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Evaluation of physiological parameters as a screening technique for drought tolerance in bread wheat

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Key words: Bread wheat, physiological indicators, drought tolerance index, principal component analysis (PCA).

Abstract

In the present study, we evaluated the ability of yield based and physiological parameters for identification of drought tolerant bread wheat genotypes. The experiment was conducted in a randomized completely block design (RCBD) with three replications under two different rainfed and irrigated conditions. The results of analysis of variance exhibited significant differences between the genotypes for grain yield (GY), cell membrane stability (CMS), proline concentration (PC), relative water content (RWC), chlorophyll fluorescence (CHF), stomatal conductance (SC), relative chlorophyll content (RCC) , excised leaf water retention (ELWR) and relative water loss (RWL) indicating the presence of genetic variation and possible screening of drought tolerant genotypes. Significant correlation was found between multiple selection index (MSI) and stress tolerance index (STI). Screening drought tolerant genotypes by physiological indicators of drought tolerance using mean rank, standard deviation of ranks and biplot analysis, discriminated genotypes (18), (15), (10), (5) and (2) as the most drought tolerant. Therefore they are recommended to be used as parents for genetic analysis, gene mapping and improvement of drought tolerance in common wheat.

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Introduction

Wheat (*Triticum aestivum* L.) is one of the main crops consumed by humans and it is cultivated in different environments (Forgóné, 2009; Shewry, 2009). Extensive and high adaptation of this plant as well as its diverse consumption in the human nutrition lead to presented as the most important cereal in the word, especially in the developing countries and it can provide 20 percent food resources of the world people (Farzi *et al.,* 2010). The negative effect of drought stress on yield performance has been well studied as a major problem in many countries of the world (Moayedi *et al.,* 2010). Drought is a complex physical-chemical process, in which many biological macromolecules and small molecules are involved, such as nucleic acids (DNA, RNA, microRNA), proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements (Bayoumi *et al.,* 2008). Yield and its component traits are controlled by polygenes, whose expression is greatly affected by environments (Ahmed *et al.,* 2007). The ability of a cultivar to produce high and satisfactory yield over a wide range of stress and non-stress environments is very important. Finlay and Wilkinson (1963) believed that stability over environments and yield potential are more or less independent from each other. The ideal situation would be having a highly stable genotype with high yield potential (Smith, 1982). Although breeders are continuing to improve the yield potential of wheat, however progress in increasing wheat yield in drought environments has been more difficult to achieve. Depending on the plant growth stages, drought stress influences morphology, anatomy, physiology and biochemistry of plants (Houshmand *et al.,* 2011). There are, however, very few examples of success obtained using physiological traits in breeding programs. The main reason for this is that few of these traits have been studied in terms of their functional significance to seed yield. In addition, screening techniques using these traits have usually proved to be laborious and costly (Turner *et al.,* 2001). Physiological attributes such as relative water content (RWC), chlorophyll fluorescence (CHF), proline accumulation, abscisic acid accumulation (ABA), osmotic adjustment, root size and stomatal resistance (SR) (Blum, 1988; Loss and Siddique, 1994) are associated with drought stress tolerance/resistance. Photosynthesis, which is the most significant process influence crop production, is also inhibited by drought stress (Ashinie *et al.,* 2011). Studies have shown that the photosynthetic rate (Pn) of leaves of both C3 and C4 plants decreases as relative water content (RWC) and water potential (Ψ) decrease (Cornic and Massacci, 1996). Zlatev and Stoyanov (2005) suggested that proline accumulation of plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism. Vendruscolo *et al.* (2007) found that proline is involved in tolerance mechanisms against oxidative stress and this was the main strategy of plants to avoid determental effects of water stress. Chlorophyll fluorescence analysis may also provide a sensitive indicator of stress condition in plants. It can also be used to estimate the activity of the thermal energy dissipation in photosystem II, which protects photosytems from the adverse effect of light and heat stress. The measurement of chlorophyll fluorescence *in situ* is a useful tool to evaluate the tolerance of the photosynthetic apparatus to environmental stress (Maxwell and Johnson, 2000). Dark-adapted values of Fv/Fm reflect the potential quantum efficiency of PSII and are used as a sensitive indicator of photosynthetic performance, with optimal values of around 0.832 measured from most plant species (Johnson *et al.*, 1993). Values lower than this are measured when the plant is exposed to stress, indicating a particular phenomenon of photo-damage to PSII reaction centers, and the development of slowly relaxing quenching process (Baker and Rosenqvist, 2004) which reduce the maximum efficiency of PSII photochemistry.

