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Genetic evaluation of bread wheat for drought tolerance indices in the field and laboratory

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Abstract

In order to study genetic variation and response of twenty bread wheat (*Triticum aestivum* L.) landraces to drought stress, two experiments were conducted in the field and laboratory using randomized complete block design and factorial experiment within completely randomized design with three replications, respectively. This experiment was under rainfed and irrigated conditions in Kermanshah, Iran during 2010-2011 cropping season. Result of combined analysis of variance exhibited genotype and environment treatments significantly affect the yield and the most of the traits. The interaction between genotype and environment was significant for grain yield, 1000-grains weight and Chlorophyll total. Germination stress index (GSI) was recorded in the laboratory. Ten quantitative criteria of drought tolerance including: stress susceptibility index (SSI), tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI), yield index (YI), yield stability index (YSI), harmonic mean (HM), relative drought index (RDI) and modified stress tolerance index (MSTI) were calculated for each landrace based on the potential (Y_p) and stress (Y_s) yields. A significant positive correlation was observed between Y_s and Y_p with MP, GMP, STI, YI, HM, MSTI indicated that these indices are the most appropriate indices to screen genotypes in drought stress conditions. A positive significant correlation between GSI with Y_s and Y_p , indicates that GSI can be considered as an early selection criterion for discriminating drought tolerant genotypes. According to all statistical procedures, genotypes No. 20, 18, 19 and 11 are superior genotypes under both stressed and non-stressed conditions.

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Introduction

Drought as the major environmental constraints is a wide-spread problem to agricultural production in arid and semi-arid regions (Amiri *et al.*, 2014). Improving drought resistance is, therefore, a major objective in plant breeding programs for rainfed agriculture in these regions (Ehdaie and Waines, 1993). Wheat production is restricted by drought and this restriction cause different problems due to great impacts on human nutrition (Sio Se-Mardeh *et al.*, 2006; Rajala *et al.*, 2009; Shiri *et al.*, 2010). So, the issue for crop science is how to improve yield production and stability under drought stress conditions (FAO, 2014). Drought adversely affects different aspects of plant growth, including seed germination as well as plant growth and development. The severe drought stress causes severe disrupting in photosynthesis and physiological processes, halting growth and eventually death of the plant (Singh and Patel, 1996). Plants change their physiological, biochemical and morphological status to tolerate or adapt to dry conditions.

One of the screening techniques based on physiological characters is stress induction in plant tissues by means of several osmotica. The most important stage of crop is seed germination in the presence of water (Ashraf and Mehmood, 1990). Growth of seed under drought condition effects on the growth of seedling (Albuquerque and de Carvalho, 2003). Seed germination in mannitol and polyethylene glycol (PEG), measurements of root length and the vigor and growth of seedlings exposed to osmotica have been proposed for drought screening (Farshadfar *et al.*, 2002). The effect of PEG was evaluated on wheat (Sapra, 1991) and on wheat – agropyron disomic addition lines (Farshadfar *et al.*, 2002). They concluded that PEG was very suitable for the adjustment of osmotic potential.

Since drought is a complex physiological reaction, its genetic basis has therefore received limited attention; hence, little information is available on genetic architecture of drought related physiological

characters, which may provide practical information to breeders during the development of drought tolerant wheat varieties (Farshadfar *et al.*, 2000, 2001; 2008). Gupta *et al.* (2001), studied the effect of drought stress on physiological traits and yield components of wheat. They stated that water stress at anthesis stage, severely decrease number of grains, grain yield, biological yield and harvest index. As Razzaq *et al.* (2013) reported physiological parameters may be considered as indicators of appropriate growth and yield under drought stress. Khakwani *et al.* (2012) studied growth and yield response of wheat varieties to drought stress at booting and anthesis. They indicated significant differences among genotypes for most of the studied trait such as, relative water content plant height, yield and yield components, biological yield, harvest index, and drought tolerance indices.

Understanding the plant response in dry environments has great importance and also a fundamental part of producing stress tolerant crops (Mohammadi *et al.*, 2011). Breeding for drought resistance is complicated by the lack of fast, reproducible screening techniques and the inability to create routinely and repeatable water stress conditions when a large amount of genotypes should be evaluated. 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 favorable environments (Talebi *et al.*, 2009). Thus, The relative yield performance of genotypes in drought stress and irrigated conditions seems to be a convenient approach to drought tolerant genotypes development (Sio Se-Mardeh *et al.*, 2006). Several selection criteria which provide a measure of drought tolerance of genotypes based on mathematical relation between stress and non-stressed conditions have been suggested. Rosielle and Hamblin (1981), proposed the stress tolerance (TOL) as the differences in yield between the stress and non-stressed environments and mean productivity (MP) as the average yield in both conditions. The stress susceptibility index (SSI)

