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Studies on General and Specific Combining Ability Effects in Onion Using Male Sterile, Maintainer and Restorer Lines and Hybrids

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ABSTRACT

An experiment was conducted on onion variety Hisar-2 in field conditions over two years to identify eight male sterile lines and two maintainer lines using pollen staining test and 24 hybrids obtained from crossing between male sterile and restorer lines. Observations were recorded on five randomly selected plants for height and attributes of bulb yield and quality. Phenotypic coefficient of variation was higher then genotypic coefficient of variation for most of the traits. The line MS 20 was found good general combiner for a greater number of traits. Line MS 34 was found good general combiner (GCA) for number of leaves per plant, average weight of bulb, total bulb yield, marketable yield, moisture content of bulb (%) and dry matter content of bulb (%). Tester Pusa Red figured good general combiner for plant height, diameter of bulb, average weight of bulb, total bulb yield, marketable yield, moisture content of bulb (%), dry matter content of bulb (%) and total soluble solids of bulb (%). Crosses MS 35 x Hisar- 3, MS 37 x Hisar-3, MS 22 x Agrifound Dark Red, MS 40 x Pusa Red and MS 21 x Pusa Red exhibited high Specific Combining Ability (SCA) suggesting the utility of heterosis breeding. Both additive and non-additive gene actions were operative to determine bulb yield and its components and quality parameters, which suggest that selection and heterosis breeding can be practice for improvement of onion.

Keywords: *Allium cepa* L., breeding, male sterility, general combining ability, specific combining ability, hybrid

Introduction

Onion (*Allium cepa* L.), 2n=16, belongs to Alliaceae family and a native of Central Asia, Near-East and Mediterranean regions (Vavilov, 1951), is an important vegetable crop after potato and tomato in the world. Globally, it is grown in mainly Asian countries, Middle-East Europe and North America. In India, the major onion producing states include Maharashtra, Karnataka, Madhya Pradesh, Gujarat, Bihar, Andhra Pradesh, Rajasthan, Haryana, and Tamil Nadu. Onion in production, productivity and area under onion were 26.6 million tonnes with average productivity 1.64 mt/ha from 16.24 million hectare area, respectively was recorded in 2020-21 (Anonymous 2020-21).

Onion crises are subjected to nexus of glut and demand and supply in the market. Therefore, increased production of onion and its availability in all seasons is most important for farmer, consumers and governments alike. One of the ways to increase onion production is to rely on hybrid breeding in onion. In view of timing, size of its flower, the traditional practices of hand emasculation and pollination is cumbersome, not practical and as serious limitation on quantity of hybrid seed production.

Onion breeders follow efficient approaches for selecting and testing parents based on general combining ability (GCA) and specific combining ability (SCA) for producing superior hybrid or varieties. There is a need to develop hybrids in onion having earliness, uniformity in bulb size, longer shelf-life, higher yield and quality.

There are two ways mainly used for identification of male sterility. The most general method is based on morphological examination of flower for impaired

development of anthers and second one is microscopic examination wherein, pollen viability test using acetocarmine is conducted (Heslop and Harrison, 1992).

Considering the above facts in view, the present study was conducted on 'Identification of male sterility line, their maintenance and evaluation of male sterile (A line), its maintainer lines (B line) and restorer lines (R line) based on general and specific combining ability estimates.

Materials and Methods

An experiment was conducted at Research Farm of the Department of Vegetable Science, CCS Haryana Agricultural University, Hisar, Haryana, India to develop male sterile lines and their maintainers (2018-19). During onion growing winter season the weather condition at Hisar are cooler and chilling temperature prevails in January. Male sterile lines (A lines) were selected from the onion plots of variety Hisar onion -2 by morphological examination and microscopic test for pollen viability using acetocarmine staining (Heslop and Harrison, 1992). Also, these selected male sterile lines and their counterpart male fertile maintainer lines (B lines) selected from the same plots were crossed in A x B manner to maintain the male sterile lines. Also, selfing was carried out to maintain B lines. The line x tester matings were attempted to produce hybrids using male sterile lines (A lines) as lines and restore lines (R lines) as testers namely verities Hisar Onion-3, Pusa Red, and Agri-found Dark Red.

The experimental material was sown in field conditions in two blocks replicated thrice. The first block was allocated to A lines and B lines and the second block was allocated to A line and R line to produce hybrids. Finally, the A, B lines and F_1 hybrids were grown in randomized block design with three replications to evaluate GCA and SCA as per line x tester design.

Plant Characters

Five randomly selected plants were studied to record observation on plant traits like plant height (cm) and number of leaves per plant and bulb characters like grade wise number and weight of bulbs, both polar and equatorial diameter of the bulb (cm), average bulb weight (g), per plot bulb yield converted to total bulb yield (q/ha), marketable bulb yield (q/ha), bulbs moisture content (%), bulb dry matter content (%), bulb total soluble solids (%).

Statistical analysis

Analysis of variance was conducted for experimental design as described by Panse and Sukhatme (1966) and for general and specific combining ability as described by Arunanchalum (1974).

Results and Discussion

Identification of male sterile lines and them maintainers

Based on morphological examination of anthers (Figure 1a and 1b) 8 male sterile lines (A line) namely MS20, MS21 MS22, MS23, MS34, MS35, MS37and MS40 were isolated from the onion variety Hisar-2 grown in field. These male sterile lines were maintained by crossing with B lines (pollinator 5 and 11) from the same field. From among 95 such crosses above mentioned 8 lines showed 100% sterility. Therefore, the sterility status of these eight lines was further confirmed by microscopic examination for pollen viability test using acetocamine stain (Figure 2a and 2b: A- fertile, B- sterile pollen).

Analysis of variance

Analysis of variance (data not given for brevity) revealed significant difference among lines (A and B) and testers (R lines) for various characters studied in the investigation. This warranted conducting the analysis of variance for combining ability. The analysis showed significant interaction effects among parents and their hybrids which revealed that different combination of A and R lines crossing would result in to different hybrids possessing different traits and thus enable selection for superior hybrids. The lines, testers and hybrids showed significant difference for various characters and therefore, the estimates of components of genetic variance and combining ability were computed.

Components of genetic variance and estimates of genetic parameters

The phenotypic and genotypic variance components were computed to estimate phenotypic (PCV) and genotypic (GCV) coefficients of variation. The results revealed that in general PCV were higher than GCV. This was evident that PCV for number of D grade bulbs (30.95) was highest among studied traits, while it was moderate for marketable bulb yield (18.86) followed by total bulb yield (18.21), number of leaves per plant (16.19), number of B grade bulb (15.99) and polar diameter of bulbs (13.75) whereas all other traits it was low to very low for example PCV for plant height (10.40) was followed by dry matter content of bulb (9.04), total soluble solids of bulb (5.59) and the lowest being for moisture content of bulb (1.02)

The high estimates of heritability were observed in characters like marketable bulb yield (99.73), total bulb yield (99.70), average weight of bulb (94.60), numbers of 'A' grade bulb (94.00), and number of leaves per plant (93.90), plant height (92.50), and diameter of bulb (polar) 91.60. Genetic advance was high for numbers of 'D' grade bulb (53.38) and 'C' grade bulb (46.69).

Combining Ability Analysis

From analysis of variance for combining ability (Table 1) it is evident that the mean sum of squares due to crosses was significant for all ten characters studied. The mean sum of square due to lines (female) was found to be significant for the characters plant height and number of leaves per plant. The mean sum of squares due to testers (male) was significant for plant height and diameter of bulbs (polar). It also revealed that mean sum of squares due to line x tester interaction effect were significant for all the characters studied except bulbs size grades (number of A grade bulb) and diameter of bulb (polar).

Combining ability effects:

General combining ability (GCA) effects of parents

The estimates of GCA effect in favour of male sterile, maintainer, tester lines for various plant and bulb traits are presented in Table 2. Male sterile line MS 20 among eight male sterile lines exhibited good general combining ability for various traits. These included plant traits like plant height and bulb traits like number of 'B' grade bulbs, total bulb yield, marketable bulb yield, bulb moisture content (%) and bulb dry matter content (%). Likewise, the line MS 34 was observed to be good general combiner for bulb traits like average weight of bulb, total bulb yield, marketable bulb yield, bulb moisture content (%) and bulb dry matter content (%) and plant trait like number of leaves per plant. On the same pattern line MS 22 recorded good general combining ability for total soluble solids of bulb (%) whereas, line MS 40 was found to be good combiner for number of 'A' grade bulb, number of 'C' grade bulb, average weight of bulb, and bulb dry matter content (%). Two male sterile lines namely MS 20 and MS 23 figured good general combiner for total soluble solids (TSS) of bulb. Our results were corroborated by earlier studies of Joshi and Tandon (1976), Hosfield et al. (1977), Divakara (2001), and Veeregowda (1988), Sundari (2003), Satyanarayana (2014), Patil and Subramaniam (2020),and Ara and Deb (2021).

Good general combining ability for plant height, polar and equatorial bulb diameter, average bulb weight, total bulb yield, marketable bulb yield, bulb moisture content, bulb diameter and total solids of bulb was recorded for tester Pusa Red among all other testers.

Specific combining ability effects

The data present in table 3 showed that good specific combining ability was exhibited by cross MS 35 x Hisar- 3 for number of 'A' grade bulb, average weight of bulb, total bulb yield, marketable bulb yield and bulb moisture content (%). Likewise, good specific combining ability in cross MS 37 x Hisar-3 was evident for plant height, number of 'A' grade bulb, average weight of bulb, total bulb yield, marketable bulb yield and bulb moisture content (%). Hybrid MS 22 x Agrifound Dark Red revealed good specific combining ability for number of 'A' grad bulb, average weight of bulb, total bulb yield, marketable bulb yield and (%), dry matter content of bulb (%).Whereas, hybrid MS 40 x Pusa Red figured to be good specific combiner for number of leaves per plant, number of 'C' grade bulb, total bulb yield and marketable bulb yield (%). Significant positive specific combining ability for total soluble solids was depicted by hybrid MS 21 x Pusa Red.These results confirm the earlier reports of Netrapal et al. (1986), Aghora and Parhak (1991), Divakara (2001), Sundari (2003), Aghora (1985), Veeregowda (1988),Satyanarayrna (2014), Patil and Subramaniam (2020) and Ara and Deb (2021).

Components of variance for GCA and SCA

The estimates of SCA (specific combining ability) variance were found to be greater than corresponding GCA (general combining ability) variance for all the characters except plant height and diameter of bulbs (polar). The estimated components of variance due to GCA (σ2GCA) were significant for plant height, number of leaves per plant, diameter of bulb (polar) and total soluble solids of bulb, while those due to SCA (σ2SCA) were highly significant in all character studied. The contribution of lines as compared to the testers was found to be more for all the characters except diameter of bulbs (polar).

Line MS 37, MS 20 and tester Hisar 3, Pusa Red were found to be good general combiner for plant height, thus indicating the predominance of additive gene effect. The crosses for SCA effect had shown significance, thus indicating the presence of non-additive gene effects. Two hybrids like MS 37 x Hisar 3 and MS 23 x Agrifound Dark Red exhibited positive and highest significant SCA effect for plant height, thus the character had both additive and non-additive gene effect. The results are in close agreement with the findings of Netrapal et al. (1980, 1986), Aghora (1985), Divakara (2001) Sundari et al. (2003).

Regarding number of leaves per plant, MS 34, MS 35 lines exhibited highest GCA effect and emerged as good combiner. Significantly positive specific combining ability effects exhibited by only one cross combination MS 40 x Agrifound Dark Red. It was generally observed that the parent hybrid having higher number of leaves resulted into higher GCA and SCA effect, respectively. These finding also revealed the fact that best general combiners may not always result in high SCA effects in

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their respective cross combination. The above findings are in accordance with Netrapal (1980).

Lines MS 23 and MS 40, lines MS22 and MS37 and lines MS 34 and MS 40 exhibited positive and significant high GCA effect for numbers of A, B and C grade bulb, respectively. In case of testers Hisar 3 showed highly positive and significant GCA effect for number of 'B' grade bulb and regarded as good combiner, thus indicating the predominance of additive gene effect.

The significant GCA variance and non-significant SCA variance indicated predominance of additive effect. However, the ratio of GCA/SCA indicated there were more of additive effects for equatorial diameter than polar diameter (Table 3). The study of GCA effect revealed that MS 35 among lines and Hisar 3 and Pusa Red among testers in case of polar diameter; line MS 35 and tester Pusa red in case of equatorial diameter, were found to be the best combiners with higher GCA effects. The maximum SCA effects was found in hybrid MS 22 x Hisar 3 for polar diameter; and MS 34 x Hisar 3, MS 21 x Pusa Red and MS 35 x Agrifound Dark Red for equatorial diameter of bulb. From the result it could be concluded that few parent having higher GCA effect contributed towards the increased bulb diameter in the hybrids, which presented higher SCA values. These results are conformity with the findings of Netrapal et al. (1986), Aghora and Parhak (1991), Divakara (2001), Sundari (2003), Aghora (1985), Veeregowda (1988) and Satyanarayrna (2014).

MS 34 and MS 40 among lines and tester Pusa Red emerged as best combiners for the average weight of bulb producing higher significant GCA effects, thus indicating the predominance of additive gene effect. While MS 35 x Hisar 3, MS 22 x Agrifound Dark Red, MS 37 x Hisar 3 and MS 23 x Agrifound Dark Red exhibited the maximum SCA effects. From these results it could be inferred that the parents having good combining ability produced heterotic hybrids with higher SCA values. Results were confirmative with Joshi and Tandon (1976), Hosfield et al. (1977), Divakara (2001), and Veeregowda (1988), Sundari (2003) and Satyanarayana (2014).

Conclusions

Thus our results revealed that the onion genotypes studied in the present investigation exhibited significant variation for different traits as corroborated by high estimates of PCA and GCA. High heritability coupled with significant variation suggested that selection would be effective for improvement of different traits associated with bulb yield and quality in onion. The analysis of variance of combining ability for line x tester design revealed significant differences among female (A and B lines) and male lines (testers/restorers) and significant interaction among parents and hybrids. Which indicated that both non additive gene effects and heterosis are involved in determining bulb yield potential. The analysis for component of variance suggested presence of additive as well as non-additive gene effects suggesting there by that the hybrids may be used for population improvement programme or direct use as hybrids. From general and specific combining ability analysis of parents and hybrids it appeared that lines; MS 20, MS 22, MS 23, MS 34 and MS 40 good general combiner for a number of traits associated with bulb yield and quality. Likewise, among testers Pusa Red figured good general combiner for number of traits contributing to bulb yield and quality. A few F_1 hybrids namely MS 35 x Hisar- 3, MS 37 x Hisar-3, MS 22 x Agrifound Dark Red, MS 40 x Pusa Red and MS 21 x Pusa Red appeared good specific combiner for a number of traits associated with total bulb yield as well as marketable bulb yield. These crosses involved parents with high GCA or average GCA indicating that both additive as well as non-additive gene effects are implied in good level of SCA. Therefore, these crosses may be effectively used in onion breeding programme either for developing improved population like composite or single cross hybrids for sustainable onion production.

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Table 1. Analysis of variance in respect of 10 characters for combining ability .

Sr. No.	Source		Replication	Crosses	Lines	Tester	Line x Tester	Error
	Degrees of freedom		**2**	**23**	**7**	**2**	**14**	**46**
1	Plant height (cm)		23.944	46.268**	59.434*	177.671**	20.914**	7.988
2	Number of leaves per plant		0.268	2.339**	4.568*	0.429	1.498**	0.361
3	Bulbs size grades (number and weight basis)	A (>70g)	8.222	165.608**	235.918	47.263	150.216	6.888
		B (40-70g)	96.055*	256.222**	233.396	130.722	285.563**	21.823
		C (<40g)	19.430	174.490**	206.880	29.347	179.029**	12.271
		D (D and B)	16.666	38.704**	35.458	20.166	42.976**	8.724
4	Diameter of bulbs (cm)	Polar	0.134	0.769**	0.365	5.898**	0.238	0.131
		Equatorial	0.151	0.527**	0.592	0.613	0.483**	0.061
5	Average weight of bulb (g)		0.152	99.198**	100.209	69.783	104.078**	6.805
6	Total bulb yield (q/ha)		16.917	3387.430**	4030.530	3351.732	3070.979**	17.552
7	Marketable bulb yield (q/ha)		5.826	3506.483**	4194.627	3476.916	3166.635**	14.565
8	Moisture content of bulb (%)		0.018	4.054**	4.597	1.852	4.097**	0.278
9	Dry matter content of bulb (%)		0.018	4.054**	4.597	1.852	4.097**	0.278
10	Total soluble solids of bulb (%)		0.350	1.228**	1.858	1.806	0.829***	0.242

*, **P ≤ 0.05 and 0.01, respectively

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Table 2. Estimates of general combining ability (GCA) effects for 10 characters in onion.

Genotypes	Plant Height (cm)	Number of Leaves Per Plant	Bulbs Size Grades (Number and Weight Basis)				Diameter of Bulbs (cm)		Average Weight of Bulb (g)	Total Bulb Yield (q/ha)	Marketable Bulb Yield (q/ha)	Moisture Content of Bulb (%)	Dry Matter Content of Bulb (%)	Total Soluble Solids of Bulb (%)
			A (>70g)	B (40-70g)	C (<40g)	D (D and B)	Polar	Equatorial						
LINES														
MS20	3.19**	-0.31	2.79*	4.66**	-8.63**	1.04	0.05	-0.03	0.09	40.42**	37.93**	-0.64**	0.64**	0.14
MS21	-0.56	-0.66**	2.79*	-2.66	-2.97*	2.59*	0.05	0.12	0.78	-7.15**	-8.36**	-0.07	0.07	-0.02
MS22	-1.53	-0.17	-4.09**	5.22**	0.69	-1.84	-0.15	-0.31**	-1.69	-16.94**	-6.08**	0.43*	-0.43*	0.64**
MS23	-3.89**	-0.10	5.23**	-4.55**	-0.19	-0.51	0.01	0.09	1.35	2.71	7.73**	-0.18	0.18	0.48**
MS34	1.71	1.25**	2.12*	-3.00	2.25	-1.40	-0.25*	-0.14	5.20**	18.22**	24.18**	-0.95**	0.95**	0.24
MS35	-0.54	0.90**	-7.43**	-2.77	7.80**	2.37*	0.32*	0.53**	-4.22**	-27.47**	-23.38**	0.93**	-0.93**	-0.41*
MS37	3.42**	-0.76**	-7.31**	8.00**	-1.75	0.59	0.20	-0.10	-4.41**	-8.16**	-13.52**	0.97**	-0.97**	-0.58**
MS40	-1.80	-0.15	4.90**	-4.88**	2.80*	-2.84**	0.23	-0.17*	2.89**	-1.64	-18.49**	-0.49**	0.49**	-0.48**
CD 5% GCA	1.89	0.40	2.11	3.13	2.35	1.98	0.24	0.17	1.75	2.81	2.56	0.35	0.35	0.33
TESTERS														
Hisar 3	1.29*	0.15	-0.73	2.69**	-1.06	-0.19	0.36**	0.01	-1.42*	-8.88**	-2.11**	0.25*	-0.25*	-0.09
Pusa Red	1.83**	-0.12	1.22	-1.30	-0.06	0.90	0.20**	0.16**	1.89**	13.41**	12.95**	-0.30**	0.30**	0.31**
Agrifound Dark Red	-3.13**	-0.03	-0.48	-1.38	1.13		-0.57**	-0.16**	-0.47	-4.53**	-10.84**	0.05	-0.05	-0.22*
CD 5% GCA	1.16	0.25	1.29	1.91	1.44	1.21	0.15	0.10	1.07	1.72	1.57	0.22	0.22	0.20

*, ** P ≤ 0.05 and 0.01, respectively

Table 3. Estimates of specific combining ability (SCA) effects for 10 characters in onion.

Genotypes	Plant Height (cm)	Number of Leaves Per Plant	Bulbs Size Grades (Number and Weight Basis)				Diameter of Bulbs (cm)		Average Weight of Bulb (g)	Total Bulb Yield (q/ha)	Marketable Bulb Yield (q/ha)	Moisture Content of Bulb (%)	Dry Matter Content of Bulb (%)	Total Soluble Solids of Bulb (%)
			A (>70g)	B (40-70g)	C (<40g)	D (D and B)	Polar	Equatorial						
CROSSES														
MS 20 x Hisar 3	-0.32	0.53	4.62*	-7.25**	2.84	-0.08	-0.26	-0.13	0.33	12.18**	4.89*	0.32	0.32	0.43
MS 20 x Pusa Red	0.08	0.09	5.33**	-1.25	-3.48	-0.66	-0.07	0.03	2.17	7.30**	8.78**	-0.11	-0.11	-0.14
MS 20 x Agrifound Dark Red	0.24	-0.63	-9.96**	8.50**	0.63	0.75	0.33	0.10	-2.50	-19.48**	-13.68**	-0.21	-0.21	-0.29
MS 21 x Hisar 3	2.77	0.65	-0.37	10.41**	-7.15**	-2.63	-0.02	-0.26	-1.00	-2.86	-1.88	-0.24	-0.24	-0.82**
MS 21 Pusa Red	-2.13	-1.22**	0.66	0.75	-1.15	-0.55	0.24	0.49**	-1.69	-6.07*	-5.45*	-0.26	-0.26	1.15**
MS 21 x Agrifound Dark Red	-0.64	0.56	-0.29	-11.16**	8.30**	3.19	-0.22	-0.23	2.69	8.93**	7.33**	0.50	0.50	-0.33
MS 22 x Hisar 3	-2.06	0.032	-2.48	7.86**	-4.15*	-1.19	0.45*	0.13	0.15	7.94**	4.73*	-0.01	-0.01	0.02
MS 22 x Pusa Red	-0.57	0.46	-9.11**	-0.80	7.51**	2.55	-0.19	-0.09	-4.72**	-23.16**	-26.23**	-0.87**	-0.87**	-0.04
MS 22x Agrifound Dark Red	2.63	-0.69	11.59**	-7.05	-3.36	-1.36	-0.25	-0.04	4.57**	15.22*	21.50**	0.87**	0.87**	0.02
MS 23 x Hisar 3	-3.79*	-0.18	-10.15**	-1.02	3.73	7.47**	-0.25	-0.43**	-4.99**	-18.08**	-35.77**	-1.03**	-1.03**	0.33
MS 23 x Pusa Red	0.44	-0.28	7.22**	-9.03**	2.40	-0.44	0.11	-0.40**	1.65	1.58	-0.11	0.41	0.41	-0.37
MS 23 x Agrifound Dark Red	3.36*	0.46	2.93	10.05**	-6.13**	-7.02**	0.15	0.03	3.34*	16.50**	35.88**	0.63*	0.63*	0.048
MS 34 x Hisar 3	-1.94	-0.06	-4.04	-2.58	4.29*	2.36	0.34	0.78**	-5.39**	-23.80**	-30.44**	-1.12**	-1.12**	0.62*
MS 34 x Pusa Red	0.96	0.54	-2.00	8.08**	-6.70	0.77	-0.01	-0.55**	2.49	7.55**	2.77	0.58	0.58	-0.279
MS 34 x Agrifound Dark Red	0.98	-0.48	6.04**	-5.50*	2.41	-3.13	-0.33	-0.24	2.90	16.25***	27.66**	0.53	0.53	-0.344
MS 35 x Hisar 3	1.68	-0.35	5.84**	-3.13	1.40	-4.08*	0.01	0.01	11.98**	60.51**	58.05**	-2.36**	-2.36**	-0.096
MS 35 x Pusa Red	0.05	-0.32	-2.44	14.19**	-12.59**	1.00	0.12	-0.47**	-4.68**	-37.98**	-36.38**	0.56**	0.56**	-0.031
MS 35 x Agrifound Dark Red	-1.73	0.66	-3.40	-11.05**	11.19**	3.08	-0.13	0.45**	-7.29**	-22.54**	-21.66**	1.50**	1.50**	0.127
MS 37 x Hisar 3	3.68*	0.321	5.06**	-3.25	-1.70	-0.63	-0.139	0.048	4.44**	5.264*	12.40**	-0.85**	-0.85**	-0.023
MS 37 x Pusa Red	0.95	-0.283	-0.88	-2.91	6.29**	-2.88	-0.159	0.156	2.46	36.33**	16.61**	-0.57	-0.57	-0.151
MS 37x Agrifound Dark Red	-4.63**	-0.04	-4.18*	6.16*	-4.58*	3.52*	0.29	-0.20	-6.89**	-41.59**	-29.02**	-1.42**	-1.42**	0.17
MS 40 x Hisar 3	-0.03	-0.96**	1.51	-1.02	0.73	-1.19	-0.11	-0.15	-5.52**	-41.15**	-11.97**	1.14**	-1.14**	-0.45
MS 40 x Pusa Red	0.24	1.01**	1.22	-9.02**	7.73**	0.22	-0.04	0.03	2.31	14.45**	39.99**	-0.54	0.54	-0.13
MS 40 x Agrifound Dark Red	-0.21	-0.05	-2.73	10.05**	-8.47**	0.97	0.15	0.13	3.21	26.70**	-28.02**	-0.60	0.60	0.59*

*, **, *** P ≤ 0.05, 0.01 and 0.001, respectively

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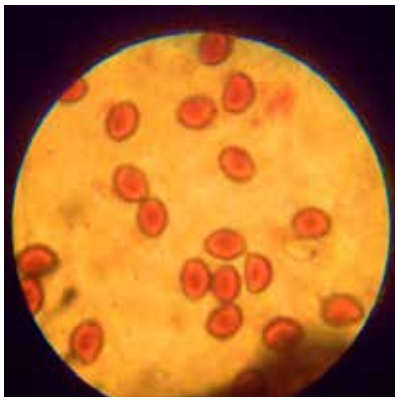
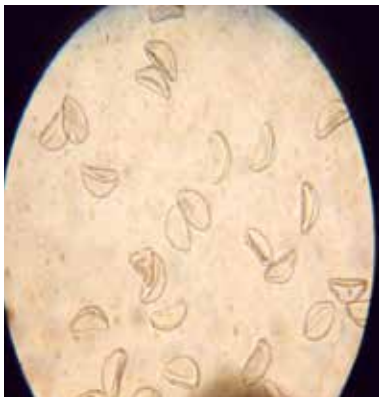
1a: A- Fertile anthers

1b: B- Sterile anthers

Figure 1a and 1b: (Original)



2a: A- Fertile pollen



2b: B- Sterile pollen

Figure 2a and 2b: (Original)

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Phenotypic Diversity of Red and White Onion Genetic Resources Collected from Different Countries

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ABSTRACT

The phenotypic diversity within onion populations is very high and occurs mostly due to bulb shape, height and diameter, neck width, weight, dry skin colour, bitterness and dry skin thickness. This study aimed to determine the phenotypic diversity of the genetic resources of red and white onions collected from different countries. Initially, a gene pool of 23 onion genetic resources was established, consisting of 14 red and 9 white onions. As a result of the research, it was determined that the red and white onion genotypes showed a high level of phenotypic diversity in terms of morphological traits. Cluster and principal component analysis (PCA) were performed to determine the relationships among the onion genotypes. As a result of the cluster analysis based on 31 variables, three groups and five subgroups were identified in the red onion genetic resources and two groups and four subgroups were clustered in the white onion genetic resources. A dendrogram was performed to evaluate the morphological similarities between the red and white onion genotypes. In the red onion genotypes, seven PC axes with an eigen value greater than 1 explained 90.2% of the total variation within the PCA. The total variation in white onion genotypes was found to be 96.4%. These results showed that the genetic variability was very high between the red and white onion genotypes. The results obtained will help onion breeders to develop high-quality, new onion varieties in the future.

Keywords**:** *Allium cepa,* genetic resources, diversity, multivariate analysis

Introduction

Onions are consumed for their bulbs and fresh green leaves. They are an appetizing vegetable species which can used in various ways, including raw in salads, cooked in meals, dried and used as spice powder in processed foods such as chips and cracker, and chopped and frozen in mass-produced food stuffs (Gökçe 2022). Onions are grown in all regions of the world except Antarctica. The global dry onion production in 2020 was approximately 104.554.458 tons, while the production of green onions was 4.452.728 tons (FAO 2022). The major onion producing countries are China, India, the USA, Egypt, Iran, and Türkiye.

The onion (*Allium cepa* var. *cepa* L.) was one of the first agricultural plants cultivated by human beings. It is a member of the Amaryllidaceae family within the *Allium* genus. There are more than 1000 species in this genus (Jones and Mann 1963; Gökçe 2011). It has been stated in the literature that 150 of these species grow naturally in Türkiye (Gökçe 2001). Edible onions and closely related species (shallot, garlic, leek, chives, Chinese onion) are part of the subfamily Allioideae (Costa et al. 2020). The gene centres of the onion are considered to be Afghanistan, Pakistan, Tajikistan, Iran, India, China, Uzbekistan, Turkmenistan and Türkiye (Karaağaç and Balkaya 2017; Bağcı et al. 2021; Gökçe 2022).

Plant genetic resources are very important in helping them adapt to the different ecologies in which they are grown, their resistance to diseases and pests,

and because they may have desirable characteristics that can be used in breeding programs (Balkaya and Yanmaz 2001; Karaağaç and Balkaya 2017). Using the existing genetic and phenotypic diversity, breeders have achieved significant success in the selection or development of new varieties with the desired characteristics in terms of adaptation, yield, quality, and resistance to diseases and pests in recent years (Taş and Balkaya 2021). The genetic diversity has occurred over time in the countries where onions are grown largely and many different landraces have occurred.

