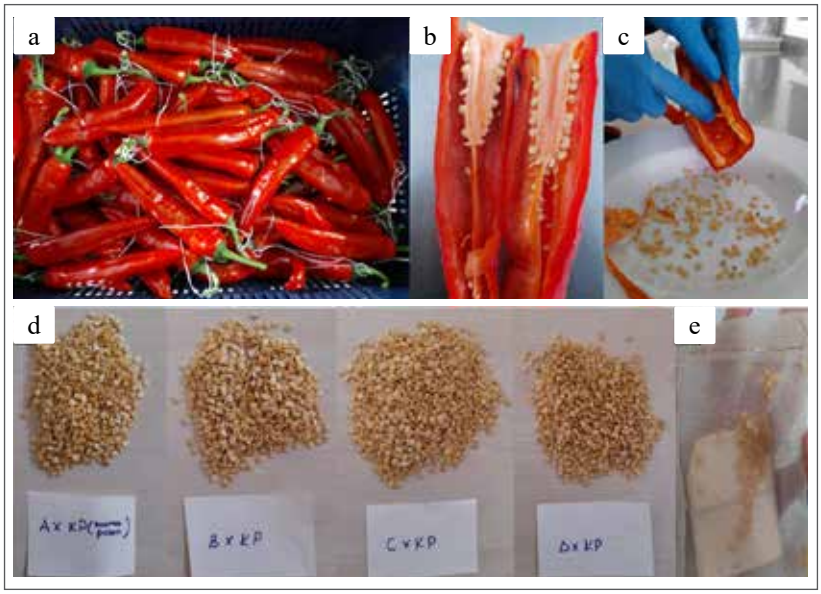

Figure 6. Cleaning and packaging of the fruits and seeds obtained as a result of the hybridization of Hatay pepper lines with Tsw-resistant peppers. (Original)

Figure 7. Development of pepper embryos from microspores and their transformation into plants (above); Growing haploid plantlets *in vitro*, transferring to soil and acclimatization to external conditions (below). (Original)

Table 1. Weighed grading observation criteria.

Observation Criteria	Weight in the Selection (%)
Bitterness	30
Yield per plant	20
Plant power	10
Plant habitus (Closed)	10
Fruit shape	10
Fruit color (dark green)	10
Brilliance in fruit	10

Table 2. Molecular marker (Tsw) test results.

Number of Plants Tested	Homozygous (RR)	Heterozygous (Rr)	Susceptible (rr)	No Tape
94	17	67	6	4
94	13	52	19	10
94	6	70	14	4
94	13	56	17	8
Total=376	**49**	**245**	**56**	**26**

Table 3. Anther numbers from plants from four different backcross (BC) combinations, their ratios with the number of embryos and plantlets obtained from them.

Genotype	Number of Anthers Cultured	Formed Embryo Number	The Number of Plantlets Obtained	Embryo Formation Rate (%)	Plant Formation Rate (%)
BC_1F_1 A	4000	82	73	2.05	1.83
BC_1F_1 B	3500	71	50	2.02	1.42
BC_1F_1 C	3500	96	81	2.40	2.31
BC_1F_1 D	3000	52	38	1.73	1.26
Total	**13500**	**301**	**242**	**2.05**	**1.71**

References

Abak K, Pochard E and Dumas De Vaulx R, (1982). Transmission of resistance to *Phytophthora capsici* on root and stems of pepper plants: Study of doubled haploid lines issued from the cross "PM 217 x Yolo Wonder" through anther culture. Capsicum Newsletter 1:62-63.

Alremi F, Taşkın H, Sönmez K, Büyükalaca S and Ellialtıoğlu Ş, (2014). Biber (*Capsicum annuum* L.)'de genotip ve besin ortamının anter kültürüne etkileri. Türk Tarım ve Doğa Bilimleri Dergisi 1 (2): 108-116, https://doi.org/10.30910/turkjans.160702 (in Turkish).

Anonymous, (2020). TÜİK kurumsal. https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim istatistikleri 2020 33737 (Erişim Tarihi: 23.02.2021)

Atasoy D, Baktemur G and Taşkın H, (2021). Bazı biber (*Capsicum annuum* L.) genotiplerinin anter kültürü performanslarının belirlenmesi. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi 31(2):282-293. (in Turkish)

Bat H, Shidfar M, Çömlekçioğlu N and Ellialtıoğlu ŞŞ, (2020). *In vitro* androgenesis in pepper and the affecting factors on success: I. Carbon source and concentrations. Biotech Studies, 29(2):62-68.

Çömlekçioğlu N and Ellialtıoğlu ŞŞ, (2018). Review on the research carried out on *in vitro* androgenesis of peppers (*Capsicum annuum* L.) in Turkey. Res. J. Biotechnol., 13(6):75-84.

Doyle JJ and Doyle JL, (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bulletin, 19:11-15.

Dumas De Vaulx R, Pochard E and Chambonnet B, (1982). Distribution of TMVpo susceptible and resistant doubled haploid lines from anther culture of heterozygous L+ /L1 hybrids. Capsicum Newsletter 1:52-53.

Ellialtıoğlu Ş, Kaplan F and Abak K, (2001). The effect of carrot extract and activated charcoal on the androgenesis of pepper. In Proceedings XIth EUCARPIA Meeting on Genetics and Breeding of Capsicum & Eggplant, (Vol.9, p.13), Antalya-Türkiye

FAOSTAT, (2020). Crop Statistics http://www.fao.org/faostat/en/#data/QC/visualize. (Accessed 09.01.2020)

German TL, Ullman DE and Moyer JW, (1992). Tospoviruses: Diagnosis. Molecular biology, phylogeny and vector relationships. Annu. Rev. Phytopathol. 30:335-348.

Griep RA, Prins M, van Twisk C, Keller HJHG, Kerschbaumer RJ, Kormelink R, Goldbach RW and Schots A, (2000). Application of phage display in selecting tomato spotted wilt virus-specific single-chain antibodies (scFvs) for sensitive diagnosis in ELISA. Phytopathology, 90(2):183-190. doi:10.1094/phyto.2000.90.2.183

Heberle-Bors E, (1985). *In vitro* haploid formation from pollen: A critical review. Theor. Appl. Genet. 71: 361-374.

Keleş D, Pınar H, Ata A, Taşkın H, Yıldız S and Büyükalaca S, (2015). Effect of pepper types on obtaining spontaneous doubled haploid plants via anther culture. Hort Science, 50(11):1671-1676.

Nar M, Albezirgan HN, Demirkaya B, Fidan H, Aktaş H and Ellialtıoğlu ŞŞ, (2022). Accelerated pepper breeding studies with MAS and androgenesis techniques. IV. International Plant Breeding Congress Plant Breeding for the Future: From Local to Global, Antalya Türkiye, 21-25 November 2022, Proceedings pp: 250-259.

Öntürk G and Çürük S, (2019). Hatay'ın farklı ilçelerinde yetiştirilen biber köy popülasyonlarında bitki ve meyve özellikleri. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 29 (4):689-701. doi: 10.29133/yyutbd. 639261. (in Turkish)

Pochard E, Dumas De Vaulx R and Florent A, (1983). Linkage between partial resistance to CMV and susceptibility to TMV in the line "Perennial". Capsicum Newsletter 2:34-35.

Sönmez K, Ellialtıoğlu ŞŞ and Oğuz A, (2015). Yerel sofralık domates gen kaynaklarının değerlendirilmesinde tartılı derecelendirme yönteminin kullanımı. Tarım Bilimleri Araştırma Dergisi (TABAD), 8(1):14-19. (in Turkish)

Şevik M, (2014). Domates lekeli solgunluk virüsü (TSWV)'nün tarımsal ürünlerde meydana getirdiği ekonomik kayıplar. Harran Tarım ve Gıda Bilimleri Dergisi, 15(1):35-42. https://dergipark.org.tr/en/pub/ harranziraat/ issue/18434/194124. (in Turkish)

UPOV (2021). https://www.upov.int/ (Accessed: 21.12.2022).

Vagera J, (1990). Pepper (*Capsicum* spp.): *In Vitro* induction of haploids. In: Biotechnology in agriculture and forestry, Vol:12, Haploids in Crop Improvement I (ed. by Y.P.S. Bajaj), Springer-Verlag Berlin Heidelberg, pp 374-392.

Vural G, Arı E, Zengin S and Ellialtıoğlu ŞŞ, (2019). Development of androgenesis studies on eggplant (*Solanum melongena* L.) in Türkiye from past to present. In book: Sustainable Crop Production (Ed: Dr. Mirza Hasanuzzaman) ISBN 978-1-78985-318-6 doi: 10.5772/intechopen.88299. pp: 1-27.

Research Article

Ekin
Journal of Crop Breeding and Genetics

9(2):150-159, 2023

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Genetic Variability and Correlation Coefficient Analysis in Wheat Genotypes for Grain Yield and Its Contributing Traits under Drought and Irrigated Condition

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Citation:
Rana P., Bishnoi OP., Chaurasia H., Behl RK., 2023. Genetic Variability and Correlation Coefficient Analysis in Wheat Genotypes for Grain Yield and Its Contributing Traits under Drought and Irrigated Condition. Ekin J. 9(2):150-159.

Received: 08.05.2023 ***Accepted: 16.06.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

ABSTRACT

Direct phenotypic selection in wheat improvement program requires preliminary knowledge of traits association degrees. In this study, a field experiment was conducted on the wheat crop in two different conditions (irrigated and drought), in order to determine the degree and direction of the association between grain yield and its attributing characters. The experimental findings indicated that correlation coefficients showed a highly significant and positive association between grain yield and harvest index followed by above ground biomass. However, other traits have a significant indirect impact on grain yield through the harvest index and above ground biomass. According to this, choosing genotypes with higher yields would be more effective if selection were based on these traits. The minimum yield reduction irrigated conditions was observed for the genotypes WH1127, WH1164, WH1105, WH1080, IC498438, EC609554, and EC609575. In the light of the fact that these genotypes have the higher yield potential under moisture stress condition and could be utilized as donors in bread wheat improvement program for drought tolerance.

Keywords: Correlation coefficient, GCV, grain yield, PCV and wheat

Introduction

The bread wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD), the third-largest cereal food crop in the world, is ranked first in terms of cultivated area and second in terms of production among cereal crops worldwide (FAO, 2020). With a projected total production of 765.53 million tons in the 2018/2019 cropping season, wheat was grown on approximately 218.22 million hectares across the world (USDA, 2022). To feed the anticipated 9.1 billion people by 2050, production of cereals should increase by 1 billion tons year, according to estimates (FAO 2015). In order to satisfy the rising demands for food supply, the current situation requires an increase in crop productivity (Iqbal et al*.,* 2017). Water scarcity is a severe drawback that limits the area under cultivation and agricultural output in arid and semi-arid regions all over the world.

Abiotic stresses have a significant impact on wheat grains' protein content, which alters the quality of baked goods (Zorb et al., 2018). According to Rakszegi et al*.,* (2019), wheat grain's protein, gliadins, glutenin, and fiber composition are all significantly impacted by drought stress. Drought stress significantly alters the crop's physiological, morphological, biochemical and molecular mechanisms which hinders plant growth (Sultan et al., 2012). Drought can happen at any stage of growth and development (Belay et al., 2021). High-yielding cultivars with superior water usage efficiency can be essential in environments with extreme drought, but progress has been delayed as a result of the unpredictable nature of drought and the complex genetic regulation of plant responses.

Wheat grain yield is a complex polygenic trait that is impacted by a wide range of variables and

can be improved based on yield components via indirect selection. In such a way, that increase in one component may affect the other components positively or negatively. Understanding the genetic makeup of yield component parts is crucial for this reason. The tillers no., grain weight (1000), length of spike and number of spikelet per spike, etc., are associated that could be utilized for estimating the grain yield (Li et al., 2020). Drought not only influences the morphological characteristics of wheat crop, but also wheat physiology. Several physiological characters have higher heritability and could be helpful in enhancing the drought tolerance of wheat crop. Evidently, stay-green phenotypes can increase their performance under moisture stress conditions with delayed leaf senescence (Lopes and Reynolds, 2012).

Evaluation of morpho-physiological changes under drought may aid in genetic improvements of wheat genotypes since bread wheat differentially respond at vegetative growth phases under water deficit situation (Chowdhury et al., 2021). Understanding the morpho-physiological traits linked to grain yields under moisture stress is crucial for improving bread wheat (Zhang et al., 2016). Wheat breeders must focus on improving drought resistance, which is genetically determined and had a significant impact in the stability of the crop. Improvement in grain yield and stress tolerance has primarily been attained through empirically or through grain yield selection.

Subhani et al., (2000) observed significant and positive association among the morphometric traits such as biomass, harvest index, tillers no. (per plant), length of peduncle, length of spike, 1000-grain weight, and number of grains per spike, grain yield per plant but significant and negative correlation of days to heading with grain yield per plant was also found. According to Khames et al., (2016) and Jan et al., (2017), grain yield had a positive phenotypic association with 1000-grain weight and number of tillers per plant. In a study, Rehman et al., (2015) examined the behavior of 100 genotypes of wheat under drought stress conditions. The findings showed that there was a positive and significant correlation between spike length, peduncle length, spikelet per spike, and grain yield. The current study's objective was to identify important drought adaption characters and variations in these traits in bread wheat grown both under irrigation and moisture stress conditions.