The present investigation was therefore carried out to discriminate drought tolerant genotypes and screening drought tolerance criteria for improvement of drought tolerant in bread wheat.

Materials and methods

Twenty genotypes 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 completely block design with three replications under two different water environments (irrigated and rainfed) during 2010-2011 growing season in th Experimental Field of Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran (47° 9′ N, 34° 21′ E and 1319 m above sea level). Mean precipitation in 2010–2011 was 509.50 mm. The soil of experimental field was clay loam with pH7.1. Sowing was done by hand in plots with three rows 2 m in length and 20 cm apart. The seeding rate was 400 seeds per m^2 for all plots. At the rainfed experiment, water stress was imposed after anthesis. Nonstressed plots were irrigated three times after anthesis, while stressed plots received no water.

Table 1. Code and name of genotypes.

Genotype	Code	Genotype	Code
WC – 5047	1	WC – 47636	11
WC – 4530	2	WC – 4584	12
WC - 4780	3	WC – 46697 – 11	13
WC – 4566	4	WC – 4823	14
WC – 47360	5	Pishtaz	15
WC – 4640	6	WC– 47341	16
WC – 47456	7	WC – 47619	17
WC - 47628	8	WC – 4931	18
WC – 47367	9	WC – 47381	19
WC – 47399	10	WC - 5053	20

Relative water content (RWC)

Relative water content was determined according to Turner (1986), where fresh leaves were taken from each genotype and each replication after anthesis stage and weighted immediately to record fresh weight (FW). Then they were placed in distilled water for 4 h and weighted again to record turgid weight (TW), and subjected to oven drying at 70°C for 24 h to record dry weight (DW). The RWC was calculated using the following equation:

$$RWC = ((FW - DW)/(TW - DW)) \times 100$$

Relative water loss (RWL)

Five youngest fully expanded leaves were sampled for each of three replications at anthesis stage. The leaf samples were weighted (FW), wilted for4 hour at 35°C, reweighed (WW4h), and oven dried for 24 h at 72°C to obtain dry weight (DW). The RWL was calculated using the following formula (Gavuzzi *et al.*, 1997):

$$RWL (\%) = [(FM - WW4h)/(FW - DW)] \times 100$$

Excised leaf water retention (ELWR)

Excised leaf water retention was determined according to Farshadfar and Sutka (2002), where the youngest leaves before anthesis stage were collected and weighed (FW), left for 4 h, then wilted at 20°C and reweighed (WW4h). ELWR was calculated using the following formula:

$$ELWR (\%) = [1 - ((FW - WW4h)/FW))] \times 100$$

Cell membrane stability (CMS)

CMS was determined according to the method described by Sullivan (1972). For this purpose, young leaves were selected at anthesis stage from each genotype and each replication. Twenty leaf discs (1 cm in diameter) were cut from leaves and washed with deionized water to remove the solution from the injured cells. For desiccation treatment, ten leaf discs were flooded in 10 ml of 30% PEG_6000 in test tubes for 24 h at 10°C and for control treatment ten leaf discs were flooded in distilled water. Then the leaf discs were washed with deionized water. Next, 10 ml of deionized water was added to tubes, and they were maintained for 24 h at 10°C. After that, the conductivity of the solutions was determined. Finally, the tubes were boiled in a water bath for 30 min, cooled to room temperature, and the conductivity of

the solutions was read again. CMS of leaf tissues was calculated using the following equation:

CMS (%)= 100 – (1 – [(1 – T1/T2)/(1 – C1/C2)]) × 100

T1 and T2 are the first and second (after boiling) measurements of the conductivity of solutions and C1 and C2 are the respective values for the controls.