defined by Fischer and Maurer (1978), for measurement of yield stability that apprehended the changes in both potential and actual yields in variable environments. These researchers reported that SSI more and less than 1 indicates above and below-average susceptibility to drought stress, respectively (Guttieri, 2001). The other value defined as relative drought index (RDI) was proposed by Fischer (1998). Also, Gavuzzi (1997), Bouslama and Schapaugh (1984) and Choukan (2006) recommended the yield index (YI), yield stability index (YSI), and yield reduction percentage, respectively. Fernandez (1992), introduced a stress tolerance index (STI) which can be used to recognize genotypes that produce high yield under stress and complementary irrigation conditions, also classified the manifestation of genotypes into four groups of (1) – genotypes that produce uniform superiority both water stress and non-stressed conditions (group A), (2) - genotypes which perform favorably only in non-stressed conditions (group B), (3) - genotypes which yield relatively higher only in stress conditions (group C) and (4) and genotypes which perform poorly in both stressed and non-stressed conditions (group D). Thus, as Fernandez demonstrated, the most suitable index for stress tolerance selection is one that is capable to distinguish the class A from other classes. The geometric mean productivity (GMP) defined by Fernandez (1992), which is frequently used by breeders interested in relative performance, since drought stress can vary in severity in field environments over years (Ramirez and Kelly, 1998). To improve the efficiency of STI a modified stress tolerance index (MSTI) was suggested by Farshadfar and Sutka (2002), which corrects the STI as a weight. However, the ideal selection criterion should distinguish genotypes that express uniform superiority in both stress and non-stressed environments from the genotypes that are favorable only in one environment. Many authors studied the associations of these indices with grain yield under stress and non-stressed conditions. Sio- Se Mardeh *et al.* (2006) reported that under moderate stress, MP, GMP and STI were more effective in A group

cultivars, while regression coefficient (b) and SSI were found to be more useful under severe stress in discerning resistant cultivars. Najaphy and Geravandi (2011) showed that YI and SSI were more appropriate selection indices to identify genotypes adapted to stress environment and SSI should be used along with yield data under stress (Ys). Amiri *et al.* (2014) suggested that that Ys and Yp with MP, GMP, STI, YI, HAM, SDI, and MSTI are the superior criteria for selection of high yielding genotypes both under stress and non-stressed conditions in durum wheat.

The objectives of present study were i) to assess drought tolerance in some bread wheat genotypes and identifying drought tolerant ones. ii) to study interrelationships among the screening methods and determining the efficiency of screening methods. iii) evaluate genetic variation for grain yield and some related traits among 20 bread wheat genotypes and iv) understanding of relationships between traits and grain yield, and their response to drought stress conditions .

Materials and methods

Field experiments

A. Plants materials

In this study twenty landraces of bread wheat (*Triticumaestivum* L.) (Table 1) which in this manuscript identified shortly as No. 1-20, were planted under rain-fed and irrigated conditions during 2010-11 cropping season in research filed of Razi University, Kermanshah, Iran (34°21'E, 47°9' N, 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. Field experiments were carried out in a randomized complete block design (RCBD) with three replications. Sowing was done by hand in plots with four rows 2 m in length and 20 cm apart. The seeding rate was 400 seeds per m² for all plots. At the rainfed experiment, water stress was imposed after anthesis. Complementary irrigation ed plots were irrigated three times after anthesis, while stressed plots received no water. At harvest time, after separation of border effects from each plot, yield

potential (Y_p) and stress yield (Y_s) were measured from 2 rows 1 m in length.

B. Physiological traits

(i) Leaf relative water content (RWC) was measured at flowering stage using Turner (1986) method

$$RWC\% = \left[\frac{(FW - DW)}{(TW - DW)} \right] \times 100$$

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).

(ii) 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:

$$RWL(\%) = \left[\frac{(Fw - WW4h)}{(Fw - Dw)} \right] \times 100$$

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:

$$\text{Chlorophyll a } (\mu\text{g/mL}) = 16.5 \times A_{665} - 8.3 \times A_{650}$$

$$\text{Chlorophyll b } (\mu\text{g/mL}) = 33.8 \times A_{650} - 12.5 \times A_{665}$$

$$\text{Total chlorophyll } (\mu\text{g/mL}) = 25.8 \times A_{650} + 4.0 \times A_{665}$$

Chlorophyll Fluorescence (CHF)

This trait was measured after 50% of flowering, therefore five leaves were selected randomly from each plot in each replication, leaf samples were put between blindy sensors of Chlorophyll Fluorimeter set (Pocket PEA model) for compatibility with darkness. Their quantumic yield was measured after 15 minutes as follows (Genty *et al.*, 1989).

$$Qy = Fv/Fm$$

Where Qy = quantum yield, Fv = variable fluorescence and Fm = maximum fluorescence.

C. Agronomical traits

After physiological maturity stage, grain yield, numbers of grain per spike 1000-grains weight (GW-gr) and spike length (SL-cm) were measured.

D. Drought indices

Drought indices were calculated using the following formulas:

$$\text{Yield index} = YI = \frac{Y_s}{Y_p} \text{ (Gavuzzi et al., 1997).}$$

$$\text{Yield stability index} = YSI = \frac{Y_s}{Y_p} \text{ (Bousslama and}$$

Schapaugh, 1984).

$$\text{Tolerance} = Tol = (Y_p - Y_s) \text{ (Rosielle and Hamblin, 1981).}$$

$$\text{Mean productivity} = Mp = \frac{Y_p + Y_s}{2} \text{ (Rosielle and Hamblin, 1981).}$$

$$\text{Harmonic mean} = HM = \frac{2(Y_p \times Y_s)}{Y_p + Y_s}$$

$$\text{Stress susceptibility index} = SSI = \frac{1 - Y_s/Y_p}{SI};$$

$$SI = 1 - \bar{Y}_s / \bar{Y}_p \text{ (Fischer and Maurer, 1978).}$$

Geometric mean productivity = $GMP = \sqrt{(Yp)(Ys)}$,

Stress tolerance index = $STI = \frac{(Ys) \times (Yp)}{(\bar{Yp})^2}$

(Fernandez, 1992).

Modified stress tolerance index = $MSTI =$

$Ki STI$, $K1 = \frac{Yp^2}{(\bar{Yp})^2}$ and $K2 = \frac{Ys^2}{(\bar{Ys})^2}$

(Farshadfar and Sutka, 2002) Where k_i is the correction coefficient.

