



## RESEARCH PAPER

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## Screening drought tolerant genotypes in wheat using multivariate and stress tolerance score methods

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### Abstract

In order to study the response of bread wheat genotypes to drought stress, an experiment was conducted in a randomized complete block design with three replications under rainfed and irrigated conditions during the growing season 2012-2013. In this study, drought tolerance indices including stress tolerance index (STI), geometric mean productivity (GMP), mean productivity index (MP), stress susceptibility index (SSI), tolerance index (TOL) and harmonic mean productivity (HMP) were calculated and adjusted based on grain yield under drought ( $Y_s$ ) and irrigated conditions ( $Y_p$ ). Drought tolerance indices discriminated genotypes 11, 13, 8 and 2 as the most drought tolerant, hence they are recommended to be used as parents for genetic analysis, gene mapping and improvement of drought tolerance in common wheat. Principal component analysis (PCA), exhibited that first and second PCA accounted for 97.66% of the variation. The result of stress tolerance score (STS) was the same as multivariate analysis, but STS equation was much easier. Therefore it can be introduced as a suitable screening technique for identification of drought tolerant genotypes.

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## Introduction

Better usage of water for all kinds of productions is one of the possible ways for food requirement warranty which need much less water and much more drought tolerance (Shao *et al.*, 2006). Drought is a huge limitation for crop beneficiary and water deficiency so most of dry and semi dry areas are affected by future weather changes toward drought (Wassmann *et al.*, 2009). The impact of water shortage and lower rainfall during the sowing period seems to be the main reason for lesser acreage under wheat crop and reduction in wheat production. Therefore, breeding for drought tolerant wheat is an important task and objective in the present scenario (Anwar *et al.*, 2011). Furthermore, in situation with lack of complete information about drought tolerance mechanism, the grain yield under drought stress can be used as a direct selection for screening drought tolerant genotypes (Farshadfar and Sutka, 2002). Breeding for drought tolerance is complicated by the lack of fast, reproducible screening techniques and the inability to routinely create defined and repeatable water stress conditions when a large amount of genotypes can be evaluated efficiently (Ramirez and Kelly, 1998). Achieving a genetic increase in yield under these environments has been recognized to be a difficult challenge for plant breeders while progress in grain yield has been much higher in favourable environments (Richards *et al.*, 2002). Therefore drought indices which are based on yield reduction of cultivars under the drought stress conditions are used as a comparison with normal condition for screening of drought tolerant cultivars (Mitra, 2001).

Based on STI index, plants are divided into the four groups: (1) – genotypes that express uniform superiority in rainfed and irrigated conditions (group A), (2) - genotypes which perform favorably only in non-stress conditions (group B), (3) - genotypes which yield relatively higher only in stress conditions (group C) and (4) - genotypes which perform poorly in non-irrigated and irrigated conditions (group D) (Fernandez, 1992). The geometric mean productivity (GMP) is often as a relative performance, since drought stress can be variable in severity fields in

over years (Ramirez and Kelly, 1998). Tolerance index (TOL) and mean productivity (MP) (Rosielle and Hamblin, 1981), stress susceptibility index (SSI) (Fischer and Maurer, 1978) and Harmonic Mean Productivity (HMP) (Fernandez, 1992) are other indices for evaluation of genotypes in drought conditions. Sardouie-Nasab *et al.* (2014) showed the STS equation is much easier to use than complicated multivariate analyses. Therefore, it is suggested as a screening tool for identification of salt-tolerant wheat genotypes. The equation STS was much easier to use than factor analysis and is suggested as a screening tool for the identification of drought-tolerant genotypes (Abdolshahi *et al.*, 2013).

The present study was undertaken to (i) assess the selection criteria of drought tolerance and (ii) for integration of drought tolerance indices in a single indicator for screening drought tolerant bread wheat genotypes.

