

## Assessment of drought tolerance and grain yield stability of rainfed winter bread wheat (*Triticum aestivum* L.) genotypes

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### ABSTRACT

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Drought-tolerance and grain yield stability are among the most important aspects in adaptation and successful performance of rainfed winter bread wheat cultivars. The main objectives of this study were (i) to assess the effectiveness of drought tolerance indices for selection of drought-tolerant winter bread wheat genotypes, and (ii) to identify high-yielding genotypes with yield stability in variable environments. In this experiment, 24 winter bread wheat genotypes were evaluated in 12 yield trials under two moisture-regimes (rainfed and supplemental irrigation) in two dryland research stations differing in winter temperature (temperate and cold agro-climatic conditions) during three cropping cycles (2018-2021). Yield-based drought tolerance indices including; stress tolerance index (STI), geometric mean productivity (GMP), mean productivity (MP), tolerance index (TOL), stress susceptibility index (SSI) and yield stability index (YSI) were used to estimate drought tolerance levels of winter bread wheat genotypes across locations and cropping cycles. GGE-biplot technique was used for grain yield stability analysis. Combined-analysis of variance revealed that the main effects of cropping season, location, moisture-regime, genotype, and their interactions effects on grain yield were significant ( $P<0.01$ ). The combined and yearly PCA-based biplots and correlation matrix analyses revealed that STI, GMP and MP were consistently correlated ( $P<0.01$ ) with grain yield in either rainfed and irrigated environments, indicating the effectiveness of these indices for selection of high-yielding genotypes in both conditions. Based on these indices, G13 (Chenab/GB-SARA-27 IRW2009-10-023-0MA-0MA-0MA-0MA-0MA-6MA), G14 (Chenab/GB-SARA-27 IRW2009-10-023-0MA-0MA-0MA-0MA-0MA-7MA), G11 (Dharwar Dry/Nesser//SARA-BW-F6-06-85-86-2-5 IRW2009-10-056-0MA-0MA-0MA-0MA-0MA-6MA) and G22 (Unknown-2) were the most drought-tolerant genotypes. GGE-biplot analysis identified G11, G13 and G14 as high yielding genotypes with yield stability across environments. In conclusion, the genotypic variation for drought tolerance and grain yield stability found in this study should be further explored in the national rainfed winter bread wheat breeding programs in Iran.

**Keywords:** winter bread wheat, rainfed, drought tolerance indices, grain yield, PCA and GGE biplot.

### INTRODUCTION

Wheat, as the most widely grown crop in the world, is currently experiencing an average yield improvement of about 0.9% per year which is much lower than the rates required to double the production by

2050 without bringing additional land under cultivation (Ray *et al.*, 2013). Wheat is the main cereal crop in Iran grown in diverse environments. Iran is prone drought country and highly vulnerable to changing climate. The mean annual rainfall is about 250 mm

with high fluctuations in amount and monthly distribution which results in mild to severe drought stresses. However, drought frequently affect the growth and productivity of major crop species such as wheat in Iran (Mohammadi, 2016).

About 65% of wheat cultivation in Iran (about four million hectares) are under rainfed conditions and contributing to about 40% of total wheat production in the country (Ahmadi *et al.*, 2020). It is expected that the demand for wheat in the country will increase due to population growth in coming years. In addition, the adverse effects of changing climate which will cause warmer temperatures and remarkable fluctuations in the amount and distribution of precipitation will affect wheat productivity in the future. One of the main strategies to improve the productivity of rainfed wheat is the development of new adapted cultivars (Foulkes *et al.*, 2011; Hernandez-Ochoa *et al.*, 2018). New adapted rainfed wheat cultivars with higher productivity and yield stability under the Mediterranean rainfed environmental conditions is found to increase grain yield of rainfed wheat (Ludwig and Asseng, 2010; Shiferaw *et al.*, 2011).

Drought is recognized as a serious constrain for crops production around the world, especially in areas with less rainfall. Grain yield is considered as a determining factor for stress tolerance in crops because water shortage leads to yield reduction (Mohammadi, 2018). Multi-environment trials (METs) are useful approach to evaluate the response of wheat genotypes to different locations and cropping seasons in order to identify the most adapted genotype for a particular environment (Singh *et al.*, 2016; Gerard *et al.*, 2020). In addition, the yield-based drought tolerance indices which are based on performance of test genotypes, in drought and irrigated conditions, have been used to increase the selection and screening efficiency of genotypes grown under drought stress (Mohammad *et al.*, 2010; Mohammad *et al.*, 2011).

Fischer and Maurer (1978) proposed a stress susceptibility index (SSI) to measure performance stability of genotypes in variable environments. Rosielle and Hamblin (1981) suggested stress tolerance (TOL) as the yield

difference between drought and irrigated environments, and mean productivity (MP) as the mean yield of genotypes in both conditions. Bouslama and Schapaugh (1984) defined the yield stability index (YSI) as the yield stability of a genotype under both stress and non-stress conditions.

Fernandez (1992) suggested the stress tolerance index (STI) as a useful tool for screening genotypes that well performed in both stress and optimum conditions. In addition, geometric mean productivity (GMP) was proposed by Fernandez (1992) to select genotypes based on their performance under drought stress and optimum environments. By using the mathematical relationship between performance under stress and non-stress conditions, genotypes can be classified, based on their responses, to four groups: (i) genotypes with good performance under stress and non-stress conditions, (ii) genotypes that only perform well in non-stress conditions, (iii) genotypes with good performance only under stress conditions, and (iv) genotypes with poor performance under stress and non-stress conditions (Fernandez, 1992).

In addition, correlation analysis between grain yield and drought tolerance indices can be a good criterion for identifying drought tolerant genotypes. The selection of different genotypes grown under environmental stress is one of the main challenges for plant breeders who utilizes genetic diversity to develop drought tolerant cultivars (Dodig *et al.*, 2012). Fernandez (1992) and Mohammadi (2016) reported that the most appropriate indicator for selecting drought tolerant genotypes was the one that had a high correlation with grain yield in non-stress and stress conditions. Grzesiak *et al.* (2019) suggested that suitable selection criteria for drought tolerance in wheat would be high MP, GMP and STI in both stress and non-stress conditions.

Wheat performance, as other crops, in the Mediterranean rainfed areas is strongly influenced by environmental factors and its grain yield varies depending on environmental conditions. Improved wheat genotypes with high grain yield stability and adaptability to these drought prone environments can help to enhance productivity in the prevailing agro-ecological conditions (Chairi *et al.*, 2020; Gerard *et al.*, 2020). Thus, evaluation of new

promising lines performance of elite lines through METs is important for assessment of adaptation and grain yield stability in rainfed wheat breeding programs (Gauch and Zobel, 1997; Yan *et al.*, 2000).

The METs' analysis would assist breeders to identify and understand genotype by environment interaction (GEI) effect on the final yield and performance ranking of a promising lines. There are several different statistical methods to estimate with reasonable precision the genotype and environment effects and their interactions, such as the GGE biplot. The GGE biplot, allows breeders, by removing the environmental impact, to focus on the genotype main effect and GEI effect (Yan and Kang, 2003). Many studies have used and recommended the GGE biplot to assess the GEI in METs (Rakshit *et al.*, 2012; Munaro *et al.*, 2014; da Silva *et al.*, 2021). To assess GEI and identify high-yielding with yield stability genotypes which also possesses high level of drought tolerance, we realized the necessity of evaluation of rainfed winter bread wheat promising lines in different locations, moisture conditions and cropping cycles, based on grain yield and yield stability.