Proline concentration (PC)

The PC was determined according to the method of Bates *et al., (*1973). Plant material (0.5 g) after anthesis stage was grinded with 10 ml of 3% sulfosalicylic acid. The homogenate was filtered and 1 ml of glacial acetic acid and 1 ml acid ninhydrin reagent were added to a 1ml of filtrate. Then the mixture was shaken by hand and incubated in boiling water bath for 1 h. After that, it was transferred to ice bath and warmed to room temperature. 2 ml Toluene was added to the mixture and the upper toluene layer was measured at 520 nm using UV spectrophotometer.

Chlorophyll fluorescence (CHF)

Chlorophyll fluorescence (Fv/Fm) was measured using a Plant Stress Meter (PM) Biomonitor Sweden as described by Oquist and Wass (1988). The photochemistry efficiency of PS II was determinate based on Fv/Fm value (the ratio of variable to the maximal fluorescence of dark-adapted leaves). Prior to measurements the leaves were dark adapted for 25 min in order to relax all energy depend fluorescence quenching. Fluorescence was induced by leaf radiation 650 umol.m^{-2}.s^{-1} for 5s. The measurements were made immediately after completing the measurements of gaseous exchange parameters. All results are represented as means (9 measurements each) from independent series for each experiment and for irrigated and drought stressed plants.

Relative chlorophyll content (RCC)

The chlorophyll content in the flag leaf was determined using a chlorophyll meter (SPAD-502, Japan). Five flag leaves of each genotype grown in both rainfed and irrigated conditions were measured after anthesis stage. Three measurements at random locations in the middle of the flag leaf were made for each plant, and the average sample was used for analysis.

Stomatal conductance (SC)

Stomatal conductance *(mmol m^{-2}s^{-1})* was measured by Porometer-AP4 (Delta Devices, Cambridge, UK).

Multiple selection index (MSI)

The value of each physiological trait (RWC, RWL, ELWR, PC, CMS, CHF and SC) was first standardized for each line, after which the MSI was calculated (Farshadfar *et al,* 2003) as:

MSI = RWCstd + RWLstd + ELWRstd + PCstd + CMSstd + CHFstd + SCstd

Stress tolerance index (STI)

At maturity, after separation of border effects from each plot, yield potential (Yp) and stress yield (Ys) were measured; stress tolerance index (STI) was calculated using the following formula (Fernandez, 1992):

$$STI = \left(\frac{Y_p}{\bar{Y}_p}\right)\left(\frac{Y_s}{\bar{Y}_s}\right)\left(\frac{\bar{Y}_s}{\bar{Y}_p}\right) = \frac{(Y_p)(Y_s)}{(\bar{Y}_p)^2}$$

where Yp and Ys are the yield of a given genotype in non-stress and stress environments, respectively, and $\bar{Y}p$ is the mean yield for all genotypes in non-stress condition.

Statistical analysis

Analysis of variance, mean comparison using Duncan's multiple range test (DMRT), correlation analysis between mean of the characters measured and principal component analysis (PCA), based on the rank correlation matrix were performed by MSTAT-C and SPSS ver. 16 and STATISTICA ver. 8. Standard deviation of ranks (SDR) was measured as:

$$S_i^2 = \frac{\sum_{j=1}^{m}(R_{ij} - \bar{R}_{i.})^2}{l-1}$$

where R_{ij} is the rank of drought tolerance indicator and $\bar{R}_{i.}$ is the mean rank across all drought tolerance criteria for the ith genotype and SDR= $(S^2_i)^{0.5}$.

Rank sum (RS)= Rank mean ($\bar{R}$) + Standard deviation of rank (SDR).

Results and discussion

The results of analysis of variance for grain yield indicated the presence of a considerable genotypic variation among the genotypes under rainfed and irrigated (P < 0.01) conditions (Table 1). Combine analysis of variance (Table 2) over both conditions for grain yield showed that drought stress reduced the grain yield significantly, and the response of genotypes to drought stress varied not significantly. Duncan multiple range test (DMRT) at 5% probability level (Table 3) showed that STI was able to identify genotypes with high yield under both stressed and non-stressed conditions and to differentiate drought tolerant from drought sensitive genotypes. Fernandez (1992) suggested STI for identification of genotypes with high yield and drought tolerance. Correlation analysis revealed that STI was positively correlated with grain yield under both conditions (Table 4). Based on STI and grain yield, genotypes no. 18, 15, 5 and 2 were found drought tolerant, exhibiting high STI and grain yield under rainfed and irrigated conditions, while genotypes no. 17, 6, 4, and 20 were found drought sensitive, displaying low STI and grain yield under both conditions. Other genotypes were identified as semi-tolerant or semi-sensitive to drought stress. Three-dimensional representation of Ys, Yp and STI is shown in Figure 1. The area of the 3D plot was divided into 4 regions, a, b, c and d (Fernandez, 1992). Genotypes 2, 5, 10, 15 and 18 were placed in a region of plot, which had the highest STI, Ys and Yp (Fig. 1).

