IRANIAN JOURNAL of GENETICS and PLANT BREEDING, Vol. 9, No. 1, Apr 2020

Research Paper / 28-40

Evaluation of rain-fed wheat (*Triticum aestivum* L.) genotypes for drought tolerance

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Received: 19 Feb 2020; Accepted: 29 Jun 2020.
DOI: 10.30479/ijgpb.2020.12734.1265

Abstract

Drought stress is one of the most important environmental stresses that have limited the production of wheat, especially in arid and semi-arid regions of the world. To recognize drought tolerant rain-fed wheat genotypes and to determine the best tolerance/susceptibility indices, a study was conducted at the Agricultural Research Station of Miandoab for two cropping years (2013-15). The experimental materials included 12 rain-fed wheat genotypes investigated in two separate field experiments based on randomized complete blocks design with three replications under both rain-fed and supplemental irrigation conditions. The combined ANOVA for grain yield and agro-physiological traits showed that there was a large genetic difference between wheat genotypes for grain yield and studied traits in response to drought stress among years and moisture regimes. The different drought tolerance/susceptibility indices were used to characterize drought tolerance of genotypes. Generally, a reduction of RWC in drought tolerant genotypes (genotypes 6, 2, 11, and 10) was lower compared to the sensitive genotypes (genotypes 4 and 8). Cluster analysis based on drought tolerance indices categorized genotypes into two main groups. The genotypes belonging to the cluster 1 could be introduced as tolerant to the drought conditions. According to MSI (Multiple scoring index), genotypes 10 (Seafallah/3/Sbn//Trm/K253) and Saein had the best combination of productivity and resistance to drought stress. The significant correlation between MSI with grain yield under drought conditions indicated the superiority of MSI as a useful tool for efficient selection of drought-tolerant genotypes. In the present study there was no significant correlation between RWC and RWL with MP, GMP, STI and MSI indices under both conditions.

Key words: Cluster analysis, Drought stress, Multiple scoring index, Productivity capacity index.

INTRODUCTION

Drought is one of the most important environmental stresses affecting agricultural production in dry and semi-dry areas (Hussain *et al.,* 2012). A recent study analyzed the data of studies published from 1980 to 2015 to report up to 21% yield reductions in wheat due to drought on a global scale (Daryanto *et al.,* 2016). Therefore, introduction of tolerant cultivars at different stages of physiological growth is one of the major challenges of worldwide wheat breeding programs (Khalili and Mohammadi, 2015).

Cereals are considered as the main food for most people in the world, and more than 70% of the world's food is prepared from cereals (Mosanaei *et al.,* 2017). Bread wheat (*Triticum aestivum* L.) is one of the most important crops in the world due to its extensive adaptation and nutrition, cultivated on 214 million hectares area with an annual production of 734 million tons (FAO, 2018). Wheat is among the most important cereal crops and large portions of human populations in many parts of the world depend on them as a source of food and animal feed (Sallam *et al.,* 2019). The bread wheat also plays an important role in the nutrient regime of Iranian people. According to the Food and Agriculture Organization of the United Nations (FAO) statistics, in Iran, wheat was cultivated on 6.7 million hectares and its total production was about 14.5 million tons (FAO, 2018). Iran with an average rainfall of 240 mm per year located in the semi-arid and arid regions. Therefore, drought stress is one of the most important factors reducing wheat production (Razegi Yadak and Tavakkol Afshari, 2010).

Use of high yielding genotypes having drought tolerance is an effective attitude to reduce drought damaging effects (Anwaar *et al.,* 2019). One of the basic strategies for overcoming the problems caused by drought is selecting resistant cultivars and breeding for adapted genotypes. The relations between the plant yield obtained under drought and optimal soil moistening was preferred among the indicators of drought tolerance in a field (Grzesiak *et al.,* 2019).

Drought tolerance is a complex quantitative polygenic trait controlled by a large number of genes and thus, it is diffcult to understand its molecular and physiological mechanisms (Senapati *et al.,* 2018). Also, plant responses to water stress are confounded by many factors such as time, severity, continuation and frequency of stress as well as by plant, soil and zone interactions (Reynolds and Tuberosa, 2008). Hence, various indices should be used for phenotyping drought tolerance (Tuberosa, 2012). Several drought tolerance indices (DTIs) based on mathematical relationships between normal and stress conditions have been proposed to identify desirable genotypes that perform well under a wide range of water treatments (Cabello *et al.,* 2013). There is evidence that phenotyping using drought stress indices, as a complement to agronomic traits, may help in identifying selectable features that accelerate breeding for yield potential and performance under drought (Mohammadi, 2016; Mwadzingeni *et al.,* 2016). Several resistance indices such as stress susceptibility index (SSI) (Fischer and Maurer, 1978), mean productivity (MP) and tolerance (TOL) (Rosielle and Hamblin, 1981), yield stability index (YSI) (Bouslama and Schapaugh, 1984), harmonic mean (HM) (Jafari *et al.,* 2009), stress tolerance index (STI) and geometric mean productivity (GMP) (Fernandez, 1992) have been described. According to Fernandez (1992) and Thiry *et al.* (2016), genotypes based on yield response to the drought stress can be divided into four groups: Group A: high yielding genotypes under both stress and non-stress conditions; Group B: genotypes with high yield under non-stress conditions; Group C: genotypes with high yield under stress conditions and Group D: genotypes with low yield under both stress and non-stress conditions. Mursalova *et al.* (2015) studied 48 bread wheat genotypes tolerant to the drought stress. They reported that yield under both stress and normal conditions had a significant positive correlation with MP, GMP and STI indices. Then, these indices were introduced as the best yield prediction indices under both stress and non-stress conditions. Similar results had been previously reported by other researchers (Bellague *et al.,* 2016; El-Hendawy *et al.,* 2017; Ben Naceur *et al.,* 2018; Halim *et al.,* 2018; Shabani *et al.,* 2018; Anwaar *et al.,* 2019; Eyni Nargeseh *et al.,* 2019; Hooshmandi, 2019). Although all these indices are mathematical derivations of the same yield data, it has been offered that a combination of stress indices (tolerance and susceptibility indices) might supply a more useful scale for improving drought tolerance selection in crop species (Thiry *et al.,* 2016). A multiple scoring index (MSI) based on scoring drought tolerance indices was expanded by Thiry *et al.* (2016). However, there are few reports on the precision of the MSI and its relationship with drought-adaptive traits under drought conditions. Mohammadi (2019) reported a significant correlation between MSI with grain yield and thousand kernel weight (TKW) under severe drought condition. This prepares evidence that MSI eventually is considered as a tool for efficient selection of drought-tolerant genotypes.