Plant genetic materials adapt to a region over time, and significant changes occur in their genetic structures as a result of environmental conditions. It is thus important to determine the level of morphological variation and phenotypic diversity in breeding programs (Yuguda et al. 2017). Many studies have been carried out by researchers in many different countries in order to collect, characterize and determine the phenotypic diversity levels of onion genetic resources. Mousavizadeh et al. (2006) investigated the morphological and agronomical diversity of Iranian onion landraces. It was determined that there were statistically significant differences between genotypes in terms of traits such as onion yield per plant, onion dry weight, dry matter ratio, bulb diameter, bulb height, shape index, number of leaves and leaf length. In another study, Mallor et al. (2011) studied the morphological and physicochemical characteristics of 86 local onion genetic resources in Spain. Their study determined that there was a high level of morphological variation in terms of bulb weight, shape, hardness, dry matter content and bitterness. Gvozdanovic et al. (2013) investigated the levels of phenotypic diversity in the Serbian onion genetic collection. It was determined that there was a high level of phenotypic diversity in onion genotypes, especially in terms of dry skin colour, the base colour of dry skin, and dry skin thickness traits. Sumalan et al. (2014) investigated phenotypic diversity levels in some local red onion cultivars in the Timiș Region of Romania. They reported that 15 red onion genotypes showed significant differences in terms of morphological characteristics. Sunil et al. (2014) determined phenotypic diversity and genetic variation in onion germplasm collected from different parts of the Indian Peninsular Region. It was determined that there was a high level of variation among genotypes in terms of plant height, number of leaves, leaf length, leaf diameter, bolting, dry matter content and bulb weight. Azimi et al. (2020) conducted a morphological characterization of onion genetic resources collected from Eastern Anatolia and South-eastern Anatolia Regions of Türkiye. As a result of their research, the dry skin colour of onion genotypes was found to be white, reddish and brownish-yellow; the bulb shape was oval or circular and flattened; and the base colour of the skin was white, reddish and yellow. In addition, it was determined that the bulb weight was ranged from 30.06 to 186.2 g and the number of leaves per plant varied between 6.1 and 16.6.

In recent years, it has become a common practice to use multivariate analysis methods in plant breeding programs (Karaağaç and Balkaya 2010). The multifaceted examination of morphological traits allows phenotypic diversity in the initial gene population to be determined. As the characters and the number of samples compared increase, classical statistical methods may be insufficient. The numerical taxonomic classification methods also called multivariate analyses used for the determination of variation and similarities require a sequence of choices, measurements, analyses and interpretations (Tan 2005). Using the data obtained from characterization studies, the existing similarities, differences and groupings are shown through cluster analysis and PCA (Balkaya and Ergün 2008; Balkaya et al. 2010; Karaağaç and Balkaya 2010; Hancı and Gökçe 2016; Kanal and Balkaya 2021). Different researchers have carried out various studies using these analyses to determine phenotypic and genetic diversity in onion populations (Manbachi et al. 2012; Sunil et al. 2014; Hancı and Gökçe 2016; Aryakia et al. 2016; Dangji et al. 2018; Manjunathagowda et al. 2021). This study aimed to determine the level of phenotypic diversity and significant variables in red and white onion genetic resources collected from different regions of the world.

Materials and Methods

This study was conducted between March 2020 and September 2021. In the first stage of the study, 14 red and 9 white onion genetic materials from different countries were obtained from the USDA ARS-National Plant Germplasm System (Table 1).

In order to determine the plant characteristics of the onion genotypes, cultivation experiments were carried out in the Faculty of Agriculture, Ondokuz Mayıs University between April and September 2021. The seeds were sown on 9 April 2021 in the plastic crates filled with a mixture of peat and perlite (v/2:1). The experiment was conducted in a randomized block design with three replications and 10 plants in each replication. All cultural treatments (irrigation, pests and diseases control, fertilization) were carried out regularly during the growing period (Gökçe 2022).

The morphological characterization criteria were used with the modification of the characteristics of the onion by the International Plant Genetic Resources

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Research Institute (IPGRI) and the International Union for the Protection of New Varieties of Plants (UPOV) (Table 2, Table 3). The characteristics for the green (fresh) onions were determined in the first week of July, and for the bulb onions between 16 August 2021 and 6 September 2021. The bulbs were harvested when the leaves and the stem of the plant had dried.

The statistical evaluation of the data obtained was carried out in the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc version 2.2; Exeter Software, New York, NY) package program (Rohlf 1993). PCA was applied to determine the amount of morphological variation among the onion genotypes examined, and factor coefficients were obtained indicating the weights of the main components that emerged based on the PC axes, their variance and cumulative variance ratios (Balkaya et al. 2009). PCA was used to determine the degree of characteristic variance between genotypes based on PCA, since one of the factor analysis methods is the reduction of a large number of variables correlated with smaller sets of variables called factors or components. Orthogonal rotation of the factor axes was used to extract factors having eigenvalues > 1 (Mohan et al. 2016). A three-dimensional (3D) PCA was constructed to provide another means of testing the relationships among genotypes using the EIGEN module in the NTSYS. After it was determined that the cumulative variation in the first three axes was of sufficient size, cluster analysis was performed to show the similarities and differences of the genotypes from each other. Row data were standardized and the SIMINT module was used to compute a distance matrix. Then, a distance matrix was used to construct a dendrogram based on the unweighted-pair group method arithmetic average (UPGMA) method in the SAHN (Sequential, agglomerative, hierarchical and nested) clustering method module. In order to see how well a cluster analysis represented the distance matrix, the COPH module was used to transform the tree matrix to a matrix of ultra-metric distances. Finally, the MXCOMP module was used to compare these ultra-metric distances and distance matrix produced for the UPGMA analysis (Huamán and Spooner 2002).

Results and Discussion

Assessment of red and white onion genotypes by principal component analysis

Knowing the existing morphological variations and phenotypic diversity levels in gene pools and applying them to breeding programs are essential elements for increasing success (Balkaya et al. 2010). PCA is performed by displaying the genotype projections on an axis or series of axes that can best represent the relationship between genotypes in vegetable breeding in a multidimensional area (Balkaya et al. 2010; Karaağaç 2013). As a result of the PCA, eight independent axes with eigenvalues greater than 1 were determined in the red onion genotypes. The coefficients of the eigenvalues of the first eight main components varied between 1.3 and 6.8 (Table 4). It has been reported in the literature that principal component axes with eigenvalues greater than 1 are very reliable in PCA analysis (Mohammadi and Prasanna 2003; Özdamar 2004; Balkaya et al. 2010; Kanal and Balkaya 2021). As a result of the analysis, the cumulative variation values of the axes and the total variation rates were also determined. The eight axes were found to represent 90.2% of the total variation (Table 4). Özdamar (2004) reported that in order for factor coefficients to be reliable in PCA, the PC axes should explain 2/3 of the total variation. In the current study, 2/3 of the total variation was more than explained by the first four PC axes (65.3%). Though clear guidelines do not exist to determine the significance of a character coefficient, one rule of thumb is to treat coefficients > 0.3 as having a large enough effect to be considered important (Karaağaç 2006; Taş and Balkaya 2021). The first PC axis accounted for 22.1% of the total variation (Table 4). Traits with a high coefficient were leaf length (-0.43), bulb height (0.32), root disc diameter (0.31), intensity of dry skin colour L (-0.91), intensity of dry skin colour b (-0.87), the position of the maximum diameter of the bulb (0.67), the shape of the bulb's stem end (0.48), time of harvest (0.75), and the base colour of the bulb's dry skin (-0.34). In the second PC axis, which represented 20.5% of the total variation, these traits were the bulb's dry matter content (0.42), the shape of the bulb's root end (0.38), and the thickness of dry skin (-0.85). In the third axis of PC, which represents 13.1% of the total variation, the number of leaves per pseudo stem (-0.93), foliage colour (0.30), intensity of foliage's green colour L (-0.41), leaf diameter (-0.50), and pseudo stem length (-0.47) were the criteria to be considered in the morphological identification. In red onion genotypes, the PC coefficients of bulb weight, diameter and shape characters were found to be low. It is thought that the constricted variation in these characters may have been because the genotypes were selected by the breeder before they were included in the seed gene bank.

As a result of PCA, the total variation was found to be 96.4% among white onion populations (Table 5). According to the analysis, there were seven PC axes with an eigen value greater than 1. The coefficients of the eigenvalues of the seven main components varied between 1.4 and 7.4 (Table 5).

The first PC axis accounted for 25.4% of the total variation (Table 5). Characters with a high coefficient were leaf length (0.69), pseudo stem length (0.30), degree of waxiness (0.36), foliage cranking (0.40), bulb weight (-0.95), bulb height (-0.52), bulb diameter (-0.86), bulb hardness (-0.62), shape of the bulb's root end (0.33), and bulb shape (0.36). In the second PC axis, which represented 21.6% of the total variation, foliage colour (-0.78), leaf diameter (0.33), degree of waxiness (-0.82), dry matter content of bulb (-0.59), intensity of dry skin colour L (0.97), shape of bulb's stem end (-0.54), degustation analysis (0.37) and the maturation time were significant. The third PC axis represented 13.5% of the total variation. In the current study, 2/3 of the total variation was more than explained by the first four PC axes (74.3%).

Traits of commercial importance, such as bulb shape, bulb firmness, and bulb weight and dimensions, for which limited variation was detected in red onions, had a very high variation in the white onion genotypes. These results indicated that white onions are subject to a lower selection severity than red onion genotypes. Many studies have been conducted to determine the levels of morphological variation and phenotypic diversity in onion populations. One study found that the first three principal axes had 83.42% of the total phenotypic diversity in Iranian onion landraces. On the first main axis, bulb dry weight, diameter, bulb dry matter, bulb yield per plant, leaf length and the number of leaves were significant (Mousavizadeh et al. 2006). In another study, Sunil et al. (2014) determined that the total variation reached 99.76% in the first four axes in an onion germplasm collection collected from different parts of the Indian Peninsular region. Hancı and Gökçe (2016) found the morphological variability ratios to be 71.84% among accessions in nine main axes with eigen values >1 in Turkish onion genotypes. Our results were similar to those discussed in the literature. In addition, these results showed that the red and white onion populations are heterogeneous.

Grouping of red and white onion genotypes by cluster analysis

Cluster analysis is more sensitive and reliable when 25% of the total variation or more is explained by the first two or three axes in PCA (Mohammadi and Prasanna 2003; Kanal and Balkaya 2021). The data to be used in cluster analysis are evaluated taking the PCA results into consideration. Genetic similarity among genotypes was estimated using UPGMA cluster analysis based on morphological traits. The dendrogram of red onions obtained from cluster analysis is shown in Figure 1. In the dendrogram, dissimilarity coefficients among genotypes ranged from -0.14 to 0.31. At the result of cluster analysis based on 31 morphological traits, the red onions were divided into three clusters (Table 6).

Group A: The largest number of genotypes were clustered in this group (Table 6, Figure 1). When compared with the genotypes in other groups, traits like intensity of foliage's green colour L (39.9), bulb weight (106.9 g), root disc diameter (13.2 mm) and the intensity of dry skin colour b (13.2) were found to be higher than in groups B and C (Table 7). A wide bulb neck width is an undesirable trait in onion varieties. The red onion genotypes in this group had the highest bulb neck width (15.2 mm). The bulb shape of genotypes in Group A were round, broadly egg-like, oval and broadly oval. Compared to other groups, foliage waxiness was not detected in this group (Table 7).

Group B: The lowest number of genotypes (two genotypes) was clustered in this group (Table 6). The average bulb height (50.6 mm) of the genotypes in this group was higher than in the other groups (Table 7). All the genotypes had dark green foliage, and were egg and oval-shaped. The pseudo stem diameter (7.9 mm) and bulb neck width (10.8 mm) of the genotypes in group B were thinner than in the other groups. The genotypes had hard bulbs. The bulb dry matter content (8.8%) was the lowest among the groups (Table 7).

Group C: Five genotypes were clustered in this group (Table 6). Traits such as the number of leaves (9.6), the intensity of green colour b (7.2), leaf length (55.4 cm), leaf diameter (12.1 mm), pseudo stem length (12.0 cm), pseudo stem diameter (9.6 mm), bulb diameter (64.1 mm), bulb dry matter content (12.0%) and the intensity of dry skin colour L (55.6) were higher than in the other groups (Table 7). The red onion genotypes in Group C were oval or broadly oval-shaped. In terms of dry skin thickness, the genotypes in this group had thin skin. All the genotypes were found to have a sweet taste according to the degustation panel test. The bulb height (44.2 mm) was lower than in group A and group B (Table 7).

The dendrogram of the white onion genotypes is shown in Figure 2. Genotypes were clustered in two groups and four subgroups (Table 8). In the dendrogram, the dissimilarity coefficients among genotypes ranged from -0.14 to 0.31. The general characteristics of these groups are given below.

Group A: Five genotypes are clustered in this group (Table 8, Figure 2). The genotypes in this group were superior to Group B in terms of traits such as the average number of leaves (9.2), the intensity of green colour b (5.0), leaf length (55.4 cm), leaf diameter (11.4 mm), pseudo stem diameter (9.2 mm), and

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bulb dry matter content (9.8%) (Table 9). The bulbs of the genotypes in this group were the hardest. The bulbs were round and broadly oval. The genotypes in Group A were harvested earliest.

Group B: This group consisted of four genotypes (Table 8, Figure 2). The genotypes in this group were superior to group A in terms of the intensity of green colour L (39.8), pseudo stem length (11.0 cm), bulb weight (128.0 g), bulb height (57.2 mm), bulb diameter (66.3 mm), root disc diameter (13.1 mm), the intensity of dry skin colour L (69.4) and the intensity of dry skin colour b (9.4) (Table 9). The bulbs were round, rhombic and oval. Foliage waxiness was present in all genotypes.

Onion populations have been grouped by cluster analysis by many researchers. Mousavizadeh et al. (2006) determined that a population formed from 20 local landraces and two hybrid varieties clustered in four main groups. Mallor et al. (2011) found three main groups in terms of morphological and physico-chemical characteristics for 86 local onion genetic resources. Similarly, Manbachi et al. (2012) classified 23 onion genotypes into three groups by cluster analysis. In another study, Manjunathagowda et al. (2021) studied the genetic diversity and variability among inbred onion lines at the S_1 level. In the cluster analysis, a high level of difference was found in group II and group IV. The results of the present study were similar to the literature mentioned above in terms of the number of groups formed in the population.

Evaluation of the principal component analysis and cluster analysis results for red and white onion genotypes

As a result of the research, it can be said that the factor coefficients of the groups that emerged in the cluster analysis and PCA for all onion genotypes were located in similar coordinates in the 3D PCA graph (Figure 3, Figure 4). It seems clear which axes' characteristics caused the distribution of onion genotypes according to the groups. The present study determined that there was a high level of variation among genotypes in terms of characteristics for the green (fresh) and dry bulb harvest periods. Genotypes were clustered in the same groups in terms of characteristics in both harvest periods. These results will assist in the elimination of closely related genotypes. In addition, it will also be possible to determine the most distant relative genotypes in terms of morphological characteristics, and a high rate of heterosis will be obtained by crossing these genotypes. There was no correlation between the origins of the onion genotypes and the similarities revealed as a result of multivariate analysis. This shows that they were introduced to their place of origin much earlier. Onion genotypes show differences as a result of natural selection in different ecologies in the same country.

Phenotypical diversity and genetic variability are very important for the achievement of breeding programs (Balkaya and Ergün 2007). The principal aim in breeding programs is to select plants with desired characteristics and a wide genetic variation. Detection of the genetic diversity of existing germplasm collections and revealing its distribution will provide significant benefits for breeding strategies. It will thus be possible to make more precise decisions using molecular breeding methods for the white and red onion genotypes studied.

This study determined phenotypic diversity using multivariate analysis in red and white onion genotypes collected from different countries. The sources of morphological variation were determined in both onion types. The research results may assist onion breeders in establishing a heterogeneous gene pool. In a further study, we plan to select superior onion genotypes through cooperation between the university and the private sector. The aim is thus to develop high-quality, new hybrid varieties in the near future.

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Table 1. Genotype code, accession number and geographical origins of 23 *Allium cepa* genotypes studied.

Genotype Code	Accession Number	Origin	Genotype Code	Accession Number	Origin
RO1	PI 546208	USA	WO1	PI 546189	USA
RO2	PI 546118	USA	WO2	PI 546106	USA
RO3	PI 344253	Türkiye	WO3	PI 546115	USA
RO4	PI 344256	Türkiye	WO4	PI 546182	USA
RO5	PI 344261	Türkiye	WO5	PI 264326	Spain
RO6	PI 264316	Spain	WO6	PI 546093	USA
RO7	PI 264325	Spain	WO7	PI 249902	Spain
RO8	PI 171475	Türkiye	WO8	PI 280554	Russia
RO9	PI 174024	Türkiye	WO9	PI 289690	Australia
RO10	PI 179627	India			
RO11	PI 220081	Afghanistan			
RO12	PI 232068	South Africa			
RO13	PI 546096	USA			
RO14	PI 357217	North Macedonia			

Table 2. List of morphological characters used in the characterization of *Allium cepa* populations.

Plant number of leaves per pseudo stem	(1) Few (2) Medium (3) Many
Foliage colour	(1) Light green (2) Green (3) Dark green
Foliage intensity of green colour L	
Foliage intensity of green colour a	This was measured with a digital colour measuring device (chromameter).
Foliage intensity of green colour b	
Leaf length, cm	Leaf lengths were measured with a ruler.
Leaf diameter, mm	Leaf widths were measured with a digital caliper.
Pseudo stem length, cm	Body lengths were measured with a ruler.
Pseudo stem diameter, mm	Body diameters were measured with a digital caliper.
Foliage attitude	(1) Erect (2) Semi-erect (3) Horizontal
Foliage waxiness	(1) Strong (2) Absent or weak
Waxiness degree	(1) Few (2) Medium (3) Many
Foliage cranking	(1) Absent or weak (2) Intermediate (3) Strong

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Table 3. List of morphological characters used in the characterization of *Allium cepa* populations.

Bulb weight, g	Three onions from each genotype were weighed with a 0.1 g precision digital scale.
Bulb height, mm	The lengths of the onions were measured with a digital caliper.
Bulb diameter, mm	The diameter of the onions was measured with a digital caliper.
Bulb width of neck, mm	The neck width of the onions was measured with a digital caliper.
Root disc diameter, mm	The root disc diameter values of onions were measured with digital caliper.
Bulb dry matter content, %	This was determined as a result of the measurement of onion juice in a digital refractometer.
Intensity of dry skin colour L	
Intensity of dry skin colour a	This was measured with a digital colour measuring device (Chromameter).
Intensity of dry skin colour b	
Bulb hardness	(1) Soft (2) Medium (3) Hard
Bulb position of maximum diameter	(1) Towards stem end (2) at middle (3) Towards root end
Bulb shape of stem end	(1) Depressed (2) Flat (3) Slightly raised (4) Rounded (5) Slightly sloping (6) Strongly sloping
Bulb shape of root end	(1) Depressed (2) Flat (3) Rounded (4) Strongly tapered
Bulb shape	(1) Elliptic (2) Medium ovate (3) Broad elliptic (4) Circular (5) Broad ovate (6) Broad obovate (7) Rhombic (8) Transverse medium elliptic (9) Transverse narrow elliptic
Degustation analysis	(1) Pain (2) Sweet
Time of harvest	(1) Early (2) Medium (3) Late
Thickness of dry skin	(1) Thin (2) Medium (3) Thick
Bulb base colour of dry skin	(1) Red (2) White

Table 4. Principal component analysis of characters associated with red onion populations.

	PC Axis							
Eigenvalues	6.8	6.3	4.1	3.0	2.4	2.3	1.7	1.3
Variation, %	22.1	20.5	13.1	9.7	7.8	7.3	5.6	4.1
Cumulative variation, %	22.1	42.5	55.7	65.3	73.1	80.5	86.1	90.2
	Eigen Vectors							
Traits	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**
Plant number of leaves per pseudo stem	-0.07	0.05	**-0.93**	-0.03	-0.02	0.10	0.04	-0.22
Foliage colour	0.20	0.02	**0.30**	0.03	-0.79	-0.07	-0.40	0.03
Foliage intensity of green colour L	0.06	0.01	**-0.41**	-0.001	0.12	0.16	-0.11	0.09
Foliage intensity of green colour a	0.01	-0.19	0.13	-0.09	-0.25	-0.05	**-0.91**	-0.15
Foliage intensity of green colour b	0.001	0.14	0.07	0.08	0.20	0.02	**0.92**	0.21
Leaf length, cm	**-0.43**	0.46	-0.26	-0.11	0.20	-0.19	0.27	0.09
Leaf diameter, mm	-0.17	0.17	**-0.50**	-0.07	0.15	-0.55	0.48	-0.02
Pseudo stem length, cm	-0.07	0.23	**-0.47**	0.18	-0.06	0.007	0.18	0.17
Pseudo stem diameter, mm	-0.07	0.05	**-0.93**	-0.03	-0.02	0.10	0.04	-0.22
Foliage attitude	-0.10	0.26	0.23	0.15	**0.62**	-0.43	0.03	0.03
Foliage waxiness	0.18	0.08	0.01	0.01	**0.93**	-0.22	0.03	0.07
Waxiness degree	-0.05	-0.01	-0.08	0.07	**-0.88**	0.10	-0.28	-0.20
Foliage cranking	-0.17	0.11	-0.21	0.27	-0.17	-0.06	0.05	-0.18
Bulb weight, g	-0.06	-0.005	0.25	0.05	0.22	-0.14	0.20	**0.88**
Bulb height, mm	**0.32**	-0.03	0.26	0.10	0.17	-0.78	-0.009	0.33
Bulb diameter, mm	-0.09	0.15	0.10	0.05	-0.006	0.21	0.17	**0.93**
Bulb width of neck, mm	-0.006	0.09	-0.07	**-0.89**	0.01	-0.14	-0.32	0.17
Root disc diameter, mm	**0.31**	0.56	0.16	-0.34	0.04	-0.11	0.06	0.61
Bulb dry matter content, %	-0.26	**0.42**	-0.27	-0.34	-0.18	0.25	-0.06	-0.50
Intensity of dry skin colour L	**-0.91**	-0.03	-0.04	-0.08	-0.03	0.22	0.11	0.02
Intensity of dry skin colour a	-0.16	-0.003	**0.47**	0.58	-0.35	-0.03	-0.20	0.28
Intensity of dry skin colour b	**-0.87**	0.14	-0.36	-0.12	0.009	0.07	-0.14	-0.04
Bulb hardness	0.19	-0.09	-0.09	**0.86**	0.05	-0.24	-0.10	0.20
Bulb position of maximum diameter	**0.67**	-0.07	-0.07	0.14	-0.25	0.13	-0.40	-0.09
Bulb shape of stem end	**0.48**	0.06	0.20	0.34	0.06	-0.35	-0.46	-0.26
Bulb shape of root end	0.15	**0.38**	-0.01	0.06	0.28	-0.59	0.30	-0.11
Bulb shape	-0.22	0.04	-0.15	-0.01	-0.12	**0.88**	0.20	0.16
Degustation analysis	-0.11	-0.07	-0.06	0.13	**-0.34**	0.31	0.08	0.11
Time of harvest	**0.75**	0.009	-0.11	-0.33	0.37	-0.34	0.07	0.02
Thickness of dry skin	0.17	**-0.85**	0.08	0.10	-0.12	-0.01	-0.39	-0.18
Bulb base colour of dry skin	**-0.34**	-0.44	-0.67	0.06	0.05	0.13	-0.07	0.06

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Table 5. Principal component analysis of characters associated with white onion populations.

	PC Axis						
Eigenvalues	7.4	6.3	3.9	3.9	2.8	2.3	1.4
Variation, %	25.4	21.6	13.5	13.3	9.8	8.0	4.8
Cumulative variation, %	25.4	47.1	60.6	73.9	83.7	91.6	96.4
	Eigen Vectors						
Trait	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**
Plant number of leaves per pseudo stem	-0.15	0.09	0.01	**-0.98**	0.07	0.03	-0.05
Foliage colour	-0.14	**-0.78**	0.30	0.30	-0.19	0.27	0.24
Foliage intensity of green colour L	-0.05	-0.08	0.23	**0.49**	0.83	-0.04	0.04
Foliage intensity of green colour a	0.01	-0.21	**-0.33**	-0.87	-0.07	-0.09	0.02
Foliage intensity of green colour b	0.19	0.29	**0.51**	0.60	0.07	0.36	-0.17
Leaf length, cm	**0.69**	-0.12	0.40	0.13	-0.33	-0.40	0.22
Leaf diameter, mm	0.23	**0.33**	0.42	-0.41	-0.33	-0.55	0.09
Pseudo stem length, cm	**0.30**	-0.21	-0.36	0.25	-0.73	-0.01	0.25
Pseudo stem diameter, mm	-0.15	0.09	0.01	**-0.98**	0.07	0.03	-0.05
Foliage attitude	0.02	-0.07	0.16	0.01	0.08	-0.03	**0.98**
Foliage waxiness	**0.36**	0.40	0.26	-0.32	0.10	0.61	-0.27
Waxiness degree	-0.07	**-0.82**	-0.40	0.14	-0.15	-0.04	-0.07
Foliage cranking	**0.40**	-0.27	0.08	0.27	0.08	-0.79	-0.09
Bulb weight, g	**-0.95**	-0.13	0.11	-0.03	0.04	0.10	0.07
Bulb height, mm	**-0.52**	-0.35	0.58	0.15	-0.32	0.23	0.26
Bulb diameter, mm	**-0.86**	-0.08	-0.33	-0.25	0.08	-0.06	-0.09
Bulb width of neck, mm	0.18	0.04	**0.83**	0.03	0.18	-0.13	0.03
Root disc diameter, mm	0.23	0.01	**0.49**	0.61	-0.34	-0.23	0.26
Bulb dry matter content, %	0.28	**-0.59**	0.06	-0.56	0.36	0.33	0.09
Intensity of dry skin colour L	-0.01	**0.97**	-0.01	0.16	0.10	0.08	0.02
Intensity of dry skin colour a	-0.28	**-0.83**	-0.45	-0.07	0.09	0.10	-0.02
Intensity of dry skin colour b	0.12	**0.79**	-0.37	0.36	-0.18	0.09	0.05
Bulb hardness	**-0.62**	0.35	0.06	-0.48	-0.15	-0.05	-0.46
Bulb position of maximum diameter	0.08	0.14	-0.19	-0.26	**0.90**	-0.04	0.22
Bulb shape of stem end	0.23	**-0.54**	0.57	0.01	-0.40	0.01	0.40
Bulb shape of root end	**0.33**	-0.14	0.83	0.29	0.14	-0.19	0.18
Bulb shape	**0.36**	-0.19	-0.88	-0.10	0.04	-0.20	-0.01
Degustation analysis	0.02	**0.37**	0.03	-0.23	0.12	-0.88	-0.01
Time of harvest	0.28	**-0.87**	0.01	0.18	-0.10	-0.01	0.29
Thickness of dry skin	-	-	-	-	-	-	-
Bulb base colour of dry skin	-	-	-	-	-	-	-

Table 6. Red onion genotype group and subgroups obtained by principal component analysis.

Groups	Subgroups	Genotypes	Total Genotype Number
A	1	RO1, RO3, RO5	7
	2	RO7, RO9, RO11, RO13	
B	1	RO2, RO12	2
C	1	RO4, RO6, RO8	5
	2	RO10, RO14	
Total	**5**		**14**

Table 7. Average values of the characteristics of red onion genotypes in PC groups.

Trait	A	B	C
Plant number of leaves per pseudo stem	9.2±2.2	7.9±2.7	9.6±1.7
Foliage colour	1, 2, 3	3	2, 3
Foliage intensity of green colour L	39.9±2.0	38.7±1.4	39.2±3.0
Foliage intensity of green colour a	-6.9±1.7	-6.2±1.5	-10.1±1.4
Foliage intensity of green colour b	3.1±2.1	2.6±1.3	7.2±2.5
Leaf length, cm	54.7±3.2	42.9±2.4	55.4±2.5
Leaf diameter, mm	11.8±1.5	9.9±3.6	12.1±0.8
Pseudo stem length, cm	10.6±2.1	8.3±1.3	12.0±0.6
Pseudo stem diameter, mm	9.2±2.2	7.9±2.7	9.6±1.7
Foliage attitude	1, 2	1	1
Foliage waxiness	1, 2	1	1
Waxiness degree	1, 3	3	2, 3
Foliage cranking	1, 2, 3	1	1, 2, 3
Bulb weight, g	106.9±16.8	78.7±5.7	99.6±41.0
Bulb height, mm	49.8±7.9	50.6±10.3	44.2±8.2
Bulb diameter, mm	63.9±4.0	54.2±4.9	64.1±11.7
Bulb width of neck, mm	15.2±3.5	10.8±1.6	12.1±1.7
Root disc diameter, mm	13.2±1.4	10.6±0.4	13.1±1.2
Bulb dry matter content, %	10.8±1.7	8.8±1.2	12.0±3.1
Intensity of dry skin colour L	54.9±18.1	37.7±11.4	55.6±24.3
Intensity of dry skin colour a	15.5±7.0	19.2±6.2	15.1±4.5
Intensity of dry skin colour b	13.2±8.5	1.5±1.1	8.2±9.0
Bulb hardness	2, 3	3	2, 3
Bulb position of maximum diameter	2, 3	3	2, 3
Bulb shape of stem end	2, 3, 4	4, 5	2, 3, 4
Bulb shape of root end	2, 3, 4	1, 3	2, 3, 4
Bulb shape	4, 5, 8, 9	2, 8	8, 9
Degustation analysis	1, 2	1, 2	2
Time of harvest	1, 3	1, 2	1, 2
Thickness of dry skin	1, 2	2, 3	1
Bulb base colour of dry skin	1, 2	1	1

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Table 8. White onion genotype group and subgroups obtained by principal component analysis.

Groups	Subgroups	Genotypes	Total Genotype Number
A	1	WO1, WO3, WO5	5
	2	WO7, WO9	
B	1	WO2, WO4, WO8w	4
	2	WO6	
Total	**4**		**9**

Table 9. Average values of the characteristics of white onion genotypes in PC groups.

