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Evaluation of the Genetic Diversity and Agro-morphophysiological Traits of Bread Wheat Varieties under Postflowering Drought Stress Using Different Statistical Methods

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ABSTRACT

Postanthesis drought stress in wheat cultivation typically occurs in semiarid regions due to limited irrigation water availability and yield loss during cereal production. The present study was designed and implemented to investigate the agronomical traits of bread wheat varieties in response to post-flowering drought stress in a randomized complete block design in Hamedan Province (Asadabad), Iran. The results of the trait variance analysis under both stress and control conditions indicated that there were significant differences ($p \leq 0.05$) among the cultivars in terms of most studied traits. The cluster analysis classified the studied varieties into three groups, and the correctness of the groupings was confirmed by the analysis of the discriminant function. According to the results of the principal component analysis, four components explained 79.6 and 79% of the variance in the total data under the control and stress conditions, respectively. Factor analysis based on principal component analysis of the control plants revealed that three factors accounted for 64.68% of the total changes, including the first factor (yield), the second factor (characteristics related to height) and the third factor (harvest index), whereas the same analysis of the stressed plants at the end of the season indicated that three factors were behind 70.361% of the changes (yield and greenness, traits related to height, and plant moisture content). Based on all statistical analyses, (both univariate and multivariate of 14 studied genotypes), three winter-type varieties (Pishgam, Zare, and Mehan) were found to have significantly better yield under the drought stress conditions.

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Introduction

Bread wheat is one of the most important crops and the main food resource in many countries. Wheat (*Triticum aestivum* L.) of the *Poaceae* family is a popular cereal crop with ancient origins. It is one of the most important commercial commodities cultivated and traded globally. Crop yield is a complex trait that is strongly influenced by environmental stresses. Identifying better-performing varieties under stress conditions is undeniably important. Under changing climate conditions, environmental stresses are becoming major threats to the production of staple crops. Recently, wheat production has been affected by progressive global climate change and the increasing water deficiency, along with environmental crises, which have endangered the food security of the growing world population (Sabagh *et al*., 2021). The growth and yield of wheat plants can be affected by drought stress in different ways, where the extent of the effect depends on the duration and intensity of the stress factors

(Guttieri *et al*., 2001). Drought stress inhibits plant growth from the time of pollination to ripening, of which the reproductive and grain-filling phases are the most sensitive stages with significant loss potential upon stress (Morsy *et al*., 2022; Elbasyoni *et al*., 2022). The extent of drought stress impact can be intraspecific, as genotype-dependent variation in the agronomical traits of cereal plants is a well-known and evident phenomenon. The genetic variations amongst the cultivars of the same species often result in genotypes with different morphological and physiological characteristics that can be used to select drought-tolerant varieties (Cai *et al*., 2020).

With the help of univariate analysis, as commonly applied in similar studies, the agronomical traits are analyzed separately. Therefore, when the measured traits are related to each other, analysis of variance methods does not fully describe the degree of difference among the subjected cultivars (Yeater *et al*., 2004). Agronomic, phenological, physiological and morphological traits are typically used for organizing germplasms, selecting suitable parents for hybridization, and creating diverging populations (Zafar *et al*., 2021).

There have been several studies on the mechanisms of drought tolerance in wheat plants, and the associated morphological and photosynthetic attributes conferring drought tolerance have been linked to genetic diversity (Ahmed *et al*., 2022; Ahmed *et al*., 2019; Ahmed *et al*., 2021; Shahid *et al*., 2022; Istanbuli *et al*., 2020). The selection of resilient varieties based on recorded agronomic traits is a justified approach that has been successfully utilized in many bread wheat breeding programs (Memon *et al*., 2022).

Multivariate statistical methods seem to be a better choice for separating populations than univariate analysis. Decomposition of the data into principal components is one of the simplest multivariate statistical methods in which the evaluation of the correlation between variables allows one to understand the overall relationships between the traits. In fact, such data reduction is performed by converting variables into principal components. Principal component analysis (PCA) was introduced in 1901 by Pearson (Pearson, 1901). In combination with cluster analysis, this approach is used to select the most distant gene bank specimens for crossing. Concerning the analysis of functional traits, in the context of multivariate statistical approaches, the opposite strategy is used where the groups are specified in advance. Determining the correlation between different traits, especially grain yield and its components, and determining the cause (e.g., stress treatment) of differences enables breeders to choose the most important combination of traits to be considered for crossings. In this approach, the diversity among breeding materials is of primary importance for proper selection.

Current research on 14 new and old varieties of bread wheat aimed to investigate and evaluate the diversity of morphological and agronomic traits to better understand trait behavior and relationships under post-anthesis drought stress conditions via univariate and multivariate statistical analyses at the end of the season. The findings of the present study can be used as a basis for recommending cultivars with the best yield for economic cultivation or in future breeding programs, along with similar studies on other varieties.