Material And Methods

Experimental Materials:

The experimental material included 40 wheat germplasm accessions whose descriptions are shown in Table 1.

Layout of Field Experiment

At the Field Research Area, CCS HAU, Hisar, seeds of all forty genotypes were sown in a randomized block design (RBD) with three replications during *rabi* 2018–19. The study was carried over the course of a year, with each plot consisting of paired rows of 2.5 m length, with row to row distance of 20 cm and plant to plant 10 cm in every replication. At 40-45 days after sowing, one irrigation was given for drought condition; however, under irrigated condition, six irrigations were given.

Observations Recorded and Statistical analysis

Except for the days to heading and days to maturity, observations were recorded on five randomly chosen plants from each genotype in each replication. The data were recorded for days to heading, plant height (cm), days to maturity, peduncle length (cm), number of effective tillers per plant, spike length (cm), number of grains per spike, harvest index (%), above ground biomass per plant (g), canopy temperature, 1000-grain weight (g), grain yield per plant (g), NDVI and SPAD. Instrument Green Seeker TM, a handheld optical sensor, was used to record NDVI carried out at Z45 stage according to Zadoks Growth Scale. Using a SPAD-502 chlorophyll meter, the mean chlorophyll of the flag leaves of five tagged plants was calculated for SPAD at Z49 stage. With a handheld infrared thermometer, CT was measured between 12:00 and 14:00 at the Z47 stage in clear and sunny weather.

Result and Discussion

According to the findings of the current study, under both conditions, grain yield per plant had the highest GCV, followed by above ground biomass per plant, harvest index, no. of effective tillers per plant, 1000-grain weight, no. of grains per spike, plant height, and so on. Compared to genotypic coefficient of variance, phenotypic coefficient of variation was higher for each of the characters under study. Usually, phenotypic variance exceeds its corresponding genotypic variance, but minimal variance must be attained to increase the heritability. Similar findings were made by Bhushan, et al., (2013) and Abinasa et al., (2011). Among both the conditions, PCV was higher in irrigated and it was found to be highest for the grain yield per plant followed by above ground biomass per plant, harvest index, effective tillers per plant, 1000-grain weight, grains per spike, and so on.

The estimates of broad sense heritability ranged from 70.20% - 96.75% under irrigated condition and 72.82%- 98.13% under drought condition. Characters which exhibited high heritability included plant height, days to heading, above ground biomass (per plant),

grains per spike, length of peduncle, grain yield per plant, and effective tillers per plant. Under moisture stressed condition, estimates of high heritability were also reported by Itam et al., 2021 for plant height (81%), days to heading (84%), and 1000-grain weight (91%); Olbana et al., 2021 for biomass yield, days to heading, plant height, days to maturity and spikelets per spike; Shamuyarira, et al., 2019 for days to heading (78.8%) and Semahegn et al., 2020 for days to heading (91.5%), days to maturity (80.5%), spike length (79.2%), spikelets per spike (78.4%), and plant height (74.1%).

Another crucial selection factor that helps breeders in a selection program is genetic advance. The highest genetic advance was found for plant height, which was followed by above ground biomass per plant, grains per spike, days to heading, harvest index, 1000-grain weight, peduncle length, grain yield per plant, days to maturity, effective tillers per plant, and spike length. In the addition, grain yield per plant had the highest genetic advance as percentage mean, followed by above ground biomass per plant. Similar findings were reported by Birhanu et al., (2017). Alemu et al., (2019) reported lowest value of genetic advance as percentage of mean for days to maturity and also in this study. High genetic advance as percentage of mean was observed by Singh et al., (2014) for grain yield and number of tillers per plant. Prior to making a selection, estimates of heritability are helpful for figuring out the characters that must be taken into account, but making a decision solely based on this aspect might restrict advancement, because it is vulnerable to environmental changes. Subsequently under drought stress condition, for grain yield per plant and above ground biomass high heritability values along with high genetic advance as percentage of mean were observed. Therefore, for the genetic enhancement under drought condition, immediate selection can be performed using such traits.

Aslani et al., (2012) and Hassan et al., (2016) also noted high variability for component traits and grain yield per plant, and Pokhrael et al., (2012), Singh et al., (2018), and Adnan et al., (2013) observed high variability for biomass yield. (Table 3, 4, 5 and 6) According to mean performance of the quantitative traits, genotypes WH1127, WH1164, WH1105, WH1080, IC498438, EC609554, and EC609575, displayed the least variation between irrigated and drought conditions. Meanwhile, genotypes such as EC178071-210, and IC529429 were found to exhibit a little high grain yield per plant and harvest index in drought condition over irrigated while genotype IC529189 displayed better expression of NDVI, C.T, and SPAD.

The Pearson's correlation coefficients between traits under stressed and non-stressed conditions are shown in Table 7, 8, 9 and 10. From the Correlation research, genetic upgradation in one character could be accomplished by choosing the other pair. Correlation analysis determines the reciprocal association between several plant characteristics and determines the individual characteristics on which selection can be centered for the enhancement of genetic yield. It is possible to predict that the least divergent genotypes will be the most stable for the given character.

According to this study, under irrigated conditions, grain yield per plant was positively associated with harvest index, above ground biomass per plant, grains per spike, spike length and days to heading, whereas under drought conditions, it was positively associated with above ground biomass followed by harvest index, effective tillers per plant, plant height, and 1000-grain weight. Similar outcomes were attained by Baye et al., (2020) and Javed et al., (2022). Effective tillers per plant associated positively with above ground biomass per plant under both conditions, but negatively with harvest index under the irrigated condition and positively but insignificantly under the drought condition. According to Munir et al., (2007), biological yield had a positive and significant relationship with grain yield and tillers per plant but a negative relationship with days to heading and harvest index. The simple correlation analysis by Ayer et al., (2017) in advanced wheat genotypes revealed a significant positive association between grain yield and 1000-grain weight, plant height, biomass yield, and harvest index. Under both conditions, plant height had a positive association with peduncle length, effective tillers per plant, 1000-grain weight, and biological yield per plant, however, a negative association with harvest index under the irrigated condition. Reza et al., (2014) provided evidence of a significant and positive association between plant height and grain yield in drought-like conditions. According to Gelalcha and Hanchinal (2013), under both conditions, there existed a positive and significant association among number of grains per spike and grain yield per plant. Days to heading showed a negative association with biological yield per plant under both the conditions.

Both the number of grains per spike and the number of spikelets per spike were found to be positively correlated with grain yield. H. Fouad (2018) reported findings that were comparable. Under both conditions, peduncle length had a positive correlation with 1000-grain weight and above ground biomass per plant but associated negatively with harvest index under irrigated condition. Meanwhile, days to maturity showed

a positive association with plant height and peduncle length in both conditions, as well as with grains per spike in case of drought. Days to maturity were found to be positively associated with above ground biomass per plant under both circumstances, though not significantly. According to Talebi (2011) and Kumari et al., (2012), chlorophyll content is associated positively with grain yield and a significant and positive association was found between SPAD and grain yield under both condition while under drought condition C.T showed a negative association with the grain yield. According to Kashif and Khaliq (2004), under drought conditions, a significant but adverse phenotypic association between canopy temperature and the number of grains per spike was found. Chlorophyll content is a crucial characteristic that can be used as a stand-in for drought resistance and increased grain yield.

The relationships between these characteristics revealed that they are governed several common genes, can be used as a selection factor in breeding projects. The other correlated traits would also get better as a result of the positive selections for one trait. According to the results of this study, genotypic correlations in drought tolerance may produce predictable correlated responses that can be used to choose wheat that is drought-tolerant in breeding programs.

Conclusions

This study showed that the traits in wheat germplasm have a wide range of variability. Under irrigated condition, PCV was higher than drought condition. Plant height exhibits high heritability and high genetic advance, suggesting that selection may be used to further improve this trait. Under both the conditions, above ground biomass per plant and harvest index showed a highly significant and positive association with the grain yield per plant. Characters namely, no. of effective tillers per plant, grains number per spike, grain weight (1000) and spike length etc. exhibited significant indirect effects on the grain yield per plant via harvest index and above ground biomass per plant in path coefficient analysis. Crop varieties with higher levels of chlorophyll and slower chlorophyll deterioration may be more drought-tolerant. Physiological characters like SPAD showed highly significant and positive association with the grain yield.

For most of the studied characters, it seemed that genotypes WH1127, WH1164, WH1105, WH1080, IC498438, EC609554, and EC609575 showed the least yield reduction under both irrigated and dry environments. Additionally, under drought conditions, genotypes IC498438, EC609575, HD2967, EC178071-210 and IC529429 demonstrated a slightly higher grain yield per plant and harvest index. It is possible to say that these genotypes have a higher yield potential and direct selection of these genotypes will be beneficial for future breeding programs.

Acknowledgement

The planting materials for this experimental work were provided courtesy of NBPGR and CCS HAU Hisar, and they are greatly appreciated. All helping hands, from planting to data analysis and interpretation, are gratefully acknowledged.

Table 1. An inventory of the wheat accessions used in the research program.

Sr. No	Genotypes	Sr. No	Genotypes	Sr. No	Genotypes	Sr. No	Genotypes
1	IC529429	11	IC539103	21	EC609575	31	WH1142
2	DT5	12	DT25	22	EC609594	32	WH147
3	IC558801	13	DT45	23	EC609563	33	WH1126
4	IC529909	14	IC539162	24	EC609550	34	WH1127
5	IC539456	15	IC145729	25	EC178071-631	35	WH1164
6	IC539543	16	IC529210	26	EC178071-210	36	WH1235
7	IC529189	17	IC296762	27	EC177816	37	WH1105
8	IC539518	18	IC128157	28	EC609589	38	HD 2967
9	IC539167	19	IC543376	29	C306	39	HD3086
10	IC498438	20	EC609554	30	WH1080	40	DPW621-50

Table 2. Statistical approaches adopted.

Sr. No	Statistical Analysis	Reference	Year
1	ANOVA	Ronald Fisher	1918
2	Correlation Coefficient	Al-Jibouri et al.,	1958
3	Variance Coefficients (GCV and PCV)	Burton	1953
4	Heritability (in broad sense)	Hanson	1956
5	Genetic advance	Johnson et al.,	1955
6	Genetic advance as percent of mean	Johnson et al.,	1955

Table 3. Mean performance, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, genetic advance and genetic advance (GA) as per cent of mean under irrigated condition.

Characters	Mean	Range	GCV (%)	PCV (%)	Heritability (h^2_{bs}) (%)	Genetic Advance	Genetic Advance as % Mean
Days to heading	103.13	92-119	5.59	5.74	94.92	11.57	11.22
Days to maturity	149.24	146-153	1.07	1.25	73.25	2.81	1.88
Plant height	106.18	85-138	12.21	12.41	96.75	26.28	24.75
Spike length	11.49	10-14	10.03	11.67	73.85	2.04	17.76
Peduncle length	37.67	31-49	10.65	11.69	83.02	7.53	20.00
Effective tillers per plant	9.75	6-13	14.62	16.78	75.93	2.54	26.25
Grains per spike	58.27	44-76	12.58	13.60	85.51	13.96	23.96
1000-grain weight	38.76	24-49	12.72	15.41	70.65	8.54	22.03
Grain yield per plant	11.38	7-19	22.65	25.68	77.79	4.68	41.16
Biological yield per plant	34.92	20-52	21.03	21.79	93.12	14.60	41.82
Harvest index	33.47	25-41	19.51	23.28	70.20	11.27	33.68

Table 4. Mean performance, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, genetic advance and genetic advance (GA) as per cent of mean under drought condition.

Characters	Mean	Range	GCV (%)	PCV (%)	Heritability (h^2_{bs}) (%)	Genetic Advance	Genetic Advance as % Mean
Days to heading	101.50	86-119	5.77	5.92	93.62	11.69	11.51
Days to maturity	143.49	141-147	0.85	0.98	74.84	2.18	1.51
Plant height	99.75	77-139	14.87	15.01	98.13	30.28	30.36
Spike length	11.26	9-14	10.20	11.51	78.52	2.09	18.63
Peduncle length	37.00	28-52	14.03	15.82	78.66	9.50	25.64
Effective tillers per plant	9.47	6-15	17.81	19.53	83.16	3.14	33.47
Grains per spike	42.42	33-58	12.75	14.65	75.68	9.69	22.85
1000-grain weight	37.87	24-48	15.35	17.99	72.82	10.21	26.98
Grain yield per plant	10.80	7-14	18.67	21.52	75.30	3.59	33.38
Biological yield per plant	30.50	24-39	15.03	15.71	91.56	9.04	29.64
Harvest index	35.59	26-41	15.61	18.00	75.20	9.92	27.89

Table 5. Mean performance, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, genetic advance and genetic advance (GA) as per cent of mean for physiological traits under irrigated condition.

Characters	Mean	Range	GCV (%)	PCV (%)	Heritability (h^2_{bs}) (%)	Genetic Advance	Genetic Advance as % Mean
NDVI	0.82	0.79-0.85	1.45	2.15	45.80	0.01	2.02
C.T	17.74	16-20	4.09	4.96	67.85	1.23	6.94
SPAD	46.87	35-54	7.54	8.63	76.24	6.35	13.56

Table 6. Mean performance, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, genetic advance and genetic advance (GA) as per cent of mean for physiological traits under drought condition.