Trait	A	B
Plant number of leaves per pseudo stem	9.2±0.4	9.1±0.6
Foliage colour	2, 3	2, 3
Foliage intensity of green colour L	39.3±3.9	39.8±2.1
Foliage intensity of green colour a	-7.8±1.5	-7.8±1.7
Foliage intensity of green colour b	5.0±2.8	3.9±2.3
Leaf length, cm	55.4±6.3	53.9±9.5
Leaf diameter, mm	11.4±1.6	10.5±2.1
Pseudo stem length, cm	10.2±2.3	11.0±1.4
Pseudo stem diameter, mm	9.2±0.4	9.1±0.6
Foliage attitude	1	1, 2
Foliage waxiness	1, 2	1
Waxiness degree	1, 2, 3	1, 3
Foliage cranking	1, 2, 3	1, 2, 3
Bulb weight, g	123.6±47.1	128.0±44.5
Bulb height, mm	56.5±13.3	57.2±11.5
Bulb diameter, mm	66.2±8.3	66.3±9.1
Bulb width of neck, mm	12.3±5.5	10.7±4.8
Root disc diameter, mm	12.7±2.1	13.1±1.4
Bulb dry matter content, %	9.8±1.8	9.7±2.3
Intensity of dry skin colour L	63.6±15.7	69.4±17.8
Intensity of dry skin colour a	-0.73±2.1	-0.4±1.7
Intensity of dry skin colour b	6.0±4.4	9.4±6.5
Bulb hardness	3	1, 3
Bulb position of maximum diameter	1, 2	2
Bulb shape of stem end	3, 4	2, 3, 4
Bulb shape of root end	2, 3, 4	1, 3, 4
Bulb shape	4, 9	4, 7, 8
Degustation analysis	1, 2	1, 2
Time of harvest	1, 2	1, 2, 3

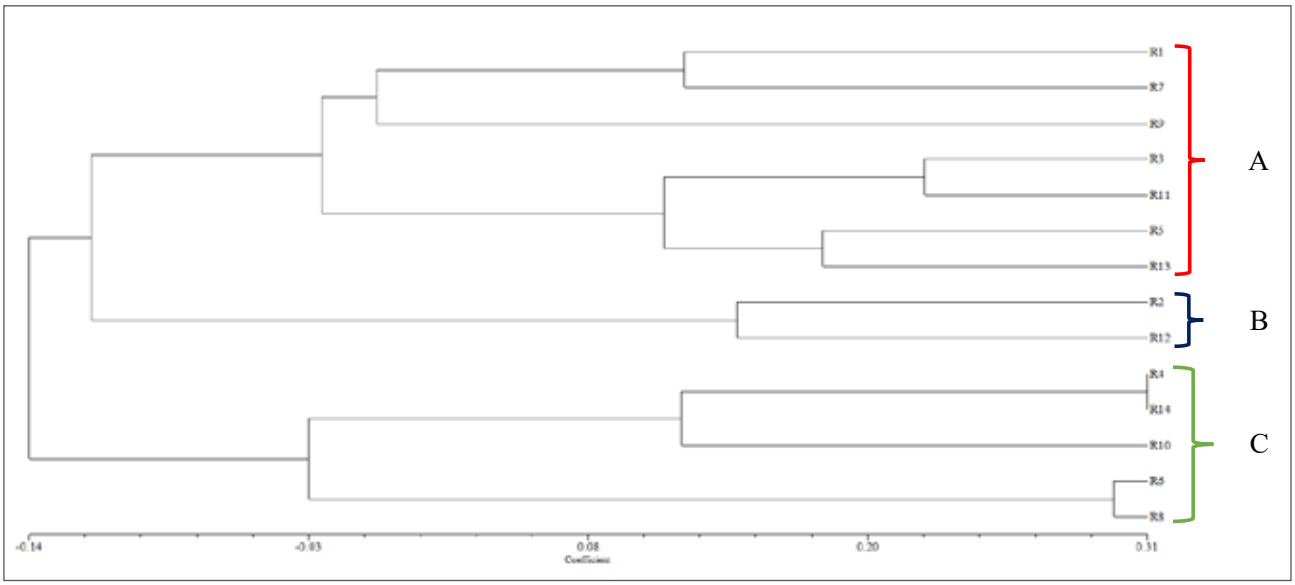

Figure 1. Phenotypical groupings of red onion genotypes according to cluster analysis.

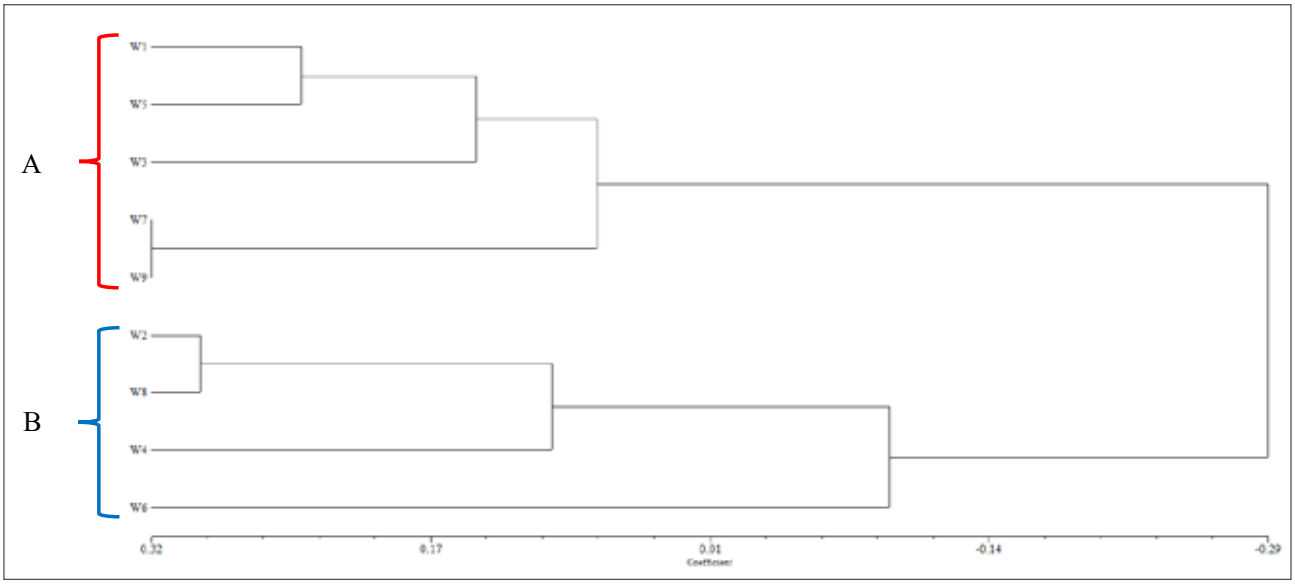

Figure 2. Phenotypical groupings of white onion genotypes according to cluster analysis.

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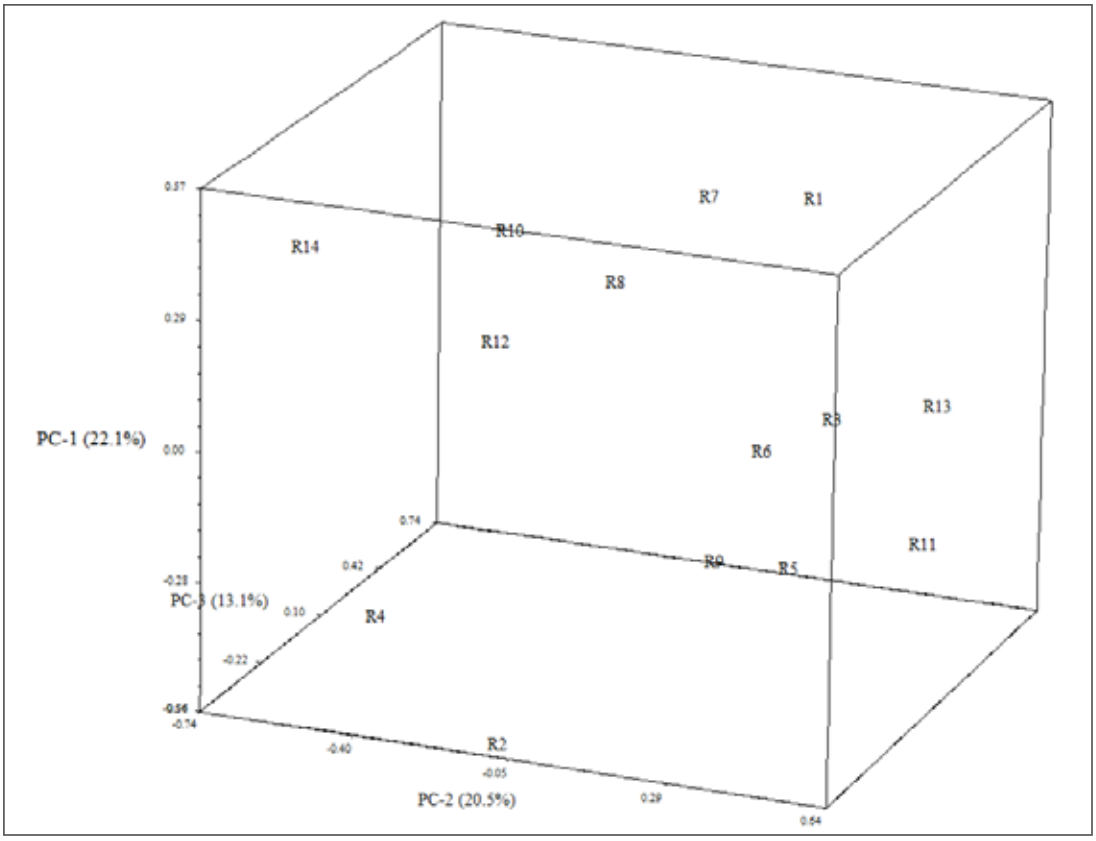

Figure 3. Three-dimensional diagram of the similarity of red onion genotypes with each other according to the first three PC values obtained by principal component analysis.

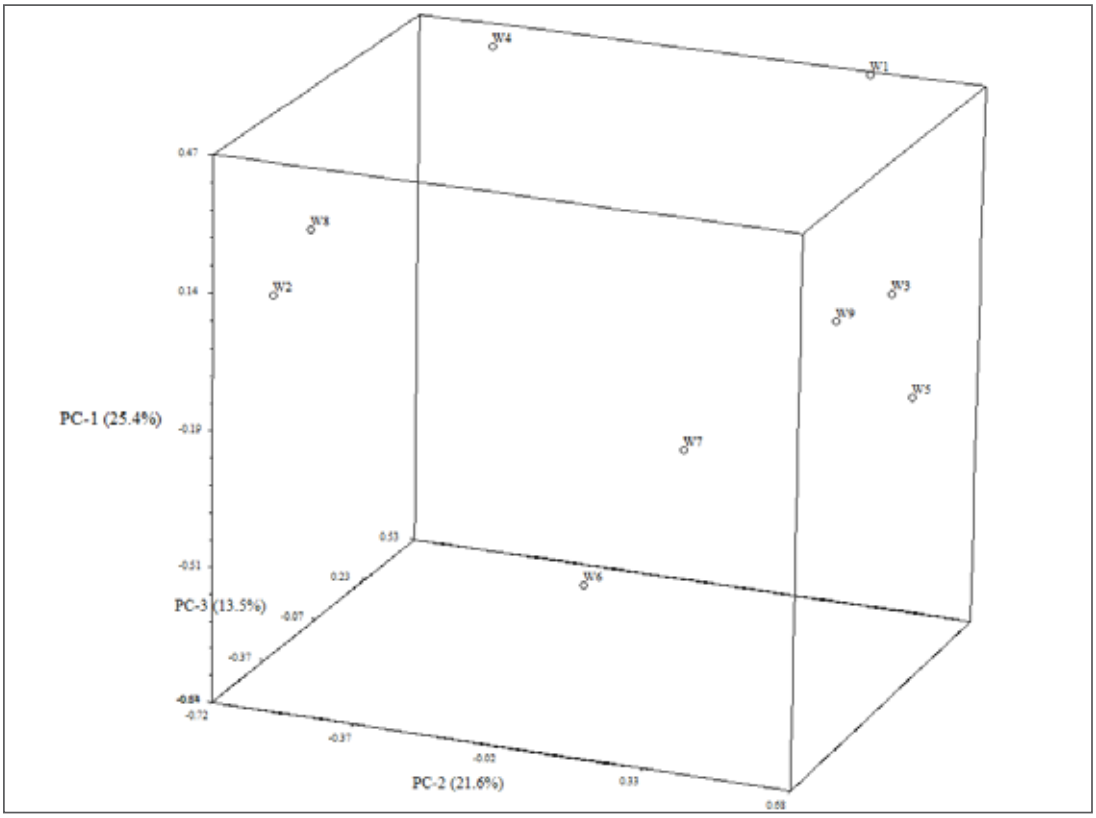

Figure 4. Three-dimensional diagram of the similarity of white onion genotypes with each other according to the first three PC values obtained by principal component analysis.

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In vitro Plant Regeneration Efficiency from Different Explants of Local Sainfoin Ecotype (*Onobrychis sativa*)

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ABSTRACT

Local populations and ecotypes are important genetic resources with genetic diversity. It is possible to protect threatened genotypes and increase biodiversity with *in vitro* tissue culture techniques. Explant type is of great importance among the factors affecting the success of *in vitro* regeneration and callus induction. In this study, Sainfoin seeds belonging to the Gözlü ecotype were used as experiment material. MS media were prepared with TDZ and combinations of IBA-NAA. Callus induction and shoot regeneration rates of hypocotyl, leaf and cotyledon explants were determined in the sainfoin ecotype. According to the results of the study, the best callus formation and shoot regeneration were obtained from the hypocotyl explant in an MS medium containing 2 mg/L TDZ. In the present study, important results were obtained that will contribute to the regeneration studies of local ecotypes and wild species, genetic transformation and increasing genetic diversity.

Keywords**:** *In vitro* regeneration, callus induction, regeneration efficiency, sainfoin ecotype

Introduction

Sainfoin (*Onobrychis sativa*) is an important forage crop used as animal feed all over the world. Anatolia-Iran-Caucasus regions constitute the gene centers of the sainfoin. Turkey is the natural habitat of the sainfoin. 52 sainfoin species are distributed in Turkey and more than half of these sainfoin species are endemic (Avcı 2010; Beyaz 2019).

Turkey is one of the gene centers of sainfoin, as in many other plant species (Harlan, 1951). Local populations and ecotypes are important genetic resource with genetic diversity. Local plant populations and wild species are of great agricultural importance due to their high stress tolerance and environmental adaptation capability (Kishii 2019; Akman and Karaduman 2021). Local ecotypes are the target of breeding studies and biotechnological approaches. Ecotypes that are genotypically adapted to certain environmental conditions have high genetic diversity within their species (Turesson, 1922). Wide genetic diversity of ecotypes is of great importance in breeding programs (Avcı et al. 2014).

In vitro propagation of local populations and genetic resources is successful and reproducible methods to preserve the germplasm of species (Ergül et al. 2018; Deb et al. 2019). In addition, tissue culture methods can be used successfully to increase genetic variation or manipulations on plant material. Successful *in vitro* regeneration protocol has been used in many fields such as somaclonal variation, *in vitro* mutation, tolerance to biotic and abiotic stress, diploid plant production, production of secondary metabolites, production of economically important transgenic plants, CRISPR genetic editing and development of somatic hybrids (Oğuz et al. 2021).

Direct or indirect plant regeneration from plant tissues and parts is possible with somatic embryogenesis and organogenesis. Callus induction, somatic embryogenesis and plant regeneration have been performed by many researchers with different *in vitro* techniques and plant growth regulators (PGR) combinations (Özcan et al. 1996; Sancak 1999; Kamalvand and Karamian 2013; Beyaz 2014; Yildiz and

Ekiz 2014; Honarmand et al. 2016; Beyaz 2019, Uzun and Yükselgüngör 2020; Uysal and Topbaş 2021). The success of *in vitro* regeneration affect by genotype, explant type, the composition of the MS medium, and PGR.

On the other hand, studies on *in vitro* regeneration of endemic and local *Onobrychis* species are very limited (Uzun and Yükselgüngör 2020). In this study, *in vitro* regeneration efficiency from different explants of the local sainfoin ecotype was determined.

Materials and Methods

In this study, Gözlü sainfoin (*Onobrychis sativa*) ecotype were used as sources of plant explant. Sainfoin seeds were sterilized with 100%, 90%, 80%, 70% and 60% commercial bleach. In all sterilization experiments, after a 15-minute treatment period, rinsing with sterile water 3 times for 5 minutes. In the experiments, a medium (MS0) containing MS mineral salts and vitamins (Murashige and Skoog 1962) and 3% sucrose, 5g/L agar (Type A, Sigma). The planted seeds in MS media were grow up at 24°C for 3 weeks in a photoperiod of 16 hours light and 8 hours dark. Cotyledon, hypocotyl and leaf explants were isolated three weeks old sainfoin plantlets.

For the callus and regeneration experiment, *in vitro* media were prepared with combinations of 0.5, 1, 2 mg/L TDZ, NAA and BAP. As Sancak (1999) stated that the rooting media was added 1 mg/L IBA. Rooted regenerants were transferred to pots with peat and soil at a ratio of 1:1. Acclimatization was carried out in the plant growth chamber under 50% constant humidity, plants with covering a transparent plastic bag.

All regeneration experiments were established according to the random plot design with three replications, and statistical analyzes were performed using the Tukey test in the "IBM SPSS 22 software" program.

Results

The results obtained from sterilization experiments using different percentage of commercial bleach are given in Table 1. Successful results were obtained in the sterilization of hulled seeds with a high rate of bleach. However, it caused a decrease in germination rate (Table 1). On the other hand, the germination rate of hulled seeds was higher at low bleach concentrations, but the contamination rate was not decreased. In addition, *in vitro* seedling growth of these seeds was deficient.

The sterilization of dehulled seeds, contamination rates was zero in 100%, 90% and 80% bleach, but germination rates were determined as 81%, 79% and 76%, respectively. The lowest contamination rate and the best germination rate were determined in the dehulled seeds with 70% bleach. The germination rate of seeds sterilized with 70% bleach was 98% (Table 1).

Effect of TDZ on callus formation and shoot regeneration

Callus induction and shoot regeneration results of sainfoin hypocotyl, leaf and cotyledon explants from MS media containing different rate of TDZ are given in Table 2. High callus formation was determined for the hypocotyl explant in MS medium containing 1 and 2 mg/L TDZ. On the other hand, the statistical difference between the callus rates of the hypocotyl explant obtained in 0.5, 1 and 2 mg/L TDZ was insignificant ($p>0.01$). The highest callus ratio from leaf explant was obtained at 0.5 mg/L TDZ ($p<0.01$). The difference between the callus rates obtained from the medium containing 1 and 2 mg/L TDZ was insignificant ($p>0.01$) (Table 2). Although there is an increase in the callus rates obtained from the cotyledon explant compared to the control ($p<0.01$), the difference between TDZ treatments are insignificant ($p>0.01$).

The highest average shoot number obtained from the hypocotyl explant was measured in MS medium containing 2 mg/L TDZ (10.76). The highest average number of shoots in the leaf explant was determined in the media containing 1 and 2 mg/L TDZ (2.40 and 2.73 respectively) (Table 2). In the cotyledon explant, the highest shoot number (6.26) was obtained from MS medium containing 2 mg/L TDZ ($p<0.01$) (Figure 1).

The effect of TDZ and BAP combinations on callus formation and shoot regeneration

In the study, hypocotyl, leaf and cotyledon explants were cultured in MS media containing a combination of TDZ and BAP. All explants were observed during 1-12 weeks after they were placed in the culture medium. Callus formations were determined 2-8 weeks after the explants transferred to the culture medium. The highest callus induction rate in hypocotyl and cotyledon explants was determined in MS media containing 1 mg/L TDZ + 0.5 mg/L BAP (respectively 83.33% and 23.30%) (Table 3). Compared to other TDZ+BAP combinations in the experiment, 1 mg/L TDZ + 0.5 mg/L BAP was found to be statistically significant ($p<0.01$).

The highest callus formation in the leaf explant was determined in MS containing 1 mg/L TDZ +1 mg/L BAP and 2 mg/L TDZ + 0.5 mg/L BAP (33.30% and 30.00%, respectively). However, the difference between these treatments are statistically insignificant ($p>0.01$). No shoot formation occurred from the cotyledon explant in MS media containing TDZ and BAP combinations. The highest average shoot number of hypocotyl explant was obtained in the nutrient medium containing 1 TDZ mg/L + 0.5 BAP mg/L ($p<0.01$) (Table 3). On the other hand, shoots were obtained from the leaf explant in medium containing only 0.5 mg/L TDZ + 1 mg/L BAP (2.60) (Table 3).

The effect of TDZ and NAA combinations on callus formation and shoot regeneration

Callus and shoot regenerations in MS media containing TDZ and NAA combinations are given in Table 3. The highest callus formation in the hypocotyl explant was determined in the medium containing 0.5 mg/L TDZ + 2 mg/L NAA. The best callus formation in leaf explant was obtained in 0.5 and 1 mg/L TDZ + NAA combinations. The difference between 0.5 and 1 mg/L TDZ + NAA combinations was statistically insignificant ($p>0.01$). Callus induction obtained from leaf explant at 2 mg/L TDS doses were less than 0.5 and 1 TDZ + NAA doses ($p<0.01$). In the cotyledon explant, the highest callus formation was obtained from MS medium containing 1 mg/L TDZ + 2 NAA mg/L (Table 4).

The highest average shoot number from the hypocotyl explant was obtained from MS medium containing 2 mg/L TDZ + 0.5 mg/L NAA (6.33). It was observed that increasing NAA negatively affected shoot formation (Table 4). In the leaf explant, the highest average shoot number was obtained from MS medium containing 2 mg/L TDZ + 1 mg/L NAA. In the experiment, shoot regeneration did not occur in the cotyledon explant (Table 4). The shoots obtained from leaf and hypocotyl explants were transferred to plastic pots after rooting in MS medium containing 1 mg/L IBA (Figure 2). All transplanted plants into the soil continued to grow and develop after acclimatization.

Discussion

Somatic embryogenesis is defined as the formation of embryos from vegetative cells *in vitro* (Hatipoğlu 2012). Developing genetic variability in existing varieties with somatic embryogenesis and *in vitro* techniques is routinely applied in the production of species that are in danger of extinction and in the production of species that are difficult to reproduce (Erişen, 2005).

Obtaining sterile explants is one of the major factors affecting success in tissue culture techniques. Contamination from plant material has to be eliminated with successful sterilization protocols. In this study, sterile plants were obtained with 70% commercial bleach. In particular, sterilization of dehulled seed was more successful. Ergül et al. (2018) reported that sterile plants were obtained by removing the seed coat with chemical and physical interventions for the sterilization of seeds of wild Beta germplasm. In this study, the seed coat was removed by physical interventions. In this way, contamination in the deep surfaces of the seed was eliminated.

The content of auxin and cytokines contained *in vitro* tissue culture media is of great importance for callus and regeneration success. Inducing hormones are needed in the growth media for the regeneration of explants isolated from the plant. Although the inducer required for organogenesis is removed from media after a certain period of time, the growth and development continue (Ikeuchı et al. 2013). On the other hand, a callus may occur in the presence of high auxin or equal auxin-cytokine hormone in the MS medium (Mohnen 1994). Besides, Oğuz et al. (2021) reported that the endogenous hormone content of plant tissues has a significant effect on *in vitro* regeneration and callus formation. In our study, callus induction and shoot regeneration were obtained from different explant types with the effect of IBA and NAA added to TDZ-containing media. In addition, it is thought that callus formation in MS medium with high TDZ is caused by the endogenous auxin level of the plant explant.

Frequency of regeneration and callus formation are hereditary characters affected by genotypes (Kagami et al. 2016). The differences between the results obtained in sainfoin *in vitro* regeneration and callus induction studies and our study are due to genotypic differences. The rate of shoot regeneration is low in MS media containing TDZ + BAP and TDZ + NAA compared to media containing only TDZ in all explants used in the study. The highest shoot regeneration was obtained from the hypocotyl explant in nutrient media containing 2 mg/L TDZ. Garshasbi et al. (2012) reported that more than 60% of shoots rooted successfully in four weeks in semi-strength MS medium supplemented with 1 mg/L IBA. In the experiment, MS media containing 1mg/L IBA were used for rooting media (Figure 1). Root formation was successfully achieved in approximately 80% of the regenerants. Besides, the fact that all plants transferred to the soil continue to grow after acclimatization is closely related to the high efficiency of *in vitro* rooting.

Conclusions

Tissue culture techniques have great importance in many areas such as the manipulation of cell tissues, biomaterial production, and elucidation of the mechanisms underlying plant growth processes. Besides, it is possible to increase and preserve the genetic diversity to be used in breeding studies with *in vitro* regeneration and callus formation of local populations and ecotypes. In this study, high degree of callus and shoot regeneration was obtained from MS media containing 2 mg/L TDZ. In addition, important results were obtained for the *in vitro* propagation and conservation of local sainfoin ecotypes. It is clear that these results could contributions to other *in vitro* based techniques such as tissue culture, genetic transformation or new biodiversity studies.

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Table 1. Effect of different bleach concentrations on sainfoin seed sterilization and germination rate.

Bleach (%)	Hulled seed		Dehulled Seed	
	Contamination Rate (%)	Germination Rate (%)	Contamination Rate (%)	Germination Rate (%)
100	0	89[b]	0	81[b]
90	0	96[a]	0	79[b]
80	14	95[a]	0	76[b]
70	20	88[b]	0	98[a]
60	26	83[b]	11.00	94[a]

* The difference between the different letters in the same column is significant at the 0.01 level (p<0.01)

Table 2. The effect of TDZ on callus and shoot formation from hypocotyl, leaf and cotyledon explants in sainfoin.

Plant Growth Regulators (mg/L)	Callus Induction (%)			Average Number of Shoots		
TDZ	Hypocotyl	Leaf	Cotyledon	Hypocotyl	Leaf	Cotyledon
0.0	55.00[b]	43.30[c]	60.00[c]	1.36[c]	1.10[b]	1.22[d]
0.5	96.66[a]	96.66[a]	96.66[a]	6.80[b]	1.43[b]	3.10[c]
1.0	100.00[a]	90.00[a]	96.66[a]	8.80[a]	2.40[a]	4.66[b]
2.0	100.00[a]	93.30[a]	96.66[a]	10.76[a]	2.73[a]	6.26[a]

* The difference between the different letters in the same column is significant at the 0.01 level (p<0.01)

Table 3. The effect of TDZ+BAP combination on callus and shoot formation from hypocotyl, leaf and cotyledon explants in sainfoin.

Plant Growth Regulators (mg/L)		Callus Induction (%)			Average Number of Shoots		
TDZ	BAP	Hypocotyl	Leaf	Cotyledon	Hypocotyl	Leaf	Cotyledon
0.5	0.5	16.00[c]	3.30[d]	0.00[d]	0.00[d]	0.00[b]	0.00
0.5	1.0	3.30[d]	3.30[d]	0.00[d]	2.60[b]	2.60[a]	0.00
0.5	2.0	0.00[f]	6.60[c]	0.00[d]	0.00[d]	0.00[b]	0.00
1.0	0.5	83.33[a]	16.60[b]	23.30[a]	2.86[a]	0.00[b]	0.00
1.0	1.0	30.00[b]	33.30[a]	3.30[c]	1.30[c]	0.00[b]	0.00
1.0	2.0	3.30[d]	10.00[c]	10.00[b]	0.00[d]	0.00[b]	0.00
2.0	0.5	0.00[f]	30.00[a]	0.00[d]	0.00[d]	0.00[b]	0.00
2.0	1.0	0.00[f]	16.60[b]	0.00[d]	0.00[d]	0.00[b]	0.00
2.0	2.0	3.30[d]	3.30[d]	0.00[d]	0.00[d]	0.00[b]	0.00

* The difference between the different letters in the same column is significant at the 0.01 level (p<0.01)

Table 4. The effect of TDZ and NAA combinations on callus formation and shoot regeneration.