This study aimed (i) to evaluate grain yield and yield stability of rainfed winter bread wheat promising lines developed by rainfed winter wheat breeding program at Dryland Agricultural Research Institute (DARI), Iran, under drought and irrigated environments, (ii) to identify superior promising lines, which exhibit drought stress tolerance, and (iii) to determine suitable drought stress indices for screening and selection of drought-tolerant genotypes.

## MATERIALS AND METHODS

Twenty-four rainfed winter bread wheat genotypes consisting of 21 promising breeding lines and three check cultivars (Table 1) were evaluated in two main research field stations of Maragheh (East Azerbaijan province) and Sararood field stations (Kermanshah province) representing national rainfed winter wheat breeding program in dryland areas of Iran, for three cropping seasons (2018-19, 2019-20, 2020-21). In each location and cropping cycle, two yield trials were carried out under rainfed (terminal drought stress) and supplemental irrigation conditions (no terminal drought

stress).

In each supplemental irrigation trial, two irrigations (each with 30 mm) were applied using sprinkler irrigation system. In Sararood field station (represented moderate cold conditions), the irrigations were applied from heading to grain filling stages to avoid terminal drought stress. In Maragheh field station (represented cold conditions), the first irrigation was applied after sowing (for better seed germination and crop establishment before approaching winter) and the second irrigation was applied at heading stage to prevent terminal drought stress. The distance between drought and irrigated trails in each location was between 200-500 m depending on cropping season and location.

More information on test locations is given in Table 2. In each environment, experimental design was randomized complete block design with three replications. The area of each experimental plot was 7.2 m<sup>2</sup> (6 rows, 6 meter-long, 20 cm row spacing). The sowing seed density was 380 seeds m<sup>-2</sup>. Experimental plots were sown using WeinterSteiger experimental plot planter. Fertilizers used were 50 kg N ha<sup>-1</sup> and 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as basal application at sowing. Weeds were controlled and managed using herbicides and hand weeding in each environment. At harvest time, the plots were harvested with WinterStieger experimental combine, and then plot grain yield was converted into kg ha<sup>-1</sup>.

## Data analysis

Kolmogorov-Smirnov test was used to verify if the probability distribution associated with the data set can be approximated by the normal distribution for grain yield data in each environment. After meeting this assumption, analysis of variance for grain yield in each environment was performed. Then, grain yield data collected from 24 genotypes grown in 12 trails were subjected to combined analysis of variance to assess the effect of year, location, moisture regime, genotype, and interactions between them. The effects due to location, moisture regimes and genotypes were considered as fixed, and years and replications as random effects.

Table 1. The code, name/pedigree, type and origin of tested rainfed winter bread wheat genotypes

Code	Pedigree/Name	Selection history	Type	Origin
G1	Sardari		Landrace	Iran
G2	Hashthrood		Improved cultivar	Iran
G3	Sadra		Improved cultivar	Iran
G4	Vee/Nac//SARA-BW-F6-06-85-86-2-5	IRW2009-10-048-0MA-0MA-0MA-0MA-1MA	Promising line	Iran
G5	Vee/Nac//Gahar	IRW2009-10-050-0MA-0MA-0MA-0MA-6MA	Promising line	Iran
G6	Maroon/3/Sardari//Ska/Aurifen	IRW2009-10-003-0MA-0MA-0MA-0MA-3MA	Promising line	Iran
G7	Maroon/3/Sardari//Ska/Aurifen	IRW2009-10-003-0MA-0MA-0MA-0MA-4MA	Promising line	Iran
G8	Maroon/Gahar	IRW2009-10-006-0MA-0MA-0MA-0MA-5MA	Promising line	Iran
G9	Debira/7/Zcl/3/Pgfn//Cno67/Son64(Es86-8)/4/Kauz/5/Trk13/6/F134.71/Nac//Sabalan	IRW2009-10-007-0MA-0MA-0MA-0MA-4MA	Promising line	Iran
G10	DharwarDry/Nesser/3/F130-L-1-12//PONY/OPATA	IRW2009-10-013-0MA-0MA-0MA-0MA-3MA	Promising line	Iran
G11	Dharwar Dry/Nesser//SARA-BW-F6-06-85-86-2-5	IRW2009-10-056-0MA-0MA-0MA-0MA-6MA	Promising line	Iran
G12	Arvand//78Zhong291/Azar2	IRW2009-10-058-0MA-0MA-0MA-0MA-4MA	Promising line	Iran
G13	Chenab/GB-SARA-27	IRW2009-10-023-0MA-0MA-0MA-0MA-6MA	Promising line	Iran
G14	Chenab/GB-SARA-27	IRW2009-10-023-0MA-0MA-0MA-0MA-7MA	Promising line	Iran
G15	Chenab//78Zhong291/Azar2	IRW2009-10-061-0MA-0MA-0MA-0MA-6MA	Promising line	Iran
G16	Wang shuibai//78Zhong291/Azar2	IRW2009-10-070-0MA-0MA-0MA-0MA-4MA	Promising line	Iran
G17	Sorkhtokhm/Desconciod-7	IRW2009-10-112-0MA-0MA-0MA-0MA-8MA	Promising line	Iran
G18	Kavir//78Zhong291/Azar2	IRW2009-10-087-0MA-0MA-0MA-0MA-2MA	Promising line	Iran
G19	Kavir//78Zhong291/Azar2	IRW2009-10-087-0MA-0MA-0MA-0MA-4MA	Promising line	Iran
G20	Systani/6/Sbn//Trm/K253/5/Anza/3/Pi//Nor/Hys/4/Sefid	IRW2009-10-134-0MA-0MA-0MA-0MA-4MA	Promising line	Iran
G21	Unkown-1	K5-0MA-0MA-0MA-0MA-4MA	Promising line	TCI
G22	Unkown-2	K50-0MA-0MA-0MA-0MA-2MA	Promising line	TCI
G23	Unkown-3	K50-0MA-0MA-0MA-0MA-3MA	Promising line	TCI
G24	Unkown-4	K50-0MA-0MA-0MA-0MA-4MA	Promising line	TCI

TCI: Turkey-CIMMYT-ICARDA winter wheat Improvement program.

Table 2. Description of cropping seasons, locations, and moisture-regimes

Cropping season	Environment		Code	Coordinates			Weather information		
	Location	Moisture regime		Longitude	Latitude	Altitude (m)	Average temperature (°C)	Rainfall (mm)	Irrigation (mm)
2018-19	Maragheh	Rainfed	MR19	46°15'N	37°15'E	1725	5.6	494.6	
2019-20			MR20				5.2	326.8	
2020-21			MR21				6.3	245.7	
2018-19	Maragheh	Supplemental Irrigation (60 mm)	MI19				5.6	494.6	60
2019-20			MI20				5.2	326.8	60
2020-21			MI21				6.3	245.7	60
2018-19	Kermanshah	Rainfed	KR19	47°17'N	34°19'E	1351	11.1	782.5	
2019-20			KR20				11.7	518.8	
2020-21			KR21				13.8	317.7	
2018-19	Kermanshah	Supplemental irrigation (60 mm)	KI19				11.1	782.5	60
2019-20			KI20				11.7	518.8	60
2020-21			KI21				13.8	317.7	60

For better evaluation of promising genotypes under different water regimes and identification of genotypes with higher level of drought tolerant than the check cultivars, five drought tolerance/susceptibility indices including; stress tolerance index (STI), geometric mean productivity (GMP) (Fernandez, 1992), mean productivity (MP)

(Rosielle and Hamblin, 1981), tolerance index (TOL) (Hossain *et al.*, 1990) and stress susceptibility index (SSI) (Fischer and Maurer, 1978) were calculated for each genotype using its grain yield under rainfed (drought stress) and supplemental irrigation (non-drought stress) environments using the following formula:

No.	Index	Reference
1	<b>Stress tolerance index (STI)</b> = $\frac{(Y_s)(Y_p)}{(\bar{Y}_p)^2}$	Fernandez, 1992
2	<b>Geometric mean productivity (GMP)</b> = $\sqrt{(Y_s)(Y_p)}$	Fernandez, 1992
3	<b>Mean productivity (MP)</b> = $\frac{(Y_s + Y_p)}{2}$	Rosielle and Hamblin, 1981
4	<b>Tolerance index (TOL)</b> = $Y_p - Y_s$	Hossain <i>et al.</i> , 1990
5	<b>Stress susceptibility index (SSI)</b> = $\frac{[1 - (\frac{Y_s}{Y_p})]}{1 - SI}$	Fischer and Maurer, 1978
6	<b>Stress intensity (SI)</b> = $[1 - (\bar{Y}_s)/(\bar{Y}_p)]$	Fischer and Maurer, 1978
7	<b>Yield stability index (YSI)</b> = $\frac{Y_s}{Y_p}$	Bousslama and Schapaugh, 1984

where  $Y_s$  and  $Y_p$  stand for grain yield of each genotype under drought and supplemental irrigation conditions, respectively;  $\bar{Y}_s$  and  $\bar{Y}_p$  represent mean grain yield of genotypes under drought and supplemental irrigation conditions.

The repeatability of relationships between genotypic mean grain yield and the six yield-based drought tolerance indices were quantified by correlation analysis in each six environments (location-year combination). This provide useful guide to check whether information gained on genotypes from one specific kind of environment was informative for other environments (Mohammadi, 2016).

Grain yield data were also subjected to GGE biplot analysis (Yan *et al.*, 2000). The GGE biplot was constructed using GEA-R package (Pacheco *et al.*, 2016). The general model for GGE biplot is as following:

$$\bar{Y}_{ij} - \mu_i - \beta_j = \sum_{n=1}^N \lambda_n \alpha_{in} \eta_{jn}$$

where  $Y_{ij}$  is the grain yield of genotype  $i$  in the environment  $j$ ,  $\mu$  is the grand mean,  $\beta_j$  is the main effect of environment  $j$ ,  $n$  is the number of principal components (PC);  $\lambda_n$  is singular value of the  $n^{th}$  PC; and  $\alpha_{in}$  and  $\eta_{jn}$  are the scores of genotype  $i$  and environment  $j$ , respectively, for  $n^{th}$  PC;  $\varepsilon_{ij}$  is the residual associated with genotype  $i$  in environment  $j$

(Yan *et al.*, 2000).

The GGE biplot visually presents the mean genotypic performances across environments, the "which-won-where" patterns for investigating crossover GEI and mega-environment identification as well as the discriminating ability and representativeness of test environments. In GGE biplot, the cosine of the angle between the vectors of two test environments indicates the correlation between them; acute angle shows strong positive correlation, obtuse angle shows negative correlation and right angle no correlation (Yan *et al.*, 2000).

The projection of a genotype or an environment on the average environment coordinate (AEC) abscissa indicating average performance of the genotype or the desirability of a test location (Yan *et al.*, 2000). The distance between genotype and AEC is used to judge the genotype's yield stability, while an acute angle between environment vector and AEC indicating high representativeness of the test location.

The vector length of a test location is a measure of its discriminating ability; thus the longer the vector, the more discriminating the location. The "which-wins-where" form of the GGE biplot divides the environments into several sectors. For each sector, the vertex genotype is the one with best mean performance for the group of environments

fell in the sector (Yan and Tinker, 2006). The "mean yield vs. yield stability" view of GGE biplot allows breeder to ranking of genotypes for both mean yield and yield stability simultaneously. The GGE biplot analysis was further applied to compare environments to a hypothetical ideal environment and compare the genotypes to an ideal genotype.

## RESULTS

### Weather conditions

The patterns of monthly rainfall and mean temperatures of the three cropping seasons in each location are presented in Fig. 1. Rainfall varied between cropping seasons, and winter bread wheat genotypes were exposed to terminal drought stress mainly during grain

filling in Sararood (Kermanshah) (mid-May to mid-June) and Maragheh (June). The amount of rainfall received in first growing season (2018-19) in both locations and in the second cropping season in Sararood (Kermanshah) was higher than the long-term average rainfall, although the majority of rainfall received in winter (tillering stage) that was not effectively used by crops. The 2019–2020 and 2020–2021 cropping seasons were characterized by abnormal low p of 323.8 and 245.5 mm in Maragheh, respectively. The 2020–21 cropping season was also characterized by abnormal low rainfall of 317.5 mm in Kermanshah. As for rainfall, marked variations in monthly temperatures, particularly in Maragheh, was observed during cropping seasons (Fig. 1).

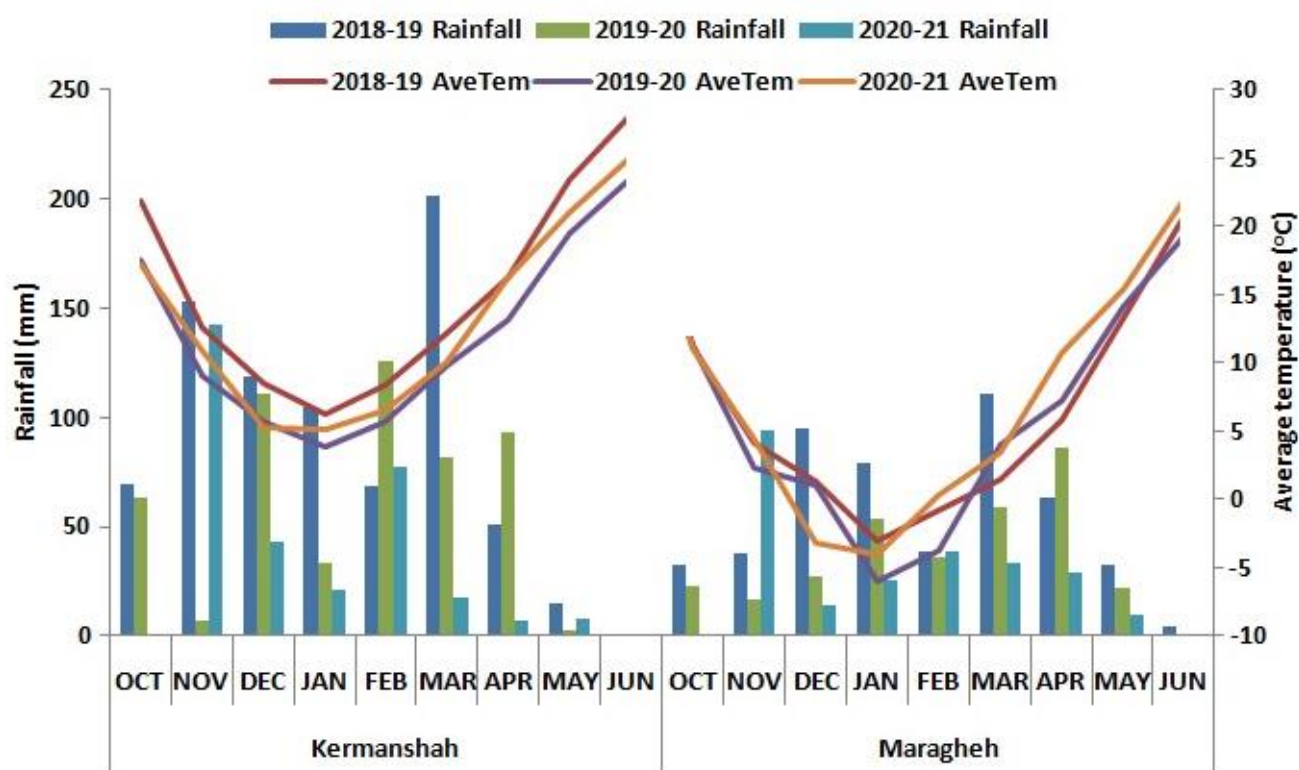


Fig. 1. Monthly rainfall and average temperature during three cropping seasons in Kermanshah (Sararood) and Maragheh