Materials and Methods

Plant materials

Old and new bread wheat varieties were obtained from the Research and Education Center for Agriculture and Natural Resources of Hamedan Province in Iran. In total, 14 different varieties (Table 1) were investigated for their growth and tolerance to drought stress by evaluating their morphological and agronomic traits (morphological, physiological, and phonological). The origin and registration information of the selected varieties is presented in the supplementary materials (Supplement 1).

Experimental design

The study was conducted at the experimental farm of Payame Noor University in Asadabad, Iran (a map and the location of the experimental farm are presented in the Supplement 2), which has moderate to semiarid weather conditions at an altitude of 1607 meters above sea level (cite coordinates: 34°47′23″N 48°07′10″ E). The monthly precipitation rates at the experimental locations are also presented in the Supplement 3.

Table 1. The investigated wheat variety names and their growth habits.

Genotype No	Variety Name	Release date
1	Bezostaya*	1968
2	Pishgam*	2008
3	Sissons*	1994
4	Gascogne*	1994
5	Shahpasand*	1942
6	Mehan*	2010
7	Omid*	1956
8	Navid*	1968
9	Roshan Backcross*	1998
10	Zare*	2010
11	Sorkh tokhm*	1957
12	Shahreyar*	2002
13	Toos**	1994
14	Alvand**	1995

*Growth habit: Winter-type, **Growth habit: Winter-Spring type.

The experiment was carried out in triplicate in a randomized complete block design. Seeds were placed on moist filter paper in Petri dishes and kept at 4°C for 1-5 weeks (depending on the variety's recommended vernalization) prior to sowing. The farm's soil texture is sandy-clay, with a pH of 7.1. The land preparation operations, including tillage and disking, were performed correctly.

The fertilizer treatments included 150 kg ha^{-1} urea, 100 kg ha^{-1} ammonium phosphate, and 100 kg ha^{-1} potassium sulfate prior to cultivation, and 100 kg ha^{-1} urea was applied before stem elongation. Weed control was performed using 20 g ha^{-1} of Granstar 50 SX® (FMC International Switzerland Sarl, Switzerland) and 0.5 L ha^{-1} plus Puma Super 069 EW (Bayer Crop Science, USA) herbicides for dicotyledonous and monocotyledonous weeds, respectively. Planting was performed manually in early November, and the regular irrigation practices of the region (14-day intervals) were applied (Controls).

To apply drought stress, irrigation was stopped at the flowering stage until the end of the growth period (stress treatment). The test plots consisted of six planting lines, each one and a half meters long and 30 cm from each other, with a planting density of 200 seeds/square meter.

The investigated traits and the applied standard methods with their abbreviations are listed in Table 2. More details about the applied methods are presented in the Supplement 4.

Statistical analysis

Univariate statistical methods, including variance and mean comparison, and multivariate statistical analyses, including factor analysis based on principal component analysis (PCA) and varimax rotation, were performed using IBM SPSS software version 24 (IBM Corp., New York, NY, USA). The normality of the data distribution was confirmed by the Shapiro–Wilk test. The main component cluster analysis was performed with the WARD method and based on the Euclidean distance similarity coefficient and analysis of the detection function with the help of Minitab 19 statistical computing software. The differences between the mean values were identified by the least significant difference (LSD) (Fischer's least significance test) at $\alpha = 0.05$.

Table 2. Abbreviations of the studied traits.

No	Studied traits	No	Studied traits
1	Seed Yield under Stress (YS)	13	Heading Percentage (HP)
2	Number of Seed Per Spike (NSPS)	14	Plant Biomass (Bio.)
3	Spike Height (SH)	15	Seeds Weight of one Spike (SWS)
4	Spike Length (PL)	16	Plant Height at the Shoots Stage (PHSS)
5	Flag Leaf Length (FLL)	17	The Height of the Plant Before Heading (HPBH)
6	Width of the Leaf (WL)	18	SPAD in Full Heading Stage (SPAD-FHS)
7	100 Kernel Weight (TKS)	19	Height After Heading (HAH)
8	Plant Height (PH)	20	SPAD at the Beginning of Heading (SPAD-SBH)
9	Relative Water Content (RWC)	21	The Length of the Second (Penultimate) Internode (LSI)
10	Soil Plant Analysis Development (SPAD)	22	Awn Length (AL)
11	Flag Leaf Area (AFL)	23	Harvest Index (HI)
12	Single Seed Weight (SSW)	24	Yield Potential (YP)

Univariate statistical methods

According to the results presented in Table 3, analysis of the variance of studied traits such as yield potential; YP, hundred kernel weight; KW, biomass; Bio., seed weight of one spike; SWS, SPAD at the beginning of heading, number of seeds per spike; and NSPS in the studied cultivars revealed significant differences at the 1% probability level ($P \leq 0.01$), whereas the heading percentage, HP; length of the second internode (Penultimate Internode) LSI; beard length, BL; relative water content, RWC; and harvest index, HI were significantly different at the 5% probability level ($P \leq 0.5$) under the control and stress conditions. Thus, these significant conditions provided the foundation for further analysis of the data obtained in our experiment.

