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Screening of Drought Tolerant genotypes in Bread Wheat (*Triticum aestivum*) using Morpho-physiological Traits and Integrated Selection Index

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ABSTRACT: Drought is one of the major factors limiting crop production in arid and semi-arid regions. In order to identify drought tolerant bread wheat genotypes using agro-morphological traits, physiological criteria and new integrated selection index, 20 bread wheat genotypes were studied in a randomized complete block design (RCBD) with three replications under irrigated and rainfed conditions. The results of ANOVA in the rainfed condition showed significant differences for all the characters investigated except for Chlorophyll a, b (Chl a, Chl b) and relative chlorophyll content (RCC), indicating the presence of genetic variation and possibility of selection for drought tolerant genotypes under drought condition. Dunkan's multiple rang test revealed that the genotype (18), (15) and (3) had higher grain's yield while genotypes (10) and (11) exhibited lower value for these trait under rainfed condition. In our study, genotypes (10), (4) and (11) displayed the lowest and genotypes (18), (3) and (19) the highest values for integrated selection index (ISI). Principal component analysis (PCA) showed that the integrated selection index (ISI) was correlated with relative water loss (RWL), Chlorophyll a (Chl a), Total chlorophyll (TChl) and grain yield under irrigation and rainfed conditions. The Results indicating that these screening techniques can be useful for selecting drought tolerant genotypes. In consideration to all indices, genotypes (18), (3) and (6) showed the best mean rank and low rank and rank sum in water deficit stress condition, hence they were identified as the most drought tolerant genotypes which is almost in agreement with the results of our new index (ISI), while genotypes (10), (4) and (11) as the most sensitive. Therefore, this genotype recommended to be used as parents for genetic analysis, gene mapping and improvement of drought tolerance in bread wheat.

Key words: Bread wheat, Morphological traits, Physiological indicators, Integrated selection index, Water deficit stress condition

INTRODUCTION

Plants are exposed to numerous stress factors during their lives, which is of a significant effect on the growth of plants. Biotic (pathogen, competition with other organisms) and abiotic (drought, salinity, radiation, high temperature or freezing etc.) stresses cause changes in normal physiological functions of all plants, including economically important cereals as well. All these stresses reduce biosynthetic capacity of plants and might cause some destructive damages on the plants (Lichtenhaler, 1996). Drought is a significant limiting factor for agricultural productivity and generally inhibits plant growth through reduced water absorption and nutrient uptake. Decreased water availability generally results in reduced growth and final yield in crop plants. Plant drought tolerance is a highly complex trait that involves multiple genetic, physiological and biochemical mechanisms (Baik and Ullrich, 20008; Erdei *et al.*, 2002). The degree of plant drought tolerance differs not only among various species but also among different varieties of the same species. Field methods of evaluating the degree of drought tolerance allow a direct or indirect estimation of the variation of determinate traits in the examined genotypes. Measurements of different physiological processes for plants responses to drought are important information on the various strategies of the plant intended to remove or to reduce the harmful effects of water deficit in soil or plant tissues. In the field indices of drought tolerance, the preference was given to the relations between the plant yield obtained under conditions of drought and that under conditions of optimum soil moistening. Agronomic traits such as grain yield and its components are the major selection criteria for evaluating drought tolerance under field conditions (Dencic *et al.*, 2000). Wheat (*Triticum aestivum L.*) is the world's widely adapted crop, providing one third of the world population with more than half of their calories and nearly half of their protein. Wheat is mainly grown on rainfed lands and about 35% of the area of developing countries consists of semiarid environments in which the available moisture constitutes a primary constraint on wheat production (Rajram, 2001).

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 (Farshadfar *et al.,* 2001; Anwar *et al.,* 2011). A physiological approach would be the most attractive way to develop new varieties (Araus *et al.,* 2008) but breeding for specific, sub-optimal environments involves a deeper understanding of yield-determining process. Generally, different strategies have been proposed for the selection of relative drought tolerance and resistance, so, some researchers have proposed selection under non-stress conditions (Richards, 1996; Rajaram and Van Ginkle, 2001; Betran *et al.,* 2003), others have suggested selection in the target stress conditions (Ceccarelli and Grando, 1991; Rathjen, 1994) while, several of them have chosen the mid-way and believe in selection under both non-stress and stress conditions (Fischer and Maurer, 1978; Clarke *et al.,* 1992; Fernandez, 1992; Byrne *et al.,* 1995). Dencic *et al.,* (2000) reported that many morphological and physiological characteristics were affected by drought stress. Gupta *et al.,* (2001) studied physiological and yield attributes of two wheat genotypes with stress at boot and anthesis. They reported that number of grains, grain yield, biological yield, and harvest index decreased to a greater extent when water stress was imposed at anthesis stage.