Plant Growth Regulators (mg/L)		Callus Induction (%)			Average Number of Shoots		
TDZ	**NAA**	**Hypocotyl**	**Leaf**	**Cotyledon**	**Hypocotyl**	**Leaf**	**Cotyledon**
0.5	0.5	93.30[a]	86.60[a]	0.30[e]	0.00[c]	0.00[c]	0.00
0.5	1.0	93.30[a]	93.30[a]	53.30[b]	0.00[c]	0.00[c]	0.00
0.5	2.0	100.00[a]	96.60[a]	43.30[b]	0.00[c]	0.00[c]	0.00
1.0	0.5	50.00[b]	90.00[a]	3.30[e]	0.00[c]	0.00[c]	0.00
1.0	1.0	36.60[c]	96.60[a]	3.30[e]	0.00[c]	0.00[c]	0.00
1.0	2.0	63.30[b]	96.60[a]	63.30[a]	0.00[c]	0.00[c]	0.00
2.0	0.5	90.00[a]	50.00[c]	16.60[c]	6.30[a]	0.00[c]	0.00
2.0	1.0	96.60[a]	73.30[b]	76.60[a]	4.60[a]	4.00[a]	0.00
2.0	2.0	63.30[b]	63.30[b]	6.60[d]	2.00[b]	1.10[b]	0.00

* The difference between the different letters in the same column is significant at the 0.01 level ($p<0.01$)

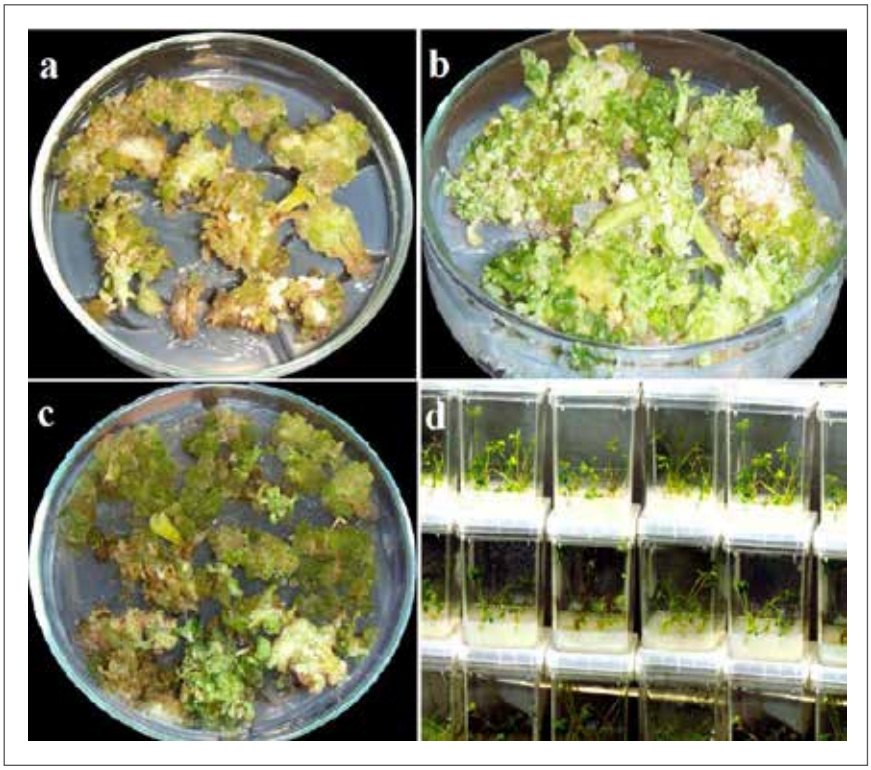

Figure 1. *In vitro* plant regeneration of sainfoin from different explants (Original)
a) Callus formation and shoot regeneration in leaf explant in MS medium containing 2.0 mg/L TDZ,
b) Callus formation and shoot regeneration from hypocotyl explant,
c) Callus formation and shoot regeneration at the end of 6 weeks from the cotyledon explant
d) Development of plantlets in rooting medium

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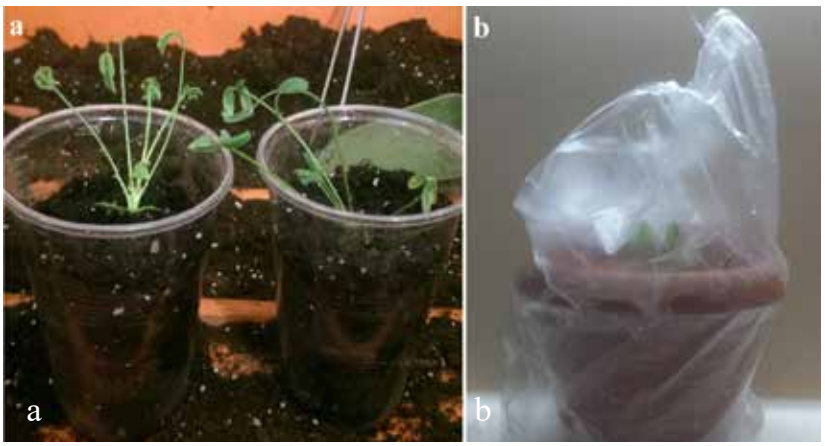

Figure 2. Acclimatization stage of *in vitro* plantlets (Original)
a) Transfer of the plant to the soil after root development,
b) Plants covered with a transparent plastic bag for acclimatization

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Phenotypic Diversity among the Virginia Breeding Lines of Groundnut

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ABSTRACT

An attempt has been made to study the genetic variability and classifying the 210 Virginia breeding lines along with five checks evaluated across two years. Significant differences observed among Virginia breeding lines for all traits except days to maturity. Moderate heritability coupled with high genetic advance as per cent of mean for hundred pod weight and hundred kernel weight suggesting the additive gene control and effectiveness for selection. Low heritability coupled with low genetic advance as per cent of mean for days to flowering and kernel characteristics suggests more environmental influence on their expression. Pod yield per plant correlated significantly and positively with primary branches per plant, hundred pod weight and kernel weight, SMK, shelling out turn, kernel length and kernel width. Cluster analysis based on Euclidean distance using Wards criteria, grouped 210 Virginia bunch breeding lines and four checks into three major clusters. Three major clusters consist each of 119 breeding lines in cluster I, 44 breeding lines and three checks in cluster II and 47 breeding lines and two check varieties in cluster III, respectively. Cluster I and cluster II contains high yielding breeding lines, where as cluster III had low yielding breeding lines. Sub cluster IIA had breeding lines with higher pod yield and kernel characteristics, which will be useful donors for Virginia groundnut improvement.

Keywords**:** Phenotypic diversity, Virginia breeding lines, hundred kernel weight, cluster analysis

Introduction

Groundnut (*Arachis hypogea* L.) is a major oilseed legume crop of arid and semi arid tropical of the world. It covers an area of 61 lakh hectares and production of 99 lakh tonnes with productivity of 1631 kg/ha in India (FAOSTAT, 2020). India has the highest area under groundnut cultivation. The six major states growing groundnut in India are Gujarat (39%), Rajasthan (15%), Andhra Pradesh (14%), Karnataka (9%), Madhya Pradesh (6%) and Maharashtra (5%) (IOPEPC, 2019). Botanically there are six distinct groups of groundnuts cultivated. The Virginia type is the predominant groundnut type grown in Gujarat and Rajasthan states of India and in small packets in southern states of India. In Gujarat, about >80% of the total area is under the six districts in the Saurashtra regions (Junagadh, Rajkot, Dwaraka, Amreli, Bhavanagar and Jamnagar). In Rajasthan, the groundnut is mainly cultivated in Bikaner, Jodhpur, Churu and Jaipur districts. In Tamil Nadu, about 10% area of groundnut area is occupied by Virginia type (TMV 10 and ALR1 of Virginia bunch and TMV 1 and TMV 4 of Virginia runner) particularly in some packets of Salem, Dharmapuri, Tiruvannamalai, Villupuram and Cuddalore districts under rainfed conditions (http://ikisan.com/tn-groundnut-history.html).Virginia runners varieties viz., Kadiri 771-1 and Kadiri-3 and Virginia bunch type Kadiri-2 are cultivated in Rayalaseema districts like Anantapur, Chitture, Cuddaph and Kurnool (Krishna, 2010). In Maharashtra, Virginia runner type grown in Marathwada region, Osmanabad and Bhid districts using the moisture gained from southeast and northwest monsoons.

Virginia groundnut belongs to sub species of *hypogaea* var. *hypogaea*, characterized by no floral axes on main stem in Virginia (*hypogaea*) compared to presence of floral axes in main stem in Spanish and Valencia types belongs to sub species *fastigiata* var. *vulgaris* and subspecies *fastigiata* var *fastigiata*

respectively. Virginia groundnut is characterized by longer maturity duration, spreading growth habit, large kernel, high oleic acid and tolerance to biotic and abiotic stresses (Erickson and Ketring 1985; ICRISAT 1992). Attempts have been made in the past to estimate the genetic variability, correlation and divergence based morpho-agronomic traits in Virginia groundnut (Gupta et al. 2015; Shinde et al. 2019 and Dudhatra et al. 2022). Using Mahalnobis D^2 statistics 35 Virginia runner genotypes were grouped into seven clusters and found lack of relationship between genetic and geographic diversity (Golakia and Makne 1992). The significant positive correlation of pod yield per plant with hundred pod weight and shelling percentage was observed by Gupta et al. (1992) and Gangadhara et al. (2020). High heritability accompanied with high genetic advance as per cent of mean was recorded by Bhargavi et al. (2017) for pod yield per plant and 100 kernel weight. Coffelt et al. (1989) studied reproductive efficiency in 14 Virginia cultivars and suggested that yield increase can be accomplished by developing the variety in combination with high harvest index and reproductive efficiency.

Hence an attempt has been made to assess the genetic variability and classifying the Virginia breeding lines based on pod yield and kernel characteristics, which will be useful for identifying the diverse parents for hybridization and genetic improvement of Virginia groundnut.

Materials and Methods

Experimental location: The material for present study consists of two hundred and ten Virginia advanced breeding lines and four checks of groundnut obtained from Plant Breeding Section of ICAR-DGR, Junagadh. An experiment was laid out in the Augmented Randomized Complete Block Design at the Experimental plots of ICAR-Directorate of Groundnut Research (DGR) Station, Junagadh, Gujarat, India during *Kharif* 2017 (E1) and *Kharif* 2018 (E2). ICAR-Directorate of Groundnut Research is situated between 21.49°N latitude and 70.44°E longitude at an elevation of 107 meters above mean sea level. Each breeding line was sown in a single row of 3 m length and with a spacing of 60 × 10 cm and standard agronomic practices was followed to raise healthy crop.

Observations recorded: The observations on days to first flowering, days to 50% plants flowering, number of primary branches per plant, days to maturity, hundred pod and kernel weight, kernel width, kernel length and pod yield per plant were recorded on five random plants from each genotype. The SPAD chlorophyll meter reading (SCMR) a surrogate trait of water use efficiency was recorded at 60 days after sowing by collecting the second to third leaves from the top of the main stem of each plant by a Minolta handheld portable SCMR meter (SPAD- 502 plus Minolta, Tokyo, Japan) using four leaflets per sample and care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina, avoiding any interference from veins and midribs. The weather conditions prevailing in growing years are presented in the Table 6.

Statistical analysis: Analysis of variance (ANOVA) for Augmented Randomized Block design was calculated using Proc GLM of SAS. Genotypic and phenotypic coefficients of variation were worked out as per the method suggested by Burton and De Vane (1953), heritability and genetic advance were calculated according to Johnson (1955) and Robinson et al. (1949). Pearson's correlation coefficients were calculated for each pair of traits to determine relationships among the yield and kernel traits. A cluster analysis was done on mean values of two years for 13 traits using Euclidean distance and dendrogram was constructed using R version 3.0.3.

Results and Discussion

Variability and genetic parameters

Analysis of variance (Table 1) showed significant differences among test genotypes for all the traits except days to maturity in two years. A wide range was observed for hundred pod weight (29-131g in E1 and 56-146 g in E2), hundred kernel weight (21-51 g in E1 and 18-63 g in E2), Sound mature kernel (26-58% in E1 and 38-80% in E2), Shelling out turned (53-83% in E1and 26-75%in E2) in both years (Fig. 1). Moderate range was observed in primary branches (3-16 in E1 and 2-20 in E2), days to 50 per cent flowering (24-40 days in E1 and 28-36 days in E2), days to maturity (112-125 days in E1 and 105-124 days in E2) and SPAD chlorophyll meter reading (25-45 in E1 and 27-44 in E2). Across years, Virginia breeding lines showed low variability for kernel width (6-9 mm in E1 and 6-10 mm in E2), kernel length (9-17 mm in E1 and 10-19 mm in E2) and kernel length to width ratio (1.2-2.4 in both E1 and E2).

The phenotypic and genotypic coefficient of variation and heritability along with genetic advance as percent of mean are presented in Table 2. Low heritability coupled with low genetic advance per cent of mean was observed for days to first flower initiation, days to 50% flowering and days to maturity in both environments suggesting that highly influence of environment and selection is ineffective for these traits. Similar results of low heritability and genetic advance as per per cent of mean was also observed

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by Saini and Sharma (2018) and Gangadhara et al. (2019). Temperature and photoperiod are two major environmental factors highly influencing the flowering and maturity duration. Cox (1979) and Nigam (1994, 1998) reported the effect of temperature and photoperiod on vegetative as well as reproductive growth of groundnut. Primary braches per plant exhibited low heritability and moderate genetic advance as per cent of mean in both E1 and E2.

Sound mature kernel (%) had high heritability in both E1 and E2 but genetic advance as per cent of mean was high in E1 and low in E2. Shelling percentage had high heritability coupled with high genetic advance as per cent of mean in E2 and high heritability with low genetic advance as per cent of mean in E1. High heritability in both E1 and E2 environments and low and high GAM in E1 and E2 indicates favourable environments in the form of rainfall in E2 compared to E1. Hundred pod and kernel weight showed moderate heritability and high genetic advance as per cent of mean both environments (E1 and E2). Moderate heritability coupled with high genetic advance as per cent of mean for hundred pod weight and hundred kernel weight suggests the additive gene control and effectiveness for selection. Pod yield per plant showed low heritability coupled with moderate genetic advance as per cent of mean in E2. A similar estimate of pod yield was recorded by Azharadheen and Gowda (2013). Kernel traits (Kernel width, Kernel length and Kernel length to width ratio) were showed low heritability coupled with low genetic advance as per cent of mean suggesting that complex nature and higher influence of environment and low scope for selection. Low heritability and genetic advance as per cent of mean for kernel width was also reported by Gangadhara et al. (2013).

Correlation and cluster analysis

Pod yield per plant correlated significantly and positively with primary branches per plant, hundred pod weight and kernel weight, SMK, shelling out turn, kernel length and kernel width. This kind of positive relationships between kernel traits with pod yield was also noticed by Gupta et al. (2015) and Gangadhara et al. (2020). Days to first flowering and 50 per cent flowering correlated significant negatively with sound mature kernel (%) and shelling out turn suggesting the influence of primary branches per plant and flowering time and duration with kernel maturity and pod yield. SCMR correlated significant positively with all kernel characteristics viz., hundred pod weight, hundred kernel weight, sound mature kernel (%), shelling out turn, kernel length and kernel length to width ratio. Kernel yield is an important economic yield, which in turn determined by hundred kernel weight, sound mature kernel (%) and shelling out turn, which are correlated positively with kernel length, kernel width and kernel length to width ratio. Size and shape of the kernel are the two important visible traits influencing the consumer preference, which in turn determined by kernel length and kernel width. Kernel length correlated positively with kernel length to width ratio, whereas kennel width correlated negatively with kernel length as well as kernel length to width ratio. Similar kind of relationship between kernel traits was also noticed by Gangadhara et al. (2019).

Cluster analysis based on Euclidean distance using Wards criteria, grouped 210 Virginia bunch breeding lines and four checks into three major clusters. Three major clusters consist each of 119 breeding lines in cluster I, 44 breeding lines and three checks in cluster II and 47 breeding lines and two check varieties in cluster III respectively. Cluster I and cluster II contains high yielding as well as higher ranges for hundred pod and kernel weight where as cluster III had low yielding breeding lines. Sub cluster IIA had breeding lines with higher pod yield and kernel characteristics, which will be useful donors for Virginia groundnut crop improvement.

Identification of trait specific breeding lines

Early flowering and high SCMR are important traits for drought tolerance in groundnut. SPAD Chlorophyll meter reading is more pertinent trait for drought tolerance associated with leaf nitrogen and drought tolerance (Kalariya et al. 2017). Breeding lines showing higher SCMR (>42) are PBS 25107, PBS 25090, PBS 25091 and PBS 25081. Breeding lines viz., PBS 21095, PBS 21089, PBS 21108, PBS 25062, PBS 25064, PBS 25076, PBS 25100, and PBS 25114 flowered early (27 days). Advanced breeding lines with high sound mature kernel (>63%) are PBS 25106, PBS 24142, PBS 24022, PBS 25077, PBS 25101, PBS 21105, PBS 21108, PBS 25116, PBS 25096, PBS 26061, PBS 26043, PBS 24148, PBS 25115 and PBS 25022. For confectionery use, large seeded type and high shelling per centage are preferred. Breeding lines PBS 21105, PBS 21120, PBS 26057, PBS 26061, PBS 24145, PBS 26056, PBS 24133, PBS 24152 and PBS 26052 had higher shelling per centage (>70%). Seed size had highly positive correlation with seed weight (Chiow and Wynne 1983), which in turn increases yield, nutritional content as well as seedling vigour. Two traits, kernel shape and size are important visible features for consumer's preference. Kernel length to width ratio (>2) are preferred as large seed with uniform shape. Advance breeding lines viz., PBS 21115, PBS 21108, PBS 25022, PBS 21086, PBS 26031, PBS 21087, PBS 25044, PBS 26047, PBS 21084, PBS 21085, PBS 26015, PBS 25059, PBS 26050 and PBS 25077 showed high kernel length to width ratio (>2mm).

Conclusions

The present study exhibited significant differences among the genotypes for pod yield and its component traits. Moderate heritability coupled with high genetic advance as per cent of mean for hundred pod weight and hundred kernel weight suggested the additive gene control and effectiveness for selection. Promising trait specific superior Virginia breeding lines identified will serve as donors for the development of large seeded and higher pod yield in Virginia groundnut.

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Table 1. ANOVA for yield and kernel characteristics 210 Virginia breeding lines.

Character	Blocks (df=6)	Entries (df=214)	Tests (df=209)	Controls (df=4)	Tests Vs Controls (df=1)	Error (df=24)
***Kharif* 2017 (E1)**						
Primary branches per plant	0.89	4.49*	4.48*	5.75*	1.2	1.65
Days to first flower initiation	3.32	6.96*	6.52*	19.19*	48.86*	2.34
Days to 50% flowering	2.72	11.39*	10.62*	46.90*	31.74*	2.87
Days to maturity	5.99	6.06	6.14	3.26	0.49	3.66
SPAD Chlorophyll meter reading	10.0	13.7*	13.9*	3.7	20.5	4.5
Hundred pod weight (g)	66.72	207.6*	179.3*	1544.1*	767.2*	39.2
Hundred kernel weight (g)	26.76*	34.3*	30.06*	159.57*	419.2*	3.15
Pod yield per plant (g)	14.52	6.61	5.83	5.75	172.8	6.69
Sound mature kernel (%)	16.44*	39.41*	33.32*	246.64*	482.28*	3.29
Shelling percentage (%)	13.82*	22.51*	20.16*	1.10	599.1*	0.98
Kernel width (mm)	0.723*	0.445*	0.413*	2.04*	0.931*	0.176
Kernel length (mm)	1.14	1.69*	1.70*	0.75	4.14*	0.59
Kernel length to width ratio	0.017	0.03*	0.029*	0.055*	0.006	0.012
***Kharif* 2018 (E2)**						
Primary branches per plant	11.45	10.03*	9.92*	14.02*	16.55	4.77
Days to first flower initiation	1.18	1.980*	1.8*	5.24*	27.75*	0.85
Days to 50% flowering	1.03	2.14*	1.9*	7.0*	32.9*	0.82
Days to maturity	18.02	17.98	17.74	32.47	10.45	13.25
SPAD Chlorophyll meter reading	3.75	5.85*	5.98*	0.30	0.31	3.07
Hundred pod weight (g)	13.85	295.0*	268.2*	1484.7*	1125.0*	28.7
Hundred kernel weight (g)	10.09	51.58*	49.4*	138.0*	158.04*	7.5
Pod yield per plant (g)	4.89	6.78*	6.44*	3.61	90.13*	2.34
Sound mature kernel (%)	0.761*	23.94*	23.17*	0.85	276.9*	1.023
Shelling percentage (%)	16.85*	72.89*	71.15*	174.5*	30.86*	5.26
Kernel width (mm)	0.70*	0.634	0.612*	1.90*	0.194	0.397
Kernel length (mm)	0.53*	2.26*	2.23*	3.86*	0.044	0.63
Kernel length to width ratio	0.038*	0.044*	0.045*	0.024	0.0054	0.021

* Indicates the statistical significance of entries or treatments at 5% level.

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Table 2. Genetic parameters for yield and kernel characteristics in Virginia breeding lines.

	Min	Max	Mean	σ^2_g	σ^2_p	GCV	PCV	$h^2_{(bs)}$	GAM
***Kharif* 2017**									
Primary branches per plant	3	16	6.94	0.40	2.06	9.16	20.68	19.63	12.01
Days to first flower initiation	22	35	27.58	0.66	3.00	2.95	6.28	22.03	4.93
Days to 50% flowering	24	40	30.80	1.22	4.08	3.58	6.56	29.82	8.15
Days to maturity	112	125	116.60	0.34	4.00	0.50	1.72	8.59	0.61
SPAD Chlorophyll meter reading	25.9	45.8	36.11	1.32	5.81	3.18	6.67	22.65	7.50
Hundred pod weight (g)	29	131	81.78	24.05	63.28	6.00	9.73	38.01	60.59
Hundred kernel weight (g)	21	51	34.23	4.45	7.60	6.16	8.05	58.55	26.78
Sound mature kernel (%)	26	58	40.70	5.16	8.45	5.58	7.14	61.05	26.12
Shelling out turn	53	83	69.33	3.08	4.06	2.53	2.91	75.78	9.14
Kernel length (mm)	9.9	17.1	13.62	0.16	0.75	2.91	6.37	20.83	2.37
Kernel width (mm)	6	9	7.76	0.04	0.21	2.53	5.97	17.92	1.02
Kernel length to width ratio	1.2	2.4	1.78	0.003	0.015	2.86	6.80	17.73	0.30
***Kharif* 2018**	**Min**	**Max**	**Mean**	σ^2_g	σ^2_p	**GCV**	**PCV**	$h^2_{(bs)}$	**GAM**
Primary branches per plant	2	20	8.07	0.75	5.53	10.77	29.22	13.58	19.22
Days to first flower initiation	25	33	28.57	0.16	1.02	1.41	3.54	15.78	1.16
Days to 50% flowering	28	36	30.68	0.19	1.01	1.41	3.28	18.54	1.26
Days to maturity	105	124	115.87	0.68	13.93	0.71	3.22	4.86	1.20
SPAD Chlorophyll meter reading	27.4	44.1	36.56	0.40	3.47	1.72	5.10	11.41	2.23
Hundred pod weight (g)	56	146	92.90	38.03	66.27	6.64	8.77	57.39	84.38
Hundred kernel weight (g)	18	63	37.49	6.32	13.29	6.71	9.73	47.59	34.77
Pod yield per plant	3	22	10.30	0.63	2.95	7.70	16.68	21.33	12.59
Sound mature kernel (%)	38	80	69.74	3.32	4.09	2.61	2.89	81.17	9.79
Shelling out turn	26	75	46.28	9.84	14.76	6.78	8.31	66.65	43.83
Kernel length (mm)	10	19	14.36	0.23	0.87	3.36	6.49	26.77	3.33
Kernel width (mm)	6	10	7.87	0.12	0.55	3.52	7.58	21.59	2.49
Kernel length to width ratio	1.26	2.4	1.84	0.0029	0.027	2.91	8.93	10.64	0.32

Min=Minimum, Max=Maximum, σ^2g=Genotypic variance, σ^2p=Phenotypic variance, GCV=Genetic coefficient of variation, PCV=Phenotypic coefficient of variation, h2(bs)=Heritability in broad sense, GAM=Genetic advance as per cent of mean

Table 3. Mean values of sub cluster for yield and kernel characteristics in Virginia breeding lines.

Cluster	No.	No.	ABL	PRIM	DFI	DFF	DM	SCMR	PYLP	HPW	HKW	SMK	SP	KL	KW	KLWR
I	119	20	Ia1	9	29	32	117	34	9	80	32	53	53	13.4	7.60	1.79
		36	Ia2	8	28	30	116	38	10	82	34	56	58	14.0	7.87	1.80
		19	Ib1	8	28	31	116	34	9	91	35	52	56	14.6	7.81	1.90
		44	Ib2	7	28	31	116	37	9	92	40	58	61	14.6	8.07	1.83
II	47	4	IIA	8	28	31	117	38	11	125	49	60	64	16.6	8.43	1.97
		27	IIb1	7	28	31	116	37	10	100	39	56	58	14.1	7.88	1.80
		16	IIb2	7	27	30	117	37	9	109	45	59	63	15.2	8.43	1.82
III	49	5	IIIA	7	28	31	115	36	7	54	23	49	49	12.2	6.94	1.76
		35	IIIb1	8	29	32	116	36	9	71	29	51	53	13.2	7.39	1.80
		9	IIIb2	6	26	29	115	34	6	64	27	53	55	11.4	7.53	1.54

PRIM=Primary branches per plant, DFI=Days to first flowering, DFF=Days to 50% flowering, DM=Days to Maturity, SCMR=SPAD Chlorophyll meter reading, PYLP=Pod yield per plant, HPW=Hundred pod weight, HKW=Hundred kernel weight, SMK=Sound mature kernel (%), SP=Shelling percentage, KL=Kernel length (mm), KW=Kernel width (mm), KLWR=Kernel length to width ratio

Table 4. Clustering pattern if Virginia breeding lines based on Euclidean distance.

Cluster	Sub Cluster	No	Advanced Breeding Lines
I	Ia1	20	PBS 24131, PBS 25086, PBS 25053, PBS 24156, PBS 25075, PBS 25078, PBS 26031, PBS 26033, PBS 24102, PBS 24106, PBS 24107, PBS 24122, PBS 25059, PBS 25051, PBS 24114, PBS 25038, PBS 25108, PBS 25048, PBS 25047, PBS 26023
	Ia2	36	PBS 25061, PBS 25064, Girnar 2, PBS 21107, PBS 26062, PBS 25033, PBS 25034, PBS 25082, PBS 24002, PBS 25081, PBS 25091, PBS 21096, PBS 25090, PBS 21109, PBS 21085, PBS 24073, PBS 25111, PBS 24151, PBS 24155, PBS 24144, PBS 24153, PBS 25117, PBS 21091, PBS 25080, PBS 25118, PBS 25032, PBS 26019, PBS 24124, PBS 24140, KDG 123, KDG 128, PBS 21087, PBS 25125, PBS 25126, PBS 21118, PBS 24141
	Ib1	19	PBS 25043, PBS 21090, PBS 25104, PBS 25044, PBS 24118, PBS 24116, PBS 21116, PBS 25060, PBS 26039, PBS 26051, PBS 24112, PBS 24113, PBS 24115, PBS 24119, PBS 25098, PBS 26038, PBS 26049, PBS 21100, PBS 21113,
	Ib2	44	PBS 21115, PBS 26052, PBS 21084, PBS 21119, PBS 25110, PBS 26058, PBS 26056, PBS 26059, PBS 25022, PBS 25101, PBS 25115, PBS 25025, PBS 25107, PBS 21093, PBS 21086, PBS 24101, PBS 25077, PBS 25116, PBS 25084, PBS 25072, PBS 26026, PBS 21111, PBS 25119, PBS 25102, PBS 26053, PBS 24150, PBS 25050, PBS 25021, PBS 25100, PBS 21114, PBS 24075, PBS 26041, PBS 25024, PBS 25028, PBS 21106, PBS 26028, PBS 24145, PBS 24152, PBS 24154, PBS 26055, PBS 26057, PBS 24142, PBS 21105, PBS 26040,
II	IIA	4	PBS 21112, PBS 25094, PBS 24022, PBS 24133
	IIb1	27	PBS 26044, PBS 25103, PBS 21117, PBS 26047, PBS 21097, PBS 25026, PBS 26015, PBS 24110, PBS 24111, PBS 21110, PBS 24109, PBS 24085, PBS 25076, PBS 24008, PBS 25015, PBS 25017, PBS 6048, PBS 24117, PBS 24126, PBS 25027, PBS 24147, PBS 24146, PBS 26060, PBS 21102, PBS 4108, PBS 21103, PBS 25097
	IIb2	16	PBS 21095, PBS 21089, PBS 21088, PBS 21092, PBS 25029, PBS 21108, PBS 26043, PBS 25095, PBS 25096, GG 20, Somnath, PBS 24148, PBS 25106, PBS 21120, PBS 25023, PBS 26061
III	IIIA	5	PBS 25067, PBS 25099, PBS 26045, PBS 24125, PBS 25113
	IIIb1	35	PBS 24121, PBS 24120, PBS 24104, PBS 24105, PBS 24103, PBS 25070, PBS 24123, PBS 24127, PBS 25069, PBS 25054, PBS 24130, PBS 26042, PBS 25037, PBS 26050, PBS 24157, PBS 24158, PBS 24076, PBS 25122, PBS 25123, PBS 25124, PBS 25120, PBS 25121, PBS 21098, PBS 24132, PBS 21099, PBS 25035, PBS 24006, PBS 24038, PBS 24129, PBS 26022, PBS 24040, PBS 25089, PBS 26025, PBS 25045, PBS 25046
	IIIb2	9	PBS 21101, PBS 25062, PBS 25066, PBS 24135, PBS 24139, PBS 24134, PBS 25114, PBS 24137, PBS 24138

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Table 5. Promising breeding lines identified for yield and kernel traits.

Trait	Promising Virginia Breeding Lines
Pod yield per plant (13g)	PBS 25111, PBS 25033, PBS 21114, PBS 24110, PBS 24085, PBS 26055, PBS 21118, PBS 21112, PBS 21095, PBS 21115
Days to 50% flowering (27 days)	PBS 21095, PBS 21089, PBS 21108, PBS 25062, PBS 25064, PBS 25076, PBS 25100, PBS 25114
SPAD Chlorophyll meter reading (>42)	PBS 25107, PBS 25090, PBS 25091, PBS 25081
Hundred pod weight (>112g)	PBS 24022, PBS 24133, PBS 21112, PBS 25094, PBS 21089, PBS 21088, PBS 21095, PBS 24148
Hundred kernel weight (>47g)	PBS 25029, PBS 24133, PBS 24022, PBS 21112, PBS 21108, PBS 25106, PBS 25096, PBS 26043
Sound mature kernel (>63%)	PBS 25106, PBS 24142, PBS 24022, PBS 25077, PBS 25101, PBS 21105, PBS 21108, PBS 25116, PBS 25096, PBS 26061, PBS 26043, PBS 24148, PBS 25115, PBS 25022
Shelling out turn (>66%)	PBS 21105, PBS 21120, PBS 26057, PBS 26061, PBS 24145, PBS 26056, PBS 24133, PBS 24152, PBS 26052
Kernel length (>15mm)	PBS 24133, PBS 24022, PBS 25095, PBS 21115, PBS 21108, PBS 26061, PBS 25044, PBS 21092
Kernel length to width ratio (>2mm)	PBS 21108, PBS 25022, PBS 21087, PBS 211086, PBS 21115, PBS 26031, PBS 26050, PBS 21115, PBS 26047, PBS 26015, PBS 25044, PBS 21084, PBS 24127, PBS 21085, PBS 25059, PBS 24022, PBS 25113

Table 6. Weather parameters recorded at Junagadh during Kharif 2017 and Kharif 2018.