Relative drought index = $RDI = \left(\frac{Ys}{Yp} \right) / \left(\frac{\bar{Ys}}{\bar{Yp}} \right)$ (Fischer *et al.*, 1979).

In the above formulas, Ys , Yp , \bar{Ys} and \bar{Yp} represent yield under stress, yield under complementary irrigation for each genotype, yield mean in stress and complementary irrigation conditions for all genotypes, respectively.

For screening drought tolerant genotypes a rank sum (RS) was calculated by the following relationship:

Rank sum (RS) = Rank mean (\bar{R}) + Standard deviation of rank (SDR) and $SDR = (S2i)^{0.5}$.

Laboratory experiment

The experiment was carried out in a completely randomized design (CRD) under two different stress (-0.8 MPa) and non - stress (0 bar) conditions created with the help of polyethylene glycol 6000 (PEG - 6000) by the method suggested by Michel and Kauffman (Khalilzade and Karbalai-Khiavi, 2002). Mature seeds were surface-sterilized in 70% (v/v) ethanol for 2.5 min, rinsed four times with sterile distilled water, incubated further in 2.5% sodium hypochlorite for 15 min, and rinsed several times in sterile distilled water. 25 seeds were then transferred into sterile Petri dishes of 25 mm

diameter containing two Whatman filter paper moistened with 10 ml of control solution (distilled water) or the same solution added with PEG-6000. Seeds were germinated in an incubator at 20 ± 0.5 c. Germination percentages were recorded daily up to 10 days using radicle extrusion (≥ 2 mm long) as a criterion. After 10 days the number of germinated seeds was recorded and promptness index (PI) and germination stress index (GSI) were calculated using the formula proposed by Sapra, (1991) and Bouslama and Schapaugh (1984).

$PI = nd2 (1.0) + nd4 (0.8) + nd6 (0.6) + nd8 (0.4) + nd10 (0.2)$.

In which $nd2$, $nd4$, $nd6$, $nd8$ and $nd10$ represent the percentage of germinated seeds after 2, 4, 6, 8, and 10 days after sowing, respectively.

$GSI (\%) = [PI (\text{in stress condition}) / PI (\text{in normal condition})] \times 100$

Statistical analysis

Analysis of variance was carried out using SAS ver.9.1 software. Duncan multiple range test (DMRT) was used for the mean comparisons. Correlation analysis and principal component analysis (PCA), based on the rank correlation matrix and biplot analysis were performed by SPSS ver. 16, and STATISTICA ver. 8.

Results and discussion

A. Anova analysis

Results of analysis of variance (ANOVA) for rainfed conditions revealed significant differences among genotypes for all the investigated traits, except for Chlorophyll a, b (Chl a, Chl b) and total chlorophyll (T Chl), indicating the presence of genetic variation and possibility of selection for drought tolerant genotypes under drought condition (Table 2). Results of ANOVA for complementary irrigation showed significant differences among genotypes for all characters except Chlorophyll b, number of grain per spike (NGS) and Chlorophyll Fluorescence (CHF) (Table 2),

indicating the *existence of sufficient* genetic variation to select them against drought stress.

Our results of the experiments, confirmed previously reported findings (Farshadfar, 2012; Kutlu and Kinaci, 2010) in bread wheat.

Results of combined analysis of variance for all tested traits over both environments exhibited significant difference between genotypes (Table 3), which is in agreement with Khakwani *et al.* (2012). Genotype × environment interaction was not significant for all studied traits with the exception of GY, GW and T Chl, indicating that genotypes for these traits had the same reaction in different environmental conditions.

Table 1. Names and codes of genotypes.

Genotype no.	Name	Genotype no.	Name
1	WC-47560	11	WC-4973
2	WC-4506	12	WC-47374
3	WC-47632	13	WC-47358
4	WC-47574	14	WC-4573
5	WC-47481	15	WC-47536
6	WC-47407	16	WC-47572
7	WC-4978	17	WC-4953S
8	WC-4860	18	WC-47536
9	WC-47620	19	WC-5050
10	WC-4992	20	WC-47359

Mean comparison of these traits in two complementary irrigation and stress conditions is shown in table 5; while, mean comparison of other traits in both conditions (combined analysis) is presented in Table 4.

B. Comparison mean

Duncan’s multiple rang test (Table 4) revealed that the genotype 20,18 and 19 had higher grain’s yield

(YG) while genotypes 12 and 13 exhibited lower value for this trait under rainfed condition. In complementary irrigation conditions, genotypes 20, 18, 19 and 7 had the highest and genotypes 2 and 13 showed the lowest grain yield, respectively. Therefore, genotypes 20, 18 and 19 gave the best performance and genotype 13 showed the worst performance in both conditions.

Table 2. Analysis of variance for studied traits under complementary irrigation and dryland conditions.

S.O.V	df	RWC		RWL		Chl a		Chl b		T Chl		CHF		NGS		GW		SL		GY	
		Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland
Rep	2	0.01	0.09**	0.13**	0.07**	2.76	3.83	0.13	2.20*	2.17	2.65	.002*	0.008**	13471.80	22546.46	106.39**	335.39**	3.45	0.50	2365.08	426.97
Gen	19	0.02*	0.02**	0.01*	0.01*	5.28*	2.06	1.06	0.92	4.65*	2.25	0.0006	0.003*	14108.92	16666.89*	165.04**	77.26**	6.18**	6.63**	52689.02**	45164.99**
Error	38	0.008	0.008	0.005	0.006	2.31	1.52	0.57	0.59	2.03	1.34	0.0005	0.001	7651.38	7342.18	11.34	14.16	1.23	1.47	1605.83	1309.39
C.V.%	-	12.04	11.85	13.11	11.22	46.72	39.25	42.18	48.93	27.70	24.27	3.05	5.51	20.05	21.63	7.50	10.47	10.46	10.90	9.87	11.56

*and ** Significant at 5% and 1% level of probability, respectively

RWC, RWL, Chl a, Chl b, T Chl, CHF, NGS , GW, SL, and GY indicate; relative water content, relative water loss, Chlorophyll a, Chlorophyll b, Chlorophyll, Chlorophyll fluorescence, , number of grains per spike, 1000-grains weight spike length, and grain yield, respectively.