Characters	Mean	Range	GCV (%)	PCV (%)	Heritability (h^2_{bs}) (%)	Genetic Advance	Genetic Advance as % Mean
NDVI	0.77	0.68-0.82	3.15	4.55	47.91	0.03	4.49
C.T	17.92	16-20	2.80	6.90	16.50	0.42	2.34
SPAD	49.15	45-52	3.07	5.41	32.25	1.76	3.59

Table 7. Phenotypic correlation coefficient among yield and yield attributes under irrigated condition.

Characters	DH	DM	PH	SL	PL	T/P	G/S	TGW	BY	HI
DH	1									
DM	0.164	1								
PH	-0.012	0.351**	1							
SL	0.071	0.144	0.192*	1						
PL	-0.070	0.262**	0.674**	0.174	1					
T/P	-0.039	0.078	0.213*	-0.091	0.218*	1				
G/S	0.029	-0.058	0.124	0.309**	0.162	-0.092	1			
TGW	-0.142	0.206*	0.476**	0.003	0.288**	-0.010	0.154	1		
BY	-0.094	0.164	0.492**	0.345**	0.476**	0.371**	0.245**	0.321**	1	
HI	0.395**	-0.023	-0.353**	0.039	-0.344**	-0.246**	0.153	-0.016	-0.338**	1
GY	0.283**	0.096	0.076	0.346**	0.070	0.082	0.371**	0.192*	0.479**	0.596**

* Significant at p= 0.05, ** Significant at p= 0.01

DH-Days to heading, DM-Days to maturity, PH-Plant height (cm), SL-Spike length (cm), PL-Peduncle length (cm), T/P- Effective tillers plant^{-1}, G/S- Grains spike^{-1}, TGW- 1000-grain weight (g), GY-Grains yield plant^{-1} (g), BY-Biological yield plant^{-1} (g), HI-Harvest index (%)

Table 8. Phenotypic correlation coefficient among yield and yield attributes under drought condition.

Characters	DH	DM	PH	SL	PL	T/P	G/S	TGW	BY	HI
DH	1									
DM	0.507**	1								
PH	0.015	0.367**	1							
SL	0.133	0.260**	-0.139	1						
PL	-0.144	0.272**	0.820**	-0.142	1					
T/P	0.229*	0.111	0.029	-0.202*	0.025	1				
G/S	0.271**	0.369**	0.087	0.338**	0.055	-0.104	1			
TGW	-0.342**	0.132	0.476**	-0.067	0.509**	-0.116	0.138	1		
BY	-0.194*	0.146	0.486**	0.201*	0.467**	0.236**	0.090	0.298**	1	
HI	0.137	0.111	-0.137	-0.097	-0.213*	0.110^{NS}	0.173	0.070	-0.280**	1
GY	-0.021	0.233*	0.258**	0.083	0.215*	0.293**	0.229*	0.248**	0.491**	0.619**

* Significant at p= 0.05, ** Significant at p= 0.01

DH- Days to heading, DM- Days to maturity, PH- Plant height (cm), SL- Spike length (cm), PL- Peduncle length (cm), T/P- Effective tillers plant^{-1}, G/S- Grains spike^{-1}, TGW- 1000-grain weight (g), GY-Grains yield plant^{-1} (g), BY-Biological yield plant^{-1} (g), HI-Harvest index (%)

Table 9. Phenotypic correlation coefficient (above diagonal) and genotypic correlation (below diagonal) among yield and yield attributes under irrigated condition for physiological traits.

Characters	NDVI	C.T	SPAD	G/S	TGW	GY
NDVI	1.000	-0.043	-0.044	0.032	-0.095	0.068
C.T	0.041	1.000	-0.004	-0.133	0.048	-0.148
SPAD	-0.106	-0.001	1.000	0.135	0.292**	0.232*
G/S	0.057	-0.228*	0.148	1.000	0.154	0.370**
TGW	-0.148	-0.009	0.404**	0.155	1.000	0.205*
GY	0.141	-0.200*	0.298**	0.476**	0.242**	1.000

* Significant at p = 0.05, ** Significant at p = 0.01

NDVI-Normalized difference vegetation index, C.T- Canopy temperature, SPAD - Soil plant analysis development

Table 10. Phenotypic correlation coefficient (above diagonal) and genotypic correlation (below diagonal) among yield and yield attributes under drought condition for physiological traits.

Characters	NDVI	C.T	SPAD	G/S	TGW	GY
NDVI	1.000	-0.168	0.145	0.093	0.007	0.171
C.T	-0.069	1.000	-0.182*	-0.182*	-0.112	-0.232*
SPAD	0.326**	-0.323**	1.000	0.169	0.027	0.223*
G/S	0.188*	-0.343**	0.480**	1.000	0.161	0.238**
TGW	0.063	-0.364**	0.015	0.125	1.000	0.263**
GY	0.214*	-0.363**	0.394**	0.307**	0.331**	1.000

* Significant at p = 0.05, ** Significant at p = 0.01

NDVI-Normalized difference vegetation index, C.T- Canopy temperature, SPAD - Soil plant analysis development

References

Abinasa M, Ayana A, and Bultosa G, (2011). Genetic variability, heritability and trait associations in durum wheat (*Triticum turgidum* L. var. *durum*) genotypes. African Journal of Agricultural Research, 6(17):3972-3979.

Adnan M, Ali A, Ali N, Ullah N, Ullah F, Ahmed Z and Swati, (2013). Effect of drought stress on the physiology and yield of the Pakistani wheat germplasms. International Journal of Advancements in Research and Technology, 2:2278-7763.

Alemu D, Firew M and Tadesse D, (2019). Genetic variability studies on bread wheat (*Triticum aestivum* L.) genotypes. Journal of Plant Breeding and Crop Sciences, 11(2):41-54.

Al-Jibouri HA, Miller PA and Robinson AF, (1958). Genetic environmental variances in an upland cotton cross interspecific origin. Journal of Agronomy, 51:515-518.

Aslani F, Mehrvar MR and Juraimi AS, (2012). Evaluation of some morphological traits associated with wheat yield under terminal drought stress. African Journal of Agricultural Research, 7(28):4104-4109.

Ayer DK, Sharma A, Ojha BR, Paudel A and Dhakal K, (2017). Correlation and path coefficient analysis in advanced wheat genotypes. SAARC Journal of Agriculture, 15:1-12.

Baye A, Berihun B, Bantayehu M, Derebe B and Moral MT, (2020). Genotypic and Phenotypic Correlation and Path Coefficient analysis for yield and yield related traits in advanced bread Wheat (*Triticum aestivum* L.) lines. Soil and Crop Sciences, 6.

Belay GA, Zhang Z and Xu P, (2021). Physio-morphological and biochemical trait-based evaluation of Ethiopian and Chinese wheat germplasm for drought tolerance at the seedling stage. Sustainability, 13:4605

Bhushan B, Bharti S, Ojha A, Pandey M, Gourav SS, Tyagi BB and Singh G, (2013). Genetic variability, correlation coefficient and path analysis of some quantitative traits in bread wheat. Journal of Wheat Research, 5(1):21-26.

Birhanu M, Sentayehu A, Alemayehu A, Ermias A and Dargicho D, (2017). Correlation and path coefficient studies of yield and yield associated traits in bread wheat (*Triticum aestivum* L.) genotypes. Advances in Plants and Agriculture Research, 6(5):00226.

Burton GW and Devane EH, (1953). Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. Agronomy Journal, 45:478-481.

Chowdhury M.K.H., M.R.M.A., Bahadur, M.M., Islam, T., Hakim, M.A., Iqbal, M.A., Javed, et al., (2021). Evaluation of drought tolerance of some wheat (*Triticum aestivum* L.) genotypes through phenology, growth, and physiological indices, Agronomy, 11:1-20

Dabi A, Mekbib F and Desalegn T, (2016). Estimation of genetic and phenotypic correlation coefficients and path analysis of yield and yield contributing traits of bread wheat (*Triticum aestivum* L.) genotypes. International Journal Natural Resource Ecology Management, 1:145-154.

FAO, (2020). Crop prospects and food situation, quarterly global report, No. 2.

FAO, (2015). World Food and Agriculture-FAO statistical pocketbook. Rome, Italy: FAO.

Fischer RA, (2007). Understanding the physiological basis of yield potential in wheat. Journal of Agricultural Science 145:99-113

Fouad H, (2018). Correlation, path and regression analysis in some bread wheat (*Triticum aestivum* L.) genotypes under normal irrigation and drought conditions. Egyptian Journal of Agronomy, 40: 133-144

Gelalcha S and Hanchinal RR, (2013). Correlation and path analysis in yield and yield components in spring bread wheat (*Triticum aestivum* L.) genotypes under irrigated condition in Southern India. African Journal of Agricultural Research, 8 (24):3186-3192

Hanson CH, Robinson HF and Comstock RE, (1956). Biometrical studies of yield in segregating population of Korean Lespedeza. Agronomy Journal, 48:268-272.

Hassan KAM and Hassan SE, (2016). Genetic variability, heritability and correlation for some morphological characters in 20 Durum wheat accessions. International Journal of Advanced Research, 2320-5407.

Iqbal M, Raja NI, Yasmeen F, Hussain M, Ejaz M and Shah MA, (2017). Impacts of heat stress on wheat: A critical review. Advances in Crop Science and Technology. 5:01-09.

Itam MO, Gorafi YSA, Tahir ISA and Tsujimoto H, (2021). Genetic variation in drought resilience-related traits among wheat multiple synthetic derivative lines: insights for climate resilience breeding. Japanese Society of Breeding, 71(4):435-443.

Jan N, Lal EP, Kashyap SC and Parr GA, (2017). Character association and Path analysis in wheat (*Triticum aestivum* L em Thell) under temperate conditions of Kashmir, India. Plant Archives, 17:43-50.

Kashif M and Khaliq I, (2004). Heritability, correlation and path coefficient analysis for some metric traits in wheat. International Journal of Agricultural Biology, 6:138-142.

Khames KM, Abo-Elwafa A, Mahmud AM and Hamada A, (2016). Correlation, path coefficient, normal and step wise regression analysis via two cycles of pedigree selection in bread wheat (*Triticum aestivum* L.). Assiut Journal of Agricultural Science 47:84-108.

Kumari M, Pudake RN, Singh VP and Joshi AK, (2012). Association of stay green trait with canopy temperature depression and yield traits under terminal heat stress in wheat (*Triticum aestivum* L.) Euphytica. 190:87-97.

Li J, Wen S, Fan C, Zhang M, Tian S, Kang W, Zhao W, Bi C, Wang Q, Lu S, Guo W, Ni Z, Xie C, Sun Q and You M, (2020). Characterization of a major quantitative trait locus on the short arm of chromosome 4B for spike number per unit area in common wheat (*Triticum aestivum* L.) Theoretical and Applied Genetics, 133:2259-2269.

Lopes MS and Reynolds MP, (2012). Stay-green in spring wheat can be determined by spectral reflectance measurements (Normalized Difference Vegetation Index) independently from phenology. Journal Experimental Botany, 63:3789-3798.

Munir M, Chowdhry MA and Malik TA, (2007). Correlation studies among yield and its components in bread wheat under drought conditions. International Journal of Agriculture and Biology, 9(2):287-290

Olbana TM, Mekbib F and Tadesse W, (2021). Assessment of genetic variability among bread wheat genotypes for agronomic and morphological traits under optimum and stress condition. Research Square.

Pokharel D and Pandey M, (2012). Genetic variability of drought adaptive traits in Nepalese wheat (*Triticum aestivum* L.) germplasm. Hydro Nepal: Journal of Water, Energy and Environment, 11:64-68.

Rakszegi M, Darkó É, Lovegrove A, Molnár I, Láng L, Bedő Z, et al., (2019). Drought stress affects the protein and dietary fiber content of whole meal wheat flour in wheat/*Aegilops* addition lines PLoS ONE, 14:1-19.

Rehman SU, Abid MA, Bilal M, Ashraf J, Liaqat S, Ahmed RI and Qanmber G, (2015). Genotype by trait analysis and estimates of heritability of wheat (*Triticum aestivum* L.) under drought and control conditions. Basic Research Journal of Agricultural Science and Riverview, 4:127-134.

Reza Nasri AK, Farzad P, Saeed V and Barary M, (2014). Correlation, path analysis and stepwise regression in yield and yield component in wheat (*Triticum aestivum* L.) under the temperate climate of Ilam province, Iran. Indian Journal of Fundamental and Applied Life Sciences, 4(4): 188-198.

Semahegn Y, Shimelis H, Laing M and Mathew I, (2021). Genetic variability and association of yield and yield components among bread wheat genotypes under drought stressed conditions. Australian Journal of Crop Science, 15(06):863-870.

Shamuryarira KW, Shimelis H, Tapera T and Tsilo TJ, (2019). Genetic advancement of newly developed wheat populations under drought-stressed and non-stressed conditions. Journal of Crop Science of Biotechnology, 22(2):169-176.

Singh KA, Singh SK, Garg HS, Kumar R and Choudhary R, (2014). Assessment of relationships and variability of morpho-physiological characters in bread wheat (*Triticum aestivum* L.) under drought stress and irrigated conditions. The Bioscan, 9(2): 473-484.