The objectives of the present investigation reported here include: (i) identification of drought tolerant wheat genotypes and (ii) assessment of a new integrated selection index (ISI) of agronomic and physiological indicators for screening drought tolerant entries.

MATERIALS AND METHODS

A. Description of the project site and growth conditions

To investigate the effects of water deficit stress in 20 genotypes bread wheat listed in Table 1 were provided from Seed and Plant Improvement Institute of Karaj, Iran. They were assessed in a randomized complete block design (RCBD) with three replications under two irrigated and rainfed conditions during 2010-2011 growing season in the experimental field of the College of Agriculture, Razi University, Kermanshah, Iran (47° 9N, 34° 21E and 1319 m above sea level). Mean precipitation in 2010–2011 was 509.50 mm. The soil of experimental field was clay loam with pH 7.1. Sowing was done by hand in plots with six 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.

Non-stressed plots were irrigated three times after anthesis, while stressed plots received no water. Fertilizer was applied at 41 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5 and planting was according to the provincial soil test recommendations before sowing. Irrigation was performed in the non-stressed site at the flowering stage. The names of genotypes showed in Table 1.

Table 1: Genotype codes.

Genotype	Name	Genotype	Name
G1	WC-47536	G11	WC-47637
G2	WC-47620	G12	WC-47400
G3	phishtaz	G13	WC-47473
G4	pishgam	G14	WC-47371
G5	WC-47374	G15	WC-47615
G6	WC-47632	G16	WC-47388
G7	WC-47358	G17	WC-5050
G8	WC-4987	G18	WC-47359
G9	WC-5045	G19	WC-47619
G10	WC-47617	G20	WC-47379

B. Crop sampling and calculation

Agronomic characteristics and physiological criteria including: relative water loss (RWL), relative water content (RWC), relative chlorophyll content (RCC), spike length (SL-cm), number of grains per spike (NGS), 1000-grains weight (GW-gr), grain yield (gr), Chlorophyll fluorescence (CHF), Stomatal conductance (SC) and Chlorophyll a, b and total (Chl a, Chl b, TChl) were measured after the physiological maturity in 10 selected plants of each experimental plot, randomly. At harvest time, yield potential (Yp) and stress yield (Ys) were measured from 2 rows 1 m in length.

Relative chlorophyll content (RCC): Physiological criteria were used for flag leaf measurement. The chlorophyll content in the flag leaf was determined using a chlorophyll meter (SPAD-502, Japan). Ten flag leaves of each genotype grown in stress and non-stress conditions were measured after tillering stage. Three measurements in the middle of the flag leaf were made randomly for each plant, and the average sample was used for analysis.

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

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

Relative water loss (RWL): Relative water loss (RWL) was determined according to Gavuzzi *et al.*, (1997) ten young fully expanded leaves were sampled for each of three replications at anthesis stage. The leaf samples were weighed (FW), wilted for 4hour 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:

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

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

Chlorophyll a, b and total (Chl a, Chl b, TChl): Chlorophylls a and b were measured by the method described by Horii *et al.*, (2007) with a slight modification after anthesis stage. 3 ml of 99.5% methanol was added to the leaf tissue (50 mg) and incubated in dark for 2h. Samples were homogenized and centrifuged at 10000 rpm for 10 min. Absorbance of the samples at 650 nm and 665 nm was measured by the UV spectrophotometer. Absolute methanol (99.5%) was used as a blank. Chl a, Chl b and TChl content was calculated using following equations:

Chlorophyll a (μg/mL) = $16.5 \times A665 - 8.3 \times A650$

Chlorophyll b (μg/mL) = $33.8 \times A650 - 12.5 \times A665$

Total chlorophyll (μg/mL) = $25.8 \times A650 + 4.0 \times A665$

Integrated Selection Index (ISI): ISI was calculated based on factor analysis of physiological traits under water deficit and the following three formulas:

(1) $Sij = (Xi j - \mu j)/ \sigma j$

(2) $MPij = (Sijd + Sijw)/2$

(3) $ISI_i = b_1MPi_1 + b_2MPi_2 + \ldots + b_jMP_{ij}$

where Sij= is the standardized physiological value of trait j (j =1 to 12) in genotype i under irrigated and drought conditions, Xij = physiological and agro-morphological value of genotype i on trait j, μ j= mean value of trait j in all genotypes, σj= the standard deviation of trait j, MPij= the mean productivity of trait j on genotype i, bj the weight value of trait j, bj was populated from the average contribution to factor 1 and ISI = integrated selection index.