Month	June		July		August		September		October	
Year	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Max temperature (°C)	37	37.1	30.9	30.7	31.3	30.5	32.4	32.1	36.5	37.4
Min temperature (°C)	26.6	28.1	25.4	25.8	24.6	25	24.5	23.5	22.9	21.5
Mean temperature (°C)	31.8	32.6	28.1	28.3	28	27.7	28.5	27.8	29.7	29.5
Relative humidity (%)	69	65	85	85	84	83	79	71	51	47
Wind speed (Km/h)	23.9	12.5	25	9.3	25.3	9.3	27.4	5.2	20.2	2.7
Evaporation (mm)	6.8	8.2	2.4	2.9	3	2.9	3.9	4.3	5.9	5.5
Total rainfall (mm)	147.8	7.4	330.5	641.9	282.6	88.6	43.5	51.5	0	0
Total rainy days	10	1	18	14	10	5	4	0	0	0

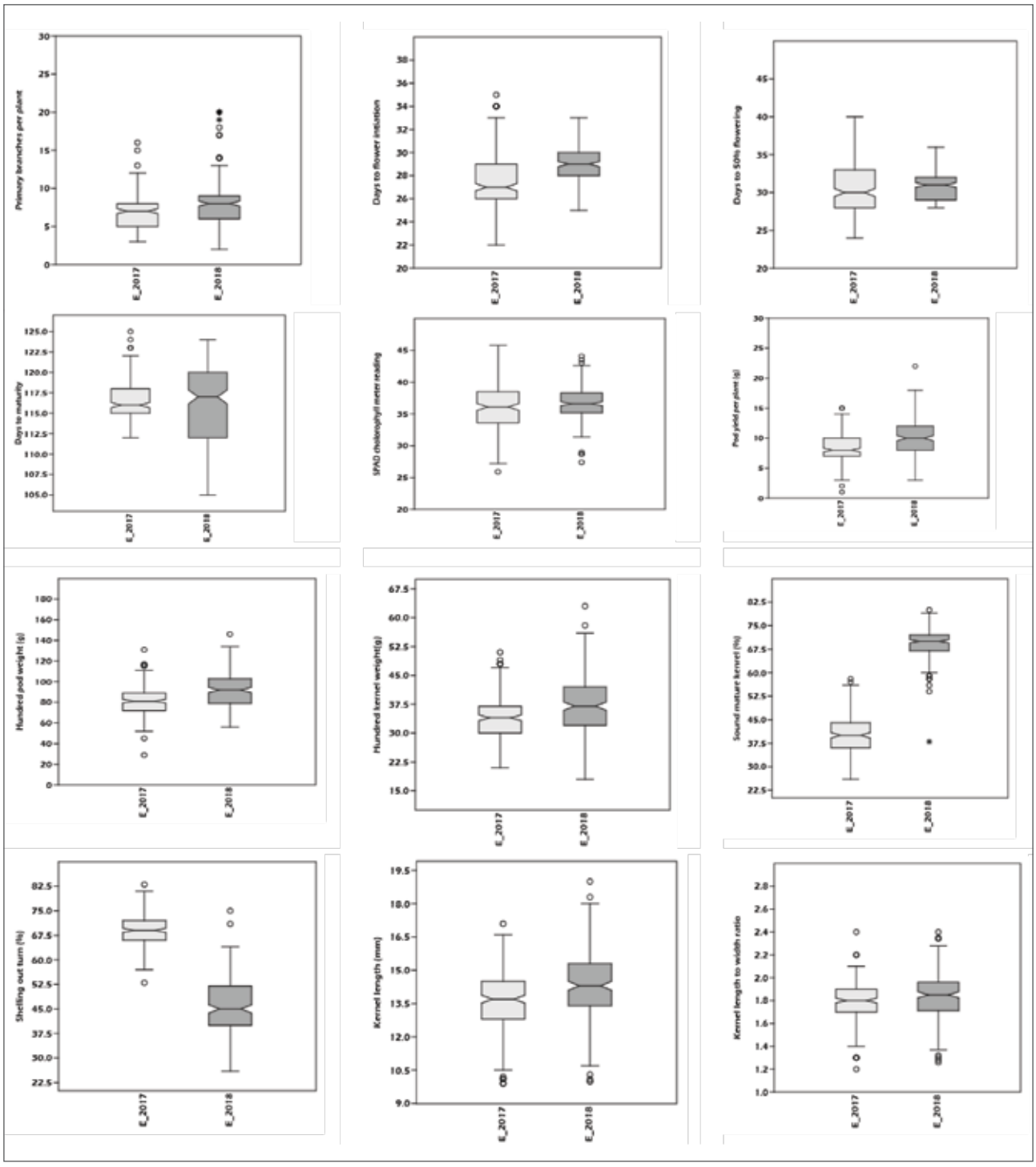

Figure 1. Box plot variation for pod yield and attributing traits in 215 Virginia breeding lines.

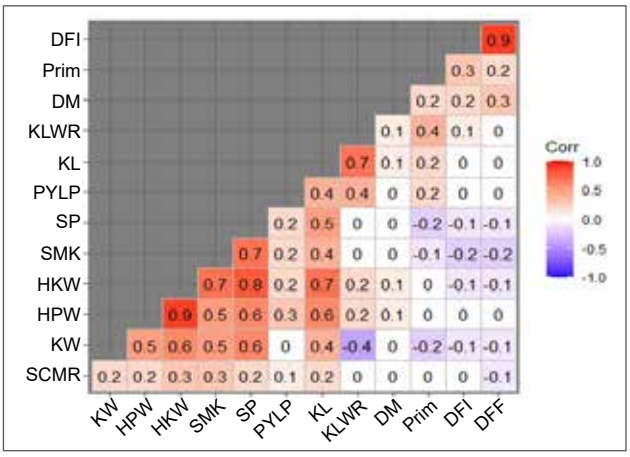

Figure 2. Relationship between pod yield, flowering and kernel characteristics.

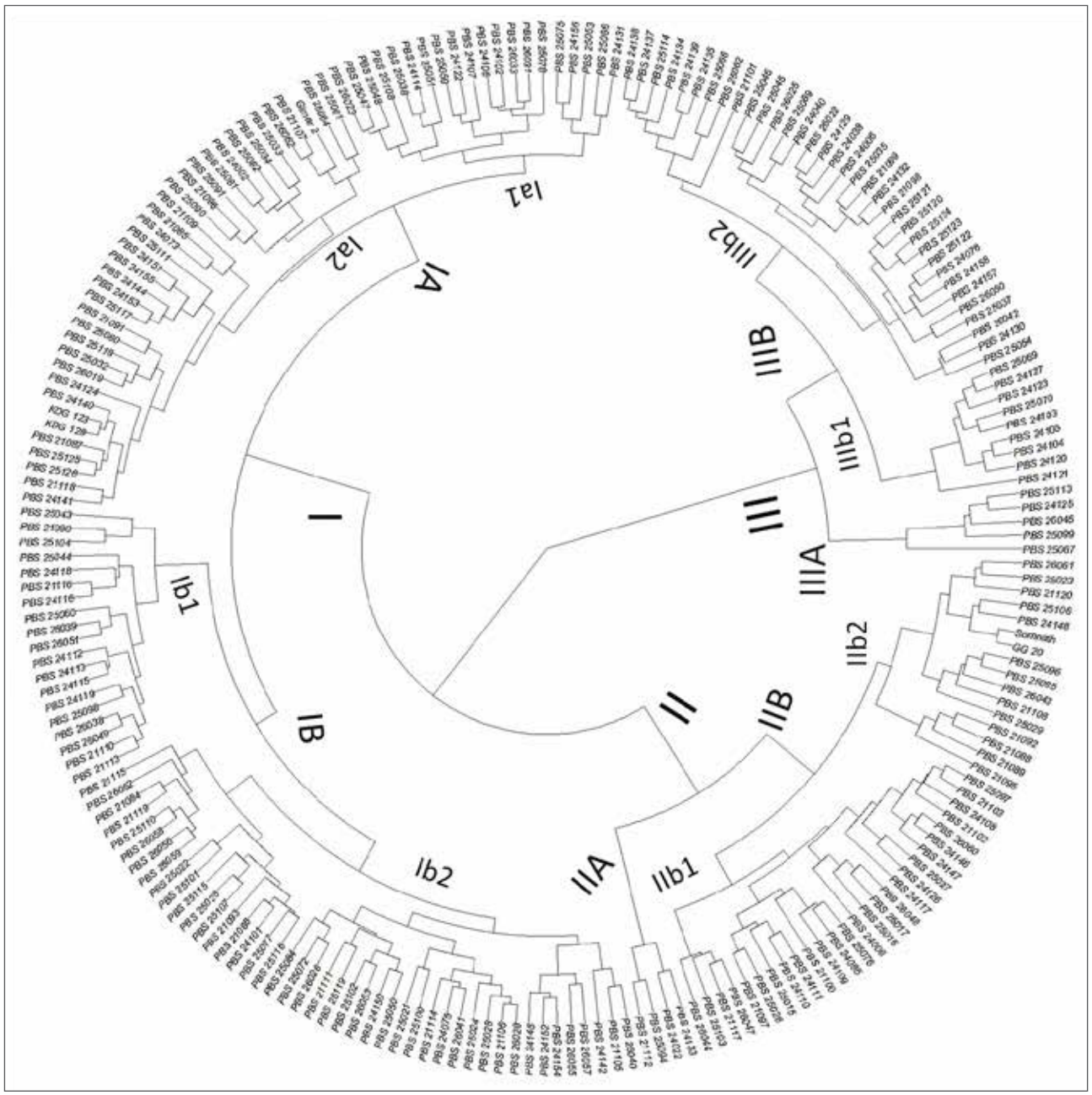

Figure 3. Dendrogram showing three major clusters among 215 Virginia breeding lines based on Euclidean distance.

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Environment by Genotype Interaction and Stability of Bread Wheat (*Triticum aestivum* L.) Genotypes under Rainfed Conditions in Trakia Region

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ABSTRACT

The study was conducted in the Trakya region, Turkey at five environments during the 2015-2016 growing cycles. Grain yield were subjected to analysis of variance (ANOVA), the additive main effect and multiplicative interaction (AMMI) and genotype and genotype-by-environment (GGE) biplot analyses. ANOVA and AMMI analysis showed highly significant ($p < 0.01$) differences among environments (E). Environment was responsible for the greatest part of the variation, followed by genotype and its interaction effects. Average yield across five environments varied from the highest 6673 kg ha^{-1} to the lowest 5008 kg ha^{-1}. Across five environment Genotypes G7 and G12 had highest grain yield. Burgaz was found near the ideal test environment of the average environment coordination. Therefore, this location should be used as the most suitable to select widely adapted genotypes. For grain yield, cultivars Gelibolu, and G17, G8, and G12 lines were well adaptable to all environmental conditions. The graphical result showed that the first principal component PC1 explained 44.71% of the interaction sum of the square while the second principal component, PC2 explained 22.57% of some of the square interaction. The result of PCA revealed that the 2 principal components contributed 67.27% of the total variability. In the study, genotypes G12 and G17 selected in 2016 and then were released in 2019 named Anafarta and Abide, respectively.

Keywords**:** Bread wheat, GE interaction, GGE biplot, yield, stability

Introduction

Bread wheat is a widely cultivated crop throughout the Trakya region of Turkey. Because of the various environmental conditions yield and quality in wheat varies in the region and GGE biplot analysis provides an easy and comprehensive solution to genotype by environment interaction (Öztürk and Korkut 2018; Öztürk 2021). The basic aim of plant breeding research is to improve genotypes for a given environment. Genotype (G) and environment (E) are thus the two explicit components that define a plant breeding research program and that also determine the potential for technology spillovers (Maredia et al. 1999). Evaluation of genotypes across various environments and several years is needed in order to identify stable genotypes that could be recommended for release as new cultivars and/or for use in the breeding programs (Sharma et al. 2010). Environmental factors play a main role in the expression of genotype characteristics (Peterson et al. 1998). The highly variable wheat growing environments provide ample opportunity for differentiation of target environments and the manifestation of genotype-environment interactions. The multiplication trials used in plant breeding are subject to two main sources of variation genotypes, location and their interaction (Petersen 1994). Genotype performance changes due to environmental stresses and differences in their ability to adapt to the stress factors. Due to various environmental conditions, abiotic stress factors can cause a reduction in yield and yield components in bread wheat genotypes (Öztürk and Korkut, 2020). Numerous methods have been developed to reveal patterns of G × E interaction, additive main effects and multiplicative interaction (AMMI) (Gauch 1992).

The AMMI stability parameters allow examining yield stability after reducing the noise from the GE interaction effect (Ajay et al. 2020). Genotype environment (G × E) interactions are of major importance because they provide information about the effect of different environments on cultivar performance and have a key role in the assessment of performance stability of the breeding materials (Moldovan et al. 2000). To develop varieties for different environments, very essential for breeders to evaluate their genotypes based on many years and several locations. Environmental variations are important in determining the performance of elite materials (Solomon et al. 2018). Performance trials have to be conducted in multiple environments because of the presence of GE. Variety trials provide essential information for selecting and recommending cultivars. Although data may be collected for many traits, an analysis may be limited to a single trait usually yield and information on other traits is often left unexplored (Yan and Tinker 2006). The success of crop improvement activities largely genotype evaluation by eliminating unnecessary testing depends on the identification of superior genotypes for sites (Letta 2009). Almost all breeding programs in the world aim to improve varieties with stable yields. The yield stability is generally grouped as static or dynamic stability (Pfeiffer and Braun 1989). Genotypes when tested across different environmental conditions often show significant variation in grain yield. This fluctuation is generally known as GE interaction. However, GE interaction is likely to be more severe in stress conditions which complicate the process of selecting high yielding stable genotypes (Cooper and Byth 1996). Therefore, breeding programs are tended to test extensively newly developed material in diverse environments to increase the chances of success (Alwala et al. 2010).

The selection of relatively high and stable yielding genotypes is a key component in wheat breeding programs to improve yield performance under various environmental conditions. Environmental variations are important in determining the performance of wheat genotypes. A variety of statistical procedures are in fact available to analyze and determine the results of multi-location trials and genotype × environmental interaction (GEI) data. In this study, two multivariate analyses such as AMMI and GGE biplot have been performed. Finlay and Wilkinson's joint regression model (1963) and Eberhart and Russel's method (1966) were applied and the regression coefficient (b), determination coefficients of the regression equations (R^2) were calculated (Finlay and Wilkinson 1963; Eberhart and Russell 1966 and 1969; Tai 1971).

The most important goal in all crop breeding programs is to increase yield, and yield improvement requires the use of efficient statistical methods to identify superior genotypes. In determining the superiority of genotype, in addition to high yield, yield stability in different environments must also be considered. AMMI and biplot analyses are good tools for selecting superior genotypes and to increase efficiency in selection (Naik et al. 2022). Therefore, the aim of this research was to assess the yield and the performance of the advanced bread wheat genotypes and to investigate their yield stability and genotype-by-environment interactions across various environmental conditions.

Materials and Methods

The experiments were carried out at five locations in the Trakya region, Turkey, in the 2015-2016 growing seasons on winter wheat (*Triticum aestivum* L.) under rainfed conditions. Each location was considered as a single environment. Twenty-five winter wheat genotypes, 5 of them were local checks and 20 advanced lines, were examined in randomized complete block design (RCBD) with four replications. Each plot was comprised of 6 rows of 6 m, spaced 0.17 meters apart. The seeds were sown at the rate of 500 seeds per square meter.

The AMMI method combines the traditional analysis of variance (ANOVA) and principal component analysis (PCA) into a single analysis with both additive and multiplicative parameters (Gauch, 1992). The first part of AMMI uses the standard ANOVA procedures to estimate the genotype and environment main effects. The second part involves the PCA of the interaction residuals. Genotype and genotype × environment (GGE) biplot analyses were conducted using GGE biplot software (Yan and Kang, 2002) to determine the performance and stability of grain yield. The biplot analysis was used to identify genotypes superior for individuals and multiple traits. GGE biplot analysis has been widely used to determine performance stability in multiplications trials when identifying superior genotypes (Yan et al. 2007; Sharma et al. 2010). Data were analyzed statistically for analysis of variance with the method described by Gomez and Gomez (1984). The significance of differences among means was compared by using Least Significant Difference (L.S.D. at a 5%) test.

Results and Discussion

The results of the variance analysis of the research are listed in Table 1. The combined analysis of variance (ANOVA) revealed significant differences among genotypes and environments for grain yield ($p<0.01$) (Table 1). Average grain yield across five locations

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varied from the highest 6673 kg ha^{-1} to the lowest 5008 kg ha^{-1}. The highest grain yield was performed by genotypes G7 followed by G12 (Table 4). The result of the AMMI model for grain yield is presented in Table 2. The data of twenty-five bread wheat genotypes in multi-location year trials were analyzed to determine whether the effect of the Genotype × Environment (GE) interaction. Data were also graphically analysed by the genotype × trait biplot method as recommended by Yan and Thinker (2005). The analysis of variance showed that the GEI was not significant. The multiplicative variance of the treatment sum of squares due to GEI was further partitioned by principal component analysis. The ordination technique revealed significant differences for IPC1 and IPC2. The factors explained, showed that wheat genotypes grain yield was affected by environment (9.11%), genotype (5.52%) and GEI (21.02%). GEI effect was responsible for the greatest part of the variation, followed by environment and then genotype.

When looking for a link between traits that could potentially help the yield breeding, it is imperative that the data be analyzed by various statistical methods (Tsenov et al. 2020). Genotype × trait biplot analysis is highlighted among the multivariate methodologies because it assesses genotypes based on multiple traits and identifies those that are superior to the desired variables; these can be used as parents in breeding programs or even as possible commercial cultivars (Yan and Tinker 2006). To visually display relations of observed traits and genotypes multivariate biplot analysis, described by Yan and Rajcan, (2002), Yan and Tinker (2006) are used. Environmental variations are important in determining the performance of wheat genotypes. So, to develop cultivars for various environments, very necessary for breeders to evaluate their genotypes based on many years and several locations. The observed G×E interactions in the AMMI model have been partitioned among the first and second IPCA accounting for 44.71% and 22.57%, respectively. The result of principal component analysis revealed that the two principal components (PC1, PC2) contributed 67.27% of the total variability. In the graphic analysis, the first principal component (IPCA1) represents genotype productivity and the second principal component (IPCA2) represents genotype stability (Yan et al. 2000).

In the GGE biplot (Figure 1a), the vectors from the biplot centre divided the graph into seven distinct sectors. The highest yielding genotypes were identified for each sector. The wheat genotypes located on vertices of polygon performed either best or poorest in one or more environments. The G7 was the highest yielding genotype in environment E1 (Edirne1). Genotype G6 and G12 was the best performer in environment E5 (Keşan) (Table 3, Figure 1a).

Discriminating ability is an important measure of a test environment. Another equally important measure of a test environment is its representativeness of a target environment. An ideal environment should be highly differentiating of the genotypes and at the same time representative of the target environment (Dehghani et al. 2006). The discrimination and representativeness of wheat genotypes according to traits are displayed in Figure 1b. This figure shows that a representative "ideal center" over the property mean values and allows evaluating genotypes according to their nearness or distance to this center (Yan et al. 2000; Yan and Tinker, 2005). Similarly to the ideal genotype, the ideal environment is located in the first concentric circle in the environment-focused biplot, and desirable environments are close to the ideal environment. The ideal environment is representative and has the highest discriminating power (Yan and Tinker, 2006). The most ideal genotypes are located in the centre, whereas genotypes located on the mean vertical axis, but far from the centre, are ideal; genotypes located below the vertical axis are undesirable. According to this statement, placed near to the first concentric circle, G7 was the ideal genotype position and it can be used as a reference for genotype evaluation in breeding study (Figure 1b).

The GGE biplot in Figure 1b shows the relative ranking of the environments relative to the ideal. The ideal environment represented by the small circle with an arrow pointing to it (Figure 1b) is the most discriminating of genotypes and yet representative of the other test environments. The environment closest to the center of the concentric circles is the most representative of the environments. An ideal genotype should have high mean performance and be absolutely stable across environments. In Figure 1b, the arrow direction of the single-arrowed line indicates the ideal genotype. Therefore, E1 (Edirne1) is a more desirable test environment than others, which had a greater value for IPC1, showing a greater power of discrimination among the genotypes in related to the other environments (Figure 1b). Therefore, the E1 location can be regarded as the most favourable environment to select extensive adapted genotypes.

The average yield performance and stability of genotypes were assessed by an average environment coordination (AEC) method (Yan 2002). In the average environmental coordinate (AEC) system, the AEC X-axis (PC1) passes through the biplot origin with an arrow indicating the positive end of the axis and indicates the mean performance axis of genotypes.

GGE Biplot graph in Figure 2a showed that the mean performance based on environments and stability of wheat genotypes in grain yield. The results in Figure 2a showed that genotypes G7, G19, and G21 were found stable. Among the stable genotypes, performances of G7, G19, and G21 were above average in generally all the environments. Based on Figure 2a, genotypes with above-average yield were from G11 to G12 and located on the right side of the biplot origin, while genotypes with blow average yield were from G10 to G3 and located on the left side of the biplot origin. Genotypes G11, G24, G10 (Bereket), and G6 were unstable. The yield performances of these genotypes significantly altered based on environmental conditions. According to results, genotypes G12 and G6 had adaptive to favorable (ideal) environments (Figure 2a).

Further information about the discriminating power of environments, together with a representation of their mutual relationships, can be obtained by the environment-vector view of the GGE-biplot (Figure 2b). The angle between the environment vectors provides further information on the correlation between environments, where an acute angle indicates a positive correlation, an obtuse angle indicates a negative correlation and a right angle indicates no correlation (Figure 2b). According to, all environments were positively correlated except E2 as all of the angles among them were smaller than 90° suggesting that indirect selection for yield can be practical across these test environments. There is also strong positive correlation between environment E3, E4 and E5 (Figure 2b). A genotype adaptable or with good performance in one environment may exhibit a similar response in another environment. With the longest vectors from the origin, environments E1 (Edirne1) and E2 (Edirne2) were the most discriminating. E5 (Keşan) was moderately discriminating, while E4 (Tekirdağ) was least discriminating (Figure 2b).

In Figure 3a, X-coordinate indicates the main effects (means) and the y-coordinate indicates the effects of the interaction (IPCA1). In the biplot, ten bread wheat genotypes (G2, G6, G7, G11, G12, G16, G17, G19, G20 and G21) and two environments (E3 and E5) located on the right side of the graph. These were considered high yielding genotypes and environments. Due to the lowest IPCA scores, genotypes G1 and G10 were least involved with the interaction, and are therefore the most stable. Furthermore, the genotypes G21 and G22 were the most unstable, G4 with the highest average yield. The most ideal genotype should combine high yield and stable performance across a range of production environments. Among the high yielding genotypes, G8 and G2 genotypes can be best evaluated based on stability and grain yield with a combined low absolute PC1 score and high yield (Figure 3a).

A stable genotype should have around unit regression coefficient over environments (bi ≈ 1) and minimum deviation from the regression (S^2d=0) in addition to higher grain yield than the population mean. The coefficient of regression for grain yield is presented in Figure 3b. According to grain yield, it was determined that genotypes Gelibolu, G17, G8 and G12 were well adaptable to all environmental conditions. Genotype G7 had higher grain yield under unfavourable conditions. Genotypes G16, G18 and G25 were medium adaptable to all environments and genotypes G2 and G6 were well adaptable to well fertile environmental conditions (Figure 3b). The adaptability of a genotype to diverse environments is usually tested by the degree of its interaction with different environments under which it is grown. A genotype is considered to be more adaptive or stable if it has a high mean yield but a low degree of fluctuation in yielding ability when grown over diverse environments.

The development of high yielding genotypes in all environmental conditions is an important result for the success of breeding studies. The stability parameters of the wheat genotypes are given in Table 5. Genotypes G7 and G12 had higher yield potential across four environments. Genotypes G4 and G2 were very stable due to their highest determinations coefficient (R^2). The regression coefficients (b) values of the wheat genotypes varied from 0.02 to 1.63. The b value showed great variation between genotypes. This result indicated that twenty-five wheat genotypes showed different performances across five environments. Cultivar Gelibolu and G12, G18, and G22 lines had optimum b value. The highest positive intercept values (a) were determined in genotypes G21, G7, G13, G19, and Aldane (Table 5). This result explained that all these genotypes were higher yields both under well fertile and unfertile environmental conditions.

The cultivar Gelibolu surpassed the average grain yield, showed minimum deviation from linear regression (S^2d=214.05), positive intercept value (a=29.51), and its coefficient of regression (b=1.01) was almost one. Therefore it would be well adapted to a better environment. The genotype G7 yielded the highest grain yield followed by cultivar Anafarta (G12) and G6, all these genotypes showed a lower coefficient of determinations and the highest deviation from linear regression. Among these, genotype G7 was well adapted to the poor environment and has above average stability; while genotypes G8 and G12 had almost unit regression coefficient, and both were well adapted to all the environments.

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The cultivar, Gelibolu had a mean value higher than the general mean, regression coefficient around unity, and minimum deviation from regression, thereby it was identified as a stable genotype across the environments (Table 5).

Conclusions

The result of the research revealed the importance of genotype-environment interaction. Therefore, genotypes reacted differently in different environments. There was a significant difference among genotypes and environment due to various environmental conditions. Genotype × environment interaction effect was responsible for the greatest part of the variation, and then followed by environment and genotype. Genotype G7 had the highest grain yield and then followed by G12. G7 was the ideal genotype and it can be used as a reference for genotype evaluation. Among locations, E1 (Edirne1) is a more desirable test environment than others, which had a greater value for IPC1, showing a greater power of discrimination between the genotypes in regards to the other environments. Therefore, the location E1should be regarded as the most suitable to select widely adapted genotypes. Among the stable genotypes, performances of G7, G19, and G21 were above average in all the environments. The yield performances of these genotypes significantly varied based on environmental conditions. According to results, genotypes G12 and G6 had adaptive to favourable (ideal) environments. E1 (Edirne1) and E2 (Edirne2) were the most discriminating environments due to the longest vectors from the origin. Environment E5 (Keşan) was moderately discriminating, while environment E4 (Tekirdağ) was least discriminating. Among the high yielding genotypes, genotypes G8 and G2 can be best evaluated based on stability and grain yield with a combined low absolute PC1 score and high yield. For grain yield, it was determined that genotypes Gelibolu, G17, G8 and G12 were well adaptable to all environmental conditions. Under unfavourable environment condition G7 had higher grain yield. Genotypes G2 and G6 were well adaptable to well fertile environment conditions across five environments. In the research, genotypes G12 and G17 selected in 2016 and were released in 2019 named Anafarta and Abide.

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Table 1. Analysis of variance for grain yield in twenty-five wheat genotypes grown across five environments.

Source of variation	DF	SS	MS	F Ratio
Environment (E)	4	640801.51	160200.37	34.67**
Genotypes (G)	24	257483.29	10728.47	2.32**
Error	96	443530.30	4620.10	
C. Total	124	1341815.10		

* and ** indicate significances, at p<0.05 and p<0.01, respectively. DF: Degree of freedom, SS: Sum of square, MS: Mean of square.

Table 2. The variance of AMMI analysis of bread wheat genotypes on yield.

Source of variation	DF	SS	MS	F	SST%
Treatments	124	12213361	98495	1.69**	
Genotypes (G)	24	1891870	78828	1.35ns	5.52
Environments (E)	4	3122716	780679	11.72**	9.11
Block	15	999083	66606	1.14	
Interactions (G × E)	96	7198775	74987	1.28	21.02
IPCA1	27	6329646	234431	4.01**	
IPCA2	25	491435	19657	0.34	
Residuals	44	377695	8584	0.15	
Error	360	21032233	58423		
Total	499	34244676	68627		

* and ** indicate significances, at p<0.05 and p<0.01, respectively. SST%: Percentage relative to the sum of squares total, DF: Degree of freedom, SS: Sum of square, MS: Mean of square, IPCA: Interaction Principal Components Axes.

Table 3. Based on AMMI selections the first four genotypes for the per environment and IPCA scores.

Environment	Mean yield (kg ha^{-1})	1	2	3	4	IPCA[1]	IPCA[2]
E1	4796	G7	G12	G17	G6	9.61	-10.71
E2	5273	G7	G12	G17	G6	9.12	-6.25
E3	6942	G7	G12	G25	G2	8.69	6.31
E4	5752	G2	G4	G25	G7	4.04	12.40
E5	6540	G9	G6	G12	G11	-31.47	-1.75

E1: Edirne 1, E2: Edirne 2, E3: Lüleburgaz, E4: Tekirdağ, E5: Keşan, IPCA: Interaction Principal Components Axes.

Table 4. Yield response across five environments and standard deviation in genotypes.

G No	Genotypes	E1	E2	E3	E4	E5	Mean
1	G1 (Aldane)	4922	5393	7136	5621	5080	5951±1051^{a-e}
2	G2	4892	5599	7633	6167	6635	6144±1071^{abc}
3	G3	4310	5336	5834	4173	5125	5008±747^{f}
4	G4	4368	4793	7512	6090	6314	5924±1169^{a-e}
5	G5 (Selimiye)	3947	5053	7062	5503	5116	5284±1147^{def}
6	G6	5670	6047	7609	5741	7737	6362±1227^{ab}
7	G7	6852	6994	7512	6602	6352	6673±556^{a}
8	G8	4956	4733	7453	5632	6339	6275±1008^{ab}
9	G9	3282	3941	5818	6105	5809	5149±1168^{ef}
10	G10 (Bereket)	4254	4454	6965	4831	5335	5065±1189^{f}
11	G11	4555	5112	7055	6300	6844	5842±1252^{a-f}
12	G12	6196	6670	7735	6164	7697	6581±1124^{a}
13	G13	4782	5450	6228	5740	6222	5928±720^{a-e}
14	G14	4075	4687	6243	5483	5307	5312±782^{c-f}
15	G15 (Pehlivan)	3821	4634	7408	5470	5341	5345±1325^{c-f}
16	G16	5532	4960	7362	5584	6688	5960±1070^{a-e}
17	G17	5839	6139	7307	5412	6760	6056±965^{a-d}
18	G18	4850	5337	7300	4326	5715	5666±1157^{b-f}
19	G19	5442	5799	6721	5719	6026	5849±556^{a-f}
20	G20 (Gelibolu)	5327	5503	7617	6000	5768	6102±882^{a-d}
21	G21	5935	5830	5686	6056	6573	5951±409^{a-e}
22	G22	3372	4844	5945	6454	6072	5535±1232^{b-f}
23	G23	3709	4182	6030	6223	5959	5353±1066^{c-f}
24	G24	4097	5179	7180	5908	5043	5282±1291^{def}
25	G25	4912	5152	7210	6498	4518	5663±1140^{b-f}
Mean		4796	5273	6942	5752	6015	5770
CV (%)		8.2	10.1	8.3	9.7	8.7	11.7
LSD (0.05)		55.8	75.1	81.6	78.8	74.1	83.30

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Table 5. The stability parameters and IPCA parameters of AMMI model of the genotypes across five environments.

