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Evaluation of water deficient stress tolerance in spring wheat lines using canonical discriminant analysis

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Abstract

Canonical discriminant analysis (CDA) in combination with cluster analysis was used to assess 296 spring wheat lines derived from a cross between Bam (drought tolerant) and Arta (drought sensitive) cultivars using stress tolerance indices, at two water regimes, well watered and cessation of irrigation at pollination. CDA results showed that the first two canonical variables explained 97% of the inter- group variation. The first canonical variable high canonical loadings for the indices Stress Tolerance Index, Harmonic Mean, Mean Productivity, Geometric Mean Productivity, Yield at normal condition, and yield at water deficit stress conditions. The second canonical variable consisted of Stress Susceptibility and Tolerance Indices. Therefore, the first canonical variable differentiated genotypes based on yield potential and stress tolerance and the second canonical variable distinguished stress tolerant genotypes from the sensitive types. Scatter plot of the first two canonical variables characterized five distinct groups and all pairwise Mahalanobis distances among groups were significant. The second group was recognized as the best group, because the genotypes of this group had the highest value in terms of the first canonical variable and most of these genotypes had negative values of the second canonical variable. Therefore, the genotypes of this group are suitable for both water stress and non-stress environments.

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Introduction

Wheat is the most important source of human nutrition in the world, and drought is one of the most common environmental stresses that reduces wheat yield. Breeding programs focus on selection of high yielding genotypes under normal condition and then under water stress environment to identify stress tolerant genotypes. To achieve this, selection of genotypes with high potential yield in normal condition is a method commonly used to identify superior genotypes (Blum, 1996). However, this method does not imply the concept of sustainability of performance and adaptation to the stress environment. This can lead to hamper the progress in breeding programs (Blum, 1996). Several stress indices have been proposed for screening of drought stress tolerance. These indices consider the relationships between yields in the stressed and stress-free environments. According to Rosielle & Hamblin (1981), Fernandez (1992) and Sarin *et al.* (2012), these indicators are divided into two groups. The first group represents sensitivity indices (TOL and SSI) that make possible differentiation between tolerant and sensitive genotypes and often have a negative relationship with yield performance. Tolerance index (TOL) was proposed by Rosielle & Hamblin (1981) and is the difference in yield under normal (Y_p) and stress (Y_s) conditions. High TOL values represent stress susceptibility. Fischer & Maurer (1978) proposed a stress susceptibility index (SSI). Low levels of SSI indicate higher tolerance to stress. The second group provides tolerance indices such as mean productivity (MP), geometric mean productivity (GMP) and especially stress tolerance index (STI) that can identify high yielding and tolerant genotypes and have a positive relationship with yield. The MP and GMP indices are respectively the mean yield and the geometric mean yield of stress-free and stressed environments (Rosielle & Hamblin, 1981). High levels of STI indicate high tolerance to stress and greater yield potential (Fernandez, 1992). STI, GMP, and MP are the most recommended indicators for identifying high-yielding genotypes in both stressed and normal environments

(Thiry *et al.*, 2016). However, tolerance and susceptibility indices may not be suitable for recognizing genotypes with high yield and stress tolerance under conditions of both normal and stressful environments (Thiry *et al.*, 2016). The combination of these two groups of indices has been proposed as a useful benchmark for improving drought stress tolerance (Ramirez-Vallejo & Kelly, 1998). For this purpose, canonical discriminant analysis (CDA) can be used to identify the superior genotypes based on the combination of both types of stress indices. As a multivariate statistical method, CDA maximizes the distinction between groups by considering all the variables simultaneously (Riggs, 1973). After determining the inter-group variation, the Mahalanbis (D^2) distance statistic can be used as an indicator of the difference between the groups (Loos, 1993). Data from the CDA can then be used to group the genotypes into smaller subgroups that are similar to each other (Loos, 1993).

Riggs (1973) used canonical discriminant analysis to select within a spring barley population and suggested that this method can be used as an alternative to the selection index when it is difficult to assign suitable economic weights to the traits under consideration. Rascio *et al.*, (2012) utilized discriminant analysis to identify physiological traits that have the ability to differentiate between durum wheat genotypes for compatibility with semi-arid environments, and showed that a function based on 13 morpho-physiological traits was effective in differentiating the genotypes into three groups: compatible, semi-compatible and non-compatible. Abdolshahi *et al.* (2015) carried out the discriminant analysis on 40 wheat genotypes and suggested the use of an index based on 10 attributes to be used as a selection criterion for drought tolerance in wheat breeding programs.