Formula (1) standardizes the value of different traits to the same unit of measure; formula (2) evaluates the appearance of genotype for each trait; and formula (3) integrates the appearance of genotypes for all traits. When defining weight values for each trait, average contribution of factor 1 to 12 major traits related to drought resistance at irrigated and water deficit stress conditions in the factor analysis were considered as bj and trait had negative functions in the final result (Table 2). Using physiological and agro-morphological data of irrigated and water deficit conditions, the formerly proposed selection index was calculated related to drought resistance.

Table 2: Contribution of factor 1 to 12 of the major traits related to drought resistance under rainfed and irrigated condition.

Trait	rainfed	irrigated
RWC	0.370	0.615
RWL	-0.588	-0.049
SC	0.261	0.053
Chl a	0.759	0.728
Chl b	0.216	0.515
TChl	0.735	0.797
CHF	0.282	0.334
RCC	0.624	0.568
NGS	0.335	0.609
SL	0.457	0.330
GW	0.425	0.274
Grain Yield	0.662	0.784

RWC, RWL, SC, Chl a, Chl b, TChl, CHF, RCC, NGS, SL and GW indicate; relative water content, relative water loss, Stomatal conductance, Chlorophyll a, Chlorophyll b, Chlorophyll total, Chlorophyll fluorescence, relative chlorophyll content, number of grains per spike, spike length, 1000-grains weight

C. Statistical analysis

Analysis of variance, principal component analysis (PCA), based on the rank correlation matrix were performed by SPSS ver. 20 and Statistica ver. 8 software's. 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 Rij is the rank of in vivo drought tolerance indicator and $\bar{R}_{i.}$ is the mean rank across all drought tolerance indicators for the its genotype and SDR= $(S^2_i)^{0.5}$, Rank sum (RS) = Rank mean ($\bar{R}$) + Standard deviation of rank (SDR) (Farshadfar and Elyasi, 2012).

RESULTS AND DISCUSSION

A. Anova analysis

The results of ANOVA in the rainfed condition showed significant differences for all the characters investigated except for Chlorophyll a, b (Chl a, Chl b) and relative chlorophyll content (RCC), indicating the presence of genetic variation and possibility of selection for drought tolerant genotypes under drought condition (Table 3). Also, except traits relative water loss (RWL), relative water content (RWC), relative chlorophyll content (RCC) and Chlorophyll a (Chl a) the results of variance analysis showed significant differences in terms of all traits expected for irrigated condition (Table 4). This indicates that the magnitude of differences in genotypes was sufficient to select them against drought. Also, similar observations have been reported in bread wheat (Farshadfar, 2012). Kutlu and Kinaci (2010) also reported similar results for agro-morphological traits and grain yield in both stress and non-stress conditions.

B. Comparison mean

Dunkan's multiple rang test (Table 5) revealed that the genotype (18), (15) and (3) had higher grain's yield (YS) while genotypes (10) and (11) exhibited lower value for these trait under rainfed condition.

Persistence in relative water content (RWC) content of genotype in water stress conditions may serve as good indicator of drought tolerance. Genotypes (14), (18) and (17) had higher relative water content (RWC) content while genotypes (5), (19) and (20) displayed lower relative water content (RWC) under water stress. In general, this genotypic variation in these characteristics may be attributed to differences in the ability of the variation to absorb more water from the soil and or the ability to control water loss through the stomata's. Merah (2001) reported that RWC % was an important indicator of water stress in leaves. The highest relative water loss (RWL) and lowest were related to genotypes (4) and (1), respectively. These results are consistent with our experiment.

The high RWC and low RWL have been suggested as important indicators of water status (Farshadfar *et al.*, 2001; Gunes *et al.*, 2008; Farshadfar *et al.*, 2011b).

Chlorophyll fluorescence (CHF), relative chlorophyll content (RCC) and stomatal conductance (SC) were decreased significantly as a consequence of drought stress (Table 5); however, the Chlorophyll fluorescence (CHF), relative chlorophyll content (RCC) for different genotypes were decreased differently.