Singh SP, Yadav MK, Singh K and Sangar RS, (2018). Effect of drought stress on the morphological and physiological characterization of Indian wheat (*Triticum aestivum* L.) genotype. International Journal of Current Microbiology and Applied Sciences, 7(08):1144-1155.

Subhani GM and Chowdhry MA, (2000). Correlation and path coefficient analysis in bread wheat under drought stress and normal conditions. Pakistan Journal of Biological Sciences, 3:72-77.

Sultan MARF, Hui L, Yang LJ and Xian ZH, (2012). Assessment of drought tolerance of some *Triticum* L. species through physiological indices. Czech Journal of Genetics and Plant Breeding, 48:178-184.

Talebi R, (2011). Evaluation of chlorophyll content and canopy as indicators for drought tolerance in durum wheat (*Triticum durum* Desf.). Australian Journal of Basic and Applied Science, 5:1457-1462.

USDA, (2022) World agricultural production and global market analysis.

Zhang Y, Xu W, Wang W, Dong H, Qi X and Zhao M, et al., (2016). Progress in genetic improvement of grain yield and related physiological traits of Chinese wheat in Henan province. Field Crops Research,199:117-128.

Zorb C, Ludewig U, Hawkesford MJ, (2018). Perspective on wheat yield and quality with reduced nitrogen supply. Trends in Plant Science, 23:1029-1037.

Review Article

Ekin
Journal of Crop Breeding and Genetics

9(2):160-171, 2023

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Accelerating Crop Breeding in the 21st Century: A Comprehensive Review of Next Generation Phenotyping Techniques and Strategies

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Citation:
Beniwal J., Dhundwal A., Goyal A., Kumari A., Behl RK., Munjal R., 2023. Accelerating Crop Breeding in the 21st Century: A Comprehensive Review of Next Generation Phenotyping Techniques and Strategies. Ekin J. 9(2):160-171.

Received: 10.04.2023 ***Accepted: 21.06.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

ABSTRACT

Biotic and abiotic stress factors significantly impede crop productivity and lead to substantial economic losses. Given the projected human population of 9 billion by 2050 and the necessity to double current food production to meet the demands of this growing populace, enhancing crop productivity has become a pressing concern. In recent years, substantial progress has been made in the field of high-throughput phenotyping technologies, enabling precise measurements of desired traits and efficient screening of large plant populations under diverse environmental conditions. These advancements involve the integration of advanced robotics, high-tech sensors, imaging systems, and computing power to unravel the genetic basis of complex traits associated with plant growth and development. Furthermore, advanced bioinformatics tools have emerged to analyze the vast amounts of multi-dimensional, high-resolution data collected through phenotyping at both the genetic and whole-plant levels, considering specific environmental conditions and management practices. The integration of genotyping and phenotyping approaches facilitates a comprehensive understanding of gene functions and their responses to various environmental stimuli. This integrated approach holds significant promise for identifying solutions to the major constraints limiting crop production. This review focuses on the recent breakthroughs in plant phenomics and various imaging techniques, emphasizing the applications of different high-throughput technologies in both controlled and natural field conditions. These advancements are crucial steps towards addressing the challenges posed by stress factors and ultimately achieving sustainable and increased crop yields to meet the demands of the growing global population.

Keywords: Genotyping, high throughput, imaging, phenotyping, sensors, stress

Introduction

In changing global climatic conditions, crop plants face various biotic and abiotic stresses throughout their life span, leading to significant losses in growth, development, and yield. As the global population continues to grow, ensuring environmental sustainability while enhancing agricultural production has become a critical goal for agricultural research. To meet the increasing food demand by 2050, the rate of yield gain must be doubled (Anonymous, 2017). The challenge of developing high-yielding, climate-resilient crop varieties has been exacerbated by deteriorating climatic conditions, such as higher CO_2 concentrations, temperature fluctuations, heat stress, and irregular rainfall (Rosenzweig et al., 2014). These stressors emphasize the need to develop new crop varieties with improved resistance against biotic and abiotic stresses. Phenotyping, which involves characterizing and quantifying plant traits, has emerged as a crucial technique for improving crop productivity based on better morpho-physiological characteristics (Furbank et al., 2011). In recent years,

high-throughput phenotyping platforms have been developed, allowing for the analysis of phenotypic responses of multiple genotypes under reproducible environmental conditions. In the pursuit of meeting the growing global demand for food, agriculture has experienced rapid transformation, particularly in crop breeding techniques. The 21st century has presented unprecedented challenges, necessitating a transformative shift in crop breeding strategies to address the needs of a rapidly expanding global population. The urgency to enhance crop productivity, resilience, and sustainability has driven researchers and breeders to explore novel approaches, leading to the emergence of next-generation phenotyping techniques as a powerful tool to revolutionize crop breeding.

To tackle the challenges posed by climate change and global population growth, crop breeding must focus on efficient resource utilization and environmental adaptability. Phenotyping, which has been an essential aspect of crop improvement since the domestication of crops, plays a crucial role in establishing the genotype-phenotype relationship. However, conventional phenotyping methods have been limited by low throughput, labor-intensiveness, and destructiveness, leading to a genotype-phenotype gap (Walter et al., 2009). To bridge this gap and accelerate crop improvement, researchers have developed next-generation phenotyping techniques, integrating advanced genomic technologies like Next Generation Sequencing (NGS) and Single Nucleotide Polymorphism (SNP) arrays (Golzarian et al., 2011). These technologies have enabled the acquisition of genotypic information at a faster and more cost-effective rate. However, the development of phenotyping methods has not kept pace with genomics, highlighting the need for improved phenotyping approaches. Next-generation phenotyping techniques are revolutionizing crop breeding by offering comprehensive and high-throughput assessments of diverse plant traits, such as growth dynamics, stress responses, nutrient uptake, and disease resistance (Kumar et al., 2015). Advancements in technology, data analytics, and genomics have played a pivotal role in reshaping the phenotyping landscape, allowing breeders to extract valuable insights from data-rich phenotyping datasets. The pressing challenges faced by agriculture demand faster and more effective crop breeding strategies. Next-generation phenotyping techniques are crucial for identifying and selecting desirable traits at an early stage of plant development which expediting the breeding process. By leveraging automation, remote sensing, imaging, and genomics, researchers can obtain vast amounts of data on crop traits with unparalleled precision and efficiency. This review aims to provide a comprehensive analysis of the next-generation phenotyping techniques, different imaging techniques, remote sensing with UAVs and their potential applications in addressing global food challenges.

Phenomics: A novel tool for next generation phenotyping

Phenomics is a multidisciplinary science that emerged from the Human Phenome Project initiated in 1997 (Freimer and Sabatti 2003). It focuses on the expression of an organism's genome as observable traits within a specific environment (Houle et al., 2010). Utilizing sensor-aided, non-destructive, and high-throughput automated methods, phenomics enables the comprehensive acquisition and analysis of high-dimensional phenotypic data on an organism-wide scale (Kumar et al., 2015). Referred to as the Next Generation Phenotyping (NGP), phenomics represents a promising solution to bridge the gap between phenotypes and genotypes (Ahmed et al., 2023). By employing non-invasive sensors, automated data processing for trait extraction, robotized delivery of plants to sensors, and vice versa, as well as robotized plant culturing, phenomics offers an automated data management pipeline for seamless and efficient analysis of processed data (Cobb et al., 2013; Arend et al., 2016). This advanced approach allows researchers to delve into the inner workings of living plants, gaining valuable insights into the relationship between genotypes and phenotypes. As such, phenomics holds great potential for advancing our understanding of plant biology and facilitating the development of improved agricultural practices.

Traditional Phenotyping

Until the last decade, plant phenotyping primarily relied on traditional agro-morphological traits, categorized into qualitative and quantitative data (Liu et al., 2010). Qualitative data served for diagnosing highly heritable traits unaffected by environmental fluctuations, regulated by major genes, while quantitative data represented traits influenced by gene interactions and affected by genotype and environment interactions (G x E) (Bogard et al., 2014). Both types of data were scaled using nominal, ordinal, continuous, or binary scales to express the degree of trait expression. Plant breeding predominantly focused on major traits related to agronomic performance, tolerance or resistance to biotic and abiotic stresses, and quality attributes such as nutritional and flavor traits. The International Plant Genetic Resources Institute (IPGRI) and other international plant research organizations established a standardized "descriptor" scheme to catalog plant traits, providing a common language for understanding plant

characteristics and facilitating successful characterization of plant genetic resources (Kumar et al., 2015). In traditional phenotyping technique, destructive sampling, manual visual/ instrument aided measurements are used. This technique is very time consuming and labor intensive [Figure 1(A)].

Modern Phenotyping

Plant phenotyping involves studying the complex interplay between genotype and environment, where genotype encompasses all genes, phenotype is the result of gene-environment interaction, and phenome refers to gene expression under existing conditions (Furbank and Tester, 2011). This rapidly evolving concept aims to connect genetic information, plant functionality, and agricultural characteristics through the measurement of phenomes, known as phenomics (Bilder et al., 2009). Crucial for the scientific accuracy of molecular breeding, phenotyping bridges the gap between genes and phenotypes, particularly in crop-environment studies. By facilitating the association among genotype, phenotype, and environment, phenotyping plays a crucial role in achieving sustainable and efficient crop production, considering changing agricultural conditions and climate change. Moreover, it allows functional studies of specific genes, forward and reverse genetic analysis, and the development of crops with desirable traits (Xiong et al., 2007).

High-throughput phenotyping platforms have gained popularity, enabling precise assessments of numerous traits in controlled environments, while recent advancements in technology have also enabled field phenotyping platforms, allowing large-scale measurements and analysis in diverse growing conditions using imaging techniques with sensors on field vehicles or flying platforms which is nondestructive in nature (Tardieu and Schurr 2009). Overall, phenotyping is indispensable in understanding gene networks, predicting global climate changes, and devising strategies for effective crop adaptation and production. In the modern phenotyping, non-destructive sampling [Figure 1(B)] and automatic machines were used. There is a visualization of multi parameter data at one time. One example of modern phenotyping given by Benamar et al., (2013). Plants grown in greenhouses are then conveyed by robotics via conveyer belt to the inspection unit for inspection. There are many kinds of imaging platforms, including visual, thermal, florescence, and others. Data will then be evaluated and interpreted after image processing whereas when plants are in the field, information is collected by using stationary or mobile sensors such as aerostats, phenocopters, etc., finally data were analyzed and interpretant it.

Exploring the Significance: Why Detailed Phenomics and Multi-Trait Analysis for Transforming Crop Breeding?

Obtaining accurate phenotypes has long been a challenge in crop breeding due to the time and cost-intensive nature of direct field measurements. However, recent developments in field phenomics have revolutionized the study of plant phenotypes across diverse environmental conditions. Modern phenomics methods, utilizing hyperspectral/multispectral cameras, now enable the acquisition of extensive reflectance data at various stages of crop development under different environments (Atkinson et al., 2018). This progress in phenotyping technology has facilitated swift and precise data collection for essential agronomic traits for the concept of high-throughput phenotyping (HTP). HTP aims to reduce data costs per plot and enhance early-season prediction accuracy by incorporating highly heritable secondary phenotypes that are closely correlated to selection phenotypes. Open-source software solutions like FieldImageR have further minimized processing expenses, making HTP data more cost-effective and accessible for agricultural research and crop improvement (Matias et al., 2020).

Furthermore, empirical evidence underscores the significance of multi-trait analysis in significantly improving prediction accuracies, especially when considering genetic and residual correlations within the modeling process. The emergence of new genomic models that incorporate multiple traits and environments has unlocked immense potential for harnessing correlations between different variables and disentangling diverse effects. These models can account for complex interactions such as trait × environment, trait × genotype, and trait × genotype × environment, leading to a more comprehensive understanding of the underlying genetic architecture (Montesinos-López et al., 2016).

By integrating contemporary Genomic best linear unbiased prediction (GBLUP) multi-trait models with those incorporating environmental information and two & three-way interaction terms, researchers can develop a potent, unified and whole genome prediction model. This holistic approach empowers them to make more precise and comprehensive predictions, offering promising avenues for advancing agricultural research and crop breeding endeavors. With the ability to consider a wide range of genetic and environmental factors, such advanced prediction models pave the way for more efficient and effective crop breeding strategies, ultimately contributing to the development of resilient and high-yielding crop varieties to meet the challenges of an ever-changing world (Crossa et al., 2021).

Advance tools for plant phenotyping in 21st Century

Image acquisition for plant phenotyping: Manual vs. Automated

Researchers can choose either a manual or automated approach for image acquisition in their image processing pipeline. The manual method involves using a standard camera on a tripod, positioned optimally to reduce distortion, with preprocessing steps to further minimize any distortion (Basak et al., 2019). The setup includes a uniformly colored wall (preferably light blue) and strategically placed light sources for appropriate illumination, enabling precise image capture of various plants.

On the other hand, transitioning to an automated image acquisition process offers significant advantages. This includes high-throughput data collection, reduced human error, and standardized imaging protocols (Li et al., 2016). The automated system employs sensors, cameras, and robotic systems to capture images of plants at different growth stages, facilitating efficient study of plant development and responses to environmental factors on a large-scale and standardized level (Hartmann et al., 2011).