The objectives of the present study were (i) to assess genetic variation among rain-fed wheat genotypes in response to drought and identify high yielding wheat genotypes tolerant to drought stress to be used in breeding programs and (ii) to investigate the association of the MSI with some drought-adaptive traits in rain-fed wheat.

MATERIALS AND METHODS

Plant material and experimental layout

Field trials were conducted in the Agricultural Research Station of Miandoab, West Azarbaijan Province, Iran

(Latitude 36° 58´N, Longitude 46° 06´E, Altitude 1314 m above the sea level). The soil texture of this site was loamy silt and soil pH was 7.9. The soil field capacity (FC) at a depth of 30 cm was 28.7. Climatic parameters (i.e., temperature, rainfall and relative humidity) are shown in Table 1 and Figure 1.

A total of twenty rain-fed wheat genotypes including 7 cultivars and 5 promising lines, listed in Table 2, were subjected to drought tolerance study. The seeds were kindly provided by the cereals department of Dryland Agriculture Research Institute (DARI) of Iran.

Two field experiments were arranged based on a randomized complete blocks design (RCBD) with three replications in two consecutive wheat

Table 1. Climatic parameters from October to June at the Agricultural Research Station of Miandoab.

Cropping season	Condition	Average temperature			Rainfall (mm)	Evaporation (mm)	Irrigation (mm)	Relative humidity (%)	Soil moisture (%)	Freezing days
		Min	Max	Mean						
2013/2014	SI	2.1	15.3	8.7	238.8	650.8	60	66.5	13.2	97
2013/2014	RF	2.1	15.3	8.7	238.8	650.8	-	66.5	13.2	97
2014/2015	SI	3.5	17.8	9.9	264.7	583.1	60	60.6	13.4	93
2014/2015	RF	3.5	17.8	9.9	264.7	583.1	-	60.6	13.4	93

SI: Supplemental Irrigation, RF: Rain-fed.

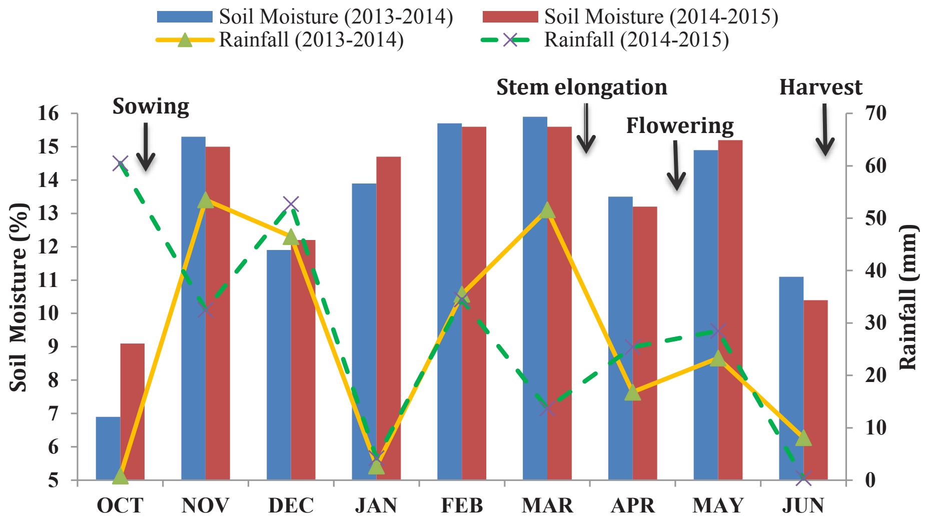

Figure 1. Monthly patterns of soil moisture and rainfall recorded during the course of the experiment (2013-15).

Table 2. Pedigree/Name of rain-fed wheat genotypes used in this study.

Genotype No.	Pedigree/Name	Type	Genotype No.	Pedigree/Name	Type
1	Sardari	Cultivar	7	Varan	Cultivar
2	Azar2	Cultivar	8	Homa	Cultivar
3	Rasad	Cultivar	9	F10S-1//ATAY/GALVEZ87	Promising line
4	Ohadi	Cultivar	10	Seafallah/3/Sbn//Trm/K253	Promising line
5	Saein	Cultivar	11	Sardari-101	Promising line
6	Azar2/87Zhong291-149	Promising line	12	Unknown11	Promising line