The purpose of this study was to use the stress sensitivity and stress tolerance indices simultaneously for identification of water deficit tolerant and high yielding genotypes based on canonical discriminant

analysis.

Materials and methods

In this study, the response of 296 spring bread wheat genotypes to water deficit stress, using stress tolerance and stress sensitivity indices, was evaluated in a split plot design based on randomized complete blocks with three replications. The experiment was conducted in the 2013 growing season, at the research field of the Faculty of Agriculture, University of Tabriz, Iran. The irrigation condition (normal irrigation through the growing season and withholding irrigation from anthesis) was arranged in main plots and the genotypes in sub-plots. These genotypes were obtained from the cross between Bam (drought tolerant) and Arta (drought susceptible) cultivars. Seed of parental cultivars were obtained from the seed bank of Seed and Plant Improvement Institute, Karaj, Iran. The length of each row was 90 cm, in which 17 seeds were planted at a distance of five centimeters. The distance between the rows was 15 cm. Sowing operation was done manually. Grain yield (g per plant) was measured on 10 plants in each row. After testing the normality of residuals, analysis of variance was performed using the SAS 9.0 (SAS, 2002).

Drought related indexes were calculated using the following formula:

$$SSI = [1 - (Y_s - Y_p)] / [1 - (Y_s - Y_p)] \quad (\text{Fischer \& Maurer, 1978})$$

$$MP = (Y_p + Y_s) / 2 \quad (\text{Hossain et al., 1990})$$

$$TOL = Y_p - Y_s \quad (\text{Rosielle \& Hamblin, 1981})$$

$$STI = (Y_p \times Y_s) / (\bar{Y}_p)^2 \quad (\text{Fernandez, 1992})$$

$$GMP = (Y_p \times Y_s)^{1/2} \quad (\text{Fernandez, 1992})$$

$$HM = 2(Y_p \times Y_s) / (Y_p + Y_s) \quad (\text{Schneider et al., 1997})$$

where, Y_s = grain yield under drought stress condition, Y_p = grain yield under normal condition, \bar{Y}_s and \bar{Y}_p = the mean yield of genotypes under stress and non-stress conditions, respectively, and

$$1 - (\bar{Y}_s - \bar{Y}_p) = \text{stress intensity.}$$

Cluster analysis for grouping of the genotypes and consequent discriminant analysis were performed using SPSS version 16.0 (SPSS, 2007). In order to carry out cluster analysis, the distances between genotypes were calculated using squared Euclidean distance and then the dendrogram was obtained by the Ward clustering method.

The cutting point of the dendrogram was determined by the discriminant analysis. Then, the accuracy of grouping by the cluster analysis was verified by the canonical discriminant analysis. SAS 9.0 software (SAS, 2002) was utilized to perform the canonical discriminant analysis (CANDISC procedure) and to draw the distribution chart of groups based on the first and second canonical variables. Furthermore, the differences between the two group vectors were determined by the Mahalanobis distance (Dillon & Goldstein, 1984).

Results and discussion

Analysis of variance of the bread wheat genotypes under two irrigation conditions showed significant differences among genotypes for grain yield. The interaction of genotype × irrigation condition was also significant for this attribute (Data not shown).

Cluster analysis based on STI, HM, MP, GMP, Y_p , Y_s , TOL, and SSI divided the genotypes into five groups (Fig. 1). Groups 1 to 5 consisted of 33, 60, 115, 48 and 42 genotypes, respectively.

The results of discriminant analysis for verifying the validity of grouping in the cluster analysis, are presented in Table 1. These results show that 97%, 98.3%, 100%, 95.8% and 95.2% of the genotypes were grouped correctly in groups 1 to 5, respectively. The success rate of the entire discriminant analysis was 98%. This indicates that the discriminant analysis was successful in differentiation of the groups obtained from the cluster analysis.

Table 1. Results of discriminant analysis for verifying the accuracy of grouping by the cluster analysis of wheat genotypes.

Groups		Predicted members in the groups					Total
		1	2	3	4	5	
Number	1	32	0	1	0	0	33
	2	0	59	0	1	0	60
	3	0	0	115	0	0	115
	4	0	0	0	46	2	48
	5	0	0	0	2	40	42
%	1	97	0	3	0	0	100
	2	0	98.3	0	1.7	0	100
	3	0	0	100	0	0	100
	4	0	0	0	95.8	4.2	100
	5	0	0	0	4.8	95.2	100
The total percentage of correct grouping							98%

Results of four methods to test the equality of the group mean vectors are presented in Table 2, which shows significant differences among vectors. CDA created linear combinations of variables that have the highest possible correlation with the groups. The canonical correlations are shown in Table 3. A

likelihood ratio test was also provided for testing the canonical correlations. An approximation of F (Rao, 2009) was used to test these correlations. The first two canonical variables explained 97% of the intergroup variation and could effectively differentiate the genotypes.