The result obtained from comparison of means exhibited that the highest amount of Chlorophyll fluorescence (CHF) and relative chlorophyll content (RCC) were attributed to genotype (18). Genotypes (14) and (8) had higher stomatal conductance (SC) content while genotype (17) showed lower stomatal conductance (SC) under water stress. The highest Chl a, Chl b and TChl belonged to the genotypes (20), (14) and (18) respectively.

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 PS II (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; Farshadfar *et al.*, 2011a).

The results exhibited that the highest amount of relative number of grains per spike (NGS) was attributed to genotypes (3), (17) and (6). Genotypes (14) and (17) displayed higher spike length (SL) while genotype (9) showed lower spike length (SL) under rainfed condition (Table 5).

Genotypes (3), (12) and 20 had higher 1000-grains weight (GW) while genotypes (7) and (10) exhibited lower value for these traits under rainfed condition.

In general, genotypes (15), (18) and (3) had the highest amount of grain yield and yield components in water deficit stress conditions. An integrated selection index (ISI), was proposed as an index of drought tolerance and used to discriminate drought tolerant genotypes. In this index, 12 traits including relative water loss (RWL), relative water content (RWC), relative chlorophyll content (RCC), spike length (SL), number of grains per spike (NGS), 1000-grains weight (GW), grain yield, Chlorophyll fluorescence (CHF), Stomatal conductance (SC) and Chlorophyll a, b and total (Chl a, Chl b, TChl) were chosen as the most relevant factors related to drought tolerance, as determined by statistical analysis. In our study, genotypes (9), (4) and (11) displayed the lowest and genotypes (3), (18) and (19) the highest values for ISI.

Table 3: Analysis of variance for agro-morphological trait and physiological criteria in rainfed condition.

S.O.V	df	RWC	RWL	SC	Chl a	Chl b	TChl	CHF	RCC	NGS	SL	GW	YS
Replication	2	1585.31**	108.95^{ns}	9823.71**	0.51^{ns}	0.83^{ns}	0.25 ^{ns}	0.012**	58.24^{ns}	2.95^{ns}	0.41 ^{ns}	2.20 ^{ns}	0.041**
Genotype	19	218.38**	596.40**	2474.42**	3.06^{ns}	0.55^{ns}	3.89**	0.015**	34.14^{ns}	140.25**	4.59**	69.54**	0.03**
Error		72.84	239.58	763.84	0.52	0.42	0.54	0.002	24.82	41.79	0.60	2.02	0.003
CV(%)		11.32%	22.70%	36.65%	19.15%	44.22%	13.88%	19.81%	10.37%	13.14%	6.90%	3.76%	8.62%

*and ** Significant at 5% and 1% level of probability, respectively
RWC, RWL, SC, Chl a, Chl b, TChl, CHF, RCC, NGS, SL, GW and YS indicate; relative water content, relative water loss, Stomatal conductance, Chlorophyll a, Chlorophyll b, Chlorophyll total, Chlorophyll fluorescence, relative chlorophyll content, number of grains per spike, spike length, 1000-grains weight and stress yield, respectively.

Table 4: Analysis of variance for agro-morphological trait and physiological criteria in irrigated condition.

S.O.V	df	RWC	RWL	SC	Chl a	Chl b	TChl	CHF	RCC	NGS	SL	GW	YP
Replication	2	858.46**	567.02**	6792.24*	0.311 ^{ns}	0.10 ^{ns}	1.50 ^{ns}	0.017**	43.96 ^{ns}	27.54 ^{ns}	0.51 ^{ns}	0.34 ^{ns}	0.02^{ns}
Genotype	19	155.64^{ns}	227.75^{ns}	1900.52^{ns}	10.006**	0.74 ^{ns} s	13.28**	0.005**	29.41 ^{ns}	82.41**	5.78**	112.38**	0.03**
Error		116.27	135.62	1630.89	1.06	0.51	1.05	0.002	31.15	14.59	0.75	1.52	0.01
CV(%)		15.31%	17.19%	36.31%	17.59%	42.01%	13.06%	23.73%	11.51%	8.04%	7.77%	2.90%	14.43%

*and ** Significant at 5% and 1% level of probability, respectively
RWC, RWL, SC, Chl a, Chl b, TChl, CHF, RCC, NGS, SL, GW and YP indicate; relative water content, relative water loss, Stomatal conductance, Chlorophyll a, Chlorophyll b, Chlorophyll total, Chlorophyll fluorescence, relative chlorophyll content, number of grains per spike, spike length, 1000-grains weight and yield potential, respectively.