The characteristics of seed yield under stress conditions at the end of the season showed significant differences for the weight of one hundred seeds, biomass, SPAD at the beginning of heading, seed weight of one spike, harvest index and length of the second internode at the probability level of 1% ($P \leq 0.01$) and for the traits of plant height, SPAD at the beginning of heading and the number of seeds per spike at the 5% probability level ($P \leq 0.01$). The traits significantly affected by drought stress at the 5% and 1% levels were selected for comparison via Duncan's test. The significant differences among the yield traits, along with functional traits such as hundred seed weight and biomass, as well as traits related to photosynthesis and greenness of the plant such as height, chlorophyll SPAD index, and relative leaf water content under both control and stress conditions, indicated the presence of remarkable diversity of the studied genotypes to be considered for selecting drought-tolerant varieties. The plant height in the stage before clustering was not significantly different under the control condition, but the differences were significant ($P \leq 0.05$) for plants subjected to drought stress at the end of the season. Regarding the characteristics of the harvest index and the length of the second internode, there were significant differences under the control condition ($P \leq 0.05$), and the differences were more pronounced after the stress treatment at the end of the season ($P \leq 0.01$). The differences in the relative leaf water content (RWC) under normal conditions and between stressed plants at the end of the season were significant ($P \leq 0.05$).

Table 4 compares the means of the studied traits under control conditions, which were assessed by Duncan's test at $P \leq 0.05$. Based on the presented data, cultivars No. 1, 10, and 2 had the highest grain yield-associated traits. The height of the plant at the stem formation stage, the SPAD at the beginning of heading and the SPAD at the full heading stage, the number of seeds in the spike, the height of the plant in the stage before heading, the length of the second internode, and the height after heading had the greatest averages. Table 4 shows the comparison of the means of traits under the applied drought stress conditions at the end of the season by Duncan's test ($P \leq 0.05$), where cultivars No. 6, 10, and 2 had the best performance in terms of the traits associated with higher yield, including the hundred seed weight, biomass, seed weight of one spike, SPAD at the beginning of heading and spike length.

Principal component analysis

Decomposition into principal components is used to reduce the number of principal variables through uncorrelated components that are combinations of variables. The basis of this analysis is that the coordinate plane changes the main X- and Y-axes so that a path is found in space, and the main components related to the data are located along that path, whichever axis is larger. This indicates that there is more variance among the data in any direction, and for this reason, it is called the first principal component. PCA biplot analysis can be used to select traits that can be classified into main groups and subgroups based on homogeneity and dissimilarity (Mohi-Ud-Din *et al.*, 2021).

Table 5 shows the breakdown of the data obtained under control and stress conditions into main components. The analysis results showed that the first four components accounted for 79.6% and 79%, respectively, of the total variation in the data.

According to Figure 1, cultivar No. 10 is located near the grain yield trait under normal conditions.

Table 3. Variance analysis of studied traits in the control group and under drought stress conditions.

Variations	df	YP	KW	Bio.	HP	SWS	HPSBS	SPAD-FHS	SPAD-SBH	NSPSS	HP-SBH	LSI	HAH	BL	SH	RWC	HI
		Mean of Squares (Control Plants)															
Replication	2	113774.95	0.784	1305194.73	0.009	1.009	11.783	13.574	0.161	10.167	25.786	21.929	150.881	3.446	4.357	1758.316	73.616
Genotype	13	4467513.36**	1.666**	7782774.9**	0.074*	4.995**	7.298ns	75.971**	6.693**	91.2**	149.701ns	21.253*	339.907ns	3.552*	2.147ns	1060.347*	293.538*
Error	26	314805.02	0.481	1105710.5	0.028	0.905	4.88	22.816	1.745	9.833	83.658	9.159	190.98	1.728	1.223	406.257	73.143
(%) CV		18	14	15	52	21	4	14	3	14	17	15	19	26	12	48	18
Variations	**df**	**Mean of Squares (Stressed Plants)**															
Replication	2	221866.667	0.309	754807.14	0.002	1.004	51.576	0.597	23.334	78.456	41.452	3.714	146.167	0.05	9.21	705.41	32.844
Genotype	13	2104032.967**	1.615**	3910863.91**	0.03*	2.148**	3.10ns	68.186**	8.143*	46.124*	62.339*	16.7**	70.51 ns	1.41ns	0.75 ns	407.88 ns	131.24**
Error	26	283215.385	0.344	510050.73	0.012	0.316	4.15	17.361	3.730	17.975	28.196	4.84	57.55	2.17	0.86	338.23	36.98
(%) CV		20	18	14	0.71	18	5	5	5	16	13	15	12	38	13	49	15

*, **, and ns: significant at the probability level of 5%, 1% and nonsignificant, respectively.