### Grain yield data analysis per location, year and moisture-regime conditions

The genotypic mean grain yield based on year, location and water-regime treatments are presented in Table 3. The genotypic mean grain yield in 2018-19, 2019-20 and 2020-21, were 3226, 2868 and 2724 kg ha<sup>-1</sup>, respectively, showing positive relationship

between total rainfall and mean yield. The genotypes across years expressed highest mean yield (3507 kg ha<sup>-1</sup>) in moderate cold location (Kermanshah location), while exhibited for lower productivity (2372 kg ha<sup>-1</sup>) in cold condition (Maragheh location). The genotypes well performed under supplemental irrigation (3209 kg ha<sup>-1</sup>) than

rainfed (2670 kg ha<sup>-1</sup>) conditions, showing 17% grain yield reduction under drought stress condition across cropping seasons and locations. Breeding lines G13 (2972 kg ha<sup>-1</sup>), G14 (2970 kg ha<sup>-1</sup>) and G11 (2966 kg ha<sup>-1</sup>) had highest mean grain yield, respectively, under rainfed conditions, and breeding lines G14 (3469 kg ha<sup>-1</sup>), 18 (3402 kg ha<sup>-1</sup>)

and 13 (3372 kg ha<sup>-1</sup>) best performed, respectively, under supplemental irrigation conditions across cropping seasons. The highest mean grain yield across all environments belonged to breeding lines G14, G13 and G11 and the lowest was correspond to breeding lines G7, G8 and G15, respectively.

Table 3. Mean grain yield of 24 rainfed winter bread wheat genotypes in different cropping seasons, locations and moisture regimes

Genotype	Cropping season			Location		Moisture regime		Mean
	2018-19	2019-20	2020-21	Kermanshah	Maragheh	Rainfed	Supplemental irrigation	
G1	3110	3172	2917	3772	2360	2784	3349	3066
G2	3164	2862	2744	3445	2402	2735	3111	2923
G3	3207	3000	2764	3504	2477	2799	3181	2990
G4	3277	2828	2697	3569	2299	2644	3224	2934
G5	3187	2555	2566	3204	2334	2439	3099	2769
G6	3013	2706	2548	3161	2350	2449	3062	2756
G7	2885	2488	2450	3018	2198	2281	2935	2608
G8	2975	2667	2376	3054	2291	2473	2872	2673
G9	3475	2963	2687	3682	2401	2826	3257	3042
G10	3204	3012	2883	3504	2562	2785	3281	3033
G11	3560	3036	2838	3811	2478	2966	3323	3145
G12	3154	2641	2548	3291	2271	2450	3112	2781
G13	3414	3333	2769	3854	2491	2972	3372	3172
G14	3298	3417	2943	3824	2615	2970	3469	3219
G15	3126	2736	2273	3317	2106	2356	3067	2712
G16	3221	3069	2710	3629	2370	2663	3336	3000
G17	3084	2733	2700	3319	2359	2635	3042	2839
G18	3106	2802	3093	3537	2464	2598	3402	3000
G19	3062	2927	2865	3575	2328	2598	3305	2951
G20	3560	2693	2753	3751	2253	2789	3215	3002
G21	2964	2426	3035	3304	2313	2547	3070	2808
G22	3539	2945	2827	3770	2437	2855	3352	3104
G23	3465	2957	2638	3594	2446	2682	3358	3020
G24	3377	2861	2754	3667	2328	2787	3208	2997
Average	3226	2868	2724	3507	2372	2670	3209	2939

### Combined analysis of variance

Combined analysis of variance for grain yield revealed significant ( $P<0.01$ ) effects of year, location, moisture regime, genotype, and most of the interactions between them (Table 4). The relative magnitudes of different sources of variation for grain yield varied greatly, as indicated by the variance components expressed as percentages of

total variation. The location effect had the highest impact on genotype performance and accounted for 41.4 % of the total variation, while 15.4% of total variation in observed grain yield was explained by location  $\times$  year interaction, 9.3% by moisture-regime, 5.7 % by differences in year, and 3.3% by differences among genotypes (Table 4).

Table 4. Combined analysis of variance for grain yield of 24 rainfed winter bread wheat genotypes across location, year, and moisture-regime

S. O. V.	MS	E(MS)	df	SS	MS	Prob	%TSS
Year (Y)	M17	M17/M14	2	38485878	19242939	0.00000	5.70
Location (L)	M16	M16/M15	1	277935488	277935488	0.00000	41.40
L x Y	M15	M15/M14	2	103696852	51848426	0.00000	15.40
R/(L x Y)	M14	M14/M1	12	7136788	594732	0.00000	1.10
Moisture regime (M)	M13	M13/M12	1	62596862	62596862	0.00000	9.30
M x Y	M12	M12/M1	2	2419762	1209881	0.00040	0.40
M x L	M11	M11/M10	1	45748	45748		0.01
M x L x Y	M10	M10/M1	2	7479836	3739918	0.00000	1.10
Genotype (G)	M9	(M1+M9)/(M3+M4)	23	21936987	953782	0.00000	3.30
G x Y	M8	M8/M1	46	15949482	346728	0.00000	2.40
G x L	M7	M7/M6	23	8982025	390523	0.00010	1.30
G x L x Y	M6	M6/M1	46	16579035	360414	0.00000	2.50
G x M	M5	M5/M4	23	3600151	156528	0.41090	0.50
G x M x Y	M4	M4/M1	46	6746918	146672	0.52130	1.00
G x M x L	M3	M3/M2	23	5386501	234196	0.04790	0.80
G x M x L x Y	M2	M2/M1	46	7785186	169243	0.26980	1.20
Error	M1		564	84837694	150421		12.60
Total			863	671601194.3			

MS: mean squares; E(MS): expected mean squares; df: degree of freedom; SS: sum of squares; Prob: probability; %TSS: percentage to total sum of squares

A heatmap-based on the ranking of genotypes for each drought tolerance indices in each location and cropping season was used to discriminate the most tolerant genotypes (Fig. 2). In all six environments the drought tolerance indices clustered in two groups, where STI, GMP and MP gave similar results

in genotypes ranking, while TOL, SSI and YSI ranked genotypes in similar order. In the case of 2018-19 cropping season, in Kermanshah, the breeding lines; G22, G11 and G9 had the highest values of STI, GMP and MP and showed as the most drought tolerant genotypes.