Table 5: Mean comparison of the traits measured in stress and non stress conditions.

Trait	RWC		RWL		SC		Chl a		Chl b		TChl	
Condition	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated
1	78.00 ABC	72.00 ABC	30.33 D	61.33 BCD	50.47 DE	144.2 AB	2.307 G	4.433 EF	0.936 C	2.040 CDE	3.270 H	7.557 DEFGH
2	73.33 BC	66.33 BC	66.33 ABC	63.67 ABCD	74.87 CDE	158.3 A	3.693 DEFG	6.950 BCD	2.530 A	2.233 BC	6.307 BC	9.237 BCD
3	78.33 ABC	74.67 ABC	48.33 CD	66.67 ABCD	57.90 DE	105.9 AB	3.677 DEFG	5.127 CDEF	1.54 ABC	1.793 FG	5.227 CDEF	7.140 EFGHI
4	71.33 BC	67.00 BC	79.67 A	71.33 ABCD	64.47 DE	101.7 AB	3.480 DEFG	4.003 F	1.220 C	2.333 B	4.737 DEFG	6.380 GHI
5	51.67 D	76.33 ABC	62.33 ABC	58.00 CD	42.60 DE	141.8 AB	2.967 EFG	5.703 CDEF	2.51 AB	2.887 A	5.530 BCDEF	8.663 BCDE
6	82.33 ABC	70.00 ABC	62.67 ABC	61.33 BCD	78.57 CDE	122.9 AB	4.187 BCDE	6.170 BCDE	1.57 ABC	1.713 GH	5.800 BCD	7.950 CDEFG
7	81.67 ABC	77.67 ABC	68.67 ABC	64.67 ABCD	80.77 CDE	85.37 AB	3.423 DEFG	4.820 EF	1.65 ABC	2.067 CD	5.110 CDEFG	6.943 EFGHI
8	79.00 ABC	74.33 ABC	68.67 ABC	73.33 ABCD	132.0 AB	132.0 AB	3.927 CDEF	5.840 BCDEF	1.75 ABC	1.653 GH	5.387 CDEFG	7.580 CDEFGH
9	75.00 ABC	67.67 BC	77.00 ABC	60.67 CD	76.67 CDE	111.6 AB	2.603 FG	4.863 EF	1.34 ABC	1.070 K	3.980 GH	6.643 FGHI
10	77.00 ABC	66.33 BC	72.33 AB	75.33 ABC	60.90 DE	109.1 AB	2.860 EFG	4.673 EF	1.47 ABC	1.190 JK	4.340 EFGH	5.910 HI
11	71.33 BC	69.33 ABC	71.33 AB	67.67 ABCD	66.00 CDE	124.9 AB	2.920 EFG	4.273 EF	1.357 ABC	1.517 HI	4.307 FGH	5.390 I
12	76.33 ABC	58.00 C	64.00 ABC	62.33 ABCD	64.30 CDE	99.40 AB	3.053 EFG	5.033 DEF	0.99 C	0.7233 L	4.070 GH	5.803 HI I
13	74.67 ABC	69.33 ABC	68.33 ABC	73.00 ABCD	44.73 DE	93.53 AB	3.513 DEFG	5.083 DEF	1.280 ABC	1.617 GH	4.827 DEFG	6.807 EFGHI
14	91.00 A	63.67 BC	59.33 ABC	59.67 CD	59.50 DE	83.53 AB	5.290 B	7.123 BC	1.187 C	1.343 IJ	6.890 B	8.533 BCDEF
15	72.00 BC	61.00 BC	69.00 ABC	84.00 AB	92.57 BCD	74.05 B	3.827 DEF	6.087 BCDE	1.510 ABC	1.830 EFG	5.377 CDEFG	10.22 B
16	71.67 BC	61.67 BC	73.67 AB	77.00 ABC	143.9 A	148.8 AB	4.490 BCD	5.517 CDEF	1.237 BC	2.000 DEF	5.770 BCDE	7.580 DEFGH
17	87.33 AB	72.00 ABC	69.33 ABC	50.33 D	33.63 E	75.00 B	3.997 BCDE	5.287 CDEF	1.017 C	1.227 JK	4.853 DEFG	6.563 FGHI
18	83.67 AB	89.00 A	53.67 BCD	85.00 A	86.07 BCDE	85.53 AB	5.177 BC	5.980 BCDE	1.130 C	1.510 HI	6.780 B	7.550 CDEFGH
19	66.00 CD	80.00 AB	69.00 ABC	68.33 ABCD	80.00 CDE	102.2 AB	3.413 DEFG	12.44 A	1.757 ABC	2.383 B	5.213 CDEFG	14.97 A
20	65.67 CD	72.00 ABC	58.00 BCD	71.33 ABCD	118.1 ABC	124.6 AB	6.547 A	7.690 B	1.570 ABC	1.750 G	8.187 A	9.513 BC