Table 4. Comparison of studied traits by Duncan's test.

Genotypes	N-NSPS	S-NSPS	N-LSI	S-LSI	N-BL	S-BL	N-SH	S-SH	N-HI	S-HI	N-KW	S-KW	N-Bio.	S-Bio.	N-RWC
1	36.66ef	30.77bc	23abc	18.33cde	5.66	3.16	10.33	7.66	66.30abc	63.18a	5.88cd	4.52c	11787.33abcd	7033.33f	61.41abcd
2	53a	35bc	22bc	23a	6.33	3.66	10.33	8.16	61.60bc	53.74abc	7.14abc	5.84ab	13725a	10733.33a	35.60cd
3	34.66ef	33bc	21.33bc	15.33e	6	2.83	9.16	7.33	65.97abc	50491bc	6.45bcd	3.83c	10550cd	8533.33cde	81.22ab
4	37.66def	27.66c	27ab	18cde	4	2.5	8.66	8	60.78bc	48.45bcd	6.33bcd	4.08c	10425cd	9066.66bcd	55.21abcd
5	38.33def	32bc	21.33bc	16.33de	7.66	2.83	10.33	9.16	59.58c	48.97bcd	6.37bcd	4.53c	11991.66abc	9233.33bcd	73.18abc
6	43.33cd	34bc	21.66bc	21.33abc	6.33	3.86	11	7.83	64.81abc	59.76ab	7.78 a	5.81ab	13633.33a	10006.66ab	33.81cd
7	37.66def	36.33ab	28.66a	16.33de	5.66	3.50	11.66	9	54.96cd	54.27abc	5.35d	4.15c	10108.33cde	7883.33def	24.77d
8	33.66f	30.66bc	23.33abc	17.66cde	4.66	3.5	10.66	8.33	69.07abc	38.40d	5.43d	4.6c	9941.66de	8150cdef	90.81a
9	44.33bc	43.33a	24.66abc	18.66bcde	5	3	11	8.66	54.05cd	53.91abc	6.34bcd	4.1c	12716.66ab	8183.33cdef	40.50cd
10	49ab	36.33ab	21.33bc	22.66ab	7.33	3.33	10	8.66	63.98bc	53.80abc	7.57ab	6.02a	13325a	10816.66a	58.44abcd
11	36.33ef	31.66bc	25.33abc	17.33cde	4.16	3	9.66	8.5	52.83cd	51.68abc	5.94cd	4.43c	10973.33bcd	7316.66ef	45.08bcd
12	37.66def	31.33bc	27ab	17.33cde	6.66	5.33	9.66	8.33	41.93d	42.42cd	6.06cd	4.21c	11210bcd	9300bc	52.38abcd
13	40.66cde	29bc	21c	19.66abcd	5.33	3.66	10.5	8.33	77.06ab	43.01cd	5.84cd	3.98c	8350e	8433.33cde	47.28bcd
14	38.33def	35.66abc	20.33c	17de	5.66	3.83	11.5	8.5	80.55a	49.66bcd	7.07abc	4.93bc	9825de	8700bcd	41.34cd
Genotypes	**N-SWS**	**S-SWS**	**N-HPSBS**	**S-HPSBS**	**N-SPAD-SBH**	**S-SPAD-SBH**	**N-HP**	**S-HP**	**N-YP**	**S-YP**	**N-SPAD-FHS**	**S-SPAD-FHS**	**N-HPSBH**	**S-HPSBH**	**S-RWC**
1	5.936cd	5.32bcde	49.83	46.36	50.23b	46.93c	0.63ab	0.3ab	7810abc	4433.33b	42.76abcd	36.26bc	57	43.66c	25.34
2	9.93a	6.17abc	52.16	49.93	53.96a	49.3abc	0.66a	0.43a	8453.33a	5736.66a	51.36a	42.56ab	49.33	45.66bc	59.98
3	7.043bcd	4.52efg	49.9	47.13	51.9ab	45.76c	0.26c	0.13b	6850cde	4306.66b	38.33bcd	34.93bc	53.33	39.66c	44.28
4	5.39d	4.7efg	51.7	46.73	50.23b	47.2c	0.23c	0.16b	6340def	4383.33b	43.06abcd	37.16abc	52	43.66c	19.05
5	7.35bc	5.393bcde	51.1	46.93	50.86b	48.26abc	0.36abc	0.1b	7136.66bcd	4520b	43.36abcd	42.4ab	67.33	48.33abc	29.34
6	6.61cd	6.29ab	54.96	48.73	53.8a	51.63a	0.6ab	0.23ab	8816.66a	5980a	46.76ab	44.56a	57	45.66bc	50.46
7	5.86cd	4.85defg	50.3	46.36	49.5b	47.5bc	0.33bc	0.13b	5498.33 fg	4280b	38.9bcd	34.93bc	63.33	46.33abc	38.90
8	6.02cd	4.206 fg	50.5	46.36	50.56b	48.16abc	0.23c	0.1b	6546.66de	3116.66c	36.53d	36.46bc	74.66	56a	28.08
9	6.19cd	5.373bcde	48.6	47.6	50.16b	47.66bc	0.46abc	0.26ab	6875cd	4380b	37.56bcd	30.93c	55.66	46.66abc	49.98
10	8.68ab	7.04a	52.76	46.86	53.66a	51.06ab	0.21c	0.1b	8525a	5800a	46.36abc	44.33a	54	46abc	32.18
11	5.32d	4.86defg	51.7	47.2	50.16b	47.9bc	0.2c	0.083b	5815ef	3783.33bc	35.5d	35.7bc	64.33	55.66ab	50.85
12	5.86cd	4 g	51.1	46.23	50.5b	47.26c	0.4abc	0.13b	4675 g	3970bc	37.43cd	29.3c	55	48.33abc	36.55
13	6.04cd	5.2cdef	52.6	47.33	50.9b	47.06c	0.4abc	0.13b	6358.33def	3566.66bc	34.83d	36.4bc	57	43.33c	31.53
14	6.38cd	5.833bcd	51.733	47.33	51.56ab	46.46c	0.38abc	0.23ab	7895.66ab	4356.66b	45.9abc	32.9c	51	42.66c	80.55a