The high RWC and low RWL have been suggested as important indicators of water status as good indicator of drought tolerance (Farshadfar *et al.*, 2001; Gunes *et al.*, 2008; Farshadfar *et al.*, 2011b). Genotypes 2, 19, 20 and 17 had higher amount of RWC content while genotypes 7 and 15 displayed lower 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.

The highest relative water loss (RWL) was related to genotypes 4, 13, 7, 9 and 11, respectively and the lowest RWL were related to genotypes 1 and 15.

Table 3. Analysis of variance for studied traits under complementary irrigation and dryland conditions.

S.O.V	Df	RWC	RWL	CHF	CHL a	CHL b	TCH	NGS	GW	SL	GY
Environment (Envi)	1	0.001	0.58**	0.17**	0.36	1.47	4.27	48160.13*	2388.95**	7.76*	259095.34**
Rep (Envi)	2	0.02	0.14**	0.003*	2.34	0.75	1.05	35110.83*	382.25**	3.24	2067.02
Genotype (Gen)	19	0.02**	0.01*	0.003**	4.13*	1.49**	2.72	21492.73**	216.89**	10.57**	92289.72**
Env× Gen	19	0.02	0.008	0.001	3.21	0.49	4.18**	9283.08	25.42*	2.24	5564.30**
Error	78	0.01	0.007	0.001	1.98	0.604	1.74	7327.82	13.95	1.33	1438.83
C.V. %	-	13.30	13.69	4.65	43.96	46.33	26.60	20.57	9.24	10.63	10.55

*and ** Significant at 5% and 1% level of probability, respectively

RWC, RWL, CHF, Chl a, Chl b, T Chl, , NGS , GW, SL, and GY indicate; relative water content, relative water loss, Chlorophyll fluorescence, Chlorophyll a, Chlorophyll b, total Chlorophyll, number of grains per spike, 1000-grains weight, spike length, and grain yield, respectively.

In a study on wheat, it was found that the drought tolerant genotypes have higher RWC and regarding to the high correlation between RWC and grain yield, it was concluded that this trait can be used for identification drought tolerant genotypes in breeding programs (Naroui Rad *et al.*, 2013). Sairam and

Srivastava (2001) observed variation in wheat genotypes for RWC and suggested that RWC is a suitable indicator for screening drought tolerant wheat genotypes. Shamsi (2010) observed a decline in wheat RWC due to drought stress and reported the highest RWC in the tolerant genotypes.

Table 4. Mean comparison of studied traits in two complementary irrigation and dryland conditions.

Gen	RWC	RWL	CHF	CHLA	CHLB	NSPS	SL
1	0.80 abcd	0.55 c	0.66 d	2.82 bc	1.43 de	439.00 abcd	8.78g
2	0.85 a	0.67 ab	0.68 cd	3.27 bc	1.61 bcde	504.67 abc	9.38 fg
3	0.67 def	0.64 abc	0.73 ab	3.22 bc	1.13 de	471.33 abcd	9.53 efg
4	0.78 abcde	0.71 a	0.73 ab	2.28 c	2.12 abcd	358.33 de	10.27def
5	0.72 abcdef	0.59 abc	0.71 abcd	3.61 bc	1.55 cde	365.33 de	12.65 ab
6	0.70 cdef	0.66 abc	0.72 ab	2.12 c	2.60 ab	447.33 abcd	11.52 bcd
7	0.64 f	0.68 a	0.71 abcd	2.33 c	1.38 de	420.00 abcd	12.05 abc
8	0.72 abcdef	0.60 abc	0.71 abcd	2.33 c	2.75 a	400.00 bcd	11.27 bcd
9	0.75 abcdef	0.69 a	0.74 ab	3.64 bc	1.01 e	383.33 de	11.22 bcd
10	0.81 abcd	0.63 abc	0.73 ab	3.70 bc	1.35 de	396.00 bcde	10.88 cde
11	0.73 abcdef	0.68 a	0.75 ab	3.11 bc	1.73 abcd	408.33 abcd	11.23 bcd
12	0.77 abcdef	0.62 abc	0.74 ab	3.01 bc	1.82 abcd	408.00 abcd	10.32def
13	0.71 bcdef	0.71 a	0.73 ab	3.07 bc	1.45 de	283.33 e	11.70 bcd
14	0.74 abcdef	0.64 abc	0.72 ab	2.33 c	2.58 abc	365.33 de	11.00 cde
15	0.65 ef	0.54 c	0.75 a	2.98 bc	1.97 abcd	354.67 de	7.42h
16	0.75 abcdef	0.62 abc	0.74 ab	3.02 bc	1.72 abcd	388.67 cde	11.67 bcd
17	0.83 abc	0.63 abc	0.73 ab	3.55 bc	1.42 de	426.67 abcd	11.18 bcd
18	0.75 abcdef	0.60 abc	0.72 abc	3.37 bc	1.49 de	471.00 abcd	11.12 bcd
19	0.84 ab	0.56 bc	0.70 bcd	4.64 ab	1.12 de	510.67 ab	13.24 a
20	0.81 abc	0.64 abc	0.75 ab	5.58 a	1.32 de	521.33 a	10.89 cde

RWC, RWL, CHF, Chl a, Chl b, NGS , and SL indicate; relative water content, relative water loss, Chlorophyll fluorescence, Chlorophyll a, Chlorophyll b, number of grains per spike, and spike length, respectively.