cropping seasons (2013-2014 and 2014-2015). Each experiment was carried out in two separate moisture regimes, i.e. non-stress (supplemental irrigation) (SI) and water-stressed (rain-fed) (RF) conditions. Under SI conditions, two irrigations each of 30 mm were employed during sowing (for seed germination) and grain filling period to reduce the effects of terminal drought stress. Chemical fertilizer application was 90 kg $CO(NH_2)_2$ ha^{-1}, 90 kg $(NH_4)_2HPO_4$ ha^{-1} and 60 kg K_2SO_4 ha^{-1} according to the local soil test advice before planting. Each plot consisted of 6 rows, 4 meters long with 20 cm row spacing (plot size=4.8 m^2). In this study, 400 grains per square meter were cultivated as the optimum density. Farm management advice for each environment was followed in every yield experiment. In each environment, evaluations were carried out for the following traits according to the assigned protocols (Pask *et al.,* 2012): days to heading (DTH), plant height (PH, cm), fertile spikelet number per spike (FSN), spike length (SPL, cm), number of kernels per spike (KPS), spike dry weight (SPDW, g), spike kernels weight (SPKW, g), thousand kernel weight (TKW, g), peduncle length (PL, cm), peduncle weight (PW, g). Finally, the plot grain yield (GY) and biomass yield (BY) were converted to productivity per hectare (kg ha^{-1}) and exposed to statistical analysis. For the evaluation of physiological traits, after anthesis stage, fresh leaves were taken from each genotype and weighed instantly to record fresh weight (FW). Then leaves were soaked in distilled water for 4 h at 25 °C, reweighed to record turgid weight (TW), and oven-dried for 48 h at 72 °C to obtain the dry weight (DW). The relative water content (RWC, %), leaf water content (LWC, %) and relative water loss (RWL, g $(g.hr)^{-1}$) for all genotypes were calculated according to the Ritchie *et al.* (1990), Ramirez-Vallejo and Kelly (1998) and Yang *et al.* (1991), respectively (Equaation 1-3):

$$(1)\qquad RWC=[(FW-DW)/(TW-DW)]\times 100$$

$$(2)\qquad LWC=[(FW-DW)/FW]\times 100$$

$$(3)\qquad RWL=(FW-ADW)/(t\times DW)$$

Where ADW is wilt weight after 2 h at 30 °C, and t is the time in an hour at the wilt weight.

Statistical analysis and calculations

Firstly, data from both years were tested for the homogeneity using Bartlett's test of homogeneity (1937) and they were found to be homogeneous. Therefore, the data were combined for analysis. A combined analysis of variance (ANOVA) for grain yield and studied traits was carried out to determine the effects of year, moisture regime, genotype and their interactions. Using the mean grain yield in two years for each genotype under SI and RF conditions, different drought tolerance/susceptibility indices were calculated based on the equations cited in Table 3 to identify the drought resistant and sensitive genotypes. In addition, Ward's cluster analysis (WCA) was also applied to recognize the rate of dissimilarity among the genotypes.

The scoring scale for MP, GMP, STI, SSI and TOL indices was computed as explained by Thiry *et al.* (2016). To enable comparison of the indices, a scale was made based on an equal reference for all indices by scoring the results from 1 to 10, where a high value always means a good response in terms of resilience or production capacity. The difference between the minimum and maximum values of each index presents the range of the scale for each index. This range is separated into ten sections and each section has a score from 1 to 10. Therefore, each section represents 10%, 20%, ..., or 100% of the range value. For STI, GMP, and MP, high values are desirable (Class 1), while for SSI and TOL, low values are desirable (Class 2). Therefore, the values of TOL and SSI were reversed,

Table 3. Drought tolerance indices used for assessment of reaction of rain-fed wheat genotypes to drought stress.

Drought tolerance indices	Formula	Reference
Stress Susceptibility Index	$SSI=1-(Ys/Yp)/1-(\bar{Y}s/\bar{Y}p)$	Fischer and Maurer (1978)
Tolerance Index	$TOL=Yp-Ys$	Rosielle and Hambling (1981)
Mean Productivity	$MP=(Yp+Ys)/2$	Rosielle and Hambling (1981)
Yield Stability Index	$YSI=Ys/Yp$	Bouslama and Schapaugh (1984)
Stress Tolerance Index	$STI=(Yp\times Ys)/(\bar{Y}p)^2$	Fernandez (1992)
Geometric Mean Productivity	$GMP=(Yp\times Ys)^{-1/2}$	Fernandez (1992)
Harmonic Mean	$HM=2(Yp\times Ys)/(Yp+Ys)$	Jafari *et al.,* (2009)

Y_p and Y_s: Potential and stress grain yield of genotypes, respectively; $\bar{Y}p$ and $\bar{Y}s$: Mean grain yield of all genotypes under non-stress and stress conditions, respectively.

so a high value obtained with the original equation will receive a lower score. This permits the two classes of indices to have the same scale, where a high score will always mean a ‘good’ genotype. Eventually, MSI was calculated as a combination of intended indices using Equation 4 (Mohammadi, 2019):

$$\text{(4)}\qquad \mathrm{MSI} = \frac{(\mathrm{MPs} + \mathrm{GMPs} + \mathrm{STIs} + \mathrm{SSIs} + \mathrm{TOLs})}{5}$$

Where STIs, GMPs, MPs, SSIs, and TOLs are score scales of these indices. All statistical analyses were carried out using SPSS ver. 16 (SPSS Inc., 2001) and Statgraphics software ver. 16.1 (StatPoint Technologies, Inc., 2009).

RESULTS

Combined analysis of variance

The results of combined ANOVA for grain yield and 14 agro-physiological traits are summarized in Table 4. Significant differences were found for most of the traits among the genotypes (G) (except for DTH and LWC), moisture regimes (M) and years (Y) effects for GY and studied traits. Except for DTH, PH, PL, TKW, and LWC, a significant G×M interaction was observed which demonstrated that genotypes responded differently to SI and RF conditions. Also a significant G×Y interaction revealed that differences between the genotypes have not been similar over years. The values of broad-sense heritability (H^2) indicated that the highest and lowest values for H^2 were observed for KPS (0.93 under SI and 0.94 under RF conditions) and SPL and BY (0.47, 0.29 under SI and 0.17, 0.25 under RF conditions), respectively.