The different letters in each column indicate significantly different values at P≤0.05, according to Duncan's test. N: normal condition; S: stress condition.

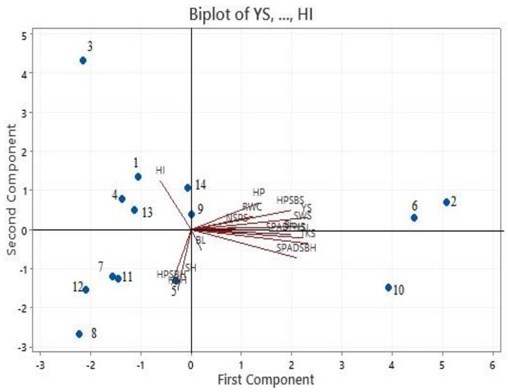

Fig. 1. Biplot of principal component analysis under control conditions.

The hundred-grain weight, plant height, harvest index, and plant height at the heading stage are traits that indicate that cultivar No. 10 (Winter Roshan Backcross) is a good candidate variety with high yield. As shown in Figure 2, genotypes No. 2 (Pishgam) and No. 6 (Mehan) are located in the vicinity of yield-related traits (including plant height, plant greenness percentage, yield under post-flowering stress conditions, relative leaf water content, and weight of the seed of a spike) in the chart. These two cultivars can be considered favorable genotypes because they experience late drought stress at the end of the season. On the other hand, cultivars No. 2 (Pishgam), No. 6 (Bahman), and No. 10 (Zare) were more desirable and more stable under control and stressed plant condition, respectively.

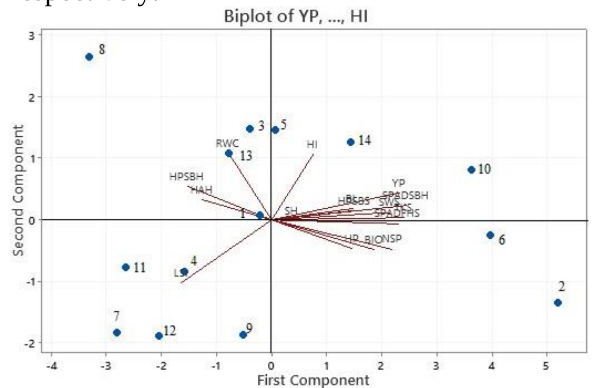

Fig. 2. Biplot of principal component analysis under drought stress

Table 6 shows the special vectors of the eight components in different varieties of bread wheat grown under normal conditions; according to Table 7, the first component had the greatest correlation with yield traits. Therefore, the first component is named the performance component, and the second component is the harvest index attribute that had the highest amount and numerical value. In Table 6, the first component (the performance component) and the second component (the harvesting index component) of the stressed plants are considered in the same manner as for the control plants, similar to Table 7.