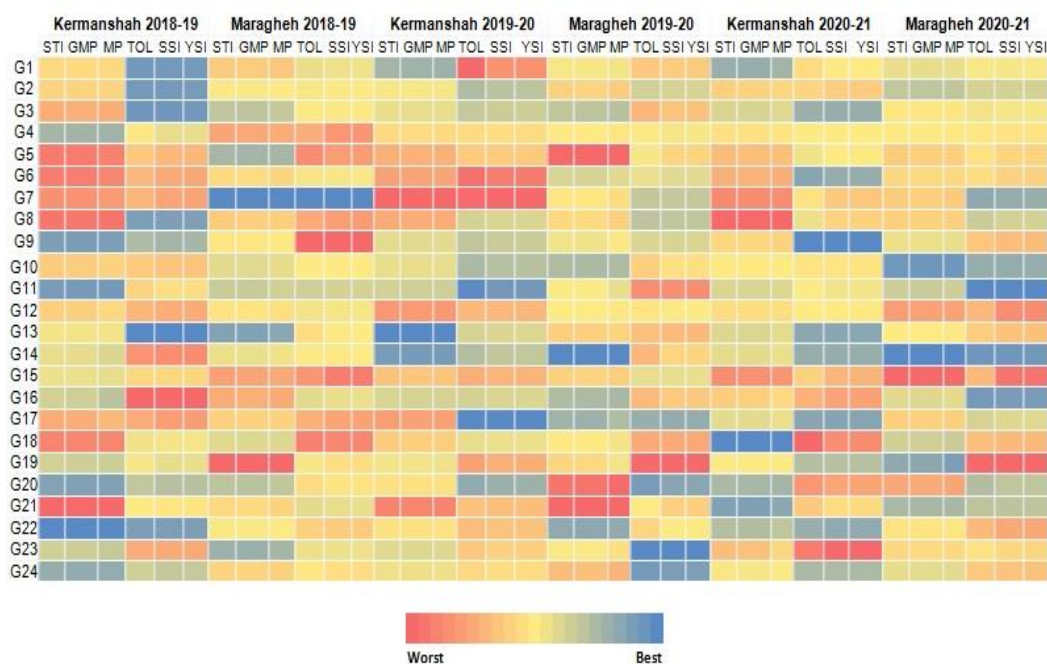


Fig. 2. Heat map of drought tolerance scores for 24 rainfed winter bread wheat genotypes across six environments



In contrast, genotypes; G16, G14 and G17 exhibited highest SSI and TOL values and were identified as the most drought-susceptible genotypes with low productivity in drought stress conditions, while genotypes; G13, G1, G2 and G3 with the lowest values of SSI and TOL characterized as the most drought-tolerant genotypes with high-yielding under drought stress conditions and poor performance under supplemental irrigation conditions as they showed high YSI values. A genotype with low TOL and SSI values shall be characterized as "resistant" (group C, Fernandez 1992) and is opposite to "susceptible" which refer to a genotype with low performance in drought conditions and high performance in supplemental irrigation conditions which led to higher TOL value (group B), while the "tolerant" refer to a genotype with higher performance in both drought and irrigated conditions (Group A). In Maragheh G7 and G13 had the highest STI, GMP and MP values and were characterized as the most drought-tolerant genotypes. In contrast, genotype G8 was characterized as resistant-genotype and G9, G18 and G16 were found to be the most susceptible genotypes (Fig. 2).

In 2019-20 cropping season in Kermanshah, the most-tolerant genotypes were G13 and G14, while the most-susceptible genotypes were G8, G7 and G1 (Fig. 2). In Maragheh, the most tolerant genotypes, according to STI, GMP and MP, were G14 and G22, while genotypes G19 and G11 with the highest SSI and TOL values showed the highest susceptibility to drought stress, and genotypes G23, G24 and G20 were drought-resistant genotypes as they had the highest YSI values (Fig. 2). In 2020-21 in Kermanshah, G18 and G21 were identified as the most-tolerant genotypes, while G23 was the most susceptible and G9 was the most resistant genotypes. In Maragheh, the most drought-tolerant genotypes were G14 and G10, the most susceptible genotypes were G19 and G15, and G11, G14 and G17 performed the most resistant genotypes (Fig 2).

#### **Relationships between grain yield and drought tolerance indices**

Pearson's correlation analysis between mean grain yield of rainfed winter bread wheat genotypes under both rainfed and supplemental irrigation conditions and drought

tolerance indices in each six environments are presented in Table 5. In four out of six environments no significant correlations was observed between grain yield under rainfed and supplemental irrigation environments and the correlation coefficients were  $r = 0.048$  (Maragheh 2018-19),  $r = 0.149$  (Maragheh 2019-20),  $r = 0.323$  (Kermanshah 2020-21),  $r = 0.360$  (Kermanshah 2018-19),  $r = 0.434$  (Maragheh 2020-21,  $P < 0.05$ ) and  $0.691$  (Kermanshah 2019-20;  $P < 0.01$ ). Positive and significant ( $P < 0.01$ ) correlations were observed between STI, GMP and MP across all six environments, showing a consistent relationship between these indices in ranking of genotypes. The STI, GMP and MP had positive and highly significant ( $P < 0.01$ ) correlation with mean grain yield under both rainfed and supplemental irrigation conditions, indicating selection by using these indices will improve productivity under both conditions. In all six environments, the significant positive ( $P < 0.01$ ) associations were also found between SSI and TOL, however, the significant negative ( $P < 0.01$ ) correlation was observed between these two indices with YSI (Table 5). In all six environments, significant and positive ( $P < 0.01$ ) correlations were observed between mean grain yield under rainfed conditions with YSI showing selection for high values of YSI would enhance productivity under drought stress conditions. In contrast, SSI showed negative and significant correlation with mean grain yield under rainfed conditions (Table 5).

#### **Principal components analysis**

To better separate and classify different genotypes based on different drought tolerance indices in each environment, principal component analysis (PCA) was employed. A biplot based on the first two PCAs was constructed (Fig. 3). The PCA biplots revealed that in all six environments STI, GMP, and MP were significantly correlated ( $P < 0.01$ ) and showed a strong positive correlation with grain yield under rainfed and supplemental irrigation conditions which revealed that these indices should be used for identifying high-yielding genotypes in both conditions. These results are also in accordance with Pearson's correlation coefficients (Table 5). A significant positive association ( $P < 0.01$ ) was observed between

SSI and TOL. The SSI and TOL showed a positive association with Yp and negative association with STI, MP, GMP, and a significant negative correlation with Ys and

YSI. However, YSI expressed positive correlation only with Ys, showing that this index can identify superior genotypes adapted to drought conditions.

Table 5. Pearson's correlation coefficients between mean grain yield of rainfed winter bread wheat genotypes under rainfed and supplemental irrigation conditions and the drought tolerance indices in six environments

Index	Ys	Yp	STI	GMP	MP	TOL	SSI
Kermanshah 2018-19							
Yp	0.360						
STI	0.872**	0.769**					
GMP	0.867**	0.776**	0.999**				
MP	0.838**	0.811**	0.997**	0.998**			
TOL	-0.607**	0.523**	-0.141	-0.131	-0.074		
SSI	-0.668**	0.447*	-0.219	-0.211	-0.157	0.991**	
YSI	0.668**	-0.447*	0.219	0.211	0.157	-0.991**	-1.0**
Maragheh 2018-19							
Yp	0.048						
STI	0.860**	0.547**					
GMP	0.846**	0.570**	0.998**				
MP	0.843**	0.578**	0.997**	0.998**			
TOL	-0.826**	0.523**	-0.426*	-0.400	-0.393		
SSI	-0.879**	0.430*	-0.515*	-0.490*	-0.487*	0.993**	
YSI	0.879**	-0.430*	0.515*	0.490*	0.487*	-0.993**	-1.0**
Kermanshah 2019-20							
Yp	0.691**						
STI	0.932**	0.902**					
GMP	0.940**	0.896**	0.998**				
MP	0.937**	0.900**	0.998**	10.000**			
TOL	-0.606**	0.155	-0.282	-0.299	-0.290		
SSI	-0.636**	0.112	-0.317	-0.337	-0.328	0.993**	
YSI	0.636**	-0.112	0.318	0.337	0.329	-0.993**	-1.0**
Maragheh 2019-20							
Yp	0.149						
STI	0.734**	0.777**					
GMP	0.736**	0.778**	0.998**				
MP	0.629**	0.863**	0.986**	0.988**			
TOL	-0.453*	0.814**	0.269	0.269	0.408*		
SSI	-0.605**	0.689**	0.092	0.091	0.233	0.977**	
YSI	0.605**	-0.690**	-0.093	-0.091	-0.233	-0.977**	-1.0**
Kermanshah 2020-21							
Yp	0.323						
STI	0.805**	0.818**					
GMP	0.823**	0.802**	0.998**				
MP	0.751**	0.867**	0.994**	0.993**			
TOL	-0.414*	0.728**	0.203	0.176	0.291		
SSI	-0.639**	0.515**	-0.065	-0.092	0.023	0.959**	
YSI	0.639**	-0.516**	0.064	0.092	-0.024	-0.959**	-1.0**
Maragheh 2020-21							
Yp	0.434*						
STI	0.902**	0.777**					
GMP	0.902**	0.780**	0.998**				
MP	0.879**	0.811**	0.997**	0.999**			
TOL	-0.660**	0.391	-0.274	-0.271	-0.221		
SSI	-0.771**	0.233	-0.423*	-0.424*	-0.377	0.982**	
YSI	0.772**	-0.232	0.424*	0.424*	0.378	-0.982**	-1.0**