Enhanced Image Processing Pipeline for High-Throughput Plant Phenotyping

The image processing pipeline for high-throughput plant phenotyping is designed to efficiently process large volumes of plant images and extract precise information for further analysis (Atkinson et al., 2018) (Figure 2). This pipeline involves several key steps, which are detailed below:

i. Region of Interest (ROI) Definition: The pipeline begins by precisely defining the regions of interest within the captured images. This step involves identifying specific areas or regions where plant analysis will take place, ensuring that only relevant parts of the images are considered for further processing.

ii. Object Segmentation: Next, it performs advanced object segmentation techniques to accurately separate the plants from the background or any unwanted elements in the image. This ensures that only the plant objects are isolated for subsequent analysis, minimizing any potential interference.

iii. Object Extraction Display and Verification: Once the objects are successfully segmented, the pipeline presents the extracted plant objects for meticulous visual inspection and verification. This feature allows users to assess the accuracy of the segmentation and make any necessary adjustments, ensuring the quality of subsequent analysis.

iv. Morphological Refinement: The pipeline applies precise morphological operations, such as dilation or erosion, to the extracted plant objects. These operations serve to refine the object boundaries, remove noise, and enhance the accuracy of the subsequent analysis, producing more reliable results.

v. Compilation of Comprehensive Analysis Results: The pipeline compiles the analysis results for all the plants into a structured and easily interpretable table format. This table consolidates quantitative measurements and derived traits for each plant, facilitating efficient data analysis and comparison.

vi. Visual Representation of Processing Steps: To aid in understanding and quality control, the processing steps performed on each plant are visually represented as an image stack. This stack presents a series of images depicting the different stages of the analysis, providing a comprehensive overview of the processing workflow and enabling better insights into the data processing steps.

By following this enhanced image processing pipeline, high-throughput plant phenotyping platforms can effectively handle large volumes of plant images, extract precise and relevant information, and present the results in a structured manner for further analysis and interpretation. The pipeline's advanced techniques ensure improved accuracy and efficiency, making it an indispensable tool for high-throughput plant phenotyping research.

Imaging technology for plant phenotyping

Plant phenotyping relies on imaging technologies that enable non-destructive and high-throughput analysis of plant traits (Omari et al., 2020) (Figure 3).

Some commonly used imaging techniques are summarized below:

i. RGB Imaging: Captures images using standard color cameras, providing visual information about plant appearance and traits related to color, size, shape, and canopy coverage. The process involves detecting reflectance from the leaf or canopy in the visible spectrum (400 to 780 nm) and generating RGB images (Basak et al., 2019). This method is low-cost, user-friendly, and visually more interpretable. However, it is susceptible to variations in lighting conditions, which can impact the accuracy of the results.

ii. Multispectral Imaging: Captures images in multiple discrete wavelength bands beyond the visible spectrum, enabling analysis of specific traits such as chlorophyll content, leaf nitrogen levels, water stress, and disease detection.

iii. Hyperspectral Imaging: Captures images across a wide range of narrow and contiguous wavelength bands, providing detailed spectral information for each pixel (Huang et al., 2012). It allows analysis of biochemical and physiological traits at a fine level of

detail, used for applications like crop disease detection and nutrient status assessment. This method provides highly precise information in narrow spectral bands, allowing for detailed analysis of specific phenomena (Perez-Sanz et al., 2017). However, the extensive image processing required for handling the large volume of data can lead to high costs associated with this approach.

iv. Thermal Imaging: Involves capturing infrared radiation emitted by plants, correlating with their temperature. Useful for detecting temperature variations, identifying stress conditions, and assessing water use efficiency. The technique entails detecting the emission of heat radiation from objects in the thermal infrared wavelength region (8-14 micrometers) (Tardieu et al., 2010) . This method provides a straightforward correlation between the acquired information and canopy temperature, making it useful for thermal analysis. However, it may be challenging to detect small changes in temperature due to its coarse resolution, which can limit its precision in some applications.

v. 3D Imaging: Utilizes techniques like stereo vision, structured light, or time-of-flight cameras to capture depth information of plant structures. This technology enables the measurement of plant height, biomass, branching patterns, and canopy architecture.

vi. Fluorescence Imaging: Captures emitted light by plants in response to excitation with specific wavelengths. Provides insights into photosynthetic activity, stress responses, and nutrient status.

vii. X-ray Imaging: X-ray imaging provides non-invasive and high-resolution visualization of internal plant structures, particularly roots (Flavel et al., 2012). This technology facilitates the study of root architecture, nutrient uptake patterns, and interactions with the soil environment. When combined with advanced image analysis algorithms, these imaging technologies enable comprehensive and quantitative assessment of diverse plant traits. As a result, researchers gain a deeper understanding of plant growth, development, stress responses, and productivity.

Remote Sensing with Unmanned Aerial Vehicles (UAVs): Expanding Horizons for Enhanced Insights

Aerial imaging, including plant, field, farm, and country scales using different systems from drones to satellites (Figure 4), has revolutionized agricultural research. Drones, also known as UAVs, offer a versatile platform capable of rapidly gathering data over expansive regions and potentially providing high spatial resolution images, with pixel sizes as small as 1 mm (Zhou et al., 2017). Leveraging advanced IT techniques like deep learning, millions of remote sensing images can be processed with remarkable accuracy and speed (Yao et al., 2017). As a result, remote sensing has found widespread application in monitoring drought stress response, evaluating nutrient status and growth, detecting weeds and pathogens, predicting crop yields, and identifying QTLs. The high-resolution imagery obtained by UAVs, capturing canopy color and texture features at remarkable spatial and temporal resolutions, has become instrumental in various phenotyping tasks (Shi et al., 2019). This wealth of detailed information enables efficient feature mining and analysis, facilitating tasks such as leaf area index estimation, wheat ear identification, weed detection, and seed performance evaluation in crop i.e. rapeseed (Nguyen and Lee 2006). Furthermore, researchers are actively investigating optimal resolution determination, highlighting the continuous efforts to harness the full potential of UAV-based remote sensing in agricultural applications**.**

Drone Mission Planning and Data Acquisition Steps for DJI Phantom Pro V2 in Agricultural Monitoring:

i. Mission Planning: Set various parameters to prepare the drone for data capturing. Develop a detailed flight plan for the drone to follow and collect images. Specify camera angle, scan line overlap, Ground sampling distance, and other parameters to obtain images with desired properties.

ii. Image Acquisition: Acquire image data, considering challenges such as illumination conditions, temperature, and in-scene parameters like background obscuring. Implement safety measures to ensure a successful flight and data capture.

iii. Image Transfer: After data acquisition, transfer the image data along with metadata (e.g., geo-locations, number of samples, flight speed, exposure, dark and white references) to a laptop or secondary storage. Regularly empty the onboard storage to avoid overlapping data from previous missions.

iv. Pre-processing: Mosaic the individual field images into a single image for further processing. Attach geo-locations to the images and ortho-rectify them to prepare for analysis.

v. Data Analytics: Utilize tools like Pix4D to calculate vegetation indices for the crop using the index calculator. Define the index formula and apply it to calculate index images from the ortho-mosaic data.

vi. Visualizing Digital Surface Model (DSM): Generate a DSM using Pix4D to calculate crop height from the soil surface. The DSM data aids in visualizing the crop's three-dimensional structure.

The Loop of Crop Phenotype-to-Genotype: Leveraging Multiomics for Crop Improvement

The integration of crop phenotyping with functional genomics studies represents a pivotal

advancement in crop improvement (Close et al., 2011). Through a high-throughput and multiscale phenotyping platform, dynamic phenotypic traits of extensive crop genetic populations can be efficiently obtained. This platform enables the merging of phenotypic data with other omics data, such as genomics, transcriptomics, proteomics, and metabolomics, facilitating comprehensive multiomics analysis (Li et al., 2018). By employing QTL mapping and GWAS, researchers can effectively mine QTL/genes and identify key genetic elements associated with desirable traits (Wing et al., 2018). Moreover, when combined with genetic transformation techniques, these findings can be harnessed to drive significant improvements in crop genetics, thereby enhancing crop yield, resilience, and quality (Figure 5). The synergistic approach of multiomics-driven phenotyping with functional genomics holds immense promise in accelerating crop breeding and ensuring food security in the face of evolving environmental challenges (Zhang et al., 2019).

High-Precision Phenotyping in Field Conditions

High-precision phenotyping in the field under natural conditions is of utmost importance as pot experiments in controlled environments may not accurately represent plant behavior in real field settings due to limited soil volume and slower moisture extraction patterns (Morisse et al., 2022). To effectively phenotype genotypes for various traits, stable and less environmentally influenced traits are preferred (Halperin et al., 2017). Key physiological traits, such as water-use efficiency, can be measured through carbon isotope discrimination using leaf sampling. Other essential parameters, including photosynthesis, chlorophyll content, thermal imaging of the canopy, transpiration, stomatal conductance, root depth, and mass, directly or indirectly reflect plant water status and functional ability under stress conditions (Figure 6) (White et al., 2012). For traits that involve a combination of multiple factors, like canopy cooling, can be influenced by high root density, stomatal conductance, and hormonal regulation, field-based evaluation becomes more pertinent. Screening for drought tolerance entails comparing yield performance and flowering under irrigated and rainfed conditions, determining the drought susceptibility index (DSI) for each genotype (Poorter et al., 2016). High-precision phenotyping for drought tolerance can be achieved through approaches such as dug-out plots with moisture gradients or rainout shelters, which prevent raindrops from reaching the plot to assess genotypes performance under extreme drought conditions (Gosa et al., 2019). Such meticulous phenotyping has led to the identification of drought-tolerant genotypes in various crops, demonstrating traits such as lower DSI and improved productivity, along with morphophysiological characteristics conferring drought resistance.

High-Precision Phenotyping in Controlled Conditions

High-precision phenotyping in controlled conditions is a crucial aspect of developing improved genotypes through breeding (Weber et al., 2012). While secondary morphological traits can be easily assessed in the field, traits associated with stresses require controlled environments for better understanding (Figure 6). Managed facilities play a vital role in increasing the accuracy of trait measurements, controlling major environmental parameters like temperature, light, and humidity (Rebetzke et al., 2013; Vadez et al., 2014). Certain traits, such as photosynthetic efficiency, can be rapidly and accurately measured using specialized imaging systems (Tardieu et al., 2017). For root-based traits and stress tolerance, controlled environments like greenhouses and growth chambers are essential, as field conditions may not adequately capture their variability (Kwon et al., 2015). Although various methods have been developed for estimating complex traits in controlled environments having some pose challenges in large-scale phenotyping. Precise phenotyping in controlled conditions is pivotal for comprehending stress response and enhancing breeding efforts to develop stress-tolerant genotypes and improve crop productivity (Deery et al., 2016).

Future Prospects and Challenges

Phenomics is poised to enter the era of 'Big Data,' presenting the crop science community with the imperative to synergize artificial intelligence technology and foster international collaborative research. This strategic fusion is fundamental to establish a novel theoretical framework for analyzing crop phenotypic information. The ultimate goal is to construct a robust technical system capable of high-throughput, multi-dimensional, and intelligent phenotyping of crops while efficiently handling vast amounts of big data. This system should seamlessly integrate data from diverse sources, encompassing multi-modality, multiscale, and phenotypic, environmental, and genotypic conditions.

Undoubtedly, the road ahead for crop phenomics entails a spectrum of challenges in the forthcoming 5-10 years, notably in the realm of phenomics, a momentous transition into the big-data era is unfolding, characterized by its high-throughput capacity, multi-dimensionality, and multi-scale nature. Our focus lies in exploring diverse phenotyping approaches encompassing crop morphology, structure, and physiological data, which exhibit three distinct multi-characteristics: multi-domain (including phenomics,

genomics, and other relevant domains), multi-level (spanning from traditional small to medium-scale data up to large-scale omics data), and multi-scale (encompassing crop morphology, structure, and physiological data at various levels, from cellular to whole-plant levels). Recognizing the limitations of single and individual phenotypic information in meeting the demands of association analysis within the emerging 'omics' era, we acknowledge that comprehensive and systematic phenomics information will form the bedrock of future research endeavors. In light of the extensive multi-domain, multi-level, and multi-scale phenotypic information available, there is an urgent imperative to harness the latest advancements in artificial intelligence, particularly in depth learning, data fusion, hybrid intelligence, and swarm intelligence. These cutting-edge approaches hold significant promise in developing robust big-data management processes, essential for supporting critical aspects such as data integration, interoperability, ontologies, shareability, and global accessibility. By strategically adopting these technologies, we can unlock the full potential of the diverse phenotypic data and pave the way for transformative advancements in agricultural research and crop science on a global scale.

The comprehensive analysis and utilization of crop genotype (G) - phenotype (P) - environment (E) information is a pivotal objective. As highlighted by Coppens et al., (2017), the future of plant phenotyping relies on collaborative synergism at national and international levels. Addressing the challenges posed by multi-omics data necessitates novel solutions, notably intelligent data-mining analytics, which can effectively unravel the intricate biological processes governing plant growth and development. Thus, in turn, advances plant breeding efforts, enabling the development of climate-resilient and high-yielding crops that are urgently required to meet evolving environmental demands.

Acknowledgement

Corresponding author Dr. Renu Munjal is thankful to Jogender, Arjoo, Alisha, Prof. Rishi Kumar Behl and Dr. Anita Kumari for technical input.