Table 5. Principal component analysis of the control and stressed plants at the end of the season.

Variations	Control plants							
	Comp. 1	**Comp. 2**	**Comp. 3**	**Comp. 4**	**Comp. 5**	**Comp. 6**	**Comp. 7**	**Comp. 8**
Eigenvalues	7.05	2.18	1.92	1.58	1.03	0.62	0.54	0.42
Cumulative variance (%)	44.1	57.7	69.7	79.6	86.1	90	93.4	96.1
	Stressed plants							
Eigenvalues	6.43	3.07	1.83	1.3	0.99	0.93	0.45	0.38
Cumulative variance (%)	40.2	59.4	70.9	79	85.3	91.1	93.9	96.4

Factor Analysis

The importance of morphophysiological traits and their positive correlation with seed weight and yield has been reported earlier in wheat. Multivariate techniques, including factor analysis (FA) and cluster analysis, showed that the amount of variation in the gene pool was satisfactory. A similar approach has been utilized in other studies with conclusive outcomes to suggest tolerant or advantageous genotypes (Ali *et al*., 2021; Wani *et al*., 2018; Mulugeta *et al*., 2022). Supplement 5 shows the eigenvalues and cumulative variance of the first, second, third, fourth, and fifth factors under control and drought stress conditions, respectively. As presented in these tables, the first three factors accounted for 64.68 and 70.361% of the variation in the total data. Tables 7 show the special vectors of the first five factors under the control treatment and the first four factors after drought stress.

Cluster analysis and detection of discriminant function

Cluster analysis is a multivariate statistical method for determining the diversity of different plant populations and categorizing them into diverse groups based on distance or genetic similarity. Figure 3 shows the dendrogram resulting from cluster analysis based on significantly distinguishable agricultural traits of control plants using the WARD method.

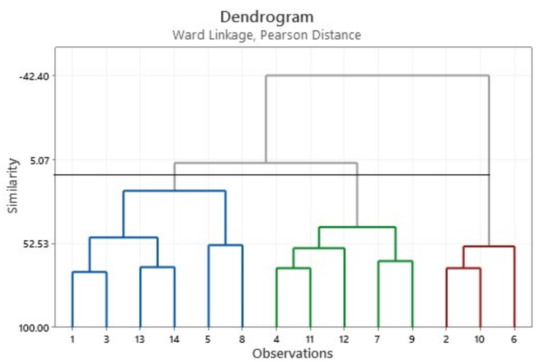

Fig. 3. Dendrogram resulting from cluster analysis based on significant agricultural traits under control conditions.

According to Figure 3, genotypes No. 1, 3, 13, 14, 5, and 8 were in the first group; genotypes No. 9, 7, 12, 11, and 4 were in the second group; and genotypes No. 2, 10, and 6 were in the third group. Cultivars No. 2, 6, and 10 had better yields under the current experimental conditions. Figure 4 shows the dendrogram obtained from the cluster analysis based on the significantly different traits under drought stress conditions by the WARD method. Cultivars No. 1, 3, 4, 9, 13, and 14 were separated from No. 5, 7, 8, 11 and 12 and cultivars No. 2, 6 and 10.

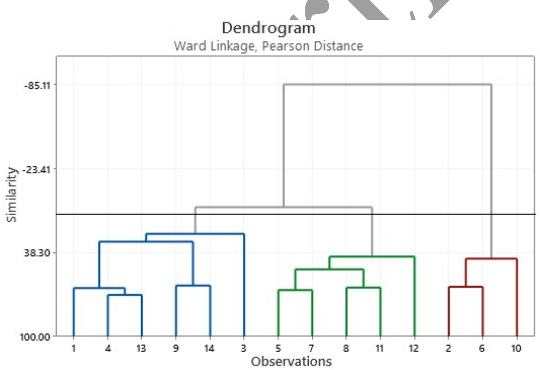

Fig. 4. Dendrogram resulting from cluster analysis based on significant agricultural traits under stress conditions.