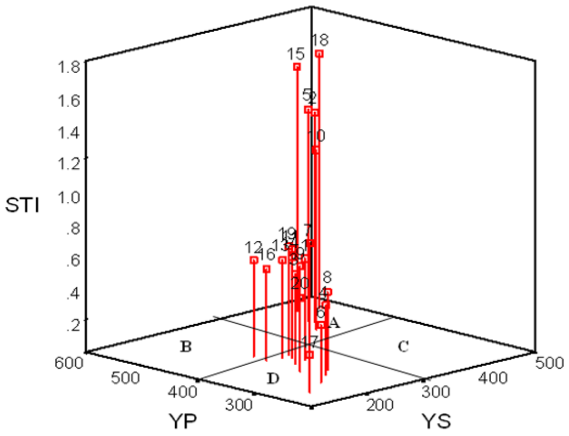

Fig. 1. Three-dimensional plot between Yp, Ys and STI.

Table 2. Analysis of variance for physiological traits in bread wheat under rainfed condition.

		Mean squares								
S.O.V	DF	Ys	RWC	PC	RWL	ELWR	CHF	RCC	SC	CMS
Replication	2	3650.52*	25.72	0.012	0.499	70.54	0.007	6.65	218.579	58.70
Genotype	19	22584.23**	262.98**	0.643**	308.88**	188.42**	0.013**	84.01**	3670.80**	295.25**
Error	38	1064.62	20.16	0.044	30.02	34.07	0.003	13.83	115.72	39.34
C. V. %		11.43	6.24	15.12	7.41	10.49	7.79	7.96	19.03	15.76

**: Significant at 1% level of probability; S.O.V: Source of variation, d.f: Degree of freedom, RWC: Relative water content, PC: Proline concentration, RWL: Relative water loss, ELWR: Excised leaf water retention, CHF: Chlorophyll fluorescence, RCC: Relative chlorophyll content, SC: Stomatal conductance, CMS: Cell membrane stability CV: Coefficient of variation.