## Materials and methods

### *Plant genetic material and experimental layout*

Nineteen landraces of bread wheat (*Triticum aestivum* L.), listed in Table 1, were provided from Seed and Plant Improvement Institute of Karaj, Iran. They were assessed using a randomized complete block design with three replications under two irrigated and rainfed conditions in the experimental field of College of Agriculture, Razi University, Kermanshah, Iran during 2012-2013 growing season. Sowing was done in mid-November in both experiments with a density of 400 plants per square meter. Each experimental plot consisted of four 2-m rows with 25 cm distance between the rows. Weeds were manually controlled during the growing season. Five plants were randomly selected from each plot to measure the number of seed per spike (NSS), 1000-grain weight (TGW), number of spike per m<sup>2</sup> (NSP) and grain yield (GY). After removing the border effect, the whole plot was harvested to calculate the grain yield (g. m<sup>-2</sup>).

### *Statistical analysis*

### *Stress tolerance indices*

Stress resistance indices were calculated using the following formulas:

Stress Tolerance Index =  $STI = \frac{Y_p \times Y_s}{\bar{Y}_p^2}$  (Fernandez, 1992).

Tolerance =  $TOL = Y_p - Y_s$  (Rosielle and Hamblin, 1981).

Stress susceptibility index =  $SSI = (1 - (Y_s / Y_p)) / (1 - (\bar{Y}_s / \bar{Y}_p))$  (Fischer and Maurer, 1978).

Harmonic Mean Productivity =  $HMP = \frac{2 Y_p \times Y_s}{Y_p + Y_s}$  (Fernandez, 1992).

Geometric Mean Productivity =  $GMP = \sqrt{(Y_s \times Y_p)}$  (Fernandez, 1992).

Mean productivity =  $MP = (Y_s + Y_p) / 2$  (Rosielle and Hamblin, 1981).

*b*: The coefficient of linear regression of grain yield of a genotype in each environment on the environmental index (mean yield of all genotypes at any environment) (Bansal and Sinha, 1991).

#### Biometrical methods

Analysis of variance (ANOVA), mean comparison and simple correlation was analyzed by SAS package (SAS 9.1.3). Spearman's rank correlation coefficient between the ranks of genotypes based on their grain yields under drought stress. Correlation analysis and principal component analysis (PCA) were calculated using Microsoft Excel 2013 and SPSS ver. 16, respectively.

#### Discriminant analysis

Indices were calculated based on weight coefficient for choosing better genotypes (Smith, 1936):

$$D = v_1 X_1 + v_2 X_2 + v_3 X_3 + \dots + v_i X_i$$

where, D is discriminant function, V is weight coefficient for each index and X is data indices. Based on this analysis genotypes 11,13,8 and 2 have the

lowest ranks (most resistant genotypes) and genotypes 16,1,5 and 18 have the highest ranks (most sensitive genotypes). Wilk's lambda for discriminant was 0.422 (non- significant).

$$D = (0.362 \times SSI) + (0.188 \times TOL) + (0.164 \times MP) + (0.336 \times STI) + (0.242 \times GMP) + (0.288 \times HMP).$$

#### STS calculation

According to the complexity of multivariate analysis, STS method was used based on the following equation (Abdolshahi *et al.*, 2013; Sardouei-Nasab *et al.*, 2014):

$$STS = GMP_{std} + STI_{std} + HMP_{std} + MP_{std} - TOL_{std} - SSI_{std} - b_{std}.$$

Indices were standardized followed as:

$$Z_{ij} = (X_{ij} - \mu) / S_i.$$

Where  $Z_{ij}$  is the standard score for  $j$ th genotype in the  $i$ th index,  $X_{ij}$  is the raw data of  $j$ th genotype in the  $i$ th index,  $\mu$  is the mean of  $X_{ij}$  and  $S_i$  is the standard deviation of the  $i$ th index. After standardization of indices, STS was calculated.

#### Results and discussion

Combined analysis of variance (Table 2) revealed significant differences between the genotypes for grain yield, spike per square meter, 1000 grain weight and genotype  $\times$  environment interaction for grain yield and 1000 grain weight. Moghadasi *et al.* (2010) also reported significant differences for number of grain per spike and 1000 grain weight between durum wheat genotypes. According to the mean comparisons (Table 3) in the irrigated condition genotypes 12,13,8 and 11 had maximum 1000 grain weight, number of grain per spike and grain yield, while in the rainfed condition genotypes 19, 2 and 9 exhibited the highest value. Talebi *et al.* (2010) and Amiri and Asade (2005) reported that number of seed per spike and grain yield reduced in the stress condition.