Functional analysis is another multivariate statistical analysis method that can be used to test the accuracy of cluster analysis results. This confirms the analysis of the clusters of control and stressed plants that are presented in Supplement 6 and 7, respectively. The distances between the centers of clusters in the control and drought-stressed plants at the end of the season are also presented in Supplement 8. Table 8 shows the number of cultivars in each cluster, the mean square of the clusters, and the average and maximum distances from the centers of the clusters for the control and stressed plants, respectively, at the end of the season of moisture stress. Similar methods were applied in earlier studies to identify better-performing genotypes of wheat plants under abiotic stress conditions (Al-Ashkar *et al*., 2023; Al-Ashkar *et al*., 2021). Water stress is one of the major environmental constraints on wheat grain yield worldwide. One way to overcome this limitation is the selection or development of wheat genotypes that are tolerant to drought stress, and that produce optimal grain yields under water deficit conditions (Yeater *et al*., 2004). We applied principal component analysis before cluster analysis to determine the relative importance of the variables involved in the cluster. In this study, it was evident that the applied drought stress caused a decrease in grain yield-associated traits. In a study on durum wheat cultivars under water scarcity conditions, Mohammadi *et al*. identified the plant height, thousand kernel weight, and potential quantum efficiency of photosystem II (Fv/Fm) as among the most promising traits for indirect selection of tolerant varieties (Mohammadi *et al*., 2019). The significant effect of genotype diversity on agronomic traits has been documented previously, showing breeding line specificity under certain stress conditions (Chaghakaboodi *et al*., 2021). Some important characteristics, such as seed weight per spike and thousand-kernel weight, which have significant effects on yield, were recognized in another study conducted recently on *Triticum* species, which is in line with the findings of our study (Lacko-Bartošová *et al*., 2022). Aghaee et al. also used multivariate statistical methods similar to the approach that was considered in our study and successfully utilized cluster analysis to separate bread wheat cultivars for grouping or varieties (Aghaee *et al*., 2010).

Table 6. Eigenvectors of the selected traits in control and stressed plants.

Traits	Control plants							
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
YP	0.328	0.201	-0.051	0.099	0.090	-0.379	-0.088	-0.128
KW	0.340	0.016	0.002	-0.137	-0.209	-0.068	0.071	-0.22
BIO	0.265	-0.223	0.341	-0.036	0.106	-0.432	0.012	0.084
HP	0.208	-0.215	-0.097	0.323	0.381	-0.206	0.534	0.237
SWS	0.305	0.055	0.239	-0.069	0.188	0.256	-0.387	0.154
HPSBS	0.212	0.069	-0.080	-0.025	-0.740	-0.031	0.292	0.229
SPADFHS	0.325	-0.030	0.070	0.022	-0.013	-0.193	-0.023	-0.586
SPADSBH	0.346	0.107	0.068	-0.113	-0.168	0.017	-0.043	0.194
NSP	0.310	-0.220	0.069	0.018	0.004	0.129	-0.457	0.297
HPSBH	-0.217	0.250	0.325	0.305	-0.169	-0.32	-0.089	0.380
LSI	-0.233	-0.469	0.111	-0.034	-0.154	-0.114	-0.118	-0.254
HAH	-0.179	0.151	0.522	0.226	-0.211	-0.030	-0.048	-0.276
BL	0.208	0.084	0.413	0.084	0.076	0.582	0.423	0.108
SH	0.052	-0.006	-0.049	0.761	-0.019	0.128	-0.158	-0.087
RWC	-0.111	0.496	0.249	-0.293	0.278	-0.171	0.089	0.022
HI	0.106	0.485	-0.409	0.171	0.015	0.027	-0.14	-0.150
	Stressed plants							
YP	0.360	0.101	-0.069	-0.058	-0.065	-0.165	0.135	-0.139
KW	0.361	-0.116	-0.073	0.081	0.032	-0.026	0.213	0.114
BIO	0.308	-0.039	-0.123	0.171	-0.043	-0.417	-0.392	-0.346
HP	0.212	0.226	0.302	0.162	-0.223	0.473	0.039	-0.429
SWS	0.348	0.029	-0.091	-0.267	-0.210	-0.003	0.112	0.321
HPSBS	0.307	0.161	0.217	0.085	0.244	0.229	-0.465	0.018
SPADFHS	0.294	-0.058	-0.409	-0.101	0.232	0.069	-0.149	-0.034
SPADSBH	0.326	-0.235	-0.138	0.002	0.166	-0.079	0.254	0.099
NSP	0.145	0.015	0.485	-0.463	-0.096	-0.245	0.370	-0.184
HPSBH	-0.061	-0.458	0.100	0.012	0.476	0.232	0.119	0.098
LSI	0.346	-0.069	-0.051	0.166	-0.090	0.170	0.132	-0.007
HAH	-0.042	-0.504	0.111	0.145	0.144	-0.063	0.115	-0.542
BL	0.032	-0.173	0.290	0.613	-0.268	-0.373	0.007	0.321
SH	0.000	-0.403	0.140	-0.455	-0.229	-0.135	-0.507	0.018
RWC	0.189	0.110	0.524	-0.018	0.416	-0.052	-0.139	0.275
HI	-0.096	0.407	-0.057	-0.011	0.442	-0.453	0.093	-0.181

Table 7. Special vectors of the 5 factors under control and 4 factors under drought stress conditions.