RWC, RWL, SC, Chl a, Chl b, TChl, CHF, RCC, NGS, SL, GW and Yield indicate; relative water content, relative water loss, Stomatal conductance, Chlorophyll a, Chlorophyll b, Chlorophyll total, Chlorophyll fluorescence, relative chlorophyll content, number of grains per spike, spike length, 1000-grains weight and Grain Yield respectively.

Table 6: Mean comparison of the traits measured in stress and non stress conditions.

Trait	CHF		RCC		NGS		SL		GW		Grain Yield	
Condition	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated	rainfed	irrigated
1	0.1400 E	0.1967 ABCD	49.33 A	50.50 ABC	46.87 ABCDE	47.33 BCDEF	10.78 BCDE	11.47 CDEF	40.68 CD	46.76 CD	0.6500 BCD	0.7967 ABCDE
2	0.1767 E	0.1300 CD	51.93 A	50.13 ABCD	46.07 ABCDE	52.53 ABC	10.17 EF	10.49 FGH	36.35 EFGH	38.12 JKL	0.5533 DEFG	0.7800 CDE
3	0.3433 AB	0.1667 BCD	46.50 AB	50.30 ABC	57.07 A	57.47 A	12.26 B	14.12 AB	47.49 A	56.89 A	0.7267 AB	0.8200 ABCDE
4	0.1600 E	0.1700 BCD	44.30 AB	43.40 CDE	44.27 BCDE	37.33 H	10.71 CDE	9.700 GH	25.88 J	29.76 N	0.4900 FGH	0.7233 DE
5	0.2100 DE	0.2633 A	47.20 AB	48.43 ABCD	48.13 ABCDE	47.80 BCDEF	12.23 B	12.38 CDE	35.83 EFGHI	40.64 HI	0.5367 EFGH	0.9500 ABCD
6	0.2133 DE	0.1600 CD	51.40 A	52.10 AB	58.27 A	51.40 ABCD	12.27 B	12.55 CD	35.66 FGHI	39.68 HIJ	0.6300 BCDE	0.8200 ABCDE
7	0.2167 DE	0.1467 CD	51.70 A	43.10 DE	56.27 AB	56.07 A	11.72 BCD	11.13 CDEFG	33.40 I	36.90 KLM	0.5500 DEFG	0.9833 ABC
8	0.2067 DE	0.1100 D	47.17 AB	49.90 ABCD	49.20 ABCDE	48.33 BCDEF	10.63 CDE	11.49 CDEF	38.09 EF	41.59 GH	0.6500 BCD	1.023 AB
9	0.3067 BC	0.2167 ABC	46.87 AB	41.23 E	36.87 E	44.80 DEFG	11.20 BCDE	10.63 FG	35.21 GHI	38.78 IJK	0.5033 FGH	0.7367 DE
10	0.1633 E	0.1567 CD	50.23 A	47.77 ABCDE	56.27 AB	45.87 CDEFG	8.967 F	8.933 H	33.54 I	35.85 M	0.4433 H	0.6733 E
11	0.3333 AB	0.2033 ABC	44.73 AB	54.18 A	36.87 E	39.67 GH	11.54 BCDE	11.05 CDEFG	42.14 BC	47.40 CD	0.4600 GH	0.7067 E
12	0.2667 BCD	0.2500 AB	45.23 AB	47.33 ABCDE	48.47 ABCDE	48.87 BCDE	11.37 BCDE	11.00 DEFG	44.04 B	48.37 C	0.5100 FGH	0.8033 ABCDE
13	0.2033 DE	0.1367 CD	39.30 B	47.37 ABCDE	55.80 ABC	46.00 CDEFG	10.21 DEF	10.00 FGH	36.19 EFGH	43.81 EF	0.5367 EFGH	0.7867 BCDE
14	0.2267 CDE	0.2033 ABC	46.87 AB	50.70 AB	56.60 AB	47.60 BCDEF	14.03 A	14.23 A	37.39 EFG	42.91 F	0.4967 FGH	0.7767 CDE
15	0.2800 BCD	0.1667 BCD	47.43 AB	50.50 ABC	46.00 ABCDE	48.13 BCDEF	11.17 BCDE	11.13 CDEFG	42.90 BC	45.85 DE	0.8033 A	0.9800 ABC
16	0.2033 DE	0.1300 CD	48.53 AB	46.93 BCDE	40.53 DE	41.13 FGH	10.13 EF	10.57 FGH	34.56 HI	36.22 LM	0.5833 CDEF	0.7767 CDE
17	0.1800 E	0.1300 CD	49.03 AB	47.63 ABCDE	58.07 A	44.07 DEFGH	13.79 A	12.70 BC	38.47 DE	44.08 EF	0.6700 BC	0.7767 CDE
18	0.4033 A	0.1433 CD	52.43 A	49.97 ABCD	49.93 ABCD	54.33 AB	11.97 BC	9.800 FGH	37.00 EFGH	51.27 B	0.7667 A	1.030 A
19	0.1700 E	0.1600 CD	47.13 AB	48.33 ABCD	49.20 ABCDE	49.00 BCDE	10.55 CDE	10.73 EFG	38.21 EF	40.81 GHI	0.5400 EFGH	0.9933 ABC
20	0.1567 E	0.1300 C	53.73 A	50.47 ABC	43.20 CDE	42.27 EFGH	10.23 DEF	10.20 FGH	44.37 B	45.70 DE	0.5700 CDEF	0.8200 ABCDE