For better evaluation of 19 bread wheat genotypes for drought tolerance, six selection indices, including MP,

STI, TOL, GMP, SSI and HMP, were calculated (Table 4). Tolerance indices were calculated on the basis of grain yield in the irrigated and rainfed conditions. The greater the TOL value, the larger yield reduction under drought stress condition and the higher

drought sensitivity. A selection based on TOL with minimum yield reduction under stress condition in comparison with nonstress condition failed to identify the most tolerant genotypes (Farshadfar *et al.*, 2014).

**Table 1.** Genotype codes.

Code	Genotype	code	Genotype
1	Ilam-zagros	11	WC-5001
2	WC-4937	12	WC-4953s
3	Pishgam	13	WC-47359
4	WC-4958	14	Ilam-Nastoor
5	Ilam- kohdasht	15	Rigav
6	WC-4888	16	Karim
7	WC-4924	17	Sardari
8	Ilam- cross sabalan	18	WC-4594
9	WC-47615	19	Azar2
10	WC-47536	---	-----

**Table 2.** Combined analysis for traits under stress and nonstress conditions.

S.O.V	DF	Mean square			
		GY	NSP	NSS	TGW
Environment(E)	1	2110404**	10258800**	124	265**
Rep/E	4	12456	17199	418**	6.49
Genotype (G)	18	130057**	72765**	54	126**
G*E	18	40966**	35798	83	9.55*
Error	72	7257	22105	66	5.034

\* Significant at the 0.05 probability level. \*\* Significant at the 0.01 probability level.

**Table 3.** Mean comparison between bread wheat genotypes for the trait under stress and non-stress.

Genotype code	GY (g. m <sup>-2</sup> )		NSP		TGW (gr)	
	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed
1	81e	22.67h	988.7abcdef	380bcd	29.9gh	26.13k
2	692.67ab	265.33bcd	1246a	524ab	40.967bc	39.1abcd
3	488.67bcd	100.67fgh	947.3abcdef	271.33d	38.567cd	35.36defgh
4	620.67abc	220cde	784.7def	385.33bcd	40.2c	39.6abc
5	78.67e	38.67gh	729.3f	308cd	35.1def	30.8ij
6	434cd	78.67gh	1199.3ab	404bcd	28.1h	28.6jk
7	501.33bcd	77.33gh	1146.7abcde	308.67cd	33.433efg	31.93hij
8	604.67abcd	290bc	1164.7abc	580.67a	46.9h	40.53ab
9	720a	119.33efgh	1158.7abcd	319.33cd	41.367bc	37.23bcdefg
10	634abc	130efg	777.3ef	302d	38.433cd	33.66fghi
11	596abcd	455.33a	902.7abcdef	480abc	37.033cde	33.23ghi
12	610abc	186.67def	842.7bcdef	286.67d	44.367ab	42.43a
13	651.33ab	346.67b	1175.3abc	572.67a	39.033cd	34.7efghi
14	428.67cd	132.67efg	1072abcdef	316cd	34.967def	35.96cdefgh
15	153.33e	136efg	810.7cdef	514ab	37.967cd	34.43efghi
16	116e	23.33h	890abcdef	370.67bcd	39.033cd	38.46abcde
17	398.67d	106fgh	1022.7abcdef	381.33bcd	39.1cd	37.73bcdef
18	494.67bcd	68gh	973.3abcdef	280.67d	32.5fg	28.86jk
19	485.33bcd	133.33efg	908.7abcdef	356bcd	47.433a	37.63bcdef