G No	Genotypes	X	R^2	S^2d	a	b	IPCAg[1]	IPCAg[2]
1	G1 (Aldane)	5951	0.27	1347.43	202.77	0.68	3.93	0.01
2	G2	6144	0.98	29.82	-151.76	1.33	0.68	1.79
3	G3	5008	0.36	591.15	176.25	0.56	1.94	-5.50
4	G4	5924	0.99	29.96	-244.87	1.45	0.39	4.50
5	G5 (Selimiye)	5284	0.93	145.78	-270.73	1.38	2.76	3.03
6	G6	6362	0.60	1009.08	-47.83	1.19	-1.31	-3.49
7	G7	6673	0.31	353.37	442.84	0.39	3.96	-5.23
8	G8	6275	0.55	764.93	89.72	0.93	0.55	1.07
9	G9	5149	0.61	883.65	-143.27	1.14	-33.79	0.04
10	G10 (Bereket)	5065	0.88	293.02	-295.82	1.39	1.73	0.50
11	G11	5842	0.76	619.30	-203.98	1.37	-0.81	3.01
12	G12	6581	0.53	981.93	65.78	1.03	-0.13	-4.47
13	G13	5928	0.28	619.23	316.11	0.48	0.42	-1.44
14	G14	5312	0.80	207.71	28.28	0.87	1.54	1.65
15	G15 (Pehlivan)	5345	0.97	89.44	-406.19	1.63	2.02	4.56
16	G16	5960	0.67	626.56	-35.99	1.10	0.20	-1.61
17	G17	6056	0.57	671.85	81.86	0.91	1.02	-5.39
18	G18	5666	0.47	1178.30	-6.65	0.99	1.94	-4.27
19	G19	5849	0.89	55.63	206.39	0.66	2.00	-3.17
20	G20 (Gelibolu)	6102	0.83	214.05	29.51	1.01	3.05	0.44
21	G21	5951	0.01	278.46	584.90	0.02	0.42	-5.68
22	G22	5535	0.43	1447.48	-26.88	1.01	-0.69	6.04
23	G23	5353	0.65	669.81	-82.36	1.07	-0.65	5.58
24	G24	5282	0.83	459.97	-321.79	1.47	3.36	4.03
25	G25	5663	0.45	1186.97	13.71	0.96	5.48	3.99

X: Mean yield, R^2: Coefficient of determinations, S^2d: Deviation from regression, a: Intercept value, b: Coefficient of regression.

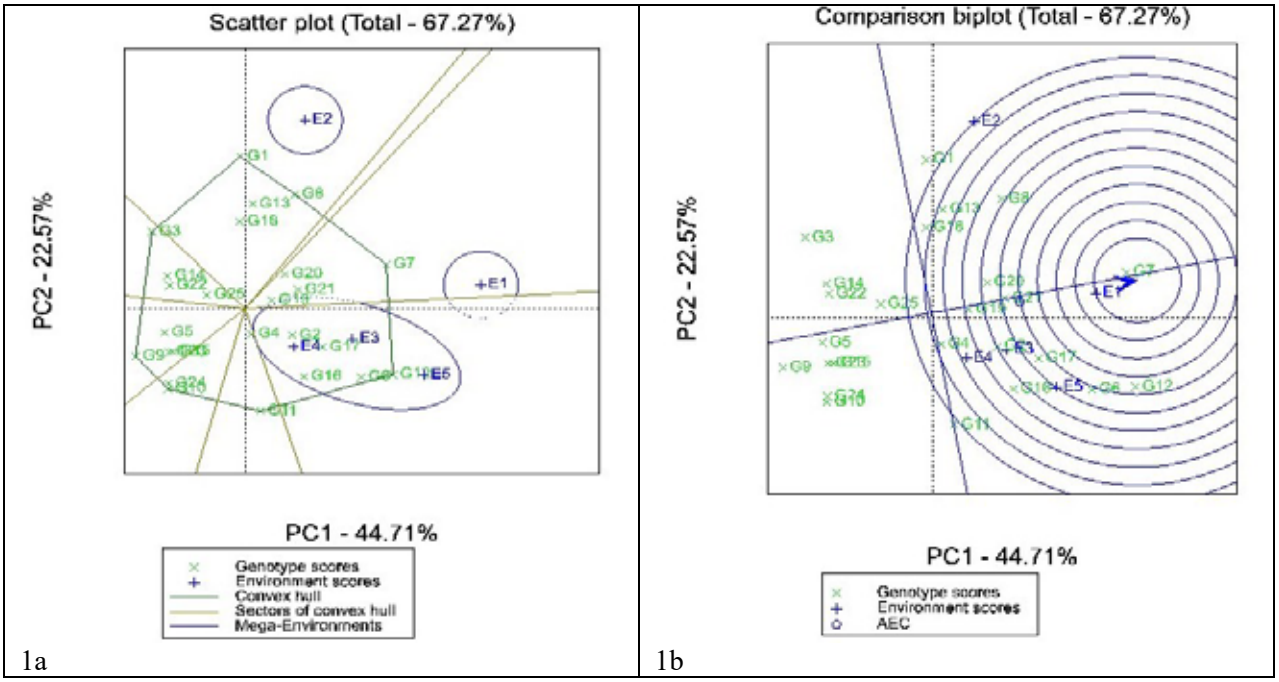

Figure 1. Polygon views of the GGE graph demonstrated that the mega-environments and the which-won-where view of the genotype according to grain yield (1a) and, GGE biplot according to genotype-focused scaling for comparison of the genotypes with the ideal genotype (Figure 1b).

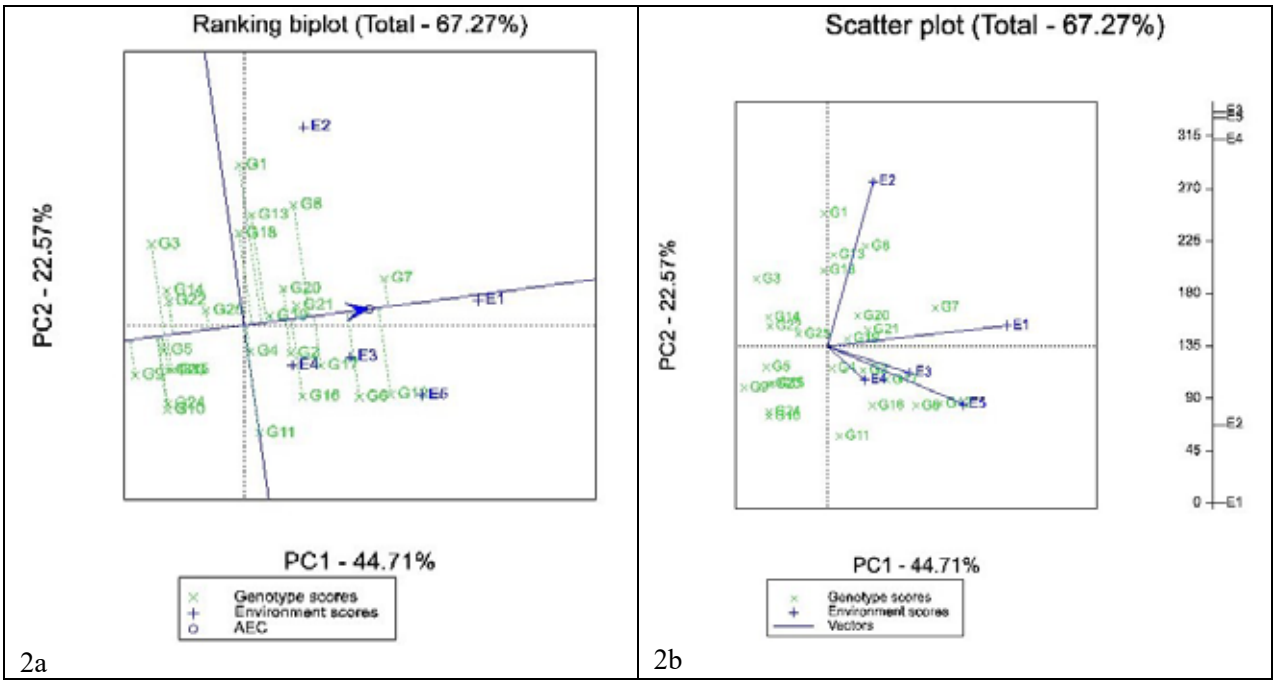

Figure 2. GGE biplot graph demonstrated ranking of the twenty-five genotypes according to mean yield and stability in yield (2a), and GGE biplot the evaluation of the relationship among environments (Figure 2b).

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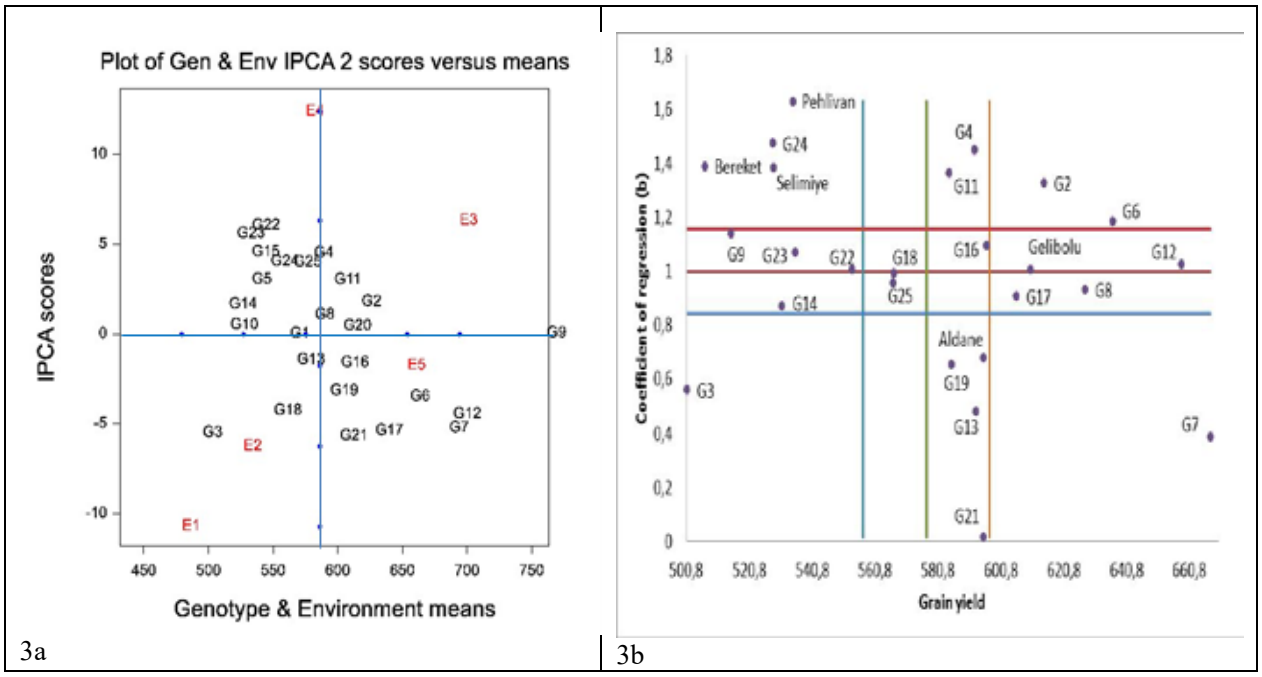

Figure 3. Genotypes environmental means and IPCA scores for twenty-five genotypes across five environments, and scatter plot of regression coefficient of 25 wheat genotypes yield (3b).

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Evaluation of Sowing Density and Agro-Ecological Conditions on Wheat (*Triticum aestivum* L.) Yield and Components

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ABSTRACT

Wheat is the strategic crop in Afghanistan. Most wheat growers broadcast wheat seeds during sowing time, and sometimes the growers sow more seeds 3 to 4 times more than appropriate rate. Variety adaptation is another concern when releasing wheat varieties adapted to a broad environment. The main objective of this study was to evaluate the effects of sowing densities at the rate of 100, 110, and 120 kg ha^{-1} seed density on the total yield of three wheat varieties, Baghlan 09, Kabul 013, and Moqwim 09 under Kabul and Khost Agro-Ecological conditions, Afghanistan. The experiment was conducted using a randomized complete block design with three replications. The results indicated that seed rate at 110 kg ha^{-1} in Kabul province for Kabul 013 and Baghlan 09 varieties and 120 kg ha^{-1} in Khost province for all three varieties produced maximum grain yield. Ranking the three varieties, Kabul 013 and Moqwim 09 produced the highest grain yield while Baghlan 09 did not perform well in either of the two locations, Kabul and Khost. Interaction effects for all traits under evaluation in the two locations were also significant. Traits like days to heading, days to maturity, plant height, and grain yield were high in Kabul province compared to Khost. Correlation analysis revealed significant positive correlations among days to heading and days to maturity (r=0.98***), spikelets spike-1 and grain yield (r=0.88*** and r=0.75***) in Kabul and Khost provinces, respectively. Analysis indicated that the seed rate at 110 kg ha^{-1} for Kabul 013 and Baghlan 09 varieties in Kabul province and 120 kg ha^{-1} for all three varieties were optimum sowing density in Khost province.

Keywords: Agro-ecological conditions, grain yield, sowing density, varieties and wheat

Introduction

Wheat (*Triticum aestivum* L.) is globally cultivated on 222,27 million hectares with production and productivity of 779,29 million tons and 3.51 ton ha^{-1}, respectively (USDA 2022). The demand of the wheat crop is more than other major food crops and approximately 35% of the food of the world population is wheat product (Ali et al. 2016). Fortunately, wheat has wider adaptability to different agro-ecological conditions (Mirbahar et al. 2009). Wheat is the main cereal crop in Afghanistan which accounts for around 70% of total cereal consumption and 60% of the total intake of calories and is produced in both irrigated and rainfall conditions (MAIL 2013). The sowing of wheat variety in a perfect environment exploits the full genetic potential of a particular variety by providing optimum growth conditions such as temperature, light, humidity, and rainfall. Unfavorable environmental conditions, created by high temperature, mostly during reproductive stages, particularly the grain filling stage, could be minimized by adjusting the sowing time to an optimum time for different varieties (Gupta et al. 2020). Various agro-ecological conditions with varying climatic conditions may influence wheat grain yield and growth (Ahmadi et al. 2021).

Appropriate sowing density is an important factor for wheat grain yield. High seed density may lead to more crops per unit area which will induce competition among them for benefiting nutrients in the soil which will lead to low grain yield. Also, using

low seed density will produce less yield because of the less number of plants per unit area (Geravandi et al. 2011). Most farmers in Afghanistan broadcast seeds manually in wheat fields that generally affects the germination of wheat by the unsuitable depth of seeds (Ahmadi and Arian 2021). Research indicated that increased seed density can also lead to different wheat diseases (Kahrizi et al. 2010; Khan and Gul 2006). Using inappropriate sowing density can lead to weeds problems in wheat fields (Hameed et al. 2003; Fahad et al. 2015) and farmers with using optimum seed density can manage this problem.

Wheat yield is conditioned by many genes and therefore then can be affected by environmental effects. To understand the impact of the environment on wheat grain yield, wheat breeders need to conduct multi-environment trials in which the wheat varieties shall be evaluated for yield performance under different agro-ecological conditions. The presence of genotype x environment interactions is often an indication of a lack of stability of genotypes across the environments. Improved varieties play an outstanding role in exploiting the yield potential of the crop under specific agro-climatic conditions (Osmanzai et al. 2008; Gupta et al. 2020).

Osmanzai and Sharma (2008) found that the genetic by environmental interaction decreases correlation between phenotype and genotype making it hard to distinguish the genotypic effects of a variety. Thus, the current research was aimed at evaluating the effect of different environments and sowing densities on the growth and grain yield of wheat varieties.

Materials and Methods

Experimental site and treatments

Two individual field experiments were carried out at two dissimilar locations of Afghanistan (Experimental farm of the Agriculture Faculty, Kabul University, Kabul Province, and in Almara Village, Khost Province). Kabul is located at 1791 m above sea level with mean annual rainfall of 300 mm. Khost is lying at 1386 m above sea level with mean annual rainfall 650 mm. Both experiments were conducted during the 2018-2019 winter growing season. Data on monthly rainfall and mean temperatures over two environments and soil properties are provided in Table 1 and Figure 1, respectively.

The treatments for this research were three sowing densities (100, 110, and 120 kg seeds ha^{-1}) and three facultative wheat (*Triticum aestivum* L.) varieties (Moqawim 09, Kabul 013, and Baghlan 09). The experiment was laid out in Randomized Complete Block Design, with three replications in a split-plot arrangement. Seed densities were the main plot and varieties were the sub plot. The combination of the treatments is presented in Table 2. The individual plot size was 6 m^2 (2 m x 3 m).

Sowing and measurements

According to the local time of environment for cultivation, wheat was sown on 24th October in Kabul Province and a month later (24th November) in Khost Province, respectively. Sowing was done with a hand drill method. A total of 120 kg ha^{-1} Nitrogen was applied three times, at sowing time, jointing, and flowering stages and Phosphorus fertilizer at 80 kg ha^{-1} was used at the time of cultivation. The data was recorded for the parameters of days to heading, days to maturity, plant height (cm), productive tillers (number m^{-2}), spikelets $spike^{-1}$ (no), grain yield (kg ha^{-1}), and harvest index (%). Harvest index (HI) was calculated using the following formula.

$$HI\,(\%) = \frac{\text{Grain yield}}{\text{Biological yield}}\, x\ 100$$

Statistical analysis

Data collected for each of the traits were subjected to analysis of variance (ANOVA), using Statistical Tool for Agriculture Research (STAR) software (version 2.0.1). To separate significant differences in the means of the treatments, Tukey's Honest Significant Difference (HSD) test was used at $p< 0.05\%$ probability level. To find the correlation among the yield and yield components of wheat varieties, the R software (R i386 4.0.2) was used.

Results and Discussion

Effects of sowing density: The effect of sowing density was not significant for the traits of days to heading, days to maturity, plant height, spikelets $spike^{-1}$, and harvest index in both provinces, while wheat crop sown at 120 kg seeds ha^{-1} performed outstandingly with respect to productive tillers (443.11m^{-2}) in Khost province as compared to 110 and 100 kg ha^{-1} but the values were not significant at any amount of sowing density in Kabul province, (Table 3 and Figure 2). The higher number of productive tillers may be due to the higher sowing density in Khost province. Our result is confirmed by (Goverdhan et al. 2018; Tilley et al. 2019) who found more number tillers in more seed rates compared to less.

In the case of grain yield, sowing density was significant in both provinces (Table 3). When the wheat crop was sown at 110 kg seeds ha^{-1} in Kabul province, the wheat produced the highest grain yield (5536.77 kg ha^{-1}) but in Khost province, the highest grain yield

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(5472.28 kg ha^{-1}) was produced at 120 kg seeds ha^{-1} compare to others sowing density, Figure 2 (c) and (d). This amount of seeds rate may be the ideal seed rate for wheat cultivation in the mentioned provinces. It is in harmony with (Goverdhan et al. 2018) who found that optimum sowing density produced high yield compared to less and more.

Effects of varieties: Data concerning days to heading, days to maturity in Kabul province, while plant height, productive tillers, spikelets spike-1, and grain yield have significant differences between the varieties for both provinces. Harvest index was not significant in either of the two locations, Table 3. Among the varieties, Kabul 013 took more days to heading and days to maturity (195.26 and 246, respectively), Figure 3 (a) and (b).

The most productive tillers (456.56) were produced by Baghlan 09 in Kabul province, while Moqawim 09 (434.33) was at the top in Khost Province. Among the varieties, Kabul 013 produced less productive tillers in both provinces, Figure 3 (c) and (d).

Data regarding spikelets spike^{-1} also had significant effects for varieties, Table 3. Higher numbers of spikelets spike^{-1} among the varieties in both locations were produced by Kabul 013. Mean values of Kabul 013 variety (16.61 and 15.38) are at the top in both provinces (Kabul and Khost, respectively) while Baghlan 09 produced the minimum values (12.80 and 11.58), Figure 3 (e) and (f).

Grain yield, the main final product, was also significant among the varieties, Table 3. A higher yield was produced by Kabul 013 among the varieties. Figure 4 (g) and (h) revealed that the Kabul 013 variety is in first-class followed by Moqwim 09 while the Baghlan09 variety is in the low (c) class. The grain value of the Kabul 013 variety (5809.66 kg ha^{-1}), was 7.28% higher than Moqawim 09 and 13.39% higher than Baghlan 09, variety in Kabul province. Similarly in Khost province, the high grain yield of the Kabul 013 variety (5493.44 kg ha^{-1}) is 6.78% and 11.82% more than Moqawim 09 and Baghlan 09 varieties. Variations among the varieties for days to heading, days to maturity, productive tillers, spikelets spike-1, and grain yield may be the genetic make-up of varieties controlled by genes. It is similar to (Tolera et al. 2008; Abd El-Lattief 2014) who found the variation for agronomic traits among the varieties without the effects of examined factors.

Effects of environments: Analysis of variance for combined data in Table 4 indicated that days to heading, days to maturity, plant height, and grain yield of the wheat crop were significantly affected due to different sowing environments. In Kabul province sowing of wheat significantly increased the days to heading (192.78 days), days to maturity (242.63 days), plant height (88.51 cm), and grain yield (5449.50 kg ha^{-1}) followed by Khost province (132.96 days, 190.8 days, 83.85 cm, and 5183.38 kg ha^{-1}, respectively) Figure 4. In Kabul province, the significantly higher of the mentioned parameters might be the accessibility of optimum environmental conditions for growth and development of the cultivated wheat varieties which could have boosted the accumulation of photosynthesis from the source of climate and land. This result is in harmony with Šíp et al. (2013) who reported the difference among the grain yield across the locations.

Interaction effects: A significantly higher number of productive tillers were observed in Khost province in all three wheat varieties when sown at 120 kg seeds ha^{-1}. The higher number of productive tillers on Moqawim 09 variety (450.33 a), closely followed by Kabul 013 (446 a) and Baghlan 09 (433 a) varieties. These varieties were top at the rate of 120 kg ha^{-1}, while at the seed rate of 100 kg ha^{-1} they produced few productive tillers, particularly Kabul 013 variety (381 b), and these differences for productive tillers in Kabul province were insignificant.

The interaction effects for grain yield among the sowing densities x varieties were significant in both provinces, Table 5. The most grain yield in Kabul province was observed on variety Kabul 013 (6017.00 a) when sown at 110 kg seeds ha^{-1}, similarly for Baghlan 09 but Moqawim 09 produced more grain yield at 120 kg seed ha^{-1} while in Khost province the most grain yield was produced when the wheat varieties were sown at 120 kg seed ha^{-1}. The Kabul 013 variety with (5845.33 kg ha^{-1} a) grain yield at 120 kg seed ha^{-1} is on the top but Baghlan 09 (4647.80 kg^{-1} c) produced the least grain yield on 100 kg ha^{-1}. The differential performance of wheat varieties for productive tillers and grain yield in different sowing densities and environments might be due to their genetic makeup and environmental effects.

Correlation among the yield and yield components of wheat varieties. The correlation among the yield and yield components of wheat varieties in this study is given in Figure 6. It is seen from the figure that there are positive and negative correlations within the yield and yield components in both provinces. There are strong positive correlations for both provinces (Kabul and Khost, respectively) among the days to heading and days to maturity (r= 0.98***, and r = 0.98***), plant height and spikelets spike^{-1} (r=0.72*** and r= 0.7^{2***}) plant height and grain yield (r= 0.64*** and r = 0.55***) and spikelets spike^{-1} with grain yield (r=0.88*** and r=0.75***) but just in Kabul province among the days

to heading with spikelets spike^{-1} (0.75***), days to heading with grain yield (r=0.83***), days to maturity with spikelets spike (r=0.69***) and days to maturity with grain yield (r=0.78***).

Conclusions

Research results indicated that the effects of sowing densities, varieties and environments were significant. The sowing of wheat with the seed rate at 110 kg ha^{-1} in Kabul province performed well for the grain yield, while in Khost province, the number of productive tillers and grain yield were maximum at 120 kg ha^{-1}. Among the varieties, the Kabul 013 variety was on the top for grain yield and its attribute (spikelets spike^{-1}). The interaction effects of densities x varieties and among the locations were also significant The most grain yield in Kabul province was observed on Kabul 013 variety (6017.00 kg ha^{-1}) when was sown at 110 kg seeds ha^{-1}, similarly for Moqawim 09 while Baghlan 09 produced more grain yield at 120 kg seed ha^{-1} while in Khost province the most grain yield was produced when the wheat varieties were sown at 120 kg seed ha^{-1}. Most days to heading and days to maturity, plant height, and grain yield were observed in Kabul province compared to Khost province. From the research results, we conclude and recommend that Kabul province is the suitable area for more grain yield of Kabul 013 and Baghlan 09 varieties at 110 kg ha^{-1} but 120 kg ha^{-1} sowing density is appropriate for Moqawim 09 while in Khost province for all three varieties, the 120 kg ha^{-1} density is suitable. Further research should be carried out in different provinces of Afghanistan besides Kabul and Khost to notice adaptation ability and appropriate sowing density for the mentioned varieties.

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Table 1. Soil and soil properties for the two environments (Kabul and Khost Provinces).

Environment		Kabul	Khost
Soil type	Property		
	Clay (%)	59.76	50.56
	Silt (%)	21	27
	Sand (%)	19.24	22.44
	Textural class	Sandy loam	Sandy clay loam
Soil analysis	pH (in water)	8.3	7.9
	N (%)	2.2	3
	P (mg kg^{-1})	6.9	7.5
	K (mg kg^{-1})	190	171
	OM (%)	0.91	0.93

Table 2. Combination of treatments from three sowing densities (S) and three wheat varieties (V).

Treatments	Description
S1 V1	100 kg seed ha^{-1} and Moqawim 09 variety
S1 V2	100 kg seed ha^{-1} and Kabul 013 variety
S1 V3	100 kg seed ha^{-1} and Baghlan 09 variety
S2 V1	110 kg seed ha^{-1} and Moqawim 09 variety
S2 V2	110 kg seed ha^{-1} and Kabul 013 variety
S2 V3	110 kg seed ha^{-1} and Baghlan 09 variety
S3 V1	120 kg seed ha^{-1} and Moqawim 09 variety
S3 V2	120 kg seed ha^{-1} and Kabul 013 variety
S3 V3	120 kg seed ha^{-1} and Baghlan 09 variety

S: Sowing densities, V: Variety.

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Table 3. Analysis of variance (ANOVA) for growth, yield and yield attributes of wheat on two environments (Kabul and Khost provinces).

Source of Variance		DH	DM	PH	SS	PT	GY	HI
	DF	MS	MS	MS	MS	MS	MS	MS
Kabul								
Replication	2	0.11	0.03	0.10	0.01	843.44	235.59	0.00
Seed Density (SD)	2	0.77	0.70	1.53	1.33	1420.33	151724.59**	3.36
Error (a)	4	0.55	0.20	0.61	0.12	365.11	16.81	1.48
Variety (V)	2	124.33**	258.92**	398.79**	34.41**	6627.11**	1068153.03**	0.70
SD x V	4	0.11	0.25	0.00	0.58	1156.61	42774.25**	2.73
Error (b)	12	0.12	0.09	0.49	0.79	394.33	13.57	2.02
CV (%)		**0.25**	**0.14**	**0.81**	**5.47**	**4.57**	**6.95**	**3.53**
Khost								
Replication	2	0.70	0.25	3.26	4.85	67.44	576.29	0.019
Seed Density (SD)	2	18.03	7.37	1.53	2.24	3444.77**	697107.73**	6.67
Error (a)	4	18.03	6.53	0.61	1.04	20.88	883.80	3.57
Variety (V)	2	0.14	0.25	398.79**	34.25**	1864.77**	769710.28**	0.85
SD x V	4	18.14	5.20	0.00	0.07	609.22**	7091.35*	2.41
Error (b)	12	11.70	3.27	0.49	0.50	50.85	2066.69	2.89
CV (%)		**2.74**	**1.06**	**0.86**	**6.03**	**1.56**	**0.81**	**4.58**

Note:* and ** Significant at p< 0.05 and p<0.01 levels, respectively, DF: Degrees of freedom, MS: Mean sum of squares, DH: Days to heading, DM: Days to maturity, PH: Plant height, SS: Spikelets spike^{-1}, PT: Productive tillers, GY: Grain yield and HI: Harvest Index.

Table 4. Analysis of variance (ANOVA) for Combine data of wheat growth, yield and yield attributes.

Source of Variance		DH	DM	PH	PT	SS	GY	HI
	DF	MS	MS	MS	MS	MS	MS	MS
Location	1	48300.46**	36244.46**	294.00**	1093.50	20.41	954620.88**	6.06
Rep within location	4	0.40	0.14	1.68	455.44	3.50*	405.94	0.00
Seed Density (SD)	2	8.46	4.05	3.07*	2718.05**	2.66*	674547.47**	9.64
L x SD	2	10.35	4.01	0.00	2147.05**	0.91	174284.85**	0.39
Error (a)	8	9.29	3.37	0.61	193.00	0.58	450.31	2.52
Variety (V)	2	65.01**	130.72**	797.58**	6493.16**	68.67**	1825257.99**	1.55
SD x V	4	8.65	3.27	0.01	480.80	0.43	15259.08**	5.09
L x V	2	59.46**	128.46**	0.00	1998.92**	0.00	12605.32**	0.00
L x SD x V	4	9.60	2.18	0.00	1285.02**	0.22	34606.53**	0.05
Error (b)	24	5.91	1.68	0.49	222.59	0.64	1040.13	2.46

Note:* and ** Significant at p< 0.05 and p<0.01 levels, respectively, DF: Degree of freedom, MS: Mean sum of squares, DH: Days to heading, DM: Days to maturity, PH: Plant height, SS: Spikelets spike^{-1}, PT: Productive tillers, GY: Grain yield and HI: Harvest Index.

Table 5. The interaction effects of sowing density, variety and environments.

Environment		Productive tillers (m^{-2})			Grain yield (kg ha^{-1})		
		Varieties					
	Sowing Density	V1	V2	V3	V1	V2	V3
Kabul	S1	411.66	408.66	434.66	5260.00 c	5609.00 c	5031.33 c
	S2	432.33	415.00	482.66	5385.00 b	6017.00 a	5208.33 a
	S3	450.33	383.33	452.33	5600.00 a	5803.00 b	5130.00 b
Khost	S1	425.00 b	381.00 b	411.00 b	4898.33 c	5205.00 c	4647.80 c
	S2	427.66 b	390.33 b	425.66 a	5137.00 b	5430.00 b	4915.40 b
	S3	450.33 a	446.00 a	433.00 a	5397.20 a	5845.33 a	5174.33 a

Note: S1, S2 and S3 describe the 100, 110 and 120 kg ha^{-1} amount of sowing density, respectively and V1, V2 and V3 show the name of varieties respectively: Moqawim 09, Kabul 013 and Baghlan 09.