RWC, RWL, SC, Chl a, Chl b, TChl, CHF, RCC, NGS, SL, GW and Yield indicate; relative water content, relative water loss, Stomatal conductance, Chlorophyll a, Chlorophyll b, Chlorophyll total, Chlorophyll fluorescence, relative chlorophyll content, number of grains per spike, spike length, 1000-grains weight and Grain Yield respectively.

C. Screening physiological and agro-morphological indicators and drought tolerant genotypes

Biplot analysis method : To better understand the relationships, similarities and dissimilarities among the indicators of drought tolerance, principal component analysis (PCA), were used based on the rank correlation matrix. 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. 1). The PCA_1 and PCA_2 axes which justify 54.56% 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 in vivo indices (Yan and Kang, 2003). The relative water loss (RWL), relative water content (RWC), spike length (SL), number of grains per spike (NGS), 1000-grains weight (GW) and Chlorophyll fluorescence (CHF) were referred to group 1= G1 indices. The relative water loss (RWL), Chlorophyll a (Chl a), Total chlorophyll (TChl), Integrated Selection Index (ISI) and grain yield under irrigation and rainfed conditions separated in a single group (G2) and Total chlorophyll (TChl), Chlorophyll b (Chl b), relative chlorophyll content (RCC) and Stomatal conductance (SC) belonged to a third group (G3). The cosine of the angle between the vectors of two indices approximates the correlation between them.

Ranking method: The estimates of indicators of drought tolerance (Table 6) indicated that the identification of drought-tolerant genotypes was contradictory based on a single criterion. The following ranking method was used to have an overall judgment. To determine the most desirable drought tolerant genotype according to the all indices rank and mean rank of ranks of all drought tolerance criteria were calculated and the most desirable drought tolerant genotypes were identified based on these two criteria. In consideration to all indices, genotypes (18), (3) and (6) showed the best mean rank and low rank and rank sum in water deficit stress condition, hence they were identified as the most drought tolerant genotypes which is almost in agreement with the results of our new index (ISI), while genotypes (10), (4) and (11) as the most sensitive. Biplot analysis and ranking methods have been used for screening drought tolerant genotypes by Farshadfar and Elyasi in wheat (2012) and Farshadfar *et al.*, (2012) in bread wheat.