Rosielle and Hamblin (1981) reported that selection based on the tolerance index often leads to selecting cultivars which have low yields under nonstress conditions. The greater SSI and TOL values, the greater sensitivity to stress, thus a smaller value of these indices is favored. Genotypes with lower SSI and TOL were genotypes 11, 13, 8 and 15 (tolerant genotypes). Genotypes 19 and 1 with the highest SSI and TOL value respectively were the most sensitive genotypes (Table 4). The tolerance indices GMP, STI, HMP and MP measure the higher stress tolerance and yield potential. Genotypes 11 and 13 were the most tolerant genotypes and had lower values of SSI and

TOL. Genotypes 11, 13, 8 and 2 were the most tolerant genotypes based on all quantitative indices (Table 4). According to positive and significant correlation between indices (Table 5) HMP, GMP, MP and STI indices with performance in both conditions (Yp and Ys). So, it can be stated that they are the best indices for identification of superior genotypes. The same conclusion was reported by Sio-se Marde *et al.* (2006), Golabadi *et al.* (2006) and Farshadfar *et al.* (2014). SSI and TOL displayed negative and significant correlation in the stress condition (Sharma *et al.*, 1980) HMP index showed a significant and positive correlation with the GMP and STI indices (Nourifarjam *et al.*, 2013).

**Table 4.** Estimates of stress tolerance attributes from potential yield and stress yield data for bread wheat genotypes.

Genotype code	Yp	Ys	SSI	TOL	MP	STI	GMP	HMP	b
1	81.333	22.667	1.082	58.667	52.000	0.009	42.937	35.453	58.66
2	692.667	265.333	0.926	427.333	479.000	0.859	428.705	383.690	427.33
3	488.667	100.667	1.191	388.000	294.667	0.230	221.794	166.943	388
4	620.667	220.000	0.968	400.667	420.333	0.638	369.522	324.853	400.67
5	78.667	38.667	0.763	40.000	58.667	0.014	55.152	51.848	40
6	434.000	78.667	1.228	355.333	256.333	0.160	184.774	133.191	355.33
7	501.333	77.333	1.269	424.000	289.333	0.181	196.900	133.997	424
8	604.667	290.000	0.781	314.667	447.333	0.819	418.752	391.997	314.67
9	720.000	119.333	1.252	600.667	419.667	0.401	293.121	204.734	600.67
10	634.000	130.000	1.193	504.000	382.000	0.385	287.089	215.759	504
11	596.000	455.333	0.354	140.667	525.667	1.268	520.940	516.256	140.67
12	610.000	186.667	1.041	423.333	398.333	0.532	337.441	285.858	423.33
13	651.333	346.667	0.702	304.667	499.000	1.055	475.179	452.496	304.67
14	428.667	132.667	1.036	296.000	280.667	0.266	238.474	202.624	296
15	153.333	136.000	0.170	17.333	144.667	0.097	144.407	144.147	17.33
16	116.000	23.333	1.198	92.667	69.667	0.013	52.026	38.852	92.66
17	398.667	106.000	1.101	292.667	252.333	0.197	205.569	167.472	292.67
18	494.667	68.000	1.294	426.667	281.333	0.157	183.405	119.564	426.67
19	485.333	133.333	1.088	352.000	309.333	0.302	254.384	209.195	352

**Table 5.** Simple correlation coefficients between tolerance and susceptibility indices of wheat genotype.

	Yp	Ys	SSI	TOL	MP	STI	GMP	HMP	b
Yp	1								
Ys	0.616**	1							
SSI	0.145	-0.593**	1						
TOL	0.836**	0.083	0.597**	1					
MP	0.951**	0.829**	-0.129	0.626**	1				
STI	0.710**	0.977**	-0.441	0.217	0.887**	1			
GMP	0.849**	0.936**	-0.324	0.422	0.969**	0.965**	1		
HMP	0.741**	0.980**	-0.459*	0.254	0.910**	0.986**	0.984**	1	
b	0.836**	0.083	0.597**	1**	0.626**	0.217	0.422	0.254	1
STS	0.22	0.883**	-0.884**	-0.338	0.502*	0.785**	0.676**	0.783**	-0.338

Significant at \*0.05 and \*\*0.01 probability level.

To employ all indices simultaneously, multivariate statistics principal components analysis was performed. The first two components explained 97.66% of total variation between the data (Table 6).