Overview of evaluated traits and indices

In this study the stress intensity (SI) was calculated according to Fischer’s method (Fischer and Maurer, 1978) that it was equal to 0.326. Descriptive statistics for agro-physiological traits under RF and SI conditions (traits with the significant G×M interaction) and the mean of two moisture conditions (traits with the nonsignificant G×M interaction) over two cropping seasons are shown in Table 5 and Table 6, respectively. According to the least significant difference (LSD) test at 5% level of probability, the genotypes significantly varied based on the measured traits. Considering all traits, no genotype was best, so genotypes should be characterized by their trait profiles. Based on GY, genotype 10, followed by 5 and 11 performed well over the years under RF condition, while 4, 12 and 8 genotypes displayed a lower yield performance under SI condition. Under RF conditions, RWC declined and

Table 4. Mean squares and broad sense heritability of rain-fed wheat genotypes over 2 years and two different moisture regimes.

Source of variation	df	DTH	PH	FSN	SPL	KPS	SPKW	SPDW	PL	PW	TKW	GY	BY	RWC	LWC	RWL
Year (Y)	1	544.44**	730.38	6.94	8.53*	27.9	0.06	0.03	96.34	0.02	238.27*	8294933.34*	5792296.89	300.02	240.27*	0.0115*
Moisture regime (M)	1	584.03**	5156.81*	394.38**	89.11*	4177.35*	12.14*	5.81*	1208*	0.99*	5285.41*	42545422.03*	777035496.34*	3709.68*	547	0.0516*
Y×M	1	2.25	1350.96*	22.02*	5.48	55.16	0.05	0.02	103.79	0.02	172.2*	69149.52	8967671.9	218.82	377.94*	0.0011
R (Y×M)	8	0.44	206.49	4.27	1.37	45.88	0.11	0.03	28.91	0.02	26.59	1144656.31	13267081.32	93.87	38.43	0.0018
Genotype (G)	11	12.16	2514.79*	80.92**	2.87**	622.91**	0.44**	0.13**	327.67**	0.16**	320.17*	6006641.77**	15756404.74*	659.09*	183.08	0.0198**
G×Y	11	19.98**	964.38**	18.93**	3.72**	40.39**	0.05**	0.02	55**	0.01	105.87**	1895112.57**	9304975.16*	212.16**	202.07**	0.0043*
G×M	11	2.19	160.1	6.27**	1.5*	84.44**	0.08**	0.07**	21.26	0.02**	11.72	1385126.59**	6908562.76**	54.19*	18.87	0.0162**
G×Y×M	11	0.93	82.08	1.21	0.43	7.14	0.01	0.004	8.8	0.002	6.71	253488.16	878668.05	23.85	13.98	0.0034
Error	88	0.58	94.39	1.88	0.5	10.88	0.02	0.03	12.03	0.01	6.77	303036.65	3491337.19	33.33	17.53	0.0021
Coefficient of variation (%)		11.2	8.34	8.89	7.16	10.43	12.53	24.74	8.76	19.16	6.41	11.25	16.28	7.28	6.54	16.54
H^2	Normal	0.64	0.84	0.83	0.47	0.93	0.65	0.73	0.78	0.92	0.7	0.8	0.29	0.73	0.51	0.62
	Stress	0.76	0.68	0.64	0.17	0.94	0.7	0.78	0.91	0.81	0.63	0.65	0.25	0.4	0.4	0.71

DTH: Days to heading, PH: Plant height, FSN: Fertile spikelet number per spike, SPL: Spike length, KPS: Number of kernels per spike, SPKW: Spike kernels weight, SPDW: Spike dry weight, PL: Peduncle length, PW: Peduncle weight, TKW: Thousand kernel weight, GY: Grain yield, BY: Biomass yield, RWC: Relative water content, LWC: Leaf water content, RWL: Relative water loss, * and **: Significant at 5% and 1% levels of probability, respectively, H^2: Broad sense heritability.

RWL increased significantly. The best genotypes in higher RWC and lower RWL were 9, 7 and 5 under RF condition, whereas the best genotypes in physiological traits were 5, 7 and 10 under SI condition. Genotypes 5 and 7 were the best in physiological traits under both experimental conditions. Genotypes 8 and 6 showed the highest and lowest TKW in average of two conditions, respectively. The KPS ranged between 23.79 (genotype 12) to 45.92 (genotype 9) among genotypes under SI condition, while SPKW varied from 0.24 (genotype 8) to 0.76 g (genotype 11) under RF condition. The genotypes with the highest SPDW were genotype 2 and genotype 3 and peduncle weight was the highest for genotype 3 under SI and RF conditions, respectively. The PH ranged between 96.07 (genotype 1) to 140.33 cm (genotype 9) among studied genotypes

Table 5. Mean values and descriptive statistics of agro-physiological traits of 12 rain-fed wheat genotypes under rain-fed and supplemental irrigation conditions and two cropping seasons.