\* and \*\*: Significant at the 5% and 1% probability levels, respectively.

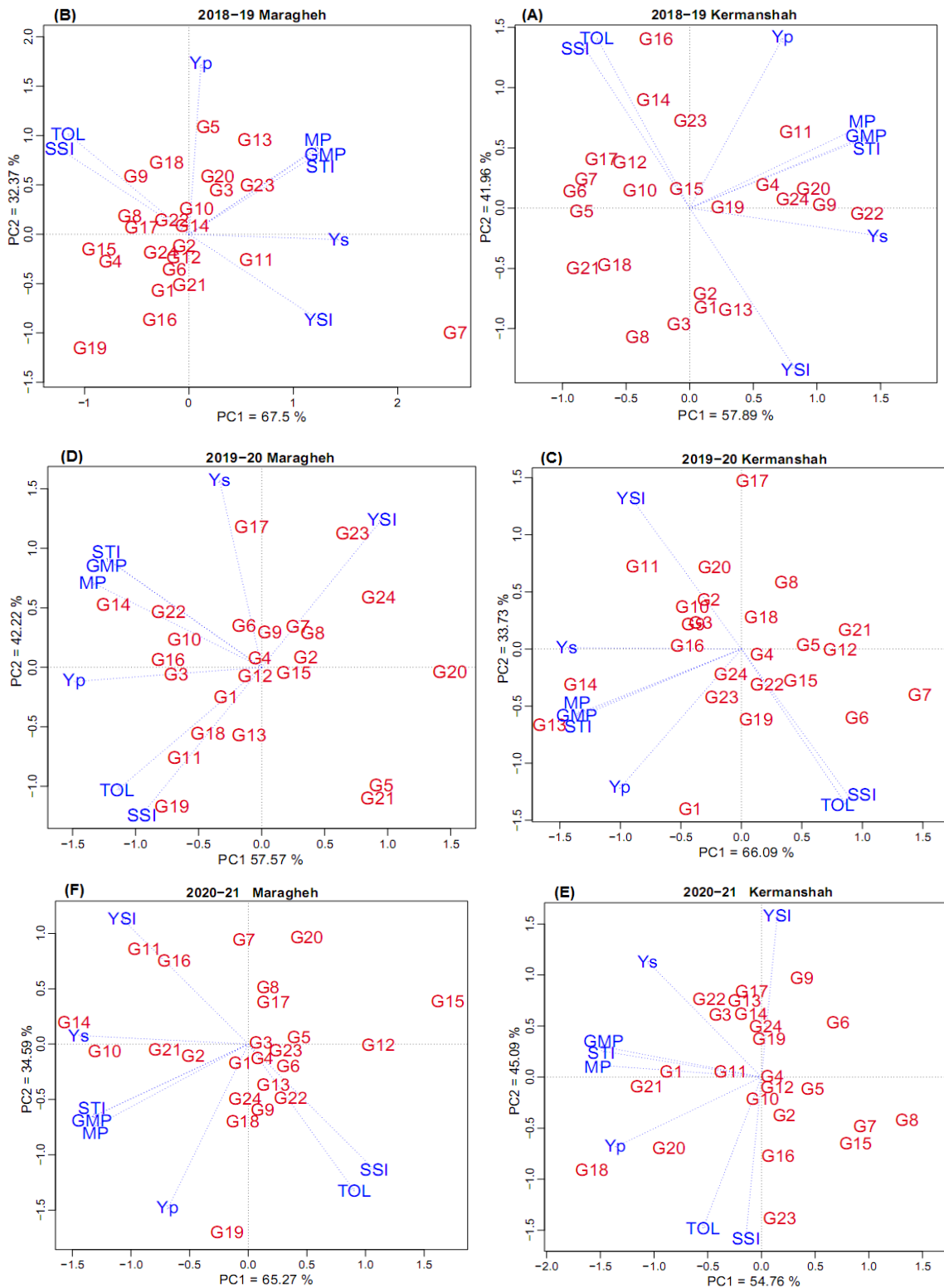


Fig. 3. Biplot-based PCA of drought tolerance indices and mean grain yield of rainfed winter bread wheat genotypes under drought and supplemental irrigation conditions in each environment

Genotypes with highest distance from the origin of biplot were more likely to be influenced by these indices, and genotypes near the origin were less influenced by the indices.

For example, in environment 2018-19 (Fig. 3a) the indices of STI, GMP and MP identified genotypes; G22, G20, G9, G11, G24 and G4 with higher confidence as best performing

genotypes. SSI and TOL identified G16, G14, G17 and G12 as the most susceptible to drought stress. According to Fig. 3b (Maragheh 2018-19), genotypes; G23, G13 positively interacted with STI, GMP and MP and were identified as the most drought-tolerant genotypes, while, in contrast, genotypes; G9, G8, G15, G4 and G17 expressed highest susceptibility to drought stress conditions.

In trails in 2019-20 in Kermanshah (Fig. 3c), G13 and G14 positively interacted with STI, GMP and MP, while G6 and G7 showed positive interaction with SSI and TOL, and G11 and G20 positively interacted with YSI. In Fig. 3d, genotypes; G14 and G21 were the most drought-tolerant genotypes as positively interacted with STI, GMP and MP; in contrast, G23 and G24 most favored YSI (high performance under drought stress conditions) and G19 and G11 with positive interaction with SSI and TOL were the most susceptible genotypes to drought stress conditions in Maragheh during 2019-20. According to Fig. 3e, STI, GMP and MP were strongly associated with each other and discriminated G21 and G1 as the most drought-tolerant genotypes, while G23 and G16 discriminated

by SSI and TOL as the most drought-susceptible genotypes, and G9 was identified by YSI as high-yielding genotype under drought stress conditions. In Fig. 3f, genotypes; G14 and G10 positively interacted with STI, GMP and MP, showing that these genotypes were the most drought-tolerant genotypes, while G11 and G16 with positive interaction with YSI showed the highest productivity under drought stress conditions.

When taking all cropping seasons and locations into consideration, the PCA biplot analysis (Fig. 4) revealed that drought tolerance indices STI, GMP, and MP significantly correlated with mean grain yield under both drought stress and supplemental irrigation environments. Based on these criteria, genotypes; G14, G13, G11 and G22 were the most drought-tolerant genotypes with high mean grain yield in both conditions. TOL and SSI were closely correlated and able to discriminate G18, G19, G15, G23 and G16 as drought-susceptible genotypes, which were suitable for supplemental irrigation conditions (Fig. 4). These genotypes negatively interacted with YSI showing poor performance under drought stress conditions.