Thus, a biplot was drawn based on the first two factors (Fig. 1). The first component (PC1), expressed 71.64% of total variation and had a high positive relationship with Ys, Yp, STI, GMP, HMP and MP, whereas a negative coefficient with SSI (Table 6).

**Table 6.** Principle component analysis for different drought resistance indices and grain yield under stress and non-stress conditions.

PC	Eigenvalues	% of variance	Cumulative %	Yp	Ys	SSI	TOL	MP	STI	GMP	HMP
1	5.732	71.648	71.648	0.863	0.929	-0.313	0.444	0.976	0.961	0.999	0.978
2	2.081	26.015	97.663	0.496	-0.36	0.898	0.879	0.211	-0.209	-0.02	-0.192

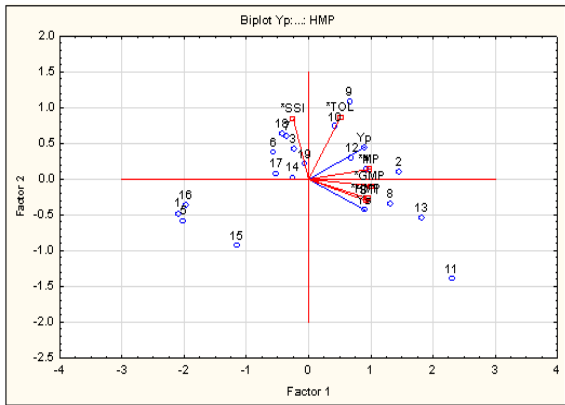
**Table 7.** analysis of STS and Discriminant.

Genotype	Discriminant	R	STS	R
1	17.71097	18	-2.97467	14
2	212.4201	4	3.540412	4
3	76.78094	13	-2.84089	13
4	176.4553	5	1.880514	6
5	30.1092	17	-1.44837	10
6	57.91927	14	-3.52442	16
7	53.58126	15	-4.11156	18
8	228.4312	3	5.040044	3
9	85.48051	11	-3.51441	15
10	99.20782	9	-2.41723	12
11	334.8112	1	11.90174	1
12	149.5297	6	0.417834	7
13	269.9714	2	7.245786	2
14	106.162	7	-0.84601	8
15	96.89894	10	2.86035	5
16	17.35391	19	-3.54274	17
17	84.00856	12	-1.88347	11
18	44.32813	16	-4.5452	19
19	106.0715	8	-1.23771	9

Therefore, the first component was named as drought tolerance. The higher scores for PC1 were in accordance with the higher rank of drought tolerance, whereas low scores for PC1 showed drought-sensitive genotypes (Table 6). The second component (PC2) accounted for 26.01% of total variation and had high communalities with TOL and SSI and a negative coefficient with Ys, GMP, STI and HMP therefore it was named as drought sensitive (Table 6). This component was able to distinguish low-yielding genotypes under stress condition with high SSI and TOL values. Regarding the principal components analysis, results for the indices and biplot were displayed based on the first two factors. The scores for the first two components for all the genotypes is in Table 6. The higher scores for PC1 and lower scores

for PC2 (part A from Fig. 1) were in accordance with the higher rank of drought tolerance. Biplot analysis (Fig. 1) showed that genotypes 11, 13, 8 and 2 were the most drought tolerant, which is in accordance with the results of discriminant analysis (genotypes with the lowest ranking were identified as the most drought tolerant and genotypes with the highest ranking were discriminated as the most drought sensitive (genotypes 18, 7, 16 and 6)).

Based on STS method, genotypes 11, 13, 8 and 4 were detected as the most drought tolerant. The results of STS and discriminant methods were the same (Table 7), but selection of superior genotypes based on STS index was easier than discriminant method.



**Fig. 1.** Biplot based on first and second components of drought tolerance indices.

Abdolshahi *et al.* (2013) used the same method for identification of drought tolerant genotypes in bread wheat. Somaye Sardouie-Nasab *et al.* (2014) employed STS for screening salt tolerant genotypes in bread wheat.

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