Traits	Rotated component matrix under control conditions					Rotated component matrix under stress conditions			
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 1	Factor 2	Factor 3	Factor 4
YP	0.656	-0.223	0.573	0.177	0.187	0.850	-0.210	0.326	-0.073
KW	0.699	-0.338	0.232	-0.022	0.486	0.910	0.126	0.197	0.118
BIO	0.872	-0.126	-0.239	0.089	0.043	0.302	-0.031	0.089	0.172
HP	0.435	-0.382	0.075	0.598	-0.202	0.310	-0.354	0.589	0.280
SWS	0.871	-0.121	0.201	-0.033	0.025	0.833	-0.049	0.328	-0.294
HPSBS	0.256	-0.068	0.174	0.036	0.898	0.584	-0.260	0.583	0.184
SPADFHS	0.749	-0.262	0.188	0.162	0.260	0.893	-0.016	-0.178	-0.238
SPADSBH	0.762	-0.222	0.325	-0.051	0.438	0.870	0.329	0.069	0.027
NSP	0.733	-0.376	-0.058	0.240	0.229	0.063	0.186	0.854	-0.278
HPSBH	-0.278	0.870	-0.007	0.046	-0.101	-0.094	0.804	-0.117	0.142
LSI	-0.4	0.061	-0.862	0.047	-0.070	0.860	0.036	0.199	0.208
HAH	-0.039	0.932	-0.229	-0.036	-0.067	-0.035	0.853	-0.144	0.302
BL	0.769	0.26	0.077	0.036	0.006	0.014	0.238	0.109	0.821
SH	0.026	0.259	0.204	0.911	0.013	-0.016	0.813	0.156	-0.346
RWC	-0.015	0.457	0.383	-0.680	-0.337	0.160	-0.060	0.839	0.202
HI	-0.138	-0.110	0.945	0.124	0.152	-0.283	-0.690	-0.004	-0.130

Table 8. The number of cultivars in each cluster and relevant distances in the control and stressed plants.

	Control plants			
	Observations	Within cluster sum of squares	Average distance from centroid	Maximum distance from centroid
Cluster1	6	3735.42	23.4221	36.5773
Cluster2	3	627.16	14.1447	18.2893
Cluster3	5	1236.88	15.0042	21.4523
	Stressed plants			
Cluster1	6	2123091435	14040.0	42033.4
Cluster2	3	429356	356.7	531.3
Cluster3	5	4160700	896.8	1070.8

The importance of grain yield under different water availability conditions was emphasized as an important trait for the selection of tolerant genotypes (Boussakouran *et al*., 2019). Although we found a positive correlation among the yield-associated traits, in a larger study of 196 sorghum accessions, grain yield had negative and significant associations with seedling vigor and plant height (Derese *et al*., 2018).

Conclusions

Low water conditions and the environment greatly affect the yield of wheat plants. Most of the areas under wheat cultivation in Iran are mostly dry land and are at least partially exposed to drought stress. Therefore, it is necessary to conduct more research in the field of breeding for optimum production by identifying genotypes or cultivars that are tolerant to drought stress. On the other hand, breeding methods that aim to increase yield as a selection index are very time-consuming. A combination of physiological, morphological, and phenological traits can be used as a selection index to help researchers reach the desired goal in a shorter time. In general, based on univariate statistical methods of analysis of variance, most of the traits (especially functional traits) were significantly ($P<0.001$) different among the investigated genotypes and in the comparison of means using Duncan's method.

Most of the distinguishing traits were found to have the highest values for cultivars 2, 6, and 10. The higher values identified for these three varieties remained after multivariate statistical methods such as factor analysis, decomposition into principal components, and cluster analysis were applied, indicating their true superiority under the applied stress. In the cluster analysis method, all the diversity between cultivars and classification traits of cultivars were used. Therefore, we can clearly say that cluster analysis is the best statistical method for grouping the genotypes. The above conclusions about the selected genotypes were quite consistent under both environmental conditions (control and drought stress). Based on this study, it can be concluded that morphological traits can be important phenotypic markers for selecting high-yielding wheat cultivars. The general conclusion is that various multivariate analyses are necessary to confirm the diversity of the subjected cultivars to select adequate genotypes in breeding programs. The results of this study can be used as a foundation for selecting cultivars with superior agronomic and morphological grain yield traits for use in wheat breeding programs.

Author contributions

Conceptualization, **M.K.**; Methodology, **M.K.**; Validation, **M.K.**, **I.M.**; Formal analysis, **M.K.**; Investigation, **M.K.**; resources, **M.K.**; Writing-original draft preparation, **M.K.**; Writing-review and editing, **I.M.**; Supervision, **M.K.**; Funding acquisition, **I.M.** All authors have read and agreed to the published version of the manuscript.

Data availability statement

Data will be available upon official request.

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Conflicts of interest

The authors declare no conflicts of interest.

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