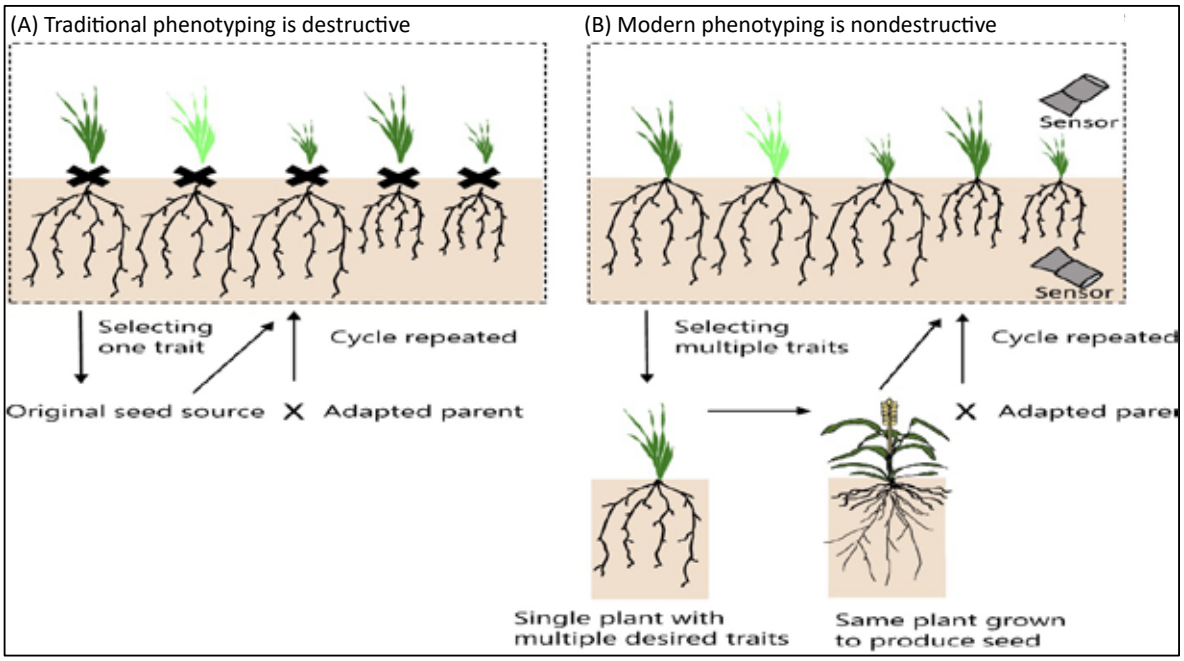

Figure 1. Traditional phenotyping vs Modern phenotyping (Saoirse et al., 2011)

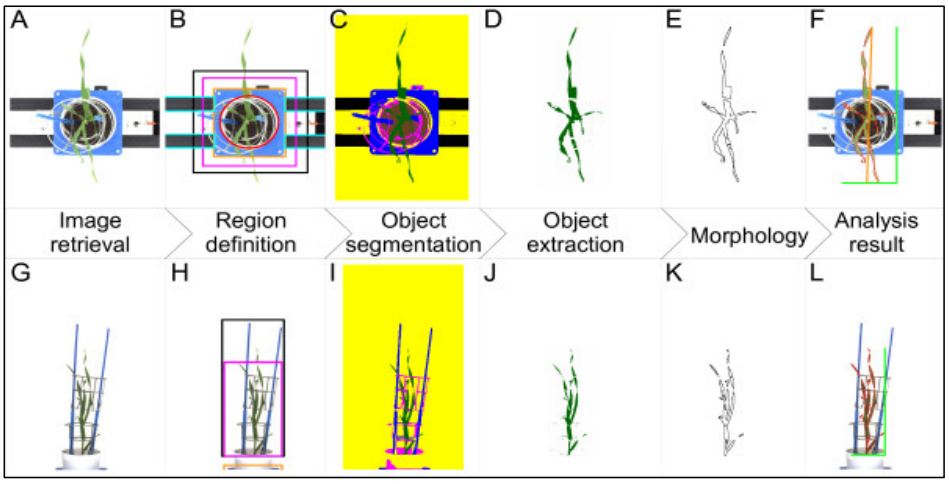

Figure 2. High-throughput image analysis pipeline for top view (A-F) and side view (G-L) images. (Hartmann et al., 2011)

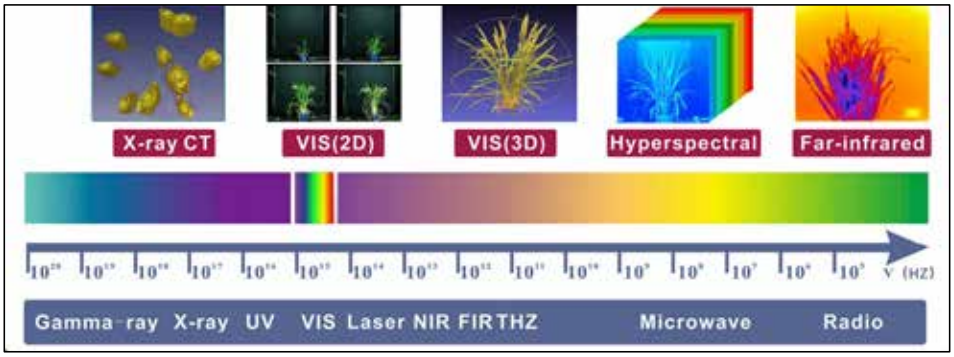

Figure 3. Crop Phenotyping and the Diversity of Spectra Utilized for Exemplification. (Yang et al., 2020)

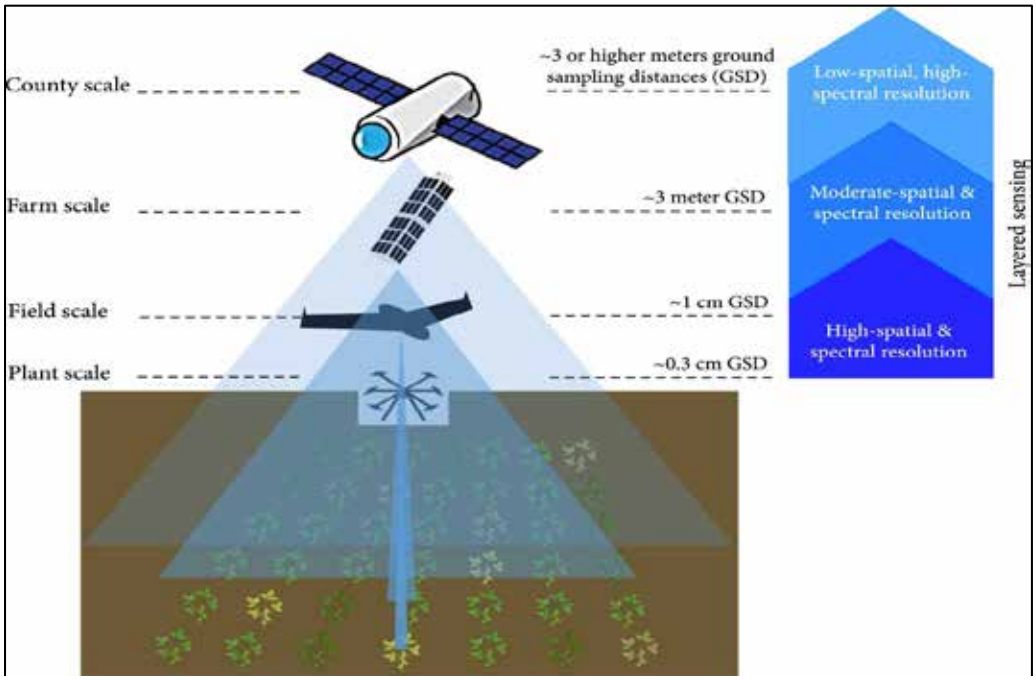

Figure 4. Using Drones for Versatile Crop Phenotyping: Different Scales and Sensing Levels. (Guo et al., 2021)

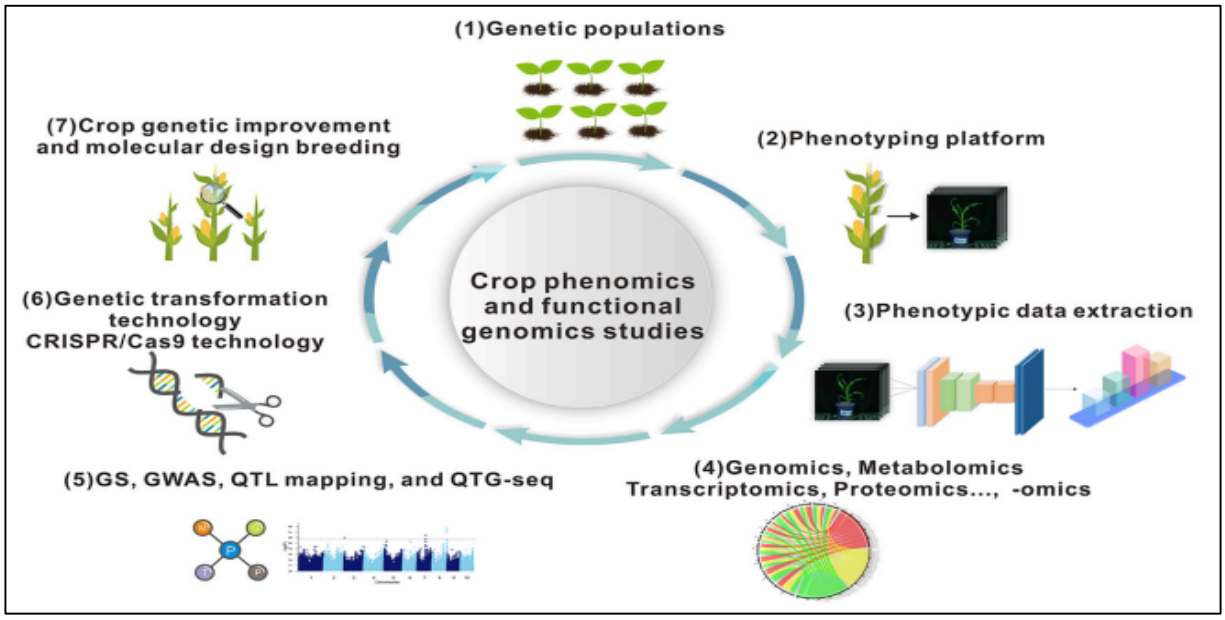

Figure 5. Crop Phenotype-to-Genotype Loop. (Yang et al., 2020)

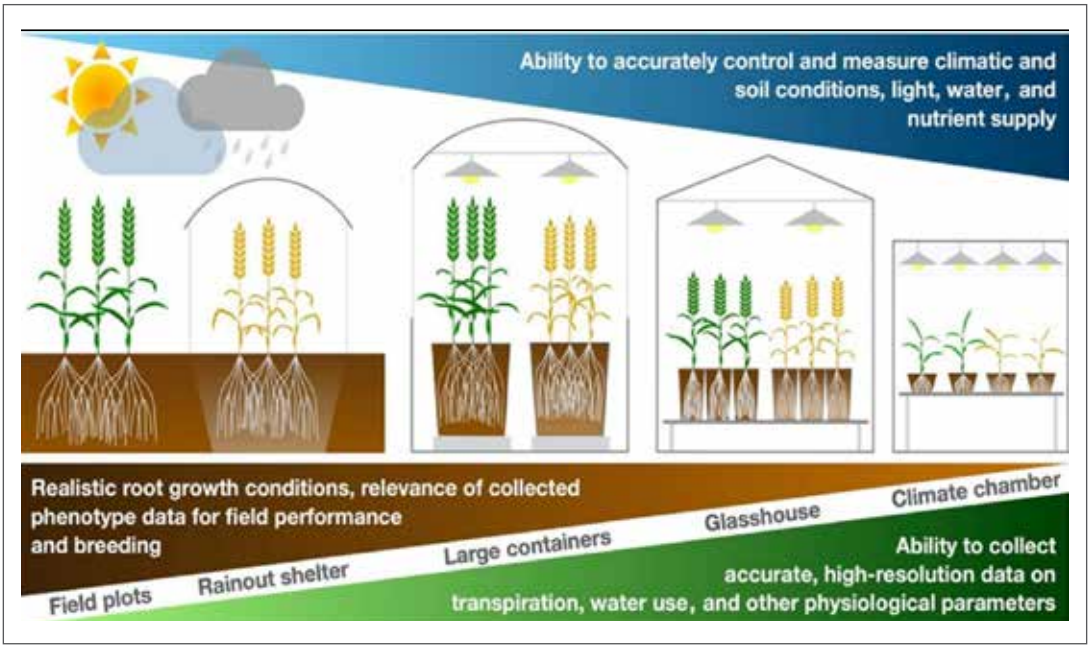

Figure 6. Field vs Controlled environment phenotyping. (Stahl et al., 2020)

References

Ahmed HG, Zeng Y, Fiaz S and Rashid AR, (2023). Applications of high-throughput phenotypic phenomics. Sustainable Agriculture in the Era of the OMICs Revolution. (pp. 119-134). Cham: Springer International Publishing.

Anonymous, (2017). United Nations Department of Economic and Social Affairs Population Division. Available online: http://www.unpopulation.org.

Arend D, Junker A, Scholz U, Schüler D, Wylie J and Lange M, (2016). PGP repository: A plant phenomics and genomics data publication infrastructure. Database (Oxford).