Physiological criteria

Based on ANOVA, there were significant differences between genotypes for RWC, PC, RWL, ELWR, CMS, CHF, RCC and SC under rainfed and irrigated conditions (Table 1). Under post anthesis drought stress conditions (rainfed), relative water content (RWC) declined significantly (Table 3). In general, a decrease of RWC in drought_tolerant genotypes (genotypes no. 2, 18, 5, and 15) was lower comparing to drought_sensitive genotype (genotype no. 17). There was significant relationship between RWC and STI under drought conditions (Table 4). Siddique *et al.* (2000) found that some of the cultivars maintained higher RWC % at anthesis, yet water-stress reduced RWC % from 88 to 45%. Changes in the RWC of leaves are considered as a sensitive indicator of drought stress and more useful integrator of plant water balance than the leaf water potential (Strauss and Agenbag, 2000; Clavel *et al.*, 2005). Proline concentration (PC) of the genotypes increased under drought stress conditions comparing to irrigated conditions. Mohsenzadeh *et al.* (2006) reported that when drought condition extended to 18 days, free proline amount increased 30 times. Increases in PC have been also reported previously for wheat under drought stress by other researchers (Kocheva *et al.,* 2009; Mafakheri *et al.,* 2010). Under rainfed conditions, some of drought_tolerant genotypes accumulated more proline in the flag leaf tissues when compared to drought sensitive genotypes. Mean comparison for proline concentration (PC) showed that genotype no. 10 had the highest amount (Table 3). There was significant relationship between PC and STI under drought conditions (Table 4). A negative, significant correlation was observed between relative water loss (RWL) and STI under drought conditions (Table 3). The highest RWL and the lowest RWL were related to genotypes 20 and 10, respectively. A significant positive correlation was found between cell membrane stability (CMS) and STI indicating that the higher the CMS, the higher is drought stress (Table 3). Cell membrane stability (CMS) is a measurement of resistance induced in plants that are exposed to desiccation created artificially by polyethylene glycol (Sullivan, 1971). This result indicated that genotypes with the higher STI were characterized by the higher membrane stability. In this study, CMS was an indicator of drought tolerance. Kocheva and Georgiev (2003) revealed that cell membranes of drought_tolerant barley genotypes injured less than membranes of sensitive genotypes. Kocheva *et al.* (2004) observed that greater water loss corresponded to greater membrane damage. This is in agreement with our experiment. The highest amount of CMS and ELWR belonged to genotype no.5, while the lowest amount of CMS and ELWR was attributed to genotypes no. 20 and 17 (Table 3). CMS and ELWR indicated high and positive correlation with STI (Table 4). The high RWC and low excised_leaf water loss (RWL) have been suggested as important indicators of water status (El-Tayeb, 2006; Gunes *et al.,* 2008). Chlorophyll fluorescence (CHF), relative chlorophyll content (RCC) and stomatal conductance (SC) decreased significantly as a consequence of drought stress (Table 2); however, RCC and SC decreased differently for different genotypes. The results obtained from comparison of means exhibited that the highest amount of CHF, RCC and SC was attributed to genotype no. 18. CHF and SC indicated positive correlation with STI. Significant correlation was not observed between STI and RCC. Similarly, the Fv/Fm ratio, which characterizes the maximum yield of the primary photochemical reaction in dark-adapted leaves and frequently used as a measure of the maximal photochemical efficiency of PSII (Krause and Weis, 1991), was reduced under water deficit condition. The patterns of changes in fluorescence parameters observed in this study are supported by the pattern of change reported by many authors under drought conditions (Zlatev and Yordanov, 2004; Ashinie *et al.,* 2011). The correlation between MSI with STI was positive and significant (Table 3). A three-dimensional representation of Ys, Yp and MSI is shown in Figure 2. The area of the 3D plot was divided into 4 regions, a, b, c and d (Fernandez, 1992). Genotypes 2, 5, 10, 15 and 18 were placed in a

region of the plot, which had the highest MSI, Ys and Yp (Fig. 2).

Table 3. Mean comparison of the traits measured in stress condition.