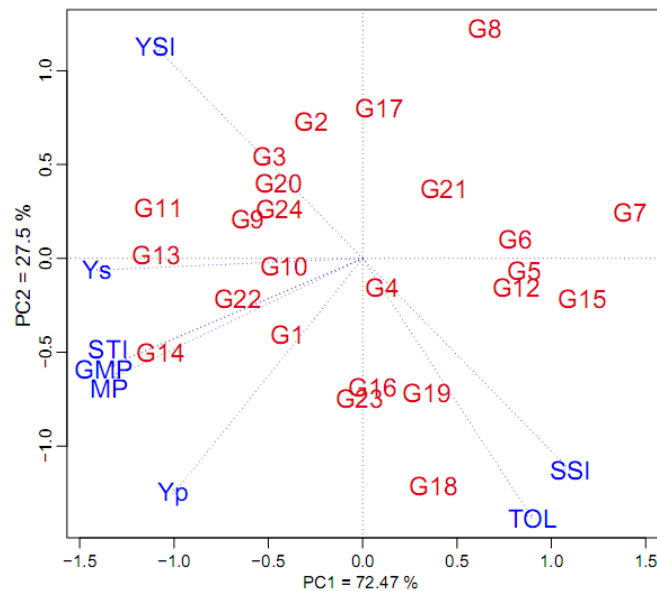


Fig. 4. Biplot-based PCA of drought tolerance indices and mean grain yield of rainfed bread wheat genotypes under drought stress and supplemental irrigation conditions across cropping seasons and locations

### Identifying high-yielding genotypes with yield stability

The "which-won-where" pattern of GGE

biplot helped to identify superior genotypes for each environment or a group of environments. The GGE biplot explained

44.37% of total variation with PC1 and PC2 for grain yield. Using the polygon view of GGE biplot, the test environments were classified into four and genotypes into six groups (Fig. 5). The first environment group, contained MI21, MR21, MI20 (Maragheh location), KI20 and KI19 (Kermanshah location); the second environment group comprised of KR20, KR21, KR19, KI21 (Kermanshah location) and MR19 (Maragheh location); the third group only consisted MI19

(Maragheh location) which is a non-stressed environment, and fourth group comprised MR20 (Maragheh location) a drought environment (Fig. 5). Genotypes that appeared in the vertex of polygon view of GGE biplot are the best/worst performers. Genotype G14 was the best performer in first environment group, while G13 well performed in second environment group, while G20 and G19 were the best yielders in third and fourth environment groups, respectively.

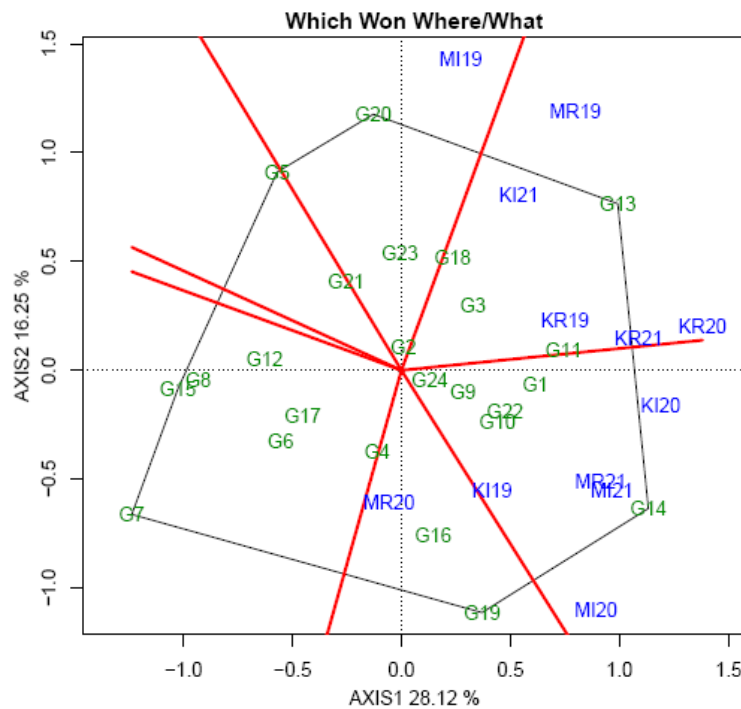


Fig. 5. "Which-won-where" pattern of GGE biplot for 24 rainfed winter bread wheat genotypes and 12 testing environments

Using the "mean vs. stability" view of GGE biplot (Fig. 6), G14 and G13 were identified as high yielding genotypes. Genotypes G14 and G13 with highest mean grain yield, respectively, but with large PC2 scores showed high yield instability and tended to have specific adaptation. G13 was adapted to most environments in Kermanshah, while G14 was the most adapted to Maragheh

environments. Genotypes G11, G1, G9 and G24 expressed highest mean grain yield and yield stability as they are positioned on the ATC abscissa with zero PC2 scores (Fig. 6). By integrating high mean grain yield and yield stability, G11 could be considered as the best genotype as it presents high level of mean grain yield and yield stability.

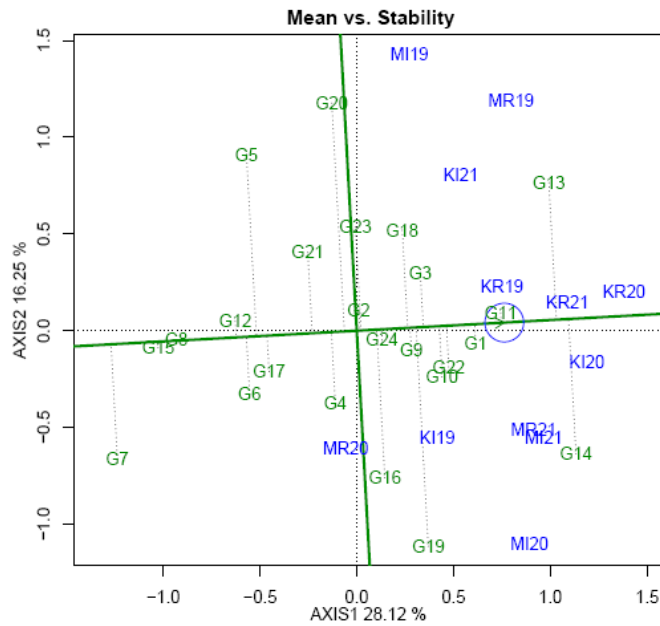


Fig. 6. Mean vs stability view of the GGE biplot of 24 rainfed winter bread wheat genotypes across 12 testing environments

In Fig. 7, genotypes are compared to an ideal genotype (the small circle on AEC). The single arrowhead line passing through the origin, the AEC abscissa, indicated high mean performance. Therefore, genotypes positioned along the arrow are considered as high yielding genotype. G13, G14, G11 and G1 had the highest mean grain yield, respectively. Moreover, the yield stability of

genotypes could be assessed through the length of the projection in both directions from the AEC abscissa, i.e., the AEC ordinate. Thus, if a genotype had greater projection from the AEC abscissa, it would have lower yield stability. Considering both mean performance and stability, G11 was an ideal genotype, having high mean grain yield and yield stability.