Atkinson J A, Jackson RJ, Bentley AR, Ober E and Wells DM, (2018). Field phenotyping for the future. Annu. Plant Rev. Online, 1:1-18.

Basak JK, Qasim W, Okyere FG, Khan F, Lee YJ, Park J and Kim HT, (2019). Regression analysis to estimate morphology parameters of pepper plant in a controlled greenhouse system. Journal of Biosystems Engineering, 44:57-68.

Benamar A, Pierart A, Baecker V, Avelange-Macherel MH, Rolland A, Gaudichon S, di Gioia L and Macherel D, (2013). Simple system using natural mineral water for high-throughput phenotyping of *Arabidopsis thaliana* seedlings in liquid culture. Int. J. High Throughput Screen, 4:1-15.

Bilder RM, Sabb FW, Cannon TD, London ED, Jentsch JD, Parker DS, Poldrack RA, Evans C and Freimer NB, (2009). Phenomics: the systematic study of phenotypes on a genome-wide scale. Neuroscience, 164:30-42.

Bogard M, Ravel C, Paux E, Bordes J, Balfourier F, Chapman SC, Le Gouis J and Allard V, (2014). Predictions of heading date in bread wheat (*Triticum aestivum* L.) using QTL-based parameters of an ecophysiological model. Journal of Experimental Botany, 65:5849-65.

Close T, Riverside UC and Last R, (2011). National science foundation phenomics: Genotype to phenotype, a report of the NIFA-NSF phenomics workshop.

Cobb JN, Declerck G, Greenberg A, Clark R and McCouch S, (2013). Next-generation phenotyping: requirements and strategies for enhancing our understanding of genotype phenotype relationships and its relevance to crop improvement. Theoretical and Applied Genetics, 126:867-87.

Coppens F, Wuyts N, Inze D and Dhondt S, (2017). Unlocking the potential of plant phenotyping data through integration and data driven approaches. Current Opinion in Plant Biology, 4:58-63.

Crossa J, Fritsche-Neto R, Montesinos-Lopez OA, Costa-Neto G, Dreisigacker S, Montesinos-Lopez A and Bentley AR, (2021). The modern plant breeding triangle: Optimizing the use of genomics, phenomics, and enviromics data. Frontiers in Plant Science, 12:651480.

Deery DM, Rebetzke GJ, Jimenez-Berni JA, James RA, Condon AG, Bovill WD and Furbank RT, (2016). Methodology for high-throughput field phenotyping of canopy temperature using airborne thermography. Frontiers in Plant Science, 7:1808.

Flavel RJ, GuppyCN TM, Watt M, McNeill A and Young IM, (2012). Non-destructive quantification of cereal roots in soil using high-resolution X-ray tomography. Journal of Experimental Botany, 63:2503-2511.

Freimer N and Sabatti C, (2003). The human phenome project. Nature Genetics, 34:15-21.

Furbank RT and Tester M, (2011). Phenomics-technologies to relieve the phenotyping bottleneck. Trends in Plant Science, 16:635-644.

Golzarian MR, Frick RA, Rajendran K, Berger B, Roy S, Tester M and Lun DS, (2011). Accurate inference of shoot biomass from high-throughput images of cereal plants. Plant Methods, 7:2.

Gosa SC, Lupo Y and Moshelion M, (2019). Quantitative and comparative analysis of whole-plant performance for functional physiological traits phenotyping: new tools to support pre-breeding and plant stress physiology studies. Plant science, 282:49-59.

Guo WCM, Singh A, Swetnam TL, Merchant NS, Soumik S, Asheesh K and Baskar G, (2021). UAS-Based plant phenotyping for research and breeding applications. Plant Phenomics, 11:253-269.

Halperin O, Gebremedhin A, Wallach R and Moshelion M, (2017). High-throughput physiological phenotyping and screening system for the characterization of plant-environment interactions. The Plant Journal, 89(4):839-850.

Hartmann A, Czauderna T, Hoffmann R, Stein N and Schreiber F, (2011). HTPheno: An image analysis pipeline for high-throughput plant phenotyping. BMC Bioinformatics, 12(1):1-9.

Houle D, Govindaraju DR and Omholt S, (2010). Phenomics: The next challenge. Nature Reviews Genetics, 11:855-866.

Huang JR, Liao HJ, Zhu YB, Sun JY, Sun QH and Liu XD, (2012). Hyperspectral detection of rice damaged by rice leaf folder *(Cnaphalocrocis medinalis*). Computers and Electronics in Agriculture, 82:100-107.

Kumar J, Pratap A. and Kumar S, (2015). Plant Phenomics: An overview. Phenomics in crop plants: Trends, options and limitations, 1-10.

Kwon TR, Kim KH, Yoon HJ, Lee SK, Kim BK and Siddiqui ZS, (2015). Phenotyping of plants for drought and salt tolerance using infra-red thermography. Plant Breed. Biotech., 3(4):299-307.

Li M, Xu J, Zhang N, Shan J, and Yao S, (2016). Study on the factors affecting grain yield measurement system. 2016 International Conference on Service Science, Technology and Engineering, 566-572.

Li Y, Xiao J, Chenn L, Huang X, Cheng Z, Han B, Zhang Q and Wu C, (2018). Rice functional genomics research: past decade and future. Molecular Plant, 11:359-380.

Liu ZY, Shi JJ, Zhang LW and Huang JF, (2010). Discrimination of rice panicles by hyperspectral reflectance data based on principal component analysis and support vector classification. Journal of Zheijang University SCIENCE-Biomedicine & Biotechnology, 11:71-78.

Matias FI, Caraza-Harter MV and Endelman JB, (2020). FIELDimageR: An R package to analyze orthomosaic images from agricultural field trials. Plant Phenome J., 3:1-6.

Montesinos-López OA, Montesinos-López A, Crossa J, Toledo FH, PérezHernández O and Eskridge KM, (2016). A genomic Bayesian multitrait and multi-environment model. G3 Genes/Genomes/ Genetics, 6:2725-2744.

Morisse M, Wells DM, Millet EJ, Lillemo M, Fahrner S, Cellini F, Lootens P, Muller O, Herrera JM, Bentley AR and Janni M, (2022). A European perspective on opportunities and demands for field-based crop phenotyping. Field Crops Research, 276:108371.

Nguyen HT and Lee BW, (2006). Assessment of rice leaf growth and nitrogen status by hyperspectral canopy reflectance and partial least square regression. European Journal of Agronomy, 24: 349-356.

Omari MK, Lee J, Faqeerzada MA, Joshi R, Park E and Cho BK, (2020). Digital image-based plant phenotyping: A review. Korean Journal of Agricultural Science, 47(1):119-130.

Perez-Sanz F, Navarro PJ and Egea-Cortines M, (2017). Plant phenomics: An overview of image acquisition technologies and image data analysis algorithms. GigaScience, 6(11):1-18.

Poorter H, Bühler J, van Dusschoten D, Climent J and Postma JA, (2012). Pot size matters: A meta-analysis of the effects of rooting volume on plant growth. Functional Plant Biology, 39(11):839-850.

Rebetzke GJ, Condon AG, Richards RA and Farquhar GD, (2013). Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Sci., 42:739-745.

Rosenzweig C, Elliott J, Deryng D, Ruane AC, Müller C, Arneth A, Boote KJ, Folberth C, Glotter M, Khabarov N and Neumann K, (2014). Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. Proceedings of the National Academy of Sciences, 111(9): 3268-3273.

Saoirse R, Tracy KA, Nagel JA, Postma HF, Anton W and Michelle W, (2020). Crop improvement from phenotyping roots: Highlights reveal expanding opportunities. Trends in Plant Science, 25(1):105-118.

Shi C, Zhao L, Zhang X, Lv G, Pan Y and Chen F, (2019). Gene regulatory network and abundant genetic variation play critical roles in heading stage of polyploidy wheat. BMC Plant Biology, 19(1):1-16.

Stahl A, Wittkop B and Snowdon RJ, (2020). High-resolution digital phenotyping of water uptake and transpiration efficiency. Trends in Plant Science, 25(5):429-433.

Tardieu F and Tuberosa R, (2010). Dissection and modelling of abiotic stress tolerance in plants. Current Opinion in Plant Biology, 13(2):206-212.

Tardieu F and Schurr U, (2009). 'White paper' on plant phenotyping. The main outcome of the EPSO workshop on plant phenotyping, Ju¨lich, 1-4.

Tardieu F, Cabrera-Bosquet L, Pridmore T and Bennett M, (2017). Plant phenomics, from sensors to knowledge. Current Biology, 27(15): R770-R783.

Vadez V, Kholova J, Medina Kakkera A and Anderberg H, (2014). Transpiration efficiency: new insights into an old story. Journal of Experimental Botany, 65(21):6141-6153.

Walter A, Silk WK and Schurr U, (2009). Environmental effects on spatial and temporal patterns of leaf and root growth. Annual Review of Plant Biology, 60:279-304.

Weber VS, Kinley SA and Nicholas AR, (2012). Efficiency of managed-stress screening of elite maize hybrids under drought and low nitrogen for yield under rainfed conditions in Southern Africa. Crop Science, 52:1011-1020.

White JW, Andrade-Sanchez P, Gore MA, Bronson KF, Coffelt TA, Conley MM, Feldmann K A, French AN, Heun JT, Hunsaker DJ, Jenks MA, Kimball BA, Roth RL, Strand RJ, Thorp KR, Wall GW and Wang G, (2012). Field-based phenomics for plant genetics research. Field Crops Research, 133: 101-112.

Wing RA, Purugganan MD and Zhang Q, (2018). The rice genome revolution: From an ancient grain to Green Super Rice. Nature Reviews Genetics, 19:505-517.

Xiong YC, Li FM, Zhang T and Xia C, (2007). Evolution mechanism of non-hydraulic root-to-shoot signal during the anti-drought genetic breeding of spring wheat. Environmental and Experimental Botany, 59:193-205.

Yang W, Feng H, Zhang X, Zhang J, Doonan JH, Batchelor WD, Xiong L and Yan J, (2020). Crop phenomics and high-throughput phenotyping: Past decades, current challenges, and future perspectives. Molecular Plant, 13:187-214.

Yao X, Wang N, Liu Y, Cheng T, Tian YC, Chen Q and Zhu Y, (2017). Estimation of wheat LAI at middle to high levels using unmanned aerial vehicle narrowband multispectral imagery. Remote Sensing of Environment, 9:1304.

Zhang H, Wang X, Pan Q, Li P, Liu Y, Lu X, Zhong W, Li M, Han L and Li Jet, (2019). QTG-Seq accelerates QTL fine mapping through QTL partitioning and whole-genome sequencing of bulked segregant samples. Molecular Plant, 12:426-437.

Zhou J, Reynolds D, Cornu TL, Websdale D, Orford S, Lister C, Gonzalez-Navarro O, Laycock S, Finlayson G and Stitt T, (2017). CropQuant: An automated and scalable field phenotyping platform for crop monitoring and trait measurements to facilitate breeding and digital agriculture. BioRxiv: 161547.

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Ekin
Journal of Crop Breeding and Genetics

9(2):172, 2023

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Registration of "Fener" Eggplant (*Solanum melongena* L.) Variety

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Citation:
Haytaoğlu MA., Mutlu S., Kahraman A., Binbir S., 2023. Registration of "Fener" Eggplant (*Solanum melongena* L.) Variety. Ekin J. 9(2):172.

Received: 24.09.2022 ***Accepted: 19.02.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

"Fener" eggplant (*Solanum melongena* L.) variety was developed by Aegean Agricultural Research Institute (AARI) and registered in 2017. Fener is pear shaped and violet-skinned hybrid eggplant variety, in other words traditional "Topan" type in Turkey. It was obtained by crossing purelines in the AARI gene pool.

Fener has vigorous, semi-erect and medium high plants. Its leaf blade size is large but sinuation of margin and intensity of green color are medium.

Fener's flower size is large, number of flowers are one to three on axil, and intensity of purple color on flower is medium. The average fruit weight is 250-300 g, and fruit shape is pear shaped. The size of pistil scar is large, and apex of fruit is flattened. On the other hand, main color of skin at harvest maturity of fruit is violet with strong glossiness (Figure 1).

Length of fruit peduncle is medium. Besides size of calyx is large and anthocyanin coloration of calyx is present but its intensity is weak. Than spininess of calyx is absent or very weak, this character is highly preferred by both farmers and consumers, and anthocyanin coloration underneath calyx is present with weak intensity.

Average yield of "Fener" variety in field test was recorded 71.460 kg ha^{-1}. The number of days from planting to first harvest are 60-65 days, and from the first harvest to the last harvest are 100-110 days. The variety Fener is recommended for commercial cultivation in open field areas. It is consumed as fresh vegetable as well as in roasted form. The seeds of the Fener variety have been produced by AARI.

Figure 1. Pictures showing fruit shape of Fener variety. (Original)

References and Notes

Anonymous (2017). Variety Registration and Seed Certification Center Directorate Technical Report, Ankara, Türkiye. (in Turkish)

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Journal of Crop Breeding and Genetics

9(2):173, 2023

Registration of "Sayım 40" Winter Barley (*Hordeum vulgare* L.) Variety

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Citation:
Sayim İ., Ergün N., Aydoğan S., Bilir M., Çetin Özkan G., Karahan E., 2023. Registration of "Sayım 40" Winter Barley (*Hordeum vulgare* L.) Variety. Ekin J. 9(2):173.