Chlorophyll fluorescence (CHF) and total chlorophyll content (T Chl) were decreased significantly as a consequence of drought stress (Tables 4-5); however, CHF and T Chl for different genotypes were decreased differently. The result obtained from comparison of means exhibited that the highest amount of CHF were attributed to genotype 15, 16, 11, 12 and 9. 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).

Table 5. Mean comparison of studied traits in two complementary irrigation and dryland conditions.

Gen	T Chl		GY		GW	
	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland
1	3.67 de	4.91 abc	3.64 df	3.32 cd	38.46 ef	32.71 def
2	5.25 abcde	4.61 abc	2.61 hi	1.90 ghij	28.37 g	23.50 g
3	4.23 bcde	4.48 bc	4.27 cd	3.02 cd	30.88 g	28.66 g
4	4.35 bcde	4.53 abc	3.12 fgh	3.54 c	42.89 def	33.51 cdef
5	6.33 abcd	4.16 bc	2.71 hi	2.26 fghi	42.97 def	33.63 cdef
6	5.70 abcde	3.82 bc	3.72 cdf	2.42 efgh	42.57 def	40.29 abc
7	3.49 e	4.01 bc	5.47 b	3.41 cd	53.47 a	45.00 a
8	4.43 bcde	5.83 ab	4.18 cd	3.30 cd	46.87 bcd	35.56 bcdef
9	5.39 abcde	4.00 bc	2.82 gh	1.77 hij	39.25 ef	30.53 ef
10	6.83 ab	3.38 c	4.45 c	3.00 cd	53.48 a	35.36 bcdef
11	4.65 bcde	5.13 abc	6.14 b	4.42 b	50.22 abc	37.99 abcd
12	3.72 de	6.03 ab	3.10 fgh	1.66 ij	37.46f	31.27 def
13	3.96 cde	5.18 abc	2.27 i	1.44 j	41.87 def	34.79 cdef
14	4.78 bcde	5.14 abc	3.07 fgh	2.12 fghi	51.47 ab	40.73 abc
15	4.94 abcde	5.06 abc	4.14 cd	2.75 def	49.51 abc	38.23 abcd
16	4.51 bcde	5.05 abc	3.47 fg	2.52 efg	55.07 a	40.07 abc
17	4.96 abcde	5.08 abc	3.48 efg	3.63 c	50.63 ab	41.10 ab
18	7.56 a	3.27 c	6.02 b	5.30 a	46.76 bcd	40.68 abc
19	6.56 abc	4.85 abc	5.59 b	5.22 a	44.08 cde	38.47 abc
20	7.55 a	6.78 a	6.93 a	5.61 a	51.27 ab	37.00 bcde

T Chl, GY and GW, indicate total Chlorophyll, grain yield, and 1000-grains weight respectively.

Genotypes 20, 12 and 8 had higher (T Chl) while genotypes 18 and 10 exhibited lower value for these traits under rainfed condition; However, Genotypes 20 and 18 had higher (T Chl) content and genotypes 7, 12 and 1 displayed lower (T Chl) under complementary irrigation condition.

The highest Chlorophyll a, b (Chl a, Chl b) belonged to the genotypes 20 and 8 respectively.

The results exhibited that the highest amount of relative number of grains per spike (NGS) was attributed to genotypes 20, 19 and 2.

Genotypes 19 and 5 displayed higher spike length (SL) while genotypes 15 and 1 showed lower spike length (SL) (Table 4).

Genotypes 7 and 17 had higher 1000-grains weight (GW) under rainfed condition while genotypes 16, 10 and 7 exhibited higher value for these traits under complementary irrigation condition; However, genotypes 2 and 3 showed lower(GW) under both environment.

In general, genotypes 18 and 19 had the highest amount of grain yield and yield components in water deficit stress conditions.

Table 6. Pearson correlation coefficients between different traits in 20 bread wheat genotypes under complementary irrigation and dryland conditions.

	RWC	CHF	RWL	Chl a	Chl b	T Chl	GY	GW	SL	NGS	
RWC	1	-.326	-.359	.321	-.309	.121	.209	-.076	.217	.608**	Dryland
CHF	.307	1	.049	.306	-.117	.254	.068	.387	.120	-.210	
RWL	.239	.488*	1	-.194	-.013	-.194	-.222	.045	.541*	-.359	
Chl a	.157	.114	-.077	1	-.359	.781**	.304	-.098	.275	.283	
Chl b	.093	.274	-.093	-.512*	1	.293	-.355	.038	-.239	-.264	
T chl	.225	.270	-.177	.869**	-.046	1	.093	-.071	.102	.107	
GY	-.088	-.011	-.093	.415	-.106	.455*	1	.399	.153	.564**	
GW	-.175	.234	-.037	.085	.195	.202	.431	1	.247	-.110	
SL	-.130	-.074	-.272	.233	.171	.366	.144	.381	1	.155	
NGS	.012	-.173	-.147	.293	-.095	.285	.394	-.208	-.019	1	

*and ** Significant at 5% and 1% level of probability, respectively.

These results coincide with the other findings which have been observed that drought caused reductions most agronomic traits such as grain yield, number of grain per spike and etc (Bayoumi *et al.*, 2008; Khakwani *et al.*, 2011).