Table 2. Multivariate statistics for testing the assumption of the equality of the mean vectors of the groups.

Statistics	Value	Numerat or df	Denominat or df	F statistic
Wilks' Lambda	0.002	32	1056.3	127.79**
Pillai's Trace	2.325	32	1156	50.17**
Hotelling-Lawley Trace	41.872	32	737.38	372.63**
Roy's Greatest Root	37.779	8	289	1364.78**

Table 3. Canonical correlations and eigen values in the canonical discriminant analysis of wheat genotypes.

Canonical variable	Canonical correlation	Eigen value	Cumulative percentage	Likelihood ratio	Approximate F value
1	0.987	37.779	0.902	0.002	127.79**
2	0.865	2.987	0.973	0.112	44.67**
3	0.704	0.987	0.997	0.450	23.54**
4	0.324	0.117	1.000	0.894	6.80**

The canonical loadings, also called canonical structures (Table 4), are linear correlations between original variables and the canonical variables. Thus, the canonical loadings indicate the relative contribution of each variable to each of the canonical

variables (Cruz-Castillo *et al.*, 1994). The first canonical variable consisted of large loadings for STI, HM, MP, GMP, Yp and Ys. The canonical loadings of SSI and TOL were positive and high in the second canonical variable (Table 4).

Table 4. Canonical loadings for the first and second canonical variables in the canonical discriminant analysis of wheat genotypes.

Variable	First canonical variable	Second canonical variable
STI	0.942	-0.294
Yp	0.973	-0.057
Ys	0.949	-0.302
HM	0.973	-0.207
GMP	0.975	-0.192
MP	0.977	-0.175
SSI	-0.060	0.596
TOL	0.359	0.596

According to Fernandez (1992), selection based on MP, GMP, and STI leads to the screening of high yielding genotypes in both stressed and non-stressed conditions. The higher amount of TOL represents the more stress susceptibility. Although genotypes with low SSI are considered as stress tolerant, because they show lower yield reduction under stress condition, but the stress tolerant genotypes determined by this index are usually low-yielding genotypes (Ramirez-Vallejo & Kelly, 1998). Therefore,

the positive and high values of the first canonical variable can select high yielding genotypes in stressed and normal environments. The second canonical variable distinguishes the tolerant genotypes from the susceptible ones. Therefore, the higher values for the first canonical variable and the lower values for the second variable may be used to select suitable genotypes for both stressed and non-stressed environments. The group means for canonical variables are given in Table 5.

Table 5. Average of the groups for the first and second canonical variables in the canonical discriminant analysis of wheat genotypes.

Group	First canonical variable	Second canonical variable
1	-0.972	-1.895
2	8.781	-2.090
3	-4.250	-0.068
4	5.009	1.366
5	1.005	3.102

Scatter diagram of the wheat genotypes based on first two canonical variables (Fig. 2) distinguished five

groups. The Mahalanobis distances between these groups are also shown in Table 6.

Table 6. Pair of Mahalanobis distances between groups (above diameter) and F* statistic (below diameter) in the canonical discriminant analysis of wheat genotypes.

	Group 1	Group 2	Group 3	Group 4	Group 5
Group 1	0	344.20	39.71	232.96	140.57
Group 2	894.12	0	175.46	27.99	88.55
Group 3	124.25	884.10	0	88.50	42.98
Group 4	555.85	91.09	365.70	0	24.45
Group 5	316.98	266.95	161.35	66.84	0

*Degrees of freedom of the numerator and denominator are 8 and 286, respectively.

All between-group distances were significant. Intra-group variation was low, because the genotypes within each group were only slightly spaced apart. Regarding Fig. 2, the genotypes of group 2 had the highest value for the first canonical variable, and most of these genotypes had negative values for the

second canonical variable. This was confirmed by Table 5 regarding the average of the first and second canonical variables. Therefore, the genotypes of this group may be regarded as suitable genotypes for both water stress and normal conditions (Table 7).

Table 7. Genotypes in each of the groups derived from the canonical discriminant analysis of the wheat genotypes.