	Ys	STI	RWC	PC	ELWR	CMS	RWL	CHF	MSI	RCC	SC
1	267.79 cd	0.62	69.99 efghi	1.32 efgh	59.86 bcde	42.68 cd	77.83 abcde	0.78 abc	0.05	37.86 f	30.67 de
2	413.84 ab	1.30	86.33 a	2.10 ab	64.11 abc	61.02 a	50.98 h	0.75 abcd	8.82	44.70 cdef	72.03 bc
3	242.38 cde	0.56	72.30 defg	1.07 ghi	56.51 cdefg	43.13 cd	80.50 abc	0.76 abc	-0.79	51.86 ab	22.13 de
4	228.25 cde	0.43	63.07 ij	1.19 fghi	51.44 defg	36.31 de	78.69 abcde	0.63 fg	-2.71	44.57 cdef	72.13 bc
5	410.82 ab	1.31	83.41 ab	1.91 bc	72.05 a	57.21 ab	68.70 efg	0.74 bcde	6.5	50.86 bcd	65.70 bc
6	199.27 ef	0.35	61.23 j	0.96 hi	56.57 cdefg	42.00 cd	83.42 ab	0.68 cdefg	-2.53	44.56 cdef	21.20 e
7	286.43 c	0.69	79.11 abcd	1.20 fghi	54.01 cdefg	32.55 def	80.70 abc	0.71 bcdef	-1.21	48.56 bcde	21.76 de
8	248.29 cde	0.49	74.09 cdef	1.14 ghi	48.59 efgh	39.97 cd	80.23 abc	0.77 abc	-1.77	54.23 ab	28.83 de
9	254.33 cde	0.59	76.63 bcde	1.03 ghi	52.49 defg	25.56 ef	73.43 bcdefg	0.64 efg	-2.17	37.76 f	24.63 de
10	383.88 b	1.12	81.46 abc	2.33 a	60.75 bcd	54.88 ab	50.18 h	0.80 ab	7.88	48.20 bcde	84.26 b
11	266.85 cd	0.66	71.61 defgh	1.38 efg	50.24 defg	33.05 def	72.21 cdefg	0.73 bcdef	-1.39	43.36 ef	42.23 d
12	230.31 cde	0.60	63.63 hij	1.53 def	45.41 gh	36.23 de	69.51 defg	0.68 cdefg	-1.99	48.73 bcde	19.93 e
13	251.76 cde	0.61	65.37 ghij	1.66 cde	52.16 defg	41.05 cd	82.07 abc	0.69 bcdef	-1.14	43.63 def	61.56 c
14	259.01 cde	0.63	60.37 j	1.40 efg	57.75 bcdef	37.24 cde	79.41 abcd	0.75 abcd	-1.74	41.06 f	117.13 a
15	435.24 ab	1.51	83.02 ab	1.87 bcd	68.50 ab	49.04 bc	63.29 g	0.74 abcd	5.66	49.10 bcde	76.20 bc
16	227.71 cde	0.57	67.16 fghij	1.13 ghi	53.77 cdefg	39.62 cd	78.07 abcde	0.58 g	-1.71	41.07 f	28.33 de
17	150.29 f	0.23	51.47 k	0.85 ij	38.89 h	33.47 def	74.17 bcdef	0.65 defg	-6.25	48.90 bcde	34.23 de
18	464.29 a	1.58	83.83 ab	1.99 abc	63.95 abc	39.56 cd	65.04 fg	0.85 a	4.26	58.06 a	123.63 a
19	267.41 cd	0.67	74.25 cdef	1.05 ghi	57.89 bcdef	27.18 ef	82.98 ab	0.79 ab	-2.23	44.20 cdef	122.63 a
20	219.42 de	0.46	70.07 efghi	0.55 j	48.35 fgh	24.11 f	87.00 a	0.69 bcdef	-5.52	51.23 bc	61.53 c
Mean	285.37	0.75	71.92	1.38	55.66	39.79	73.92	0.72	0.001	46.62	56.53

Note: Means followed by the same letter(s) in each column are not significantly different ; STI: Stress tolerance index, RWC: Relative water content, PC: Proline concentration, ELWR: Excised leaf water retention, CMS: Cell membrane stability, RWL: Relative water loss, CHF: Chlorophyll fluorescence, MSI: Multiple selection index, RCC: Relativ chlorophyll content, SC: Stomatal conductance

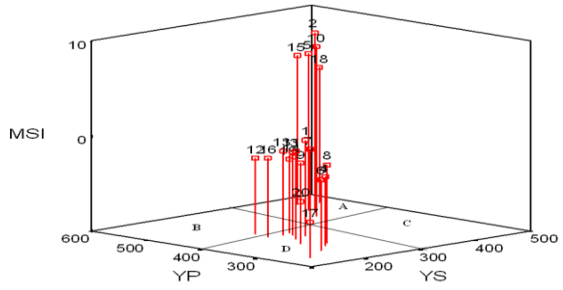

Fig .2. Three-dimensional plot based on Ys, Yp and MSI.

Screening physiological indicators and drought tolerant genotypes

(i) Ranking method

The estimates of physiological indicators of drought tolerance (Table 4) indicated that the identification of drought-tolerant genotypes based on a single criterion was contradictory. For example, according to RWC%, the desirable drought-tolerant genotype was (2), while according to PC and CHF the desirable drought-tolerant genotypes were no. (10) and (18). To have an overall judgement the following rank method was used. To determine the most desirable drought tolerant genotype according to all indices mean rank and standard deviation of ranks of all drought tolerance criteria were calculated and based on these two criteria the most desirable drought tolerant genotypes were identified. In consideration to all indices, genotypes (18), (15), (10), (5) and (2) showed the best mean rank and low standard deviation of ranks (minimum rank sum = RS) in stress condition, hence they were identified as the most drought tolerant genotypes, while genotypes (17), (20) and (6) as the most sensitive.