Rain-fed

Genotype	FSN	SPL	KPS	SPDW	SPKW	PW	GY	BY	RWC	RWL
1	13.95	9.67	22.61	0.84	0.50	0.37	2071.42	5014.94	68.85	0.481
2	13.16	9.26	26.32	1.01	0.61	0.48	2713.08	7267.04	76.54	0.375
3	14.6	9.55	27.80	1.03	0.50	0.66	1920.53	7943.08	75.49	0.189
4	11.76	9.35	21.03	0.79	0.49	0.41	2073.84	6016.79	62.90	0.553
5	16.46	9.39	31.37	0.87	0.53	0.44	2988.5	8127.77	83.82	0.266
6	14.41	8.70	29.20	0.80	0.45	0.47	2717.84	6818.43	76.34	0.314
7	15.25	8.83	28.01	0.74	0.40	0.47	2238.66	7155.81	81.62	0.348
8	10.89	8.02	14.53	0.48	0.24	0.28	2107.34	7633.73	66.84	0.339
9	18.85	9.56	40.02	0.92	0.53	0.44	1853.08	9949.46	84.39	0.5
10	13.64	9.27	26.99	0.95	0.52	0.51	3332.42	6726.90	81.82	0.317
11	11.80	9.11	28.47	0.99	0.76	0.43	2793.58	7820.66	70.39	0.423
12	10.70	8.35	18.52	0.63	0.43	0.30	1978	5336.89	72.31	0.711
LSD$_{5\%}$	1.70	1.52	5.5	0.12	0.12	0.12	1118.67	2110.61	11.5	0.42
Mean	13.79	9.09	26.24	0.84	0.50	0.44	2399	7150.96	75.11	0.401
Min	10.70	8.02	14.53	0.48	0.24	0.28	1853.08	5014.94	62.90	0.189
Max	18.85	9.67	40.02	1.03	0.76	0.66	3332.42	9949.46	84.39	0.711
SE	0.69	0.15	1.89	0.05	0.04	0.03	140.42	384.73	2.02	0.041

Supplemental irrigated

Genotype	FSN	SPL	KPS	SPDW	SPKW	PW	GY	BY	RWC	RWL
1	13.8	10.11	27.52	1.33	1.00	0.51	3747.42	10817.46	74.53	0.395
2	17.35	11.63	42.80	1.81	1.32	0.71	3380.25	11973.79	82.91	0.15
3	16.3	10.46	38.71	1.70	1.08	0.90	3197.92	12609.22	83.71	0.109
4	14.8	10.80	29.66	1.17	0.84	0.48	2557.50	10058	77.46	0.321
5	19.7	10.82	45.55	1.62	1.03	0.72	3921.75	14273.93	94.62	0.184
6	18.87	9.79	42.57	1.49	1.13	0.65	3801.75	10548.01	81.84	0.244
7	20.21	10.90	45.69	1.45	1.06	0.75	4938.50	11024.61	93.40	0.226
8	13.79	10.37	30.71	1.08	0.74	0.48	2775.00	12155.04	76.94	0.171
9	22.85	10.41	45.92	1.47	0.99	0.55	3230.66	12269.61	95.26	0.244
10	18.61	12.03	44.17	1.61	1.18	0.68	4941.00	14291.4	90.90	0.241
11	14.74	10.90	28.13	1.22	0.82	0.44	3643.16	10343	77.96	0.302
12	14.11	9.71	23.79	1.09	0.74	0.45	2605.50	11198.18	78.87	0.268
LSD$_{5\%}$	3.99	1.82	6.4	0.18	0.16	0.1	1290.41	2118.23	13.12	0.221
Mean	17.09	10.66	37.10	1.42	0.99	0.61	3561.7	11796.85	84.03	0.238
Min	13.79	9.71	23.79	1.08	0.74	0.44	2557.5	10058	74.53	0.109
Max	22.85	12.03	45.92	1.81	1.32	0.90	4941	14291.4	95.26	0.395
SE	0.86	0.2	2.44	0.07	0.05	0.04	227.69	408.36	2.18	0.023

FSN: Fertile spikelet number per spike, SPL: Spike length (cm), KPS: Number of kernels per spike, SPDW: Spike dry weight (g), SPKW: Spike kernels weight (g), PW: Peduncle weight (g), GY: grain yield (Kg ha^{-1}), BY: Biomass yield (Kg ha^{-1}), RWC: Relative water content (%), RWL: Relative water loss (g (g.hr)$^{-1}$), Min: Minimum, Max: Maximum, SE: Standard error.

Table 6. Mean values and descriptive statistics of agro-physiological traits of 12 rain-fed wheat genotypes in average of two moisture conditions and two cropping seasons.

Genotype	DTH	PH	TKW	PL	LWC
1	199	96.07	45.94	34.60	61.73
2	198.42	121.68	45.91	41.56	63.63
3	200	117.40	44.42	41.68	61.52
4	199	103.54	43.56	35.92	60
5	199.17	129.47	37.05	45.60	68.50
6	198.59	122.42	32.27	41.76	62.59
7	199.34	135.96	33.71	45.60	68.96
8	197.50	107.75	46.74	34.75	58.86
9	199.83	140.33	34.88	44.36	71.82
10	199.83	119.88	38.51	45.36	67.24
11	201	99.42	40.17	32.20	61.75
12	198.00	103.74	43.61	33.71	65.15
$LSD_{5\%}$	5.68	29.17	8.33	5.23	12.08
Mean	199.14	116.47	40.56	39.76	64.31
Min	197.50	96.07	32.27	32.20	58.86
Max	201	140.33	46.74	45.60	71.82
SE	0.28	4.18	1.49	1.49	1.16

DTH: Days to heading, PH: Plant height (cm), TKW: Thousand kernel weight (g), PL: Peduncle length (cm), LWC: Leaf water content (%), Min: Minimum, Max: Maximum, SE: Standard error.