Received: 20.03.2023 ***Accepted: 07.04.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

The Central Research Institute for Field Crops developed the "Sayım 40" winter feed barley (*Hordeum vulgare* L.) variety using the bulk selection method after a bi-parental cross. The cross of the variety was made in the 2001-2002 growing season and its pedigree includes Sunrise/Tokak 157/37 varieties. The segregating materials between the F_2 and F_5 progenies were subjected to natural selection in the ecological conditions of Ankara, then single spike selection was performed on the F_5 progeny. Preliminary and regional yield trials were conducted under different environmental conditions throughout of Central Anatolia and Transitional regions. Due to its outstanding tillering capacity and high grain yield potential, it was selected among the other lines and offered for registration with line number 'Anka-09'. The Variety Registration and Seed Certification Center carried out 2019 and 2020 years registration trials. After two years of testing, the line 'Anka-09' ranked first place with a 5.68 t ha^{-1} grain yield, which is 22.2% more than checks average (4.64 t ha^{-1}) in the Central Anatolian Dry Barley Registration Trials. Based on these results, it was registered as 'Sayım 40' and included in the National List of Varieties in 2021 (Anonymous, 2021).

Sayım 40 is a two-rowed feed barley cultivar, having winter growth habit. It has a moderately late and uniform heading date. Spikes have medium length, and their plant heights are 80-100 cm depending on the growing conditions. The kernels are white and highly homogeneous (Figure 1). According to the quality results obtained from the registration trials, hectoliter weight varies between 63.4-70.8 kg hl^{-1}, 39.0-42.3 g for 1000-kernel weight, 13.4-14.2% for protein content, and the ratio of plump kernels (>2.5 mm sieve) is between 48.2% and 69.0%. Sayım 40 variety has been moderately resistant to scald and barley leaf stripe diseases. Sayım 40 is recommended for winter sowing in dry and semi-dry areas of Central Anatolia and Transitional regions. Certified seed production rights of the variety have been transferred to The General Directorate of Agricultural Enterprises (TİGEM).

Figure 1. General view, spike, and kernels of Sayım 40 variety. (Original)

Acknowledgments

Sayım 40 variety was developed within the framework of the Central Anatolia Region Barley Breeding Research Project numbered TAGEM/TBAD/B/18/A7/P40/172 and financially supported by the General Directorate of Agricultural Research and Policies (TAGEM).

References and Notes

Anonymous (2021). Report of Central Anatolian Dry Barley Registration Trials, Ankara, Turkiye. (in Turkish)

Petersen, R.G. (1994). Agricultural field experiments design and analysis. Marcel Dekker, Inc., 409 p., Corvallis, Oregon.

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9(2):174, 2023

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Registration of "Sinanbey" Winter Feed Barley (*Hordeum vulgare* L.) Variety

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Citation:
Aydoğan S., Ergün N., Sayim İ., Bilir M., Karahan E., Çetin Özkan G., 2023. Registration of "Sinanbey" Winter Feed Barley (*Hordeum vulgare* L.) Variety. Ekin J. 9(2):174.

Received: 22.03.2023 ***Accepted: 10.04.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

Sinanbey is a winter feed barley (*Hordeum vulgare* L.) variety developed by the Central Research Institute for Field Crops (CRIFC). In 2021, the variety was released primarily for its high yield and wide adaptation to rainfed and semi-arid barley production areas. The pedigree was derived from the multi-cross of ADAY-2/4/ROHO//ALGER/CERES/3/ALPHA/DURRA/5/ANADOLU-98, made in 2002.

The bulk breeding method was used after crossing and segregating material was subjected to natural selection between F_2-F_5 progenies. The single-head selection was made in F_5 progeny based on breeder's aims. Sinanbey was assigned the line number ANKA 08 in yield trials conducted in randomized complete block design. In regional yield trials, observations were made in multiple environments over Central Anatolia for two growing seasons. In 2019, ANKA 08 was submitted to the Variety Registration and Seed Certification Centre to be tested in the Central Anatolia Barley Registration Trials. As the candidate variety performed above the check varieties (4.64 t ha^{-1}) yielding competitively better about 5.08 t ha^{-1}, ANKA 08 was registered as Sinanbey in 2021 (Anonymous 2021).

Sinanbey is a two-rowed, white-kernelled, and uniformly tillered with a moderately early heading date (Figure 1). No significant susceptibility is detected to economically important barley diseases. Based on the registration trials, grain yield varies between 3.77 and 6.80 t ha^{-1}. Additionally, Sinanbey has a hectoliter weight ranging from 62.4 to 70.8 kg while 1000-kernel weight varies between 40.7 and 47.9 g with a protein content ranging from 13.0 to 14.6 %. The ratio of plump kernels is between 37.4 and 77.6% for a 2.5 mm sieve. Sinanbey is recommended for winter sowing in dry and semi-arid areas of Central Anatolia and Transitional regions.

Acknowledgments

Sinanbey variety was released via the Central Anatolia Region Barley Breeding Research Project numbered TAGEM/TBAD/B/18/A7/P40/172 and funded by the General Directorate of Agricultural Research and Policies (TAGEM).

Figure 1. General view of field, spike, and kernels of Sinanbey variety. (Original)

References and Notes

Anonymous (2021). Report of Central Anatolian Dry Barley Registration Trials, Ankara, Türkiye (in Turkish).

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Journal of Crop Breeding and Genetics

9(2):175, 2023

Registration of "Samsoy" Soybean [*Glycine max* (L.) Merr.] Variety

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Citation:
Erdoğmuş M., Ergin M., Yılmaz S., Erol SA., Bayram C., 2023. Registration of "Samsoy" Soybean [*Glycine max* (L.) Merr.] Variety. Ekin J. 9(2):175.

Received: 12.10.2022 ***Accepted: 14.03.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.2023***

Samsoy is a Soybean [*Glycine max* (L.) Merr.] variety (Figure 1) developed by Black Sea Agricultural Research Institute, Samsun, Türkiye and registered in 2019 Samsoy was developed by crossing (Sprite 87 x Macon).

In terms of morphological characteristics, Samsoy variety has determinate plant growth type, semi-upright, medium early (143 days) with white flowers. It can grow up 90-115 cm in favorable conditions and first pod height is 15 cm. Seeds of Samsoy variety are round flat and light brown (Figure 1). Moreover, its pods are resistant to grain cracking and lodging. Agricultural characteristics of Samsoy variety are that number of pods/plant is 90 and weight of 1000 grain is 202 g. The yield of Samsoy variety is varying between 370-500 kg/da. Its protein and fat ratios vary between 36-42% and 19, 5-21.7%, respectively. Resistance or tolerance to pests and diseases has not been tested. However, disease and harmful contamination were not found in field experiments.

It can be grown as the main commodity in the Black Sea Region, Marmara Region and Transitional Zones, as a second crop in the Mediterranean Region and Southeastern Anatolia in Türkiye.

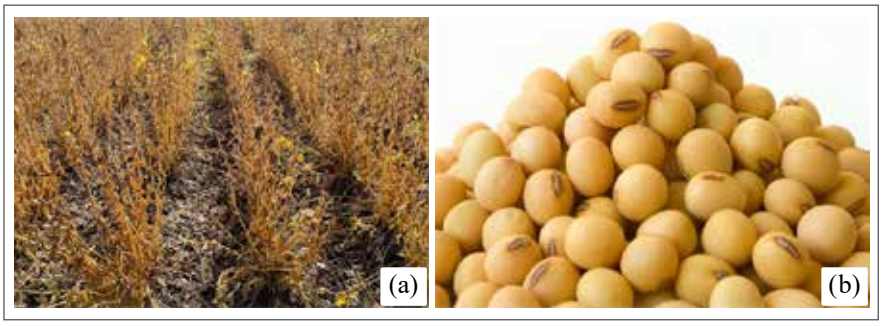

Figure 1. (a) Plant(b) Seeds. (Original)

References and Notes

Anonymous, (2019). Ana ürün soya tescil raporu, Ankara. (in Turkish, unpublished)https://www.tarimorman.gov.tr/BUGEM/TTSM/Sayfalar/Detay.aspx?SayfaId=132

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Ekin
Journal of Crop Breeding and Genetics

9(2):176, 2023

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Registration of "Bossa 159" Cotton (*Gossypium hirsutum* L.) Variety

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Citation:
Nasırcı Z., Dolançay A., 2023. Registration of "Bossa 159" Cotton (*Gossypium hirsutum* L.) Variety. Ekin J. 9(2):176.

Received: 13.10.2022 ***Accepted: 09.02.2023*** ***Published Online: 31.07.2023*** ***Printed: 31.07.20223***

Bossa 159 is upland cotton (*Gossypium hirsutum* L.) variety developed by Eastern Mediterranean Agricultural Research Institute (EMARI) registered in 2021. Bossa 159 is developed by single crossing as Cerdo 45/GS 93 in pedigree method. Crossing was made in 2010 in Adana. F_1 generation was grown in Adana and all individual plant selections, progeny rows and line selections were made in Adana. Elite line performance tests were carried out in Adana, Hatay and Şanlıurfa in 2017-2018.

Bossa 159 is in the medium-early maturity group, besides having a semi-pyramidal plant form and upper medium tall cultivar. Petal colour is cream and pollen colour yellow. Leaf and stem pubescence is moderate.

Bossa 159 variety has high seedcotton yield, lint yield and adaptability as well as superior fiber quality properties according to upland cotton. Average of % Lint is 42-43. Some of important fiber quality values are micronaire 4.8, length 32-33 mm, strength 33 g/tex, Rd 78 and Trash ID 4.

Plant height is between 130 and 160 cm depending on the growing conditions and cotton regions. It is medium early and as it has very good adaptation ability, it has been grown throughout Çukurova, Aegean and South Eastern Anatolia regions of Turkey. It gives high seedcotton yield both on different soil and under high temperature conditions. It is tolerant to verticillium wilt. Its yield potential is high however, high yield can be obtained if environmental conditions are applied with good agronomic practices by farmers. In the trials conducted by Varıety Registration and Seed Certification Center (TTSM) in the Aegean-Mediterranean regions in 2019 and 2020, Bossa 159 variety ranked third in terms of seedcotton yield and fourth terms of lint yield.

The highest seedcotton yield obtained was 6450 kg/ha^{-1} in Adana and Şanlıurfa in 2017-2018 growing years. Average of mean yield of the variety testing experiment was 6150 kg/ha^{-1} in all cotton regions and growing conditions.

Pre-basic seeds of the Bossa 159 cultivar have been produced by Eastern Mediterranean Agricultural Research Institute. Seed sales and marketing rights of Bossa 159 variety were transferred to Caso Seed Industry company from Diyarbakır for 10 year period in March 2020. Seed lots are currently produced and marketed by the Caso Seed Company.

Figure 1. Before blooming, blooming and harvesting stage of the Bossa 159 cultivar. (Original)

References and Notes

Anonymous (2020). Ege-Akdeniz Pamuk Tescil Raporu, Ankara, Türkiye (in Turkish).

About the Journal

Ekin, Journal of Crop Breeding and Genetics, is an international journal owned and edited by the Plant Breeders Sub-Union of Turkey (BISAB). Ekin is aimed at keeping information among plant breeders about new advances in the plant breeding and genetics as well as genetic diversity of plant species. Ekin publishes research papers and critical reviews on all aspects of plant breeding, genetics and plant registrations cover; old and new cultivars, local populations and introduction materials, germplasm, resistance sources for biotic and abiotic stresses, parental lines, genetic stocks, breeding materials, mapping populations. All manuscripts submitted for publication are reviewed by at least two referees and accepted for publication by editors based on advice from referees.

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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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The list of references should include cited works in the text. Personal communications (Personal com. with Prof./Dr./Mr./Ms. Ucar, Ankara, Turkey, 2012) should only be mentioned in the text. The works under consideration, submitted and unpublished works should not be listed in the References section. References should be chronologically alphabetized by the surnames of the first author of each work. Some examples;

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.

Toker C and Canci H (2003). Selection of chickpea (*Cicer arietinum* L.) genotypes for resistance to ascochyta blight [*Ascochyta rabie*i (Pass.) Labr.], yield and yield criteria. Turk J Agric For27: 277-283.

Toker C, Canci H and Ceylan FO (2006). Estimation of outcrossing rate in chickpea (*Cicer arietinum* L.) sown in autumn.Euphytica 151: 201-205.

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

Book:

Toker C (2014). Yemeklik Baklagiller. BISAB, Ankara.

Book chapter:

Toker C, Lluch C, Tejera NA, Serraj R and Siddique KHM (2007). Abiotic stresses. In: Chickpea Breeding and Management,Yadav SS, Redden B, Chen W and Sharma B (eds.),CAB Int. Wallingford, pp: 474-496.

Online document:

FAOSTAT J (2013) http://faostat.fao.org/site/567/default.aspx# anchor. Accessed 15 May 2013.

Dissertation (Thesis):

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

Acknowledgments

Acknowledgments of people, grants, funds, etc. could be placed before the reference list. The names of funding organizations should be written.

Abbreviations

Abbreviations should be defined at first mention and used consistently.

bisab
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ISSN 2149-1275

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