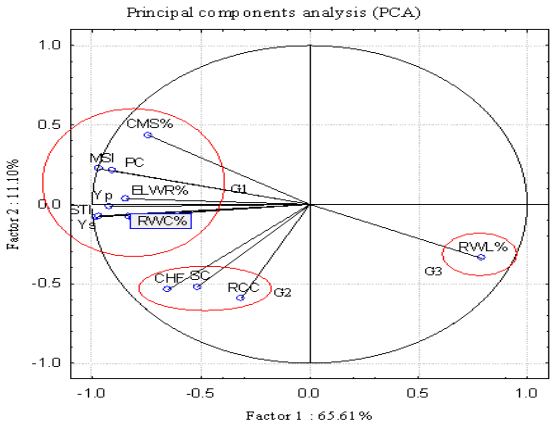

Fig 3. Biplot analysis of physiological indicators of drought tolerance.

(ii) Biplot analysis method

To better understand the relationships, similarities and dissimilarities among the physiological indicators of drought tolerance, principal component analysis (PCA), based on the rank correlation matrix was used. The main advantage of using PCA over cluster analysis is that each statistics can be assigned to one group only (Khodadadi *et al.,* 2011). The relationships among different indices are graphically displayed in a biplot of PCA_1 and PCA_2 (Fig. 3). The PCA_1 and PCA_2 axes which justify 76.71% of total variation, mainly distinguish the indices in different groups. One interesting interpretation of biplot is that the cosine of the angle between the vectors of two indices approximates the correlation coefficient between them. The cosine of the angles does not precisely translate into correlation coefficients, since the biplot does not explain all of the variation in a dataset. Nevertheless, the angles are informative enough to allow a whole picture about the interrelationships among the indices (Yan and Kang, 2003). Ys, Yp, STI, CMS%, RWC%, ELWR%, PC and MSI we refer to group 1= G1 indices. The PCs axes separated CHF, RCC and SC in a single group (G2) and RWL% in a single group (G3). The cosine of the angle between the vectors of two physiological indices approximates the correlation between them. For example, G1 indices were positively correlated (an acute angle), the same conclusion was obtained for the G2 indices, while G1 was negatively correlated with G3 indices (an obtuse angle). Independence (right angle), negative (obtuse angle) or very weak correlation (almost right angle) was observed between G1 with G2 and G2 with G3 physiological indices.

Table 4. Simple correlation coefficients among physiological traits with grain yield and STI in bread wheat genotypes.

	RWC	PC	CMS	RWL	ELWR	RCC	CHF	SC	Ys	STI	MSI
RWC	1										
PC	0.612**	1									
CMS	0.463*	0.783**	1								
RWL	-0.575**	-0.845**	-0.697**	1							
ELWR	0.733**	0.662**	0.671**	-0.461*	1						
RCC	0.298	0.175	0.155	-0.147	0.77	1					
CHF	0.550*	0.511*	0.337	-0.351	0.524*	0.442	1				
SC	0.279	0.426	0.116	-0.256	0.465*	0.156	0.557*	1			
Ys	0.865**	0.844**	0.639**	-0.731**	0.842**	0.346	0.625**	0.529*	1		
STI	0.822**	0.840**	0.626**	-0.734**	0.822**	0.354	0.585**	0.526*	0.990**	1	
MSI	0.798**	0.916**	0.855**	-0.842**	0.829**	0.197	0.537*	0.368	0.918**	0.898**	1

*, **: Significant at 0.05 and 0.01 level of probability, respectively; RWC: Relative water content, PC: Proline concentration, CMS: Cell membrane stability, RWL: Relative water loss, ELWR: Excised leaf water retention, RCC: Relative chlorophyll content, CHF: Chlorophyll fluorescence, SC: Stomatal conductance, Ys: grain yield under rain-fed, STI: Stress tolerance index, MSI: Multiple selection index.

Table 5. Ranks (R), ranks mean ($\overline{R}$) and standard deviation of ranks (SDR) of physiological indicators of drought tolerance.