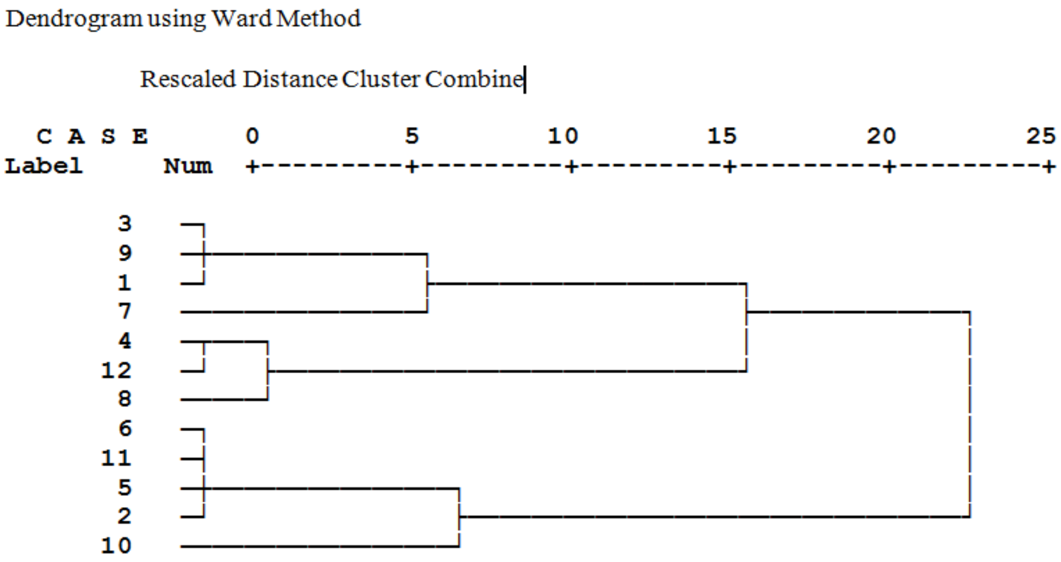

Figure 2. Dendrogram from cluster analysis of 12 rain-fed wheat genotypes based on MP, GMP, HM and STI indices and grain yield under both non-stress and drought stress conditions. Numbers are genotypes codes (See Table 2).

in means of two conditions and the highest SPL was obtained in genotype 10 and genotype 1 under SI and RF conditions, respectively (Table 5 and Table 6). The values of different tolerance indices are presented in Supplementary Table 1. SSI and TOL varied from 0.58 to 1.68 (genotype 4) and 483.66 to 2699.84 (genotype 7), respectively. The highest MP, GMP and STI were observed for genotype 10, while the lowest values of these indices were obtained in genotype 4.

Cluster analysis

In order to assign the genetic diversity between the studied genotypes and category of genotypes based on MP, GMP, HM and STI drought indices and grain yield in both experimental conditions, the cluster analysis was performed based on squared Euclidean distance using Ward's method to classify the similar genotypes in one group. Figure 2 shows the dendrogram resulted from cluster analysis of 12 rain-fed wheat genotypes.

Cutting dendrogram based on discriminate analysis categorized genotypes into two main clusters with 5 and 7 genotypes, respectively (Figure 2). The first group consisted of the genotypes 10, 5, 6, 11 and 2 that had a high performance under stress conditions (Table 7). On the other hand, considering the results of principal component analysis and bi-plot diagram (data not shown), these genotypes could be introduced as tolerant to the drought conditions. As it appears in Figure 2, genotypes 4, 12, 8, 3, 9, 1 and 7 were classified in the second cluster. The second group was divided into two subgroups. Subgroup A included genotypes 3, 9, 1 and 7 and subgroup B consisted of genotypes 4, 12 and 8. Based on the results from other statistical analyses, genotypes in subgroup A had high grain yield under non-stress conditions, indicating specific compatibility to the irrigated conditions. Hence, the subgroup A genotypes should be introduced as semi-sensitive to the drought stress conditions. The genotypes in subgroup B had low yield under both stress and non-stress conditions and showed the least value for tolerance criteria. Therefore, they should be introduced as susceptible to the drought stress conditions (Table 7). Generally, results from this study were in agreement with PCA analysis (data not shown).

Scoring index

The scores of drought stress indices are given in Table 8. The 10-score indices prepare an explanation of small differences between SSI and TOL. On the other hand, MP and GMP were very similar, but both were slightly different from STI. The score indices represent small differences, but they hold the same importance into each class, where class 1 inclines to detect genotypes with stress tolerance and high mean yield and Class 2 inclines to discern between the tolerant and the susceptible genotypes (Thiry *et al.,* 2016). The genotypes with the lowest score in class 1 (SSI and TOL) were 2, 4, 8, 12 and 11 whereas the highest scores were found for genotypes 7 and 1. Similarly, in terms of class 2 (MP, GMP and STI) index scores, genotype 10 was the superior genotype, while 12, 8, 4, 9 and 3 were the inferior genotypes.

Table 7. Mean values of grain yield under both non-stress (Yp) and stress (Ys) conditions and drought tolerance indices based on the groups separated in cluster analysis.

Group	Subgroup	Mean					
		Yp	Ys	MP	GMP	HM	STI
1		3937.6	2909.1	3423.3	3382.8	3342.9	0.910
2	A	3778.6	2020.9	2899.8	2759	2646	0.608
	B	2646	2053.06	2349.53	2330.47	2311.58	0.428

MP: Mean productivity, GMP: Geometric average productivity, HM: Harmonic mean, STI: Stress tolerance index.

Table 8. The score index, Multiple Scoring Index (MSI) and rank values of MSI for 12 rain-fed wheat genotypes based on drought tolerance indices.

Genotype	SSI	TOL	MP	GMP	STI	MSI	Rank
1	8	6	7	8	8	7.4	8
2	1	1	6	6	7	4.2	4
3	6	4	9	9	9	7.4	8
4	1	1	10	10	10	6.4	6
5	2	3	4	4	5	3.6	2
6	3	3	5	5	6	4.4	5
7	10	10	3	5	5	6.6	7
8	2	1	10	10	10	6.6	7
9	7	5	9	9	10	8	9
10	4	6	1	1	1	2.6	1
11	2	2	5	5	6	4	3
12	2	1	10	10	10	6.6	7

SSI: Stress susceptibility index, TOL: Tolerance index, MP: Mean productivity, GMP: Geometric average productivity, STI: Stress tolerance index, MSI: Multiple scoring index. Numbers are genotypes codes (See Table 2).