Preventing irrigation at the heading stage may cause a great decrease in some important processes affecting yield production to the formed kernels. It was reported that drought stress conditions decreased wheat yield significantly and most of its components such as NGS (Khaled *et al.*, 2015). Elhafid *et al.* (1998) stated drought leads to reducing inoculation of flower and this affects number of produced grain. Foulkes *et al.* (2002) reported that the grain yield in stress conditions has significant reduction at anthesis stage and after that relative to complementary irrigation conditions.

Among all studied traits under both conditions, T Chl and NGS revealed positive and significant correlation with GY at complementary irrigation and stress conditions respectively (Table 6).

Decreased or unchanged chlorophyll level during drought stress has been reported in other species, depending on the duration and severity of drought (Kpyoarissis *et al.*, 1995; Ahmadi, 2000; 2005; Jung, 2004; Nayyar & Gupta, 2006). A decrease of total

chlorophyll with drought stress implies a lowered capacity for light harvesting. It seems that the decomposition of chlorophyll is the reason for its decrease under drought stress (Kulshreshta *et al.*, 1987). Ashraf *et al.* (1994) related the decrease in chlorophyll concentration under drought stress to the increase in activity of the enzyme chlorophyllase. Nonetheless, some reports show that drought stress had no effect on chlorophyll concentration and/or the resistant and sensitive wheat cultivars showed no difference in response to drought stress (Kulshreshta *et al.*, 1987). One reason for these inconsistent findings may be the difference in study conditions such as stress intensity and duration (Jagtap *et al.*, 1998).

In this study genotypes with higher T Chl gave higher yield than others at complementary irrigation condition.

In order to evaluate drought tolerance of the genotypes, grain yield under both conditions and also different indices including SSI, TOL, MP, GMP, STI, MSTI, YSI, HM, RDI and GSI were calculated. Results of correlation analysis between grain yield in both conditions and calculated drought resistance indices (Table 7) showed that STI, MP, GMP, HM, YI and MSTI had positive and significant correlations with Yp and Ys. Therefore these indices were able to

discriminate group A genotypes from other genotypes. The similar results were proposed by Amiri *et al.* (2014) in bread wheat.

Ys had significantly positive correlation ($r=0.833^{**}$) with (Yp) showing that stress intensity was mild.

Therefore, indirect selection in mild drought stress will be efficient based on the results of complementary irrigation conditions for wheat genotypes (Fernandez,1992, Mohammadi *et al.*, 2010). It could be due to high stress intensity in their experiments.

Table 7. Correlations between different drought resistance indices with grain yield complementary irrigation and dryland conditions.

	Yp	YS	TOL	MP	GMP	STI	YI	YSI	SSI	HM	K1STI	K2STI	RDI	GSI
Yp	1													
YS	.833**	1												
TOL	-.391	.107	1											
MP	.962**	.923**	-.183	1										
GMP	.962**	.923**	-.183	1.000**	1									
STI	.959**	.925**	-.174	.998**	.998**	1								
YI	.833**	1.000**	.107	.923**	.923**	.925**	1							
YSI	.205	.656**	.752**	.403	.403	.415	.656**	1						
SSI	.205	.656**	.752**	.403	.403	.415	.656**	1.000**	1					
HM	.931**	.947**	-.105	.989**	.989**	.991**	.947**	.481*	.481*	1				
K1STI	.986**	.872**	-.299	.986**	.986**	.985**	.872**	.295	.295	.964**	1			
K2STI	.881**	.980**	.003	.962**	.962**	.964**	.980**	.567**	.567**	.982**	.925**	1		
RDI	-.205	-.656**	-.752**	-.403	-.403	-.415	-.656**	-1.000**	-1.000**	-.481*	-.295	-.567**	1	
GSI	.767**	.395	-.668**	.639**	.639**	.633**	.395	-.298	-.298	.568**	.714**	.483*	.298	1

*and ** Significant at 5% and 1% level of probability, respectively; Yp=grain yield under normal condition; Ys=grain yield under stress condition; TOL=Tolerance Index; MP=Mean Productivity; GMP=Geometric Mean Productivity; STI=Stress Tolerance Index; YI=Yield Index; YSI=Yield Stability Index; SSI=Stress Susceptibility index; HM=Harmonic Mean, K1STI and K2STI= Modified stress tolerance index; RDI= Relative drought index; GSI= Germination stress index.

The highest correlation ($r^2 = 1.00^{**}$) was observed between Ys and YI which confirmed results of other reported studies (Amiri *et al.*, 2014, Farshadfar and Elyasi, 2012).

YSI and SSI had positive and significant correlation with Ys. The correlation of YSI and SSI with Yp was not significant. So, they can not select high yielding genotypes in both stressed and complementary irrigation environments. This result inconsistent with Amiri *et al.* (2014).

RDI was negatively correlated with Ys but there wasn't significant correlation between this index with Yp. Therefore, it can not distinguish A group genotypes from other groups.

GSI revealed a significant and positive correlation with yield under complementary irrigation condition, while no significant correlation was observed between GSI and YS. However the results of correlation analysis showed that MP, GMP, STI, MSTI and RDI had positive and significant correlations with GSI. There for this index can be considered as an early selection criterion for discriminating drought tolerant genotypes, which is in agreement with Vaisi and Farshadfar (2011).

Based on MP, GMP and STI values (Table 8), genotypes No 20, 18 and 19 identified as drought tolerant genotypes. These genotypes had greater values for these indices, While Genotypes No. 13, 2 and 9 identified as susceptible genotypes. Based on

HM, YI and K2STI, genotypes 20, 18 and 19 were the most and genotypes 13, 12 and 9 were the least tolerant genotypes. Genotypes 4, 17 and 19, displayed high YSI and SSI while Genotypes 12, 13 and 7

showed the lowest amount. The highest amount of modified stress tolerance index (K1STI) was attributed to genotypes 20, 18 and 11 while genotypes 13, 2 and 9 had the lowest K1STI.