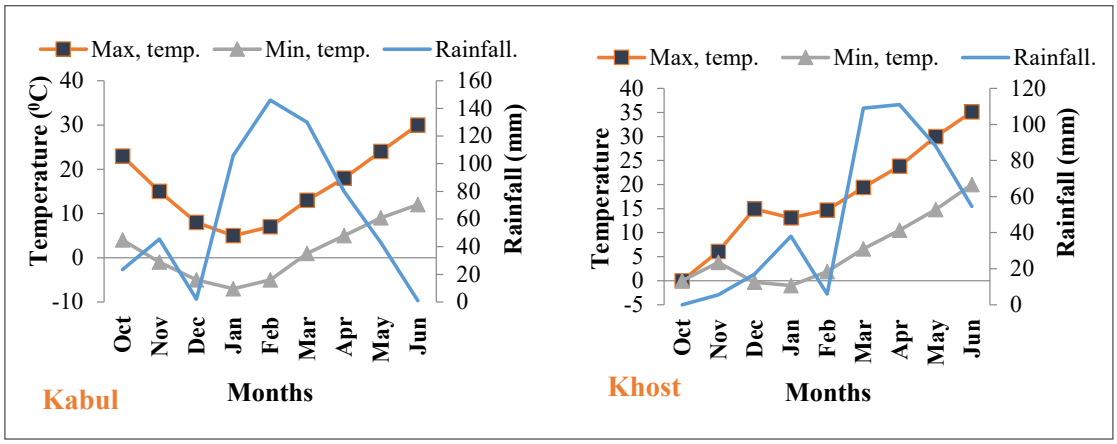
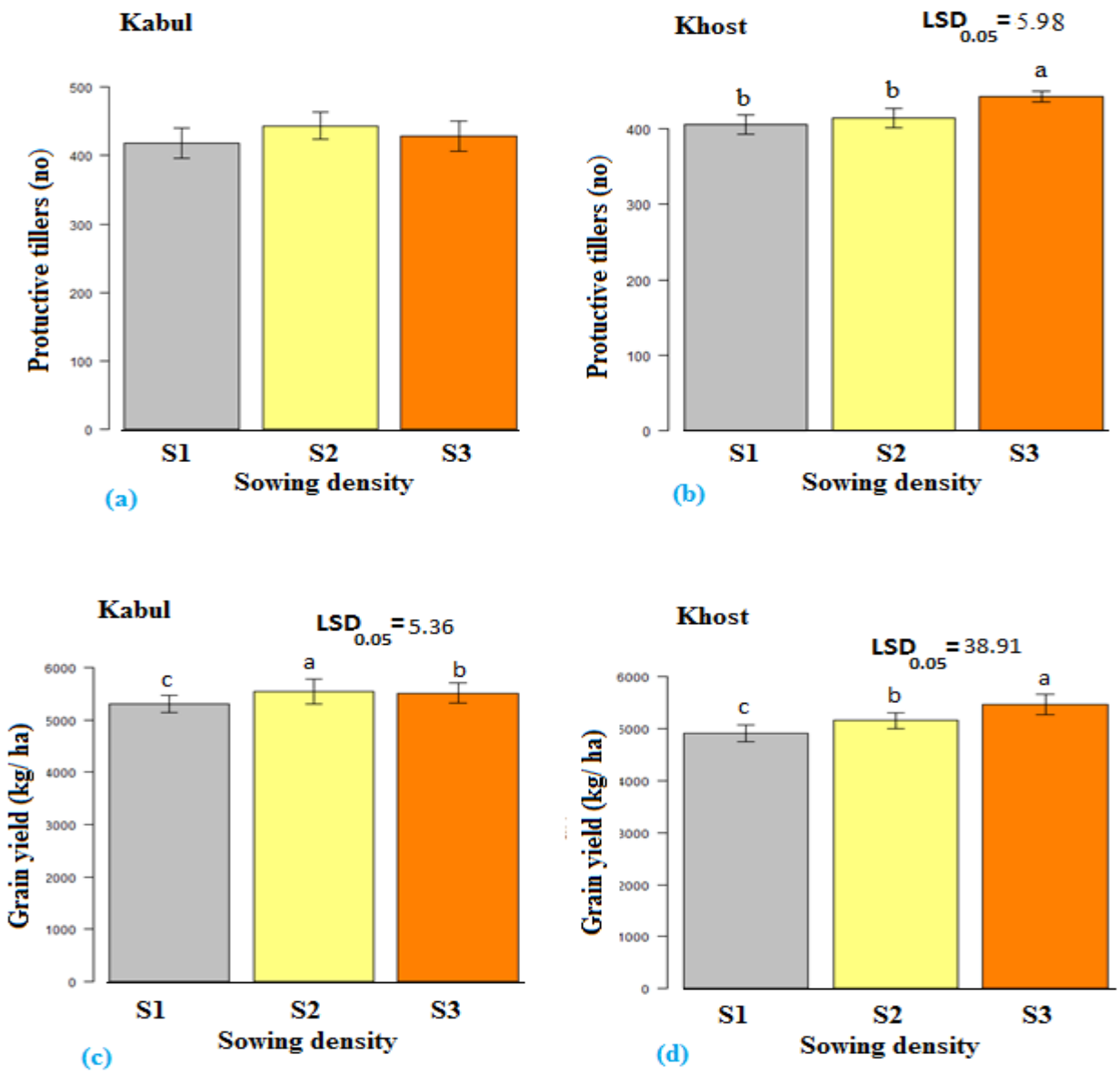
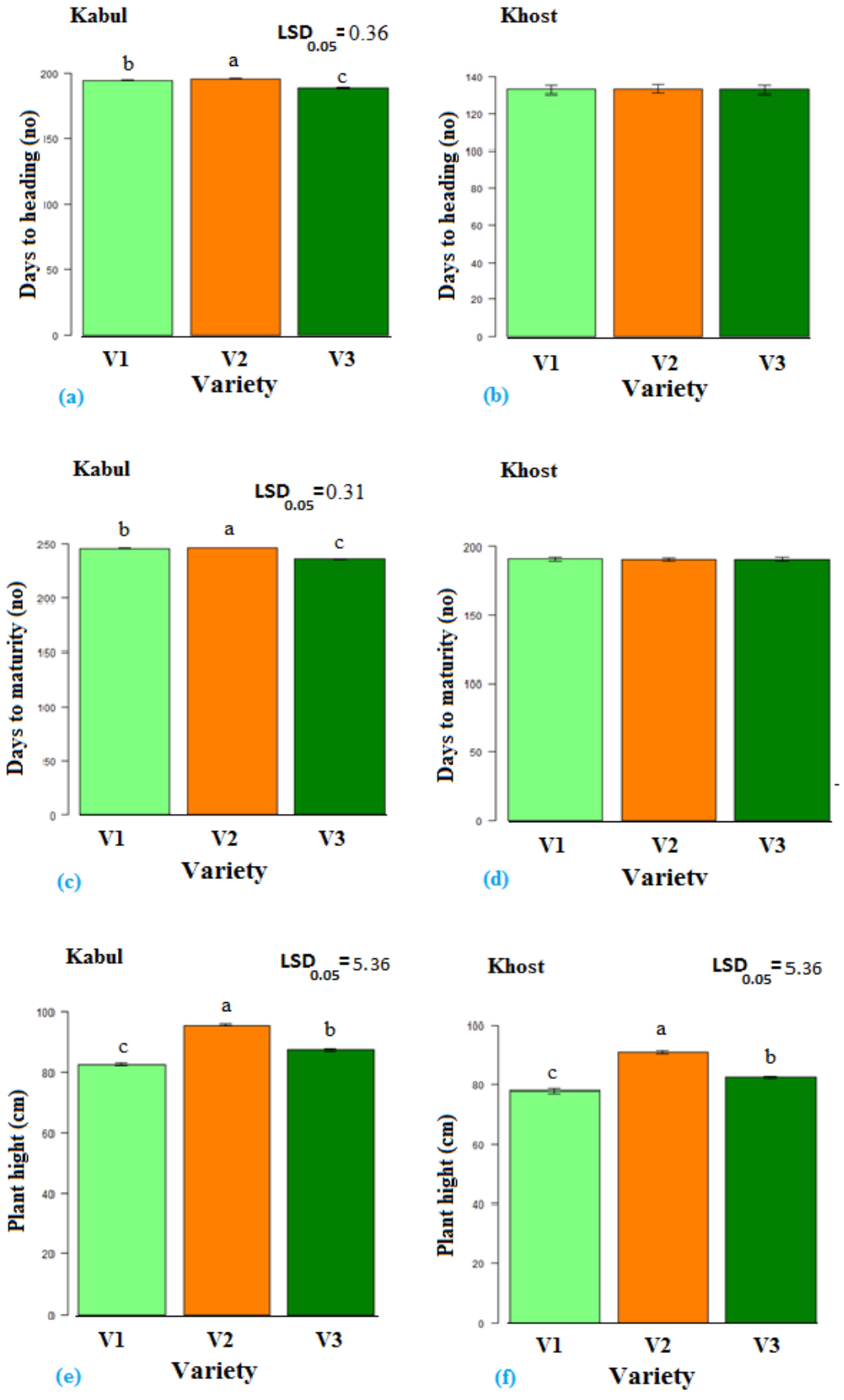


Figure 1. Rainfall and temperature during the growing period.



Note: S1, S2 and S3 describe the 100, 110 and 120 kg seeds ha^{-1}, respectively.

Figure 2. Effects of sowing density on productive tillers and grain yield of wheat.



Note: V1, V2 and V3 describe the name of varieties Moqawim 09, Kabul 013 and Baghlan 09, respectively.

Figure 3. Effects of varieties for days to heading, days to maturity and plant height.

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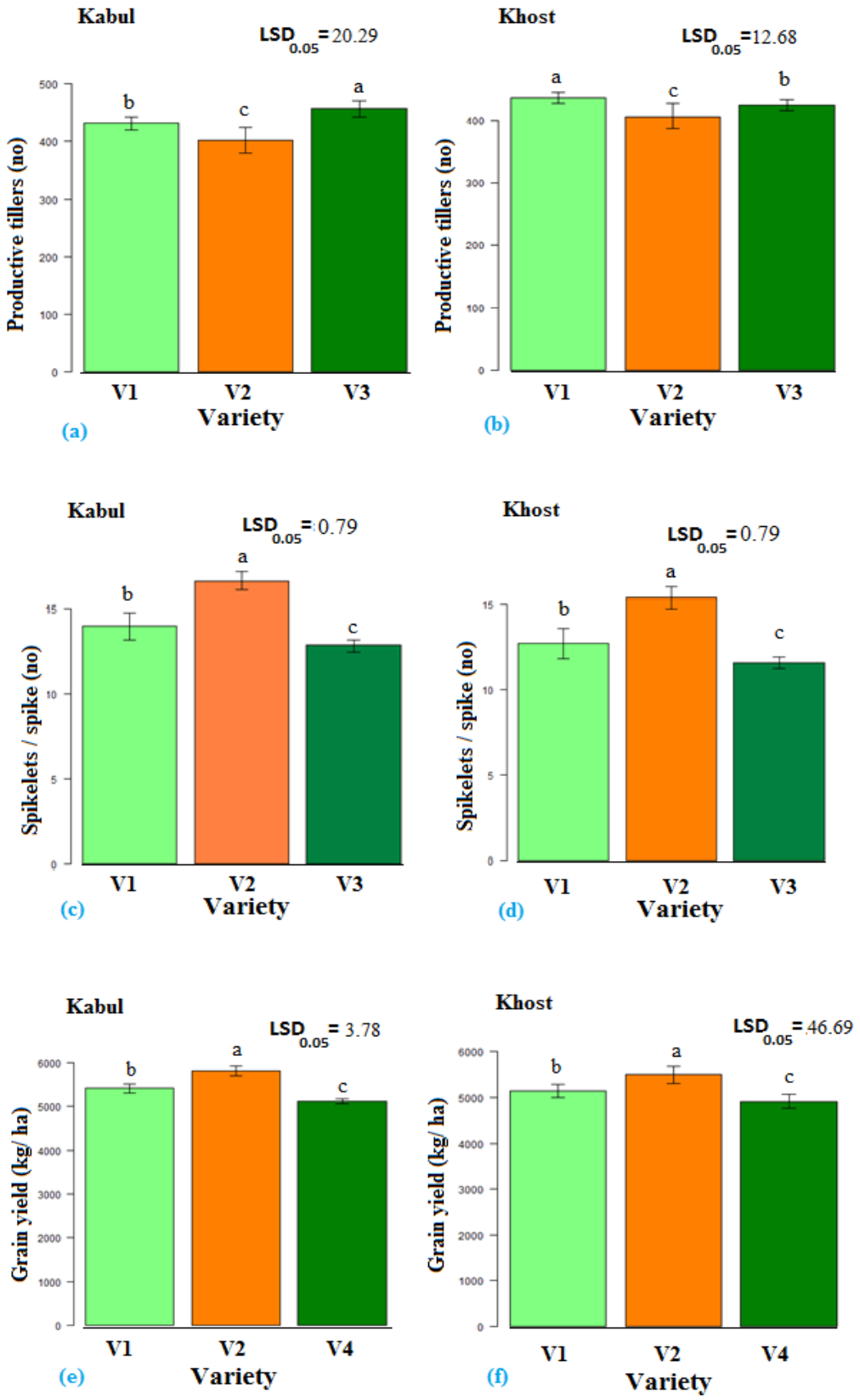


Note: V1, V2 and V3 describe the name of varieties Moqawim 09, Kabul 013 and Baghlan 09, respectively.

Figure 4. Effects of varieties for productive tillers, Spiklets spike^{-1} and grain yield.

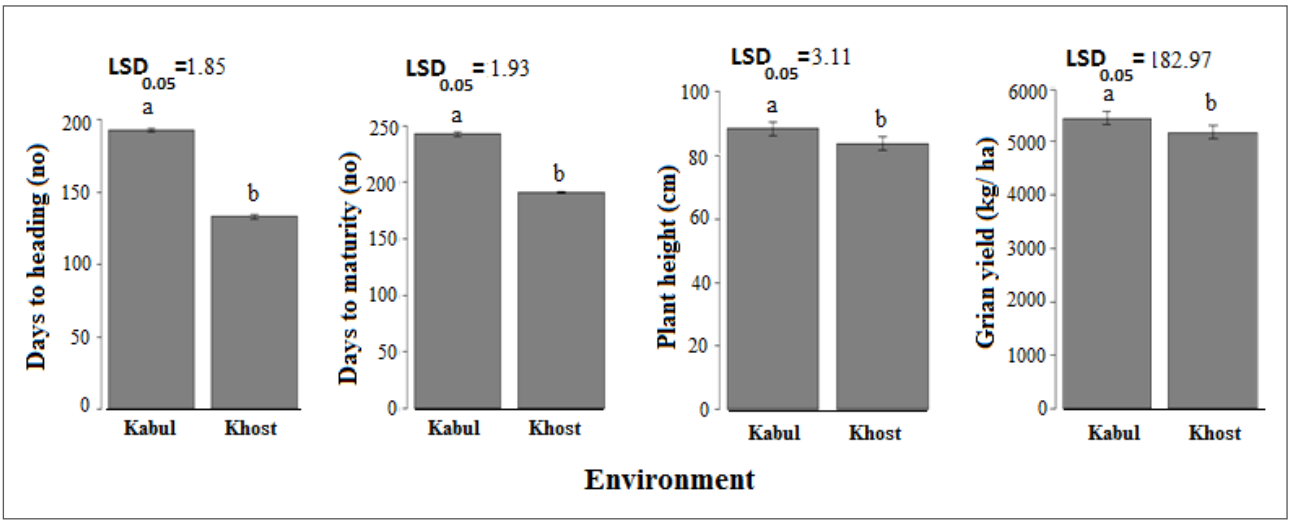
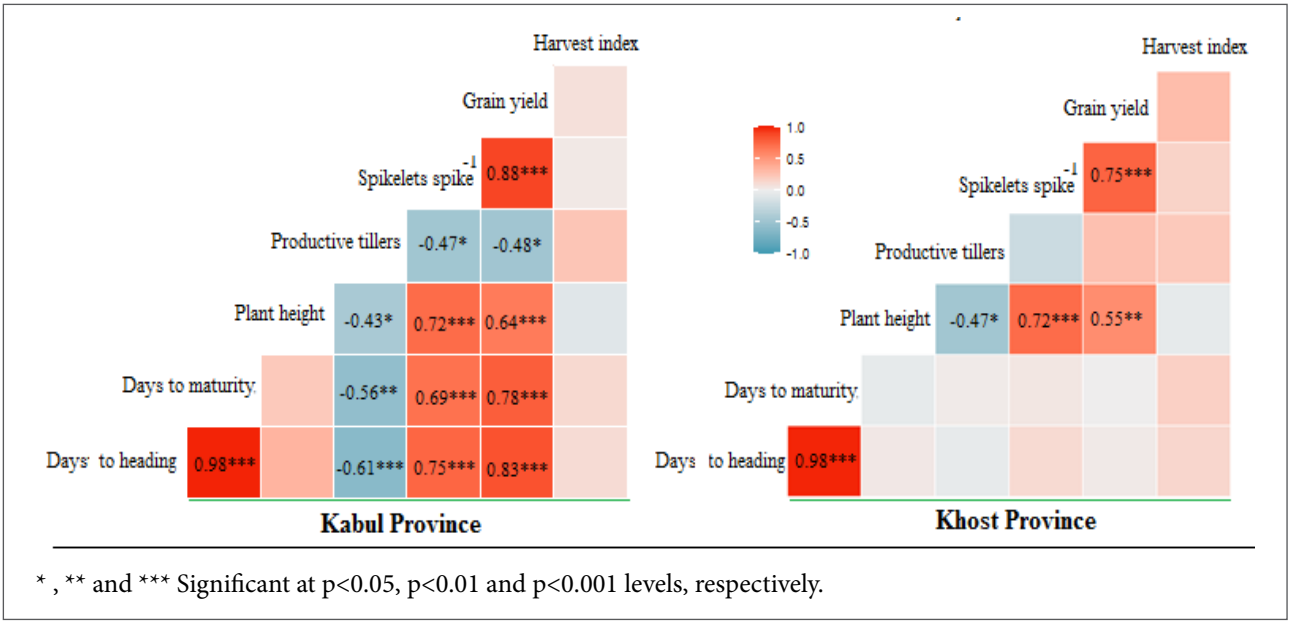


Figure 5. Effects of environments for days to heading, days to maturity, plant height and grain yield on wheat crop.



* , ** and *** Significant at p<0.05, p<0.01 and p<0.001 levels, respectively.

Figure 6. Correlation among the yield and yield components of wheat varieties on two different provinces.

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Genetic Variability Analysis and Correlation Studies of Bread Wheat (*Triticum aestivum* L.) Genotypes

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ABSTRACT

In the present investigation, fifty four bread wheat (*Triticum aestivum* L.) genotypes were evaluated for estimation of the genetic variability and its potential of each genotype under normal spring condition in Kabul Afghanistan in two years consequently (2020 and 2021). Analysis of variance revealed that genotypes possess significant genetic variability among all traits. For all the studied traits, mean squares showed the presence of significant variation among the genotypes. Higher values of PCV and GCV indicated that there was high variability exiting among the genotypes. The higher values of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (GCV) were recorded for grain yield (GCV = 11.93% and PCV = 16.15%) followed by thousands grains (GCV=7.53% and PCV=8.15%). The highest heritability (0.94) was indicated in grain yield in the year 2021 followed by thousand grains (0.85) whereas the plant height (0.70) observed in (2020). The values of genetic advance were recorded low to moderate for all parameters. A highly significant positive correlation was observed among yield and harvest index and showed the significant difference for others except number of effective tiller per m^2, on contrary the negative non-significant correlation was observed for effective tiller per m^2 (-0.07). Grain yield with biological yield revealed a non-significant correlation (0.04), similarly days to heading revealed a non-significant negative correlation with grain yield (-0.05) respectively. From the study suggested that all the traits showing significant correlation with grain yield needs better attention for increasing yield in future wheat breeding programs.

***Keywords*:** Bread wheat, Broad sense heritability, Genetic advance, Correlation coefficients

Introduction

Wheat (*Triticum aestivum* L.) is the most important grain and is being used as a staple food for more than one third of the world. Development of the varieties with high potential that having desirable combination of characters is always the main objective of wheat breeding programme. The studies of the genetic association and identification of genetic variability between various genotypes are essential for wheat improvements as well for breeders, because the crosses between dissimilar parents permit a huge segregation and the grouping of various favorable alleles (Bered et al. 2002). Wheat is the second important cereal crop after rice in the world and that is a source of nutrition for 35% of the world population and currently ranks first among cultivated plants in terms of cultivation area and production. Wheat is the first most important cereal crop of Afghanistan and plays a vital role in food and nutritional security.

Wheat cultivated under diverse agroclimatic conditions and showed wide variations in productivity from region to region, as crop becomes restricted due to sudden fluctuations in environmental changes. The main objective of a crop breeding programme is to develop varieties that perform well over a broad spectrum of environments. Further, a variety having wide or good adaptability is one which gives consistently superior performance over several environments (Frey 1964). The quantitative trait like yield mainly dependent on Genotype × Environment interaction as it obscure

the interpretation of genetic experiments and makes predictions difficult. In such circumstances it is difficult to select and suggest one better genotype across various locations. A wider adapted genotype performs consistently over a wider range of environment. To ensure valid genotype recommendation and to identify promising genotypes, a breeder should conduct multi location yield trials across different environments (Soleman et al. 2018).

Grain yield in wheat is a complex character and is depend on its component traits. For genetic manipulation of grain yield, quality and other characters in wheat, there is a need to examine the nature of genetic variability for the yield related attributes and quality traits. Estimation of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) helps to choose the potential genotype and heritability along with genetic advance would be more useful tool in predicting the resultant effect for selection of best genotypes for yield. Path coefficient analysis was used by plant breeders to help identify traits that could be useful as a selection criterion for improving crop yield. The path coefficient divides correlation coefficients into direct and indirect effects within the correlation system of traits. When there is a genetic correlation between two traits, the selection for one of them will produce a change in the other trait. In other words, the response of the correlation to the act of selection will take place.

Path parameters show a direct effectof the independent variable in the dependent variable, as well as an indirect effect of the independent variable in the dependent variable through another independent variable (Hadi et al. 2018). Keeping these things in the view, the present investigation was made to assess genotypes with the objectives, to estimate the variability, heritability and genetic advance for yield and yield components traits in wheat.

Materials and Methods

The experimental materials consisting of 54 genotypes including checks. The experiment was conducted at Research Station of Agricultural Research Institute of Afghanistan in spring season of the year 2020 and 2021. The experiment was carried out in a Randomized Block Design (RBD) under irrigated conditions with two replications. Each line was sown in 6 rows of 3 meter long and 20 cm apart. The recommended packages of practices were followed to raise the good crop. Sowing was done by hand drilling.

The observations, namely, days to heading, days to maturity, grain yield and biological yield were recorded on plot basis whereas, data for the traits such as plant height, spike length, number of spikelets per spike, number of grains per spike were recorded on randomly selected five plants per genotype per replication. The number of effective tillers was recorded by counting effective tillers of per meter square row length. The thousand grain weights were recorded by counting 1000 seeds per genotype per replication and weigh.

The mean performance of individual genotypes was used for statistical analysis. Statistical analysis of recorded phenotypic data was performed using a computer based statistical package program. To test the difference among the genotypes, the analysis of variance was worked out separately for each character as per method suggested by Fisher (1954). Genotypic coefficient of variation (GCV) and Phenotypic coefficient of variation (PCV) were calculated as per the standard formula suggested by Burton and Devane (1953). Heritability in broad sense was calculated as the ratio of genotypic variance (Vg) to phenotypic variance (Vp) and expressed in percentage (Falconer, 1981). Genetic advance as percent of mean of each character was worked out adopting the formula given by Johnson et al. (1955). Genetic gain (GG) is the genetic advance expressed as per cent of mean. It was estimated by using the formula of Johnson et al. (1955). Similarly, path analyses were carried out using the method suggested by Dewey and Lu (1959).

Results and Discussion

Analysis of variance

The analysis of variance was worked out to test the differences among genotypes by F-test. It was carried out according to the procedure of Randomized Complete Block Design.The analysis of variance (ANOVA) indicated for both years highly significant differences among the genotypes for days to heading flowering, days to maturity, effective tillers/m^2, plant height, spike length, number of spikelets per spike, number of grains per, grain yield, 1000 grain weight, biological yield, and harvest index (Table 1.) revealed the existence of sufficient genetic variability in all the traits the genotypes under study. The genotypes showed significant different in characters suggested for the next year as the best genotypes. Significant differences among the genotypes for different morphological and quality traits were also earlier reported by Singh et al. (2013), Singh et al. (2014), Kumar et al. (2016) and Gauravrajsinh (2021) in wheat crop.

Heritability (Broad sense) and genetic advance

The estimates of broad-sense heritability in percent have been presented in Table 2. The heritability estimates in broad sense were quite high for most of the characters indicated that strong genetic nature for all the traits. The higher heritability implied that selection for most of the traits might be effective in this set of genotypes. The characters namely, grain yield per plot was showed highest value (h^2=0.94) in 2021, and followed by 1000 grains yield (h^2=0.85) in 2020 number of spikelets per spike, plant height and weight per ear have high heritable.

The high heritability indicate that the characters were less influenced by environment, the similar results were also found by Bharat et al. (2012), Liu and Ma (1994), Deswal et al. (1996). High estimates of heritability coupled with high genetic advance were observed for plant height, thousand grain weight biological yield per plant and grain yield which indicated that above characters was governed by additive gene action and as such expected to exhibit improvement by direct selection. Similar findings were also reported by Bhushan et al. (2013) and Nukasani et al. (2013).

The estimates of genetic advance as percent of the mean are presented in Table 2. It revealed that the highest genetic advance as percent of mean was recorded for grain yield (27.7%) followed by biological yield (25.6%) during the year 2021.

Genotypic and phenotypic variability

The study revealed, phenotypic coefficient of variation (PCV) were higher than their corresponding genotypic coefficient of variation (GCV) for all the quantitative traits among the genotypes in both the years i.e. 2020 and 2021. This indicates that the characters were influenced by the environment. The selection on the basis of phenotype alone can be effective for the traits where variation between PCV and GCV were less means less influenced by the environment. (Table 3). Higher values of PCV and GCV indicated that there was high Variability exiting among the genotypes that consequently showed.

The higher values of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were recorded for grain yield (11.9%) and (PCV= 16.2%) followed by thousands grain weight (GCV=7.54% and PCV=8.16%), biological yield (GCV=7.20% and PCV=7.20%), effective tillers per plant (GCV=6.63% and PCV=11.5%), plant height (GCV=6.28% and PCV=7.47%), number of grain per spike (GCV=5.44% and PCV=8.63%), number of spikelets per spike (GCV=5.25% and PCV=8.07%), spike length (GCV=4.43% and PCV=6.54%) in 2020, similarly for 2021 also higher value of PCV and GCV were recorded in grain yield (PCV=13.9%) and (GCV=14.4%) followed by effective tiller per m^2 (GCV=9.43%) and (PCV=11.5%) indicating better opportunity for improvement in these traits through selection. A range of GCV (6.93% to 28.24%) and PCV (7.62% to 28.57%) were reported by Girnam et al. (2018). Though a range of PCV (0.81% to 9.07%) and GCV (0.50% to 8.08%) reported by Kumar et al. (2016). The other researchers namely Singh et al. (2013) and Singh et al. (2014) also reported high value of PCV and GCV for grain yield and tillers per plant in wheat. High phenotypic and genotypic coefficient of variation for grain yield, harvest index and tillers per plant were also reported by Kumar et al. (2016).

Correlation coefficient analysis

Correlation coefficient is the mutual association between variables without implying any cause and effect relationship. Simple correlation coefficients were computed at genotypic and phenotypic levels between pair of characters adopting following formulae given by Al- Jibouri et al. (1958). Genotypic correlation analysis was conducted between different morphological traits and it was observed that most of them showed a significant correlation coefficient at 0.01 or 0.05 level of significance (Table 3). The yield and related traits was expressed different trends in relationship among the traits.

Grain yield showed significant positive association with harvest index (0.97**), plant height (0.73**), number of grain per spike (0.3*) spike length (0.21*), number of spikelets per spike (0.48*), thousand grains weight (0.21) and similarly, noticed there was no significant positive relationship between effective tillers per m^2 (-0.07) and biological yield (0.04), other researchers also reported the results like Ahmad Khan, et al. (2017). Ahmad et al. (2016), reported significant and positive association between grain yield and flag leaf area, tillers per plant and 1000-grain weight. Significant positive association of grain yield with tillers per plant and plant height was previously noticed by Masood et al. (2014).

Similarly, Iftikhar et al. (2013) also noticed positive association of yield with flag leaf area, grains per spike and 1000-grain weight. Similarly, significant positive relationship of grain yield and flag leaf area was also observed by Hussain et al. (2013). Rehman et al. (2015) also reported positive association between1000-grain weight and plant height, spike length, grains $spike^{-1}$ and grain yield.

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Conclusions

The results of the study showed that significant variations were present among the genotypes for different traits.

The heritability values were observed high for grain yield per plot in 2021 and thousands grain weight in 2020 and moderately showed for others while the lower heritability observed for days to heading. The genetic advance were observed low to moderate, which is an indicator that non-additive gene action was controlling the expressions of these characters.

Furthermore, the genotypic correlation revealed positive correlation of grain yield with harvest index and plant height at highly significant and shows the significant correlation with number of grain per spike, number spikelets per spike and spike length. It suggesting that more importance should be given to these traits for improving the yield in wheat.

The analysis of variance for grain yield and its contributing components namely days to 50% flowering, days to maturity, productive tillers, plant height, spike length, spikelets pet spike, grains per spikelet, biological yield, harvest index, 1000 grain weight, grain yield and gluten content showed highly significant differences (at<1% level of significance) among the genotypes under present study. High heritability along with high genetic advance and high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) for grain yield (g), biological yield (g), harvest index (%), spike length (cm) and 1000 grain weight (g) indicated substantial contribution of additive gene action in the expression and thus selection would be effective for genetic improvement of these traits for improving grain yield in wheat.