Multiple Scoring Index (MSI) and agro-physiological traits correlated with MSI

Data presented in Table 8 showed that the studied 12 wheat genotypes varied in their resilience capacity index (RCI) and productivity capacity index (PCI) under the conditions of imposed drought stress. According to MSI, genotypes 10 and 5 had the best combination of productivity and resilience to drought stress. The repeatability and validity of MSI for the estimating of tolerance/susceptibility under drought stress previously confirmed by Mohammadi (2019). Correlation coefficients between MSI and measured traits displayed in Table 9. The MSI was highly significantly correlated with grain yield in drought stress conditions. No significant correlation was found between MSI with RWC, RWL and LWC under both experimental conditions.

DISCUSSION

In order to improve the wheat drought tolerant genotypes without increasing the area of cultivated land, emphasis must be concentrated on key traits related to plant productivity and adaptation to environmental challenges. Genetic improvement and developing drought-tolerant wheat cultivars are critically important and the main aim for wheat breeders (Khan *et al.,* 2019). Several and various mechanisms are associated with tolerance to adverse conditions. Selecting a genotype with such characteristics is not an easy task and is most difficult in the early stages of plant breeding. Evaluation of genotypes under stress and non-stress conditions are generally used in stress studies (Amini *et al.,* 2015; Mursalova *et al.,* 2015; Norouzi *et al.,* 2017; Ben Naceur *et al.,* 2018). Drought stress often reduces grain filling period, reducing grain weight and causing yield loss in wheat experiments under rain-fed conditions. Concerning the importance of drought in the country, it is necessary to provide different strategies to reduce the effects of this stress. In the present study, promising lines in late stages of rain-fed breeding programs along with seven rain-fed bread wheat cultivars were evaluated for response to stress conditions. According to combined ANOVA, the expression and the quantity of grain yield and some studied traits were affected by year, moisture regime and genotype. The highly significant genotypic differences were detected among the agro-physiological traits show that the genetic materials used in the present study could be a wealthy source of genetic diversity for improving drought tolerance and identify genotypes with high levels of drought tolerance in wheat. Similar results were found by Grzesiak *et al.* (2018) and Halim *et al.* (2018). Selecting for improved grain yield under both drought and irrigated conditions allow genotypes to maintain ranks for high yields since the same genotypes will be expected to perform well in either condition (Mohammadi, 2019). The high yield obtained for genotypes 10 and 5 under RF and SI conditions confirm the reports of Grzesiak *et al.* (2018) that genotypes performing well under normal conditions hold high yield under stress. The KPS, PW, PL, SPDW, and SPKW had high heritability estimates. These traits also displayed noticeable coefficients of variation, which represented the main role of additive gene action in the inheritance of these traits and the possibility of improving them through breeding programs. Selecting traits with higher heritability other than yield can be helpful for indirect selection. For this reason, several researchers have offered that selection under the rain-fed conditions may be developed by selecting traits associated with yield under the drought stress (McIntyre *et al.,* 2010; Gizaw *et al.*, 2016). High RWC and low RWL for genotypes grown under the drought condition are suitable for selection. Under RF conditions, RWC decreased and RWL increased significantly. Generally, a reduction of RWC in drought tolerant genotypes (genotypes 6, 2, 11, and 10) was lower comparing to sensitive genotypes (genotypes 4 and 8). It has been reported that drought tolerant genotypes displayed the higher RWC rather than drought sensitive genotypes (El-Tayeb, 2006). Also, under RF conditions, genotypes 6, followed by 10, 11 and 5 (drought tolerant genotypes) had the least increase in RWL compared to SI conditions. There was no significant correlation between RWC and RWL with MP, GMP, STI and MSI under both conditions (data not shown). This result is in agreement with Geravandi *et al.* (2011) that reported RWC is not an indicator of drought tolerance. Cluster analysis has been widely used for the evaluation of genetic diversity of genotypes and grouping based on drought tolerance indices. Grouping the genotypes by the Ward method using desirable drought tolerance indices classified them into two main groups. Hence, by using genotypes that are placed in distinct groups and show the maximum genetic distance, it is feasible to analyze genetic parameters of these drought tolerance indices (Mursalova *et al.,* 2015). Since group 1 and subgroup B genotypes displayed the maximum genetic distance and dissimilarity, they are recommended for the genetic analysis using diallel test and QTLs mapping of drought tolerance indices. There was no significant relationship between GY with SPL, KPS, PL, PW, RWC and RWL, which offers that grain yield could potentially be improved without reducing these traits

Table 9. Correlation coefficients between agro-physiological traits and Multiple Scoring Index (MSI) of 12 rain-fed wheat genotypes under stressed (lower diagonal) and irrigated (upper diagonal) conditions.