Table 8. Drought tolerance criteria and ranks (R), ranks mean (\bar{R}) and standard deviation of ranks (SDR) and rank sum (RS) of drought tolerance indicators.

Gen	YP		YS		TOL		MP		GMP		STI		YI		YSI	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank
1	3.64	11	3.32	8	32.13	3	3.48	10	3.48	10	0.48	10	0.85	8	0.91	4
2	2.61	19	1.90	17	70.48	6	2.26	19	2.22	19	0.20	18	0.49	17	0.73	9
3	4.27	7	3.02	10	124.35	13	3.64	8	3.59	8	0.51	8	0.77	10	0.71	12
4	3.12	14	3.54	6	-41.75	1	3.33	12	3.32	12	0.44	12	0.90	6	1.13	1
5	2.71	18	2.26	15	45.06	5	2.49	16	2.48	16	0.24	16	0.58	15	0.84	6
6	3.72	10	2.42	14	129.73	14	3.07	13	2.99	13	0.36	13	0.62	14	0.65	16
7	5.47	5	3.41	7	206.29	20	4.44	5	4.32	5	0.74	5	0.87	7	0.62	18
8	4.18	8	3.30	9	88.41	9	3.74	6	3.71	6	0.56	6	0.84	9	0.80	8
9	2.82	17	1.77	18	105.12	12	2.29	18	2.23	18	0.20	19	0.45	18	0.63	17
10	4.45	6	3.00	11	144.81	18	3.73	7	3.65	7	0.53	7	0.77	11	0.68	14
11	6.14	2	4.42	4	171.99	19	5.28	4	5.20	4	1.08	4	1.13	4	0.72	11
12	3.10	15	1.66	19	144.24	17	2.38	17	2.27	17	0.20	17	0.42	19	0.54	20
13	2.27	20	1.44	20	82.63	8	1.85	20	1.80	20	0.14	20	0.37	20	0.62	19
14	3.07	16	2.12	16	95.04	11	2.59	15	2.54	15	0.26	15	0.54	16	0.68	13
15	4.14	9	2.75	12	138.63	16	3.44	11	3.37	11	0.45	11	0.70	12	0.66	15
16	3.47	13	2.52	13	95.01	10	3.00	14	2.96	14	0.35	14	0.64	13	0.73	10
17	3.48	12	3.63	5	-15.03	2	3.55	9	3.55	9	0.50	9	0.93	5	1.04	2
18	6.02	3	5.30	2	72.18	7	5.66	2	5.64	2	1.27	2	1.35	2	0.88	5
19	5.59	4	5.22	3	37.28	4	5.41	3	5.40	3	1.16	3	1.33	3	0.93	3
20	6.93	1	5.61	1	132.02	15	6.27	1	6.23	1	1.54	1	1.43	1	0.81	7

Gen	SSI		HM		K1STI		K2STI		RDI		GSI		R	SDR	RS
	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank			
1	0.40	4	3.47	10	0.25	10	0.35	9	1.17	17	0.34	17	9.36	4.14	13.50
2	1.23	9	2.19	17	0.06	19	0.05	17	0.94	12	0.45	16	15.29	4.39	19.68
3	1.32	12	3.54	9	0.37	8	0.31	11	0.91	9	0.67	9	9.57	1.83	11.40
4	-0.61	1	3.31	11	0.17	14	0.36	8	1.45	20	-0.03	20	9.86	6.35	16.20
5	0.75	6	2.46	16	0.07	17	0.08	16	1.07	15	0.30	18	13.93	4.58	18.51
6	1.59	16	2.91	14	0.20	12	0.15	14	0.83	5	0.65	10	12.71	2.84	15.55
7	1.71	18	4.20	5	0.91	5	0.58	5	0.80	3	1.07	5	8.07	5.84	13.91
8	0.93	8	3.68	6	0.46	6	0.45	6	1.02	13	0.61	11	7.93	2.16	10.09
9	1.70	17	2.17	18	0.06	18	0.04	18	0.80	4	0.60	12	16.00	4.08	20.08
10	1.46	14	3.58	7	0.42	7	0.31	10	0.87	7	0.73	7	9.50	3.65	13.15
11	1.27	11	5.13	4	1.66	3	1.43	4	0.92	10	1.45	3	6.21	4.77	10.99
12	2.11	20	2.17	19	0.08	16	0.04	19	0.69	1	0.74	6	15.86	5.53	21.39
13	1.73	19	1.74	20	0.03	20	0.03	20	0.80	2	0.60	13	17.21	5.62	22.83
14	1.43	13	2.50	15	0.11	15	0.09	15	0.88	8	0.54	14	14.07	2.23	16.31
15	1.52	15	3.30	12	0.32	9	0.23	12	0.85	6	0.69	8	11.36	2.79	14.15
16	1.25	10	2.92	13	0.17	13	0.15	13	0.93	11	0.52	15	12.57	1.65	14.22
17	-0.20	2	3.55	8	0.24	11	0.43	7	1.34	19	0.16	19	8.50	5.50	14.00
18	0.54	5	5.63	2	1.83	2	2.34	2	1.13	16	1.57	2	3.86	3.84	7.70
19	0.30	3	5.40	3	1.44	4	2.06	3	1.20	18	1.27	4	4.36	3.95	8.31
20	0.85	7	6.19	1	3.01	1	3.19	1	1.04	14	2.35	1	3.79	5.03	8.81

Low value of TOL index reveals the tolerance of the genotype; therefore, the tolerant genotypes were selected based on low TOL. As shown in Table 8, the lowest value of this index was calculated for genotypes 4, 17 and 1. The highest TOL value was calculated for genotypes 7, 11 and 10. According to

RDI genotypes 12, 13 and 7 were the most and genotypes 4, 17 and 19 the least tolerant genotypes.