Genotype no.	Ys	R	Yp	R	STI	R	RWC	R	PC	R	CMS	R	RWL	R
1	267.79	7	377.71	13	0.62	10	69.99	13	1.32	10	42.68	6	77.83	11
2	413.84	3	507.12	4	1.30	4	86.33	1	2.10	2	61.02	1	50.98	1
3	242.38	14	370.39	15	0.56	15	72.30	10	1.07	15	43.13	5	80.5	15
4	228.25	16	301.39	18	0.43	18	63.07	17	1.19	12	36.31	13	78.69	12
5	410.82	4	516.40	3	1.31	3	83.41	3	1.91	4	57.21	2	68.70	5
6	199.27	19	279.75	19	0.35	19	61.23	18	0.96	18	42.00	7	83.42	19
7	286.43	6	388.17	12	0.69	6	79.11	6	1.20	11	32.55	17	80.70	16
8	248.29	13	317.46	17	0.49	16	74.09	9	1.14	13	39.97	9	80.23	14
9	254.33	11	372.61	14	0.59	13	76.63	7	1.03	17	25.56	19	73.43	8
10	383.88	5	472.81	5	1.12	5	81.46	5	2.33	1	54.88	3	50.18	2
11	266.85	9	400.34	10	0.66	8	71.61	11	1.38	9	33.05	16	72.21	7
12	230.31	15	429.76	6	0.60	12	63.63	16	1.53	7	36.23	14	69.51	6
13	251.76	12	401.62	9	0.61	11	65.37	15	1.66	6	41.05	8	82.07	17
14	259.01	10	391.74	11	0.63	9	60.37	19	1.40	8	37.24	12	79.41	13
15	435.24	2	560.58	1	1.51	2	83.02	4	1.87	5	49.04	4	63.29	3
16	227.71	17	404.84	8	0.57	14	67.16	14	1.13	14	39.62	10	78.07	10
17	150.29	20	250.78	20	0.23	20	51.47	20	0.85	19	33.47	15	74.17	9
18	464.29	1	547.87	2	1.58	1	83.83	2	1.99	3	39.56	11	65.04	4
19	267.41	8	406.95	7	0.67	7	74.25	8	1.05	16	27.18	18	82.98	18
20	219.42	18	337.35	16	0.46	17	70.07	12	0.5566	20	24.11	20	87.00	20

Table 5 continued.

Genotype no.	ELWR	R	RCC	R	CHF	R	SC	R	MSI	R	RS	$\overline{R}$	SDR
1	59.86	6	37.86	19	0.78	4	30.67	13	0.05	8	14.15	10.00	4.15
2	64.11	3	44.70	11	0.75	7	72.03	7	8.82	1	6.88	3.75	3.13
3	56.51	10	51.86	3	0.76	6	22.13	17	-0.79	10	15.88	11.25	4.63
4	51.44	15	44.57	12	0.63	19	72.13	6	-2.71	13	17.92	14.25	3.67
5	72.05	1	50.86	5	0.74	9	65.70	8	6.50	3	6.48	4.16	2.32
6	56.57	9	44.56	13	0.68	15	21.20	19	-2.53	18	20.31	16.08	4.23
7	54.01	11	48.56	9	0.71	12	21.76	18	-1.21	12	15.49	11.33	4.16
8	48.59	17	54.23	2	0.77	5	28.83	14	-1.77	15	16.78	12.00	4.78
9	52.49	13	37.76	20	0.64	18	24.63	16	-2.17	17	18.59	14.41	4.18
10	60.75	5	48.20	10	0.80	2	84.26	4	7.88	2	6.48	4.09	2.39
11	50.24	16	43.36	16	0.73	11	42.23	11	-1.39	11	14.38	11.25	3.13
12	45.41	19	48.73	8	0.68	16	19.93	20	-1.99	16	17.92	12.91	5.01
13	52.16	14	43.63	15	0.69	13	61.56	9	-1.14	9	14.87	11.50	3.37
14	57.75	8	41.06	18	0.75	8	117.13	3	-1.74	6	15.03	10.41	4.62
15	68.50	2	49.10	6	0.74	10	76.20	5	5.66	4	6.41	4.00	2.41
16	53.77	12	41.07	17	0.58	20	28.33	15	-1.71	14	17.13	13.75	3.38
17	38.89	20	48.90	7	0.65	17	34.23	12	-6.25	20	21.34	16.59	4.75
18	63.95	4	58.06	1	0.85	1	123.63	1	4.26	5	5.89	3.00	2.89
19	57.89	7	44.20	14	0.79	3	122.63	2	-2.23	7	15.1	9.59	5.51
20	48.35	18	51.23	4	0.69	14	61.53	10	-5.52	19	20.56	15.66	4.90

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