Group 1	P1-F3 (25*) - BC1S1 (2-13) - BC1S2 (1-8, 10-20)
Group 2	P2-F3 (9, 28, 33, 59, 74, 79, 85) -F4 (16, 19, 26, 28, 29, 35, 41) -F5 (1, 2, 4, 6, 8, 9, 11, 12, 15, 42,44, 52) - BC1S2 (9) - BC2S1 (1-12) - BC2S2 (1-20)
Group 3	F3 (1-8, 10-22, 24, 26, 27, 30-32, 35, 37, 58, 60-73, 75-78, 80-84, 86-126) - F4 (42)
Group 4	F3 (23, 29, 34, 36) - F4 (1, 5, 24, 50) - F5 (3, 5, 7, 10, 13, 14, 16-41, 43, 45-51)
Group 5	F4 (2, 3, 4, 6-15, 17, 18, 20-23, 25, 27, 30-34, 36-40, 43-49, 51-53) - BC1S1 (1)

P1= Parent 1; P2= Parent 2; *=Represent NO.

In general, using canonical discriminant analysis, made it possible to better understand the behavior of genotypes under water stress condition. The first canonical variable consisted of high loadings for

stress tolerance indices under water stress and normal conditions and the second canonical variable had high loadings for the stress sensitivity indexes (SSI and TOL).

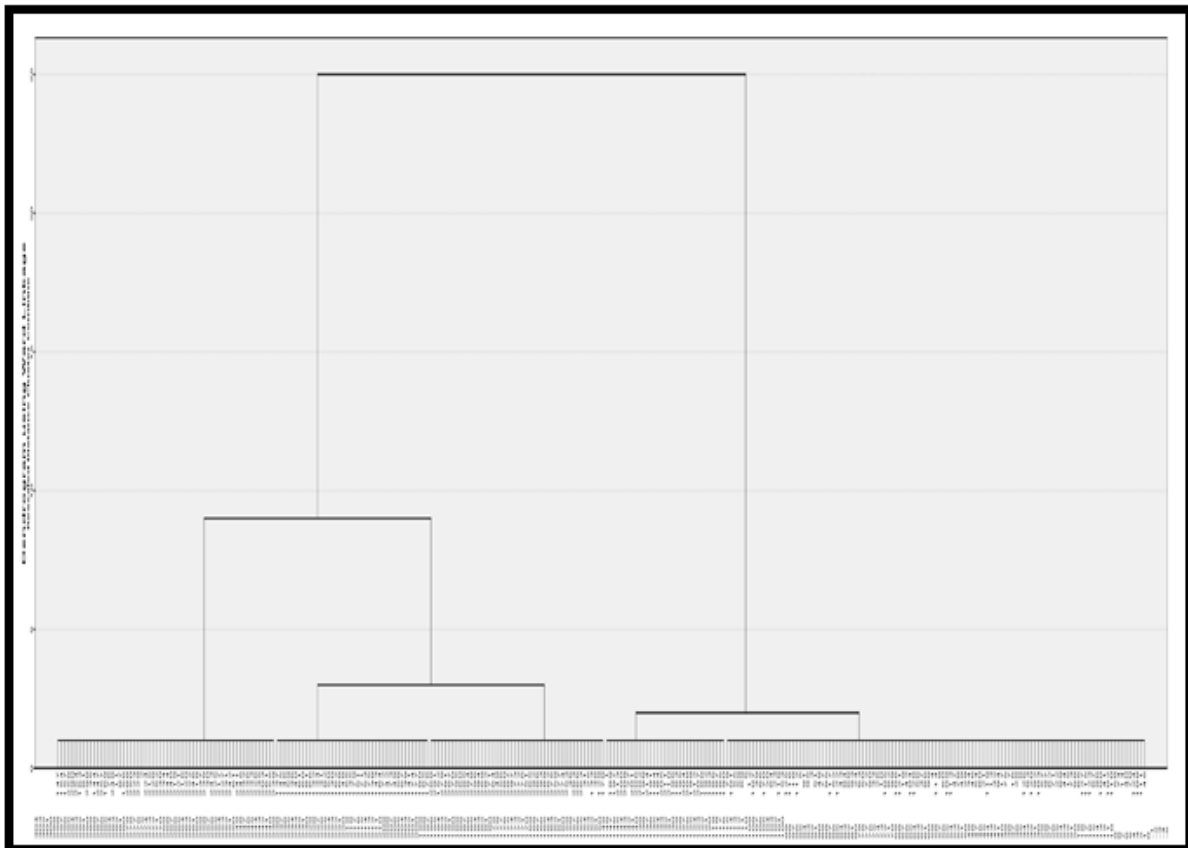


Fig. 1. Dendrogram of the cluster analysis of genotypes obtained from crossing of wheat cultivars Artta and Bam.

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