The highest amount of GSI was attributed to genotypes 20, 18 and 11 while genotypes 4, 17 and 5 had the lowest GSI.

Table 9. Principal components analysis for drought tolerance indices.

Component	Eigen value	Cumulative (%)	Yp	YS	TOL	MP	GMP	STI	YI	YSI	SSI	HM	K1STI	K2STI	RDI	GSI
1	9.709	69.354	-0.911	-0.976	0.015	-0.979	-0.979	-0.981	-0.976	-0.568	-0.568	-0.989	-0.947	-0.989	0.568	-0.524
2	3.855	96.892	0.390	-0.135	-0.953	0.184	0.184	0.172	-0.135	-0.815	-0.815	0.093	0.301	-0.019	0.815	0.756

For more understanding and visualizing the relationships between calculated indices and genotypes performance, principal component analysis (PCA) was performed. Result of this analysis showed that the first two components explained about 96.89% of the total variance (Table 9). The first component (PC1) was mostly affected by Ys, Yp, MP, GMP, STI, YI, HM and MSTI. Therefore this component was related to yield potential and drought

tolerance. Second component explained 27.54% of the total obtained variation and can be named drought susceptible dimension with high yield in complementary irrigation and low yield in stressed conditions. Hence, selection of genotypes with high PCA1 and low PCA2 are suitable for both stress and complementary irrigation environments (Shahryari and Mollasadeghi, 2011; Amiri *et al.* 2014).

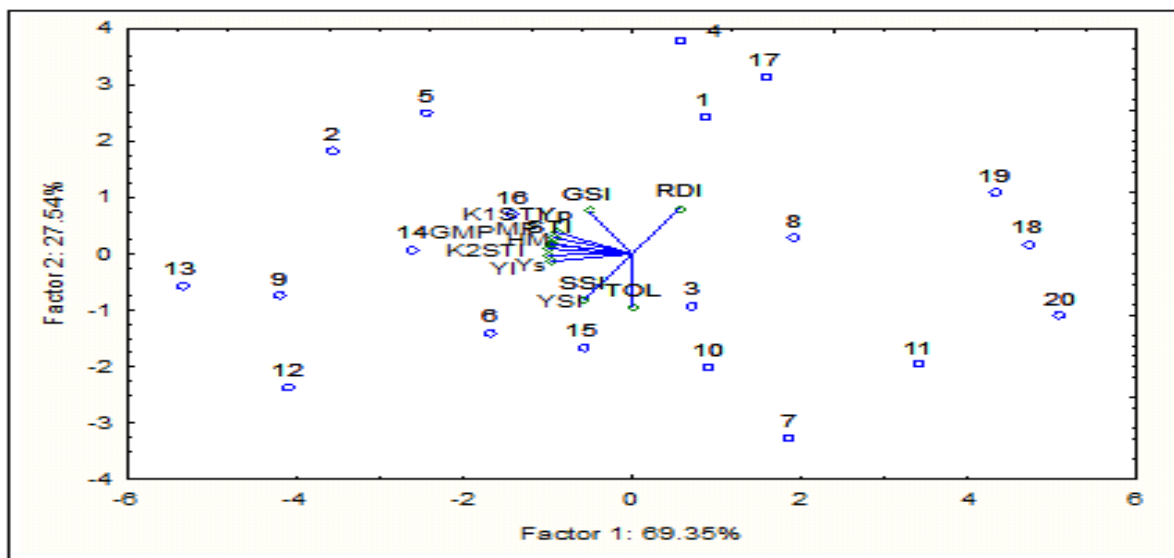


Fig. 1. Biplot display tolerance and sensitivity to drought in 20 wheat genotypes based on first two principal components.

Based on biplot graph (Fig. 1) genotypes No. 20, 18, 19 and 11 with rather higher PCA1 and lower PCA2 are superior genotypes under both stressed and complementary irrigation conditions. These genotypes had stable performance in the circumstances of low sensitivity to drought stress. So, they are belong to Group A. These genotypes also had high Yp, Ys, GMP, MP, STI, HM, YI, K1STI , K2STI and GSI. These indices are able to select and identify genotypes with high grain yield in both conditions (Fernandez, 1992). Genotypes 8, 7, 8 and 10 could be known as Group B. These genotypes are suitable for non-stressed conditions, While genotypes No. 4, 17

and 1 had high yielding performance in complementary irrigation conditions, so they are related to group B. Genotypes 7, 3,10 and 15 with high amount of YSI had a relatively low yield in both conditions, but they were more stable genotypes than the others (Group C). Genotypes 13, 12, 9, 2, 5, 14, 6 and 16 are drought susceptible and had low yield in both conditions (Group D).

Conclusion

At the present study, a genotypic variation was observed for grain yield and the other studied traits under both conditions, especially complementary

irrigation conditions. Results indicated that genotype and environment treatments significantly affect the yield and the most of the other evaluated traits. This study emphasis that total chlorophyll and number of grain per spike can be utilized to screen wheat genotypes for drought tolerance. Result of correlation analysis between grain yield in both conditions and calculated drought resistance indices showed that MP, GMP, STI, YI, HM, MSTI and GSI were the best indices for identifying high yielding genotypes in both conditions. According to all statistical procedures, genotypes No. 20, 18, 19 and 11 are superior genotypes under both stressed and non-stressed conditions.

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