Traits	MSI	DTH	PH	FSN	SPL	KPS	SPDW	SPKW	TKW	PL	PW	GY	BY	RWC	RWL	LWC
MSI		-0.087	-0.026	-0.121	-0.596*	-0.294	-0.349	-0.392	0.188	-0.351	-0.141	-0.473	-0.377	-0.181	0.126	-0.023
HD	-0.273		0.287	0.459	0.245	0.291	0.214	0.099	-0.409	0.098	0.135	0.388	0.053	0.45	0.163	0.413
PH	-0.061	-0.158		0.929**	0.262	0.889**	0.57*	0.482	-0.653*	0.847**	0.603*	0.444	0.493	0.945**	-0.502	0.868**
FSN	0.132	0.09	0.818**		0.241	0.908**	0.584*	0.537	-0.79**	0.774**	0.479	0.505	0.41	0.921**	-0.3	0.893**
SPL	0.02	0.614*	0.152	0.573*		0.44	0.508	0.523	0.075	0.477	0.321	0.5	0.472	0.375	-0.24	0.202
SPSN	-0.095	0.341	0.743**	0.887**	0.596*		0.792**	0.752**	-0.628*	0.921**	0.721**	0.602*	0.553	0.866**	-0.522	0.722**
SPDW	-0.311	0.787**	0.22	0.429	0.817**	0.659*		0.932**	-0.189	0.767**	0.851**	0.476	0.547	0.565*	-0.545	0.408
SPSW	-0.412	0.749**	-0.062	0.141	0.609*	0.505	0.829**		-0.26	0.737**	0.735**	0.569*	0.37	0.447	-0.351	0.344
TKW	0.287	-0.136	-0.686*	-0.622*	-0.102	-0.738**	-0.234	-0.185		-0.513	-0.178	0.558*	-0.028	-0.65*	-0.072	-0.681*
PL	-0.131	0.014	0.931**	0.83**	0.395	0.769**	0.422	0.054	-0.656*		0.776**	0.664*	0.695*	0.84**	-0.537	0.69**
PW	-0.16	0.633*	0.405	0.452	0.585*	0.544	0.78**	0.375	-0.357	0.603*		0.465	0.5	0.543	-0.688*	0.305
GY	-0.98**	0.32	0.142	0.1	0.071	0.187	0.355	0.403	0.572*	0.218	0.211		0.344	0.567*	0.008	0.487
BY	0.021	0.148	0.706*	0. 631*	0.179	0.697*	0.314	0.178	-0.464	0.583*	0.34	0.022		0.629*	-0.55	0.472
RWC	-0.263	0.074	0.855**	0.788**	0.264	0.767**	0.383	0.143	0.697*	0.845**	0.447	0.365	0.554		-0.393	0.926**
RWL	0.34	-0.347	-0.431	-0.337	-0.175	-0.271	-0.346	0.014	0.278	-0.452	-0.658*	-0.418	-0.425	-0.385		-0.115
LWC	-0.115	0.018	0.65*	0.637*	0.199	0.673*	0.247	0.211	-0.621*	0.629*	0.148	0.191	0.391	0.863**	0.096	

DTH: Days to heading, PH: Plant height (cm), FSN: Fertile spikelet number per spike, SPL: Spike length (cm), KPS: Number of kernels per spike, SPDW: Spike dry weight (g), SPKW: Spike kernel weight (g), TKW: Thousand kernel weight (g), PL: Peduncle length (cm), PW: Peduncle weight (g), GY: grain yield (Kg ha^{-1}), BY: Biomass yield (Kg ha^{-1}), RWC: Relative water content (%), LWC: Leaf water content (%), RWL: Relative water lose (g (g.hr)$^{-1}$), * and **: significant at 5% and 1% probability levels, respectively.

under drought stress conditions. The MSI had a highly significant correlation with GY under RF conditions. Thus the genotypes with both high yield and resilience to drought under drought stress conditions can be considered as tolerant ones. This displays the competence of this method for selecting tolerant genotypes in wheat. It has been also confirmed that a combination of stress indices might supply a more useful scale for improving drought tolerance selection in crop species. These results are in agreement with a similar study reported by Mohammadi (2019). The MSI method supplies more information than the use of drought tolerance indices *per se*, because it synchronically selects the genotypes for both high productivity and resilience to drought.

In conclusion, score indices leading to creation easy-to- use methods (such as MSI) to categorize quickly which are the best or the worst crop genotypes within a population, in terms of resilience and production. Results gained from this study offered potential lines that may be used as parents for the future breeding programs, for expanding drought tolerant wheat cultivars to enhance productivity under drought environments in Iran.

ACKNOWLEDGEMENTS

The authors would like to extend his thanks to Agricultural Research Station of Miandoab for its support in implementing the project.

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SUPPLEMENTARY DATA

Supplementary Table 1. The mean grain yield and amounts of different tolerance drought indices for 12 rain-fed wheat genotypes over two years.

Genotype	Yp	Ys	SSI	TOL	MP	GMP	HM	STI	YSI
1	3747.42	2071.42	1.37	1676.00	2909.42	2786.12	2668.05	0.61	0.55
2	3380.25	2713.08	0.61	667.17	3046.67	3028.35	3010.14	0.72	0.80
3	3197.92	1920.53	1.23	1277.39	2559.23	2478.25	2399.83	0.48	0.60
4	2557.50	2073.84	0.58	483.66	2315.67	2303.01	2290.42	0.42	0.81
5	3921.75	2988.50	0.73	933.25	3455.13	3423.47	3392.11	0.92	0.76
6	3801.75	2717.84	0.87	1083.91	3259.80	3214.43	3169.69	0.81	0.71
7	4938.50	2238.66	1.68	2699.84	3588.58	3325.00	3080.78	0.87	0.45
8	2775.00	2107.34	0.74	667.66	2441.17	2418.24	2395.52	0.46	0.76
9	3230.66	1853.08	1.31	1377.58	2541.87	2446.77	2355.22	0.47	0.57
10	4941.00	3332.42	1.00	1608.58	4136.71	4057.77	3980.33	1.30	0.67
11	3643.16	2793.58	0.72	849.58	3218.37	3190.21	3162.30	0.80	0.77
12	2605.50	1978.00	0.74	627.50	2291.75	2270.17	2248.80	0.41	0.76

Yp: Yield under non-stress condition, Ys: Yield under stress condition, SSI: Stress susceptibility index, TOL: Tolerance index, MP: Mean productivity, GMP: Geometric average productivity, HM: Harmonic mean, STI: Stress tolerance index, YSI: Yield stability index.