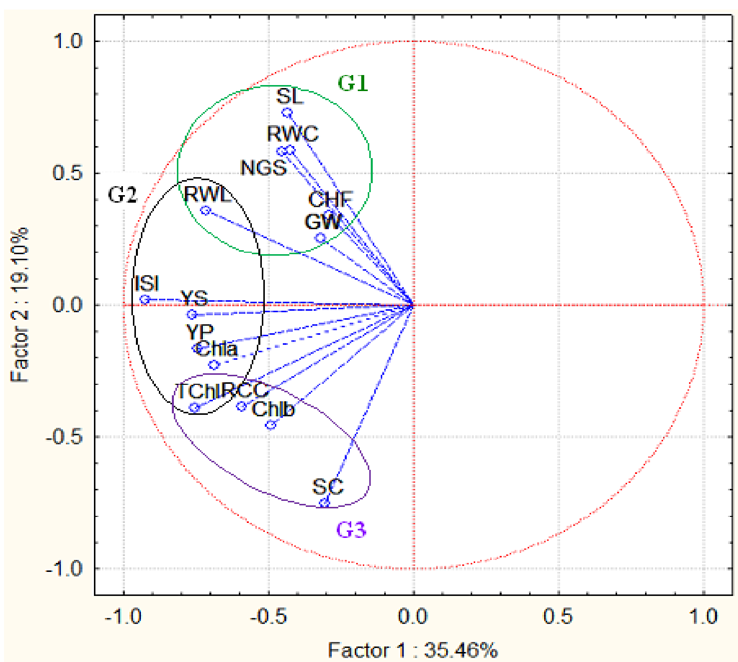

Fig. 1. Biplot analysis of agro-morphological and physiological indicators of drought tolerance.

RWC, RWL, SC, Chl a, Chl b, TChl, CHF, RCC, NGS, SL, GW, YS, YP and ISI indicate; relative water content, relative water loss, Stomatal conductance, Chlorophyll a, Chlorophyll b, Chlorophyll total, Chlorophyll fluorescence, relative chlorophyll content, number of grains per spike, spike length, 1000-grains weight, stress yield, yield potential and Integrated Selection Index respectively.

Table 7: Ranks mean ($\bar{R}$), standard deviation of ranks (SD) and rank sum (RS) of agro-morphological and physiological characteristics of drought tolerance.

Genotype	RWC	RWL	SC	Chl a	Chl b	TChl	CHF	RCC	NGS	SL	GW	YS	YP	ISI	$\bar{R}$	SD	RS
1	8	1	17	20	20	20	20	7	13	12	6	6	11	13	12	6	19
2	13	11	10	9	1	4	15	3	14	18	12	10	13	11	10	5	15
3	7	2	16	10	8	10	2	16	3	4	1	3	9	2	7	5	12
4	17	20	12	12	15	15	18	19	16	13	20	18	18	20	17	3	20
5	20	7	19	16	2	7	10	11	12	5	14	13	6	8	11	5	16
6	4	8	8	5	7	5	9	5	1	3	15	7	8	4	6	3	10
7	5	6	6	13	5	12	8	4	6	7	19	11	4	10	8	4	13
8	6	16	2	7	4	8	11	12	10	14	9	5	2	9	8	4	12
9	11	14	9	19	12	19	4	15	20	10	16	16	17	17	14	5	19
10	9	18	13	18	10	16	17	6	5	20	18	20	20	18	15	5	20
11	16	17	11	17	11	17	3	18	19	8	5	19	19	19	14	6	20
12	10	13	14	15	19	18	6	17	11	9	3	15	10	15	13	5	17
13	12	15	18	11	13	14	13	20	7	17	13	14	12	16	14	3	17
14	1	5	15	2	16	2	7	14	4	1	10	17	16	6	8	6	14
15	14	12	4	8	9	9	5	10	15	11	4	1	5	7	8	4	12
16	15	19	1	4	14	6	12	9	18	19	17	8	15	14	12	6	18
17	2	9	20	6	18	13	14	8	2	2	7	4	14	12	9	6	15
18	3	3	5	3	3	3	1	2	8	6	11	2	1	1	4	3	7
19	18	10	7	14	17	11	16	13	9	15	8	12	3	3	11	5	16
20	19	4	3	1	6	1	19	1	17	16	2	9	7	5	8	7	15

RWC, RWL, SC, Chl a, Chl b, TChl, CHF, RCC, NGS, SL, GW, YS, YP and ISI indicate; relative water content, relative water loss, Stomatal conductance, Chlorophyll a, Chlorophyll b, Chlorophyll total, Chlorophyll fluorescence, relative chlorophyll content, number of grains per spike, spike length, 1000-grains weight, stress yield, yield potential and Integrated Selection Index, respectively.

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