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Table 1. Analysis of variance (ANOVA) for yield and yield contributing traits in bread wheat genotypes for the year 2020 and 2021

Traits	Replication (d.f=1)		Treatments (d.f=53)		Error (d.f=53)		F. ratio	
Years	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
DH	0.75	0.453704	4.204228*	3.0330**	2.61792	0.3971	1.6059	7.6379
DM	2.37	1.564815	1.452131**	3.1273**	0.57791	0.62141	2.5127	5.0326
ET	7566.8	984.037	2393.301**	2015.6*	1191.75	183.433	2.0082	10.988
PH	3.703	11.3425	62.50874**	41.321**	10.7791	6.62561	5.799	6.2367
SL	0.280	1.33333	0.563636**	0.3962**	0.20933	0.06918	2.6925	5.7273
NSS	0.593	6.11564	3.695318**	1.8792**	1.49825	0.84696	2.4664	2.2188
NGS	27.0	0.59259	19.18239**	6.1481*	8.26415	1.10202	2.3212	5.5789
GY	0.046	0.00128	0.208105**	0.15986*	0.06116	0.00506	3.4025	31.536
TSW	28.99	0.00037	22.3988**	17.5744*	1.77361	2.88923	12.628	6.0827
BY	0.0048	0.006379	0.745992*	0.92374*	0.42661	0.06587	1.7486	14.023
HI	39.64	4.304371	22.2473***	15.7751*	8.74909	3.29260	2.5428	4.7911

DH= Days to heading, DM= Days to maturity, ET= Effective number of tillers per meter square, PH= Plant height, SL= Spike length, NSS= Number of spikelets per spike, NGS= Number of grains per spike, GY= Grain yield per plot, BY= Biological yield per plot, HI= Harvest index

Table 2. Mean, range, heritability (BS), genetic advance and coefficient of variation for yield and yield attributing traits of bread wheat genotypes for the year 2020 and 2021.

Traits	Mean		Range				Heritability (Broad sense)		Genetic Advance as % of Mean		Coefficient of Variation (%)			
			Maximum		Minimum						GCV		PCV	
Year	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
DH	72	69	78	73	67	66	0.23	0.77	1.22	2.99	1.23	1.65	2.56	1.89
DM	114	112	116	115	113	110	0.43	0.67	0.78	1.69	0.58	1.00	0.88	1.23
ET	370	321	419	389	293	242	0.34	0.83	7.91	17.7	6.63	9.43	11.5	10.3
PH	81.0	78.9	92.5	90.0	62.5	70	0.71	0.72	10.86	9.25	6.28	5.28	7.47	6.20
SL	9.5	9.2	10.5	10.3	8.3	8.3	0.46	0.70	6.18	7.62	4.43	4.41	6.54	5.26
NSS	20.0	17.8	22	20.5	16	16	0.42	0.38	7.03	5.13	5.25	4.05	8.07	6.58
NGS	42.9	40.5	51.5	46.5	38	37	0.40	0.70	7.07	6.75	5.44	3.93	8.63	4.71
GY	2.27	2.00	2.86	2.43	1.53	1.37	0.55	0.94	18.2	27.7	11.9	13.9	16.2	14.4
TSW	42.6	41.3	49.2	48.0	34.9	36.0	0.85	0.72	14.3	11.4	7.54	6.56	8.16	7.74
BY	5.55	4.92	6.93	6.40	4.14	3.36	0.27	0.87	7.75	25.6	7.20	13.3	7.20	14.3
HI	40.9	40.8	47.1	47.6	31.8	32.3	0.44	0.65	8.64	4.16	6.36	6.13	9.63	7.57

DH= Days to heading, DM= Days to maturity, ET= Effective number of tillers per meter square, PH= Plant height, SL= Spike length, NSS= Number of spikelets per spike, NGS= Number of grains per spike, GY= Grain yield per plot, BY= Biological yield per plot, HI= Harvest index

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Table 3. Genotypic correlation coefficients of yield and yield related traits

DH	DM	ET	PH	SL	NSS	NGS	TSW	BY	HI	GY
DH	1	-0.30	0.02	-0.04	0.48**	0.029	0.44*	-0.00	0.15*	-0.05
DM	-0.30*	1	-0.14	-0.07	-0.12	-0.47	-0.31	-0.06	-0.41*	0.23*
ET	0.02	-0.14	1	0.66**	0.37*	0.34*	0.26	0.18*	0.93**	0.26*
PH	-0.04	-0.07	0.66**	1	0.17	0.41*	-0.22	0.41*	0.59**	-0.32*
SL	0.48**	-0.12	0.37*	0.17	1	0.60*	0.20	0.24*	0.78**	-0.07
NSS	0.02	-0.47	0.34*	0.41*	0.60**	1	-0.16	0.12	0.51*	-0.18
NGS	0.44	-0.31	0.26*	-0.22	0.20*	-0.16	1	-0.26	0.43*	-0.11
TSW	-0.00	-0.06	0.18*	0.41*	0.24*	0.12	-0.26	1	0.19*	-0.10
BY	0.15	-0.41	0.93**	0.59*	0.78**	0.51	0.43*	0.19	1	0.28*
HI	-0.05	0.23*	0.26*	-0.32	-0.07	-0.18	-0.11	-0.10	0.28	1
GY	0.17*	-0.07	0.73**	0.21*	0.48*	0.30*	0.21*	0.04	0.97**	0.73**

The significant was tested at 0.05% and 0.01%

DH= Days to heading, DM= Days to maturity, ET= Effective number of tillers per meter square, PH= Plant height, SL= Spike length, NSS= Number of spikelets per spike, NGS= Number of grains per spike, GY= Grain yield per plot, BY= Biological yield per plot, HI= Harvest index

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Identification of Novel Five Variants of *Caladium* Species Through Multi-Environmental Evaluation

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ABSTRACT

Caladiums, commonly termed 'angels wings' are high valued ornamental plants known for their attractive multi-coloured foliage. The plants are toxic to human but the leaves are known to possess antimicrobial, antiangiogenic, antioxidant and antitoxic properties. *Caladium* species show wide range of variations in the length, width and number of leaves per plant and their market value depends on the type and colour of leaf. So, development of unique Caladium varieties with attractive foliage thus makes them a highly priced plant in the market. The present study therefore focuses on identification and development of unique Caladium varieties for commercialization using twenty-five germplasm collected from different parts of Northeast region. The study was conducted during spring 2019 and 2020, following which five novel variants were identified, *viz-Caladium red flash* (Jor-Lab CL-36), *C. bicolour* var. *florida clown* (Jor-Lab CL-12), *C. hunboldtii* var. *mini white* (Jor-Lab CL-54), *C. bicolor* var. *florida sweetheart* (Jor-Lab CL-115), *C. bicolor* var. *red star* (Jor-Lab CL-24). Jor-Lab CL-36 showed olive green foliage, Jor-Lab CL-12 and Jor-Lab CL-24 each showed green foliage. Jor-Lab CL-54 exhibited green and white contrast foliage, while Jor-Lab CL-115 showed pink foliage with green margin. Multilocation trial study conducted during spring 2021 for the identified lines at four locations of North East India further confirmed their stability performance and found stable for different agronomical traits and colour combinations.

***Keywords*:** Angel wings, foliage variability, high priced, novel variants, multilocation study

Introduction

Caladiums (*Caladium* Vent.),also called as angels wings and elephant ears are a highly valued ornamental plants that are grown as an indoor plant for their beautiful multi-coloured and variably-shaped foliage (Deng, 2012; Cao et al. 2017). It belongs to the family Araceae and indigenous to Central and South America (Mayo et al. 1997). Caladiums are heat-loving tropical perennials with large, heart- or arrow-shaped, paper-thin leaves and have striking array of colours and patterns that display amazing colour combinations of white, pink, red and green (Mayo et al. 1997). They rarely flower, but the beautiful foliage guarantees a colourful show wherever they are planted and thrive for few months during spring and summer season till their leaves starts to die and the plant goes to dormant condition. When Caladiums bloom, they produce a single (rarely 2-3) typical arum-type flower with a green or pinkish spathe surrounding a short white spadix and its fruits are white berries with several to many seeds (Wilfret and Hurner, 1982).

Caladiums are an excellent garden and shady yards plant. While certain Caladium varieties are more sun tolerant than others, most prefer moderate shade from the hot evening sun (Wilfret and Hurner, 1982; Wilfret, 1993; Laliberte, 2015). The growth of the plant is affected by the amount of water received during developmental period. They are known to grow best in fertile and well-drained soil with consistent moisture (Vanzile, 2022). Among different species of Caladium, *C. bicolor*, a Brazilian species, is the most common in this genus that are used as ornamental plants.

Although they are toxic, the leaves were found to possess antimicrobial (Essien et al. 2015; Uche et al. 2019), antiangiogenic, antioxidant and antitoxic properties (Tosoc et al. 2016).It has been seen among various species of Angiosperms that variegation of leaves is the main characteristics among understorey herbs of temperate and tropical forests (Givnish, 1990). It has been contemplated that the variegated leaves that have partial loss of photosynthetically active surface affects the utilisation and absorption of light therefore effecting their growth and reproduction as well as the net photosynthesis (Deng, 2012). Caladium cultivars show a wide range of variations in the length, width of mature leaves and the number of leaves per plant (Wilfret and Hurner, 1982). Notably, leaves of Caladium can be classified into three types: lance, strap, and fancy. Firstly, the lance leaves are intermediate between the strap and fancy types, basal lobes if present are broadly separated by sinus and have a broad sagittate to cordate-lanceolate and usually grow less than 12 inches tall. Secondly, strap leaves have ribbon-like and narrow leaves with one main vein and no obvious basal lobes. Thirdly, fancy type have triangular heart-shaped leaves with three main veins, two obvious basal lobes and separated by a short narrow sinus as well as a peltate petiole attachment and it grows between 12 and 30 inches tall (Russ and Polomski, 1999; Deng, 2012). The marketing price and value of the plant in the market depends mainly on the type and colour of leaf the plant attains (Deng, 2012; 2018). The method for introducing new cultivars of Caladium is through hybridization between elite lines and cultivars to produce variants with different plant growth, leaves pattern, leave size, number of leaves and venation pattern (Wilfret, 1993). These entire characters make Caladium a highly priced plant in the ornamental market. It is also necessary to develop suitable stable cultivars which are favourable and is well adapted in all environments. So far to the best of our knowledge this is the first report on systemic evaluation of collected germplasm and identified some unique traits lines which were again tested in multi-locational trial.

Materials and Methods

Plant sample

During the year 2019, a total of 25 Caladium germplasm was collected from different North East region and planted at experimental farm (CSIR-NEIST, Jorhat, Assam) following RBD (Randomized Block Design) with three replications. Necessary agro-practices were followed, *viz*- a 4×4 meters plot size with 60×60 cm spacing, application of NPK in dose of 40:30:30 and consistent moisture to raise a good plant. Nitrogen was given in two split doses, while phosphorus and potassium applied at the time of planting. Caladiums prefer acidic soil and so the experimental field was ideal in this regard with a pH of 4.9. Identification of germplasm was performed by breeder of the institute and voucher specimen submitted in the departmental herbarium.

Observation recorded

Agronomical and other morphological observations were recorded for two consecutive seasons, i.e. *spring* 2019 and 2020. Characters taken for study includes plant height (cm), leaf length (cm), leaf width (cm), tillers/plant, leaf colour, midrib colour and leaf spot.

Assessment

After two years of initial plant trial five unique germplasm were selected and taken for multilocation trial at four locations of North East region- Lakhimpur (Assam), Pasighat (Arunachal Pradesh), Nongpoh (Meghalaya) and Madang (Assam) during spring 2021. These germplasm (*Caladium red flash, C. bicolour* var. *florida clown, C. hunboldtii* var. *mini white, C. bicolor* var. *florida sweetheart, C. bicolor* var. *red star*) were selected from the initial trial on the basis of leaf size, colour variations, plant height and comparison were done taking the locally grown cultivars as check varieties.

Results and Discussion

Twenty-five Caladium germplasm were subjected to morphological studies during *spring* 2019 and 2020, which showed variation in leaf colour and size, leaf spot, colour of mid vein and tillers per plant (Table 1). In *Caladium red flash*, plant height ranged from 39-54 cm, leaf length from 17.2-30.7 cm, leaf width from 15.5-25.2 cm and tillers/plant from 2-6 (Table 1). The colour of leaf showed olive green with pink spots and red colour midrib. *C. bicolour* var. *florida clown* showed 30-42 cm plant height, 14-20 cm leaf length, 10-18 cm leaf width and 5-20 tillers/plant and showing green coloured leaves with distinct pink, red and white spot. *C. hunboldtii* var. *mini white*, *C. bicolour* var. *florida sweetheart* and *C. bicolour* var. *red star* each showed 10-25, 23-43, 18-27 cm plant height, 4-10, 13-30, 10-19 cm leaf length, 3-8, 7-13, 8-16 cm leaf width and 4-18, 2-5, 2-4 tillers/plant respectively. *Mini white* variety showed green and whitish coloured leaf with white midrib and leaf spots. *Florida sweetheart* variety have bright pink leaves and green margin with dark pink midrib colour and no noticeable leaf spot, whereas *red star* variety have green leaves, red colour midrib with distinct white and indistinct pink spots. The development of elite lines with unique leaf traits

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is the most preferred criterion in Caladium breeding programme (Wilfret, 1993; Deng, 2012). But due to increasing population size in this genus the efforts to find unique leaf characteristics has possibly been increased, thus making difficult for successful breeding. This may be due to lack of variability in Caladium gene pool as also reported by Deng (2018).The process for obtaining new cultivars with unique leaf traits in Caladium is through hybridization between elite lines, which makes them highly valued in the market (Cao et al. 2016a). This can be achieved when there is high variability in the experimental material. The germplasm used for the study showed great variations in leaf characteristics and other studied traits and this variability can be utilized for Caladium improvement and varietal development programme. It is also necessary to develop suitable stable cultivars which are favourable and can be adopted to all growing environments. Therefore, identifying lines with unique leaf traits from its wild collection will be a boost in Caladium breeding programme and the present study is attempt to the same. Deng (2018) in Caladium breeding study also observed highest variation in leaf colour, followed by leaf patterns and leaf types. Hartman et al. (1972) reported that Caladium showed high variability when propagated by seeds, but for commercial cultivation tuberous corm is mainly preferred. It is because although they show high variability, they tend to lose their germination capacity easily and rapidly (Carpenter, 1990). Zhu et al. (1984) and Ali et al. (2007) obtained excellent shoot multiplication during rapid micropropagation of *Caladium* species on *in vitro* study, thereby describing the method to be successful for large scale quality planting material propagation. Cao et al. (2016b) observed much variability (25%) in terms of leaf shape and size, midrib colour and leaf spots among the somaclones of red flash variant. Chu and Yazawa (2001) also observed high variation frequency (65- 79%) in somaclones produce through tissue culture. Hussain et al. (2016) reported that application of potting mixers is the best method for improving crop productivity and providing best environment for Caladium growth.

Based on two years assessment, lines with best leaf traits for each of the five varieties were identified and named as- Jor-Lab CL-36 (RRL-C-36) for *Caladium red flash,* Jor-Lab CL-12 (RRL-C-12) for *C. bicolour* var. *florida clown,* Jor-Lab CL-54 (RRL-C-54) for *C. hunboldtii* var. *mini white,* Jor-Lab C-115 (RRL-C-115) for *C. bicolor* var. *florida sweetheart* and Jor-Lab CL-24 (RRL-C-24) for *C. bicolor* var. *red star* (Fig. 1). Jor-Lab CL-36 was found to obtain a height of 53.80 cm, 25.30 cm leaf length, 21.20 cm leaf breadth and 4 tillers/plant (Table 2). Similarly, Jor-Lab CL-12 obtained a height of 41.30 cm, 19.20 cm leaf length, 15.80 cm leaf breadth and 15 tillers/plant; Jor-Lab C-54 obtained a height of 23.00 cm, 8.80 cm leaf length, 6.70 cm leaf breadth and 13 tillers/plant. Jor-Lab C-115 and Jor-Lab CL-24 each showed 42.11, 25.40 cm plant height, 25.70, 16.01 cm leaf length, 9.90, 15.70 cm leaf width and 4, 3 tillers/plant respectively. These lines were further evaluated through multilocation trial performed at Lakhimpur, Pasighat, Nongpoh and Madang of Northeast India. Multilocation trial is an important aspect of plant breeding programme for developing superior lines with stable performances (Lal et al. 2021). The present study was performed taking these criteria into mind with one check variety for each identified line. The average multilocation trial data of identified lines along with check varieties were presented in Table 3. These lines showed superior performances than their respective check counterpart for the studied traits. Moreover, it was also observed that these lines did not show any variation in colour when multilocation trial study was conducted, a common phenomenon when there is a change in environmental condition (Sevik et al. 2014). Further the lines showed consistent performance at multilocation studies as reflected by three years average data. Selection of stable lines with consistent performance is very important in formulating targeted breeding programme as this would ultimately help in varietal development of the species (Lal et al. 2020).

Conclusions

The present study makes an attempt to identify unique five novel variants of Caladium- Jor-Lab CL-36 for *Caladium red flash,* Jor-Lab CL-12 for *C. bicolour* var. *florida clown,* Jor-Lab CL-54 for *C. hunboldtii* var. *mini white,* Jor-Lab C-115 for *C. bicolor* var. *florida sweetheart,* Jor-Lab CL-24 for *C. bicolor* var. *red star*. Since these plants are highly valued for their attractive colour and beauty, it is therefore necessary to develop suitable stable cultivars which are favourable and can be adopted to all growing environments.

This varietal development study is by far the first report on Caladium as far our knowledge. These newly identified lines can be adopted for commercial cultivation as evaluated from multilocation studies and found stable performance.

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Table 1. Range of traits taken for study among Caladium germplasm used in initial varietal trial during spring 2019 and 2020.

Traits	Range				
	Caladium red flash	*C. bicolour* var. *florida clown*	*C. hunboldtii* var. *mini white*	*C.bicolor* var. *florida sweetheart*	*C. bicolor* var. *red star*
Plant height (cm)	39-54	30-42	10-25	23-43	18-27
Leaf length (cm)	17.2-30.7	14-20	4-10	13-30	10-19
Leaf width (cm)	15.5-25.2	10-18	3-8	7-13	8-16
Tillers/plant	2-6	5-20	4-18	2-5	2-4
Leaf colour	olive green	green	green/white contrast	pink with green margin	green
Midrib colour	red	green	white	dark pink	red
Leaf spot	pink	pink/red/white	white	absent	pink/white

Table 2. Morphological traits of identified five unique lines of *Caladium*.

Traits	Jor-Lab CL-36	Jor-Lab CL-12	Jor-Lab CL-54	Jor-Lab C-115	Jor-Lab CL-24
Plant height (cm)	53.80	41.30	23.00	42.11	25.40
Leaf length (cm)	25.30	19.20	8.80	25.70	16.01
Leaf width (cm)	21.20	15.80	6.70	9.90	15.70
Tillers/plant	4	15	13	4	3
Leaf colour	olive green	green	green/white contrast	pink with green margin	green
Midrib colour	red	green	white	dark pink	red
Leaf spot	pink	pink/red/white	white	absent	pink/white

Table 3. Average multilocation trial data of identified lines along with check varieties at Lakhimpur, Pasighat, Nongpoh and Madang during spring 2021.

Variety	Plant Height	Leaf Length	Leaf Width	Tillers/ Plant	Leaf Colour	Midrib Colour	Leaf Spot
Jor-Lab CL-36	51.80	24.16	19.61	4	olive green	red	pink
Check-1	47.30	20.20	15.50	3	olive green	red	pink
Jor-Lab CL-12	39.60	17.28	13.50	13	green	green	pink/red/white
Check-2	35.70	13.30	9.70	8	light green	green	pink/red/white
Jor-Lab CL-54	20.70	7.29	6.00	11	green/white contrast	white	white
Check-3	15.70	6.40	5.30	9	green/white contrast	white	white
Jor-Lab C-115	39.30	22.70	9.30	3	pink with green margin	dark pink	absent
Check-4	34.90	18.90	8.70	3	pink with green margin	dark pink	absent
Jor-Lab CL-24	23.40	14.90	13.71	3	green	red	white/pink
Check-5	23.30	12.30	10.20	2	green	red	white/pink

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Figure 1. Identified Caladium varieties (a) Jor-Lab CL-36, (b) Jor-Lab CL-12, (c) Jor-Lab CL-54, (d) Jor-Lab CL-115, (e) Jor-Lab CL-24. (Original)

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Released Varieties

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Registration of "Orak 22" Barley (*Hordeum vulgare* L.) Variety

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Orak 22 is two rowed barley (*Hordeum vulgare* L.) variety developed by Trakya Agricultural Research Institute and registered in 2022. Orak 22 is developed by crossing Sllo/Robust//Quına/3/Chıa.73T/4/Cabuya/5/NS 529 with TEA2370-0T-0T-31T-9T-0T in pedigree method. Crossing was made in 2007 and yield test began in 2014-2015 growing year.

Orak 22 is two rowed cultivar and spike is moderately long, and compact. It resembles cultivar Harman. Orak 22 is a medium-tall cultivar, similar to Sladoran, Yaba and Ocak. Plant height is between 75 and 110 cm depending on the growing conditions. It is medium early and as it has good adaptation ability, it has been grown throughout Trakya-Marmara and transitional zone region of Turkey. It gives high yield both on fertile and less fertile soils. It has resistance to winterkilling and is tolerant to medium drought conditions. Orak 22 is highly tolerant to net blotch (*Pyrenophora teres*), scald (*Rhynchosporium commune*), and powdery mildew (*Blumeria graminis* f. sp. *hordei*).

Its yield potential is high however, high yield can be obtained if environmental conditions are favorable and applied good agronomic practices. The highest grain yield obtained was 10.560 kg ha^{-1} in Edirne location in 2019-2020 growing years. Mean yield of the variety testing experiment was 7.575 kg ha^{-1} in Trakya growing conditions. Suggested planting rate is between 450-500 seeds/m^2.

Its grain feeding quality is good. The mean values of some qualities of the variety testing experiment (2020 and 2021) are; test weight 63.7-71.6 kg/hl, thousand kernel weight 31.4-46.5 g, protein content 10.2-14.8%, and sieve value 37.6-95.0%. The highest quality values during 2018-2019 growing seasons before of the variety testing experiment were; 1000-kernel weight 48.3 g, test weight 72.0 kg, protein content 12.4%, and sieve value 87.2%.

Pre-Basic and Basic seeds of the Orak 22 cultivar have been produced by Trakya Agricultural Research Institute. Certified seed of the Orak 22 are produced by both private companies and state farms.

Figure 1. Spike (a and b) and grain (c) of the Orak 22 cultivar. (Original)

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Registration of "Harman 22" Bread Wheat (*Triticum aestivum* L.) Variety

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Harman 22 is winter bread wheat (*Triticum aestivum* L.) variety developed by Trakya Agricultural Research Institute and registered in 2022. Harman 22 is developed by crossing Tekirdağ//GK Kalasz/ Beabourg with TE6748-0T-0T-9T-2T-2T-0T through pedigree method. Crossing was made in 2008 and yield test were began in 2016-2017 growing year.

The spike of the Harman 22 cultivar is moderately long, white, smooth, with awn and medium compact. The flag leaf is medium dark-green and with medium-low glaucousity. Grain is oval, hard and red colour. Harman 22 is a medium-tall cultivar, similar to Eylül, Abide and Saban. Plant height is between 70 and 85 cm depending on the growing conditions. It is medium early and as it has good adaptation ability; it has been grown throughout the Trakya-Marmara region and some other transitional-zone parts of Turkey. It gives high yield both on fertile and less fertile soils. It has resistance to winterkilling and is tolerant to medium drought conditions. Harman 22 is highly tolerant to stripe rust (*Puccinia striiformis* f. sp. *tritici*) and leaf rust (*Puccinia triticina*). It has tolerant to powdery mildew (*Blumeria graminis* f. sp. *tritici*), and it has susceptible to septoria leaf disease.

Figure 1. Spike and grain of the Harman 22 cultivar. (Original)

Its yield potential is high however, high yield can be obtained if environmental conditions are favorable and applied good agronomic practices. The highest grain yield obtained was 8985 kg ha^{-1} in variety testing experiment (Edirne location in 2020-2021 cycle). Mean yield of the variety testing experiment was 7750 kg ha^{-1} in Trakya growing conditions. Suggested planting rate is between 450-500 seeds/m^2.

Its grain quality is extremely good. The mean values of some bread-making qualities of the variety testing experiment (2020 and 2021) are; test weight 73.2-75.1 kg hl, thousand kernel weight 35.7-37.3 g, protein content 14.5-18.4%, absorption 55.5-57.9%, sedimentation (Zel) 61-73 ml, gluten index 93.2-99.6%, gluten value 25.7-34.8%, alveograph energy value (W) 267-385 and flour yield 60-71%. The highest quality values in 2018-2019 growing seasons application of the variety testing experiment were; thousand kernel weight 44.7 g, test weight 82.4 kg, protein content 13.8%, gluten value 35.7%, gluten index 91.2% and sedimentation (Zel) 56 ml.

Pre-Basic and Basic seeds of the Harman 22 cultivar have been produced by Trakya Agricultural Research Institute. Certified seed of the Harman 22 are produced by both private companies and state farms.

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

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Kirve is a bread wheat (*Triticum aestivum* L.) variety developed by Black Sea Agricultural Research Institute and registered in 2020 (Figure 1). Kirve was developed by crossing MILAN/6/KAUZ*2/4/CAR///KAL/BB/3/NAC/5/KAUZ.

Kirve is a spring cultivar with awn, white spike, white grain colour and medium early maturing. It can grow up 96-114 cm in favorable conditions. It has high flour yield, straw yield, protein content and SDS sedimantation value. Threshing character is good. Resistant to shattering. It shows a good response to nitrogen fertilization and irrigation. The grain yield is changing between 620-760 kg/da in different conditions. The variety is resistant to rusts (stripe, leaf and stem rust), powdery mildew and lodging. It can be recommended to spring sowing conditions.

Its quality properties is high. The average values of some bread-making qualities of Kirve are; test weight 79-82 kg, thousand kernel weight 44-51 g, protein content 15-16%, water absorption 57-62%, SDS sedimentation 46 ml, gluten value 32-38%, alveograph energy value (W) 196-337 and flour yield 68%.

Figure 1. Plant, spike and grain of Kirve.

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Registration of "Yörem 55" Forage Legume (*Trifolium meneghinianum* Clementi) Variety

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Yörem 55 (*Trifolium meneghinianum* Clementi) is an annual (aerial-seeding) temperate legume developed and registered by the Black Sea Agricultural Research Institute in 2020. It can be grown in the Black Sea region, coastal areas and some other transitional regions of Turkey. It can be made use for hay, silage, grazing, and as a cover or green manure crop (for soil improvement), and pollinators.

Gelemen clover (Yörem 55) is developed through within-half sibling family selection as an early flowering and diploid variety. It was tested between 2016-2019 at growing season.

Gelemen clover, which has a strong flowering tendency, develops semi-prostrate and is moderately height (101.3 cm) depending on the growing conditions. Its stem is moderately thick and has anthocyanin. It is weak in terms of winter hardiness. The shape of central terminal leaflet is rounded, its length and width are medium. The leaf color is light green, and there is no anthocyanin. Its stem is glabrous and hollow, and leaflet margins are serrated.

The flower color of the plant is white, the ramification is dense and the number of flowers is many. The seed is multi-colored (yellowish and greenish), and shaped like oval (kidney). It produces substantial amounts of seeds (70 kg da^{-1}). The inflorescence consists of a long cylindrical or conical flower head including many small white flowers. Average 1000-seed weight is 1.5-2 gr. Gelemen clover is open pollinated, therefore it is also a good source of nectar and pollen for bumblebees and honeybees. Because it is an aerial seeding species, the seed of gelemen clover can easily harvested using conventional machines.

Sowing can be done by hand or by spreading in October and November while the soil is tempered in the coastal part, but sowing with a seeder is recommended.

Since the seeds are very small, the sowing depth should be 1-2 cm and the row spacing should be 20-40 cm. Suggested sowing rate is between 750 gr-1000 gr/da. Fertilizer should be applied according to soil analysis.

Yörem 55 can provide excellent herbage production (7731 kg/da fresh yield-1528 kg/da dry matter) if ecological requirements are suitable and convenient management.

Yörem 55 also performs very well in terms of forage quality required for the productivity of ruminant animals.

Its forage quality is good. The crude protein and cellulose content are 15.02% and 34.40%, respectively. Also, NDF, ADF, ADL, and RFV values were calculated as 43.17%, 33.24%, 7.26%, and 119, respectively.

Although it depends on the environmental conditions in which it grows, the disease does not occur.

Figure 1. (a) Plant of *T. meneghinianum* (b) Flower (c) Seeds. (Original)

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Registration of ''Yılmaz" Fiber Linen (*Linum usitatissimum* L.) Variety

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Yılmaz is fiber linen (*Linum usitatissimum* L.) variety improvemented by Black Sea Agricultural Research Institute and registered in 2022. Yılmaz was improvemented by crossing (Avangard x Linda.)

Yılmaz fiber flax variety is a variety with high fiber yield. Average fiber yield is 150 kg per decare. Average seed yield is 150 kg per decare. The flower color is purple and the plant height is 80 cm. The adaptability of Yılmaz fiber flax variety is high and the average oil yield is 38-40%. Its seed color is brown. According to the results of the combined analysis of the region yield experiment carried out in Gelemen and Black Sea region, the tallest plant height was obtained from Yılmaz variety with 92 cm. Yılmaz variety passed in terms of plant height against 3 standard varieties (Antares, Nareum, Barbara) in the experiment. In terms of technical stem length (56.6 cm) and fiber yield (125.6 kg/da), Yılmaz variety surpassed all standards and lines and reached the highest value. When examined in terms of 1000 grain weight, the highest value was obtained from Yılmaz variety with 6 g according to the results of the combined region yield test.

Figure 1. General view of the plant. (Original)

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Anonymous, (2022). Yazlik keten tescil raporu, Ankara. (in Turkish, unpublished)