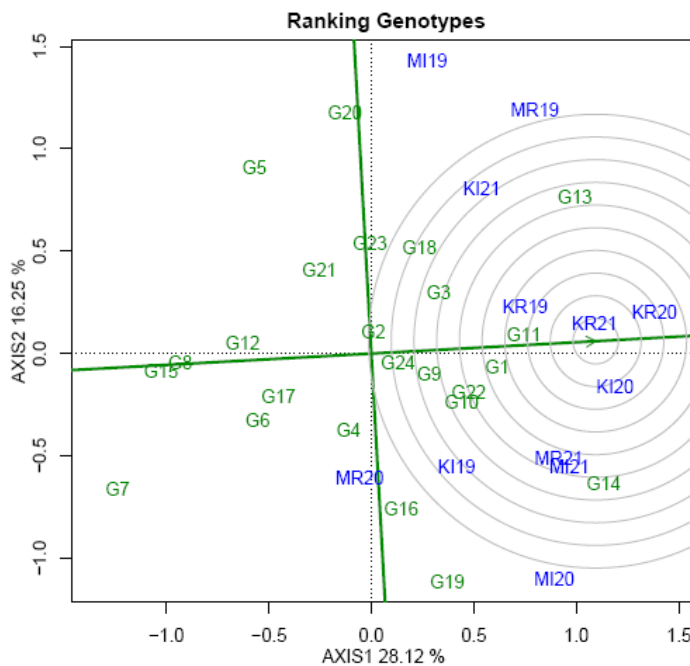


Fig. 7. GGE biplot showing genotype ranking view of the 24 rainfed winter bread wheat genotypes across 12 testing environments

Negative and positive correlations were observed between the environments under study since the angle between the environments showed acute ( $<90^\circ$ ) to obtuse ( $>90^\circ$ ) angles (Fig. 8). The environments belonged to Kermanshah showed positive correlations. They also showed positive correlation with other two environments; MR21 and MI21 (corresponded to

Maragheh). Some negative correlations were observed between environments belonged to Maragheh, showing different responses of genotypes to environmental conditions in this location. The study indicated that the environments KR20, MI20, MR19 and MI19 with the longest vector had more discriminating power compared to the other environments (Fig. 8).

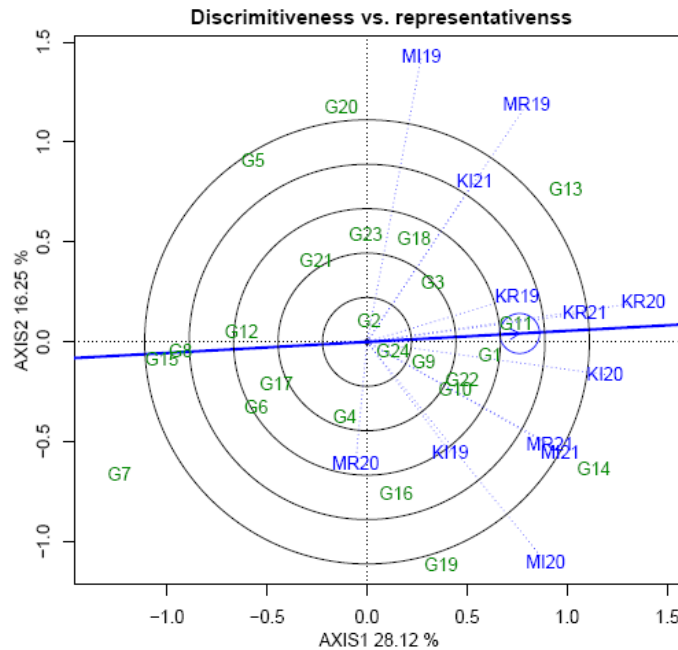


Fig. 8. Discriminativenss vs. representativenss view of test environments based on GGE biplot for 24 rainfed winter bread wheat genotypes across 12 testing environments

AEC view compared the environments in relation to an ideal environment. The environment KR20 followed by KR21 and KI20 had the smallest angle with the AEC, hence these three environments belonged to Kermanshah were highly representative. Fig. 9 presents the AEC view comparing environments relative to an ideal environment (the small circle on AEC). It indicates that environments KR20 followed by KR21 and KI20 were located in the direction of the ideal environment.

### DISCUSSION

Drought-tolerance is one of the most important traits of interest in rainfed winter bread wheat breeding programs (Mohammadi, 2018). The effect of climate change such as

frequent seasonal drought, rising temperatures, and variation in pests and diseases incidnces and damages provide additional challenges for ensuring yield stability across diverse dryland environments and meeting the future demand for wheat products. Under this situation, wheat breeding can play significant role to global food security through the development of high-yielding and stress tolerant genotypes, which adequately respond to expected future changing climatic conditions (Crespo-Herrera *et al.*, 2018; Gerard *et al.*, 2020). Selection of genotypes that should be grown as commercial cultivars needs to be evaluated for compliance with not only their yield under drought stress and optimum conditions but also adaptability to their growing environments.

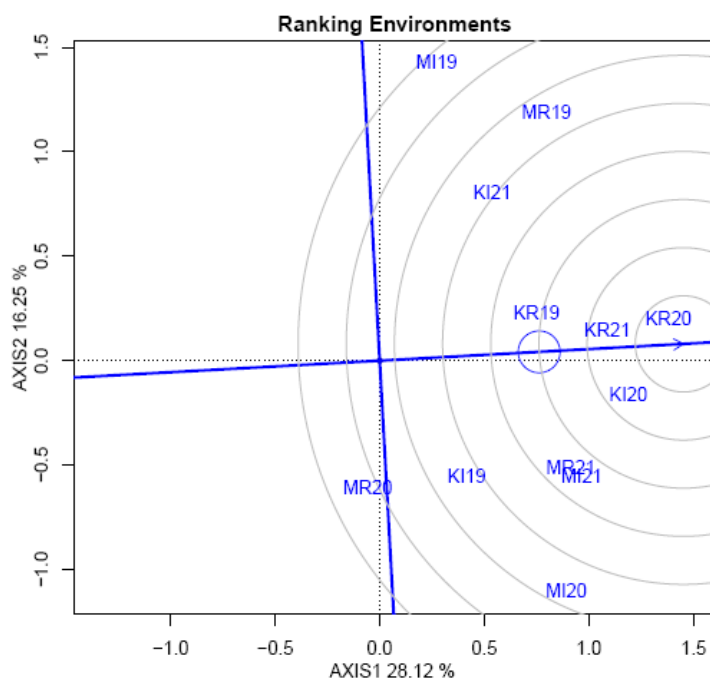


Fig. 9. The AEC view of GGE biplot comparing environments relative to an ideal environment (the center of the concentric circles)

Variation in grain yield is strongly dependent on environmental conditions (Singh *et al.*, 2016; Crespo-Herrera *et al.*, 2018). In our study, moisture-regime had significant effect on grain yield, in addition to the effect of location and location  $\times$  year interaction which were significant due mainly to environmental variation. Variation in annual weather conditions can affect the level of stress experienced by a crop (Sio-Se Mardeh, 2006; Mohammadi, 2016). In the present study, rainfall (amount and distribution) and temperature varied considerably from cropping season to cropping season, especially during critical periods of crop development (heading and grain filling), as marked in the second and the third cropping seasons, where low rainfall and extreme minimum temperatures were recorded (Fig. 1).

It has been confirmed, in many studies, that drought tolerance indices are suitable criteria to select high-yielding drought-tolerant genotypes (Fischer and Maurer, 1978; Fernandez, 1992; Sio-se Mardeh *et al.*, 2006; Mohammadi *et al.*, 2010; Singh *et al.*, 2016). Our results revealed that STI, GMP, and MP indices had highly significant positive correlations with grain yield in both conditions and can be used for identifying high-yielding winter bread wheat genotypes adapted to both

rainfed and supplemental irrigation conditions. In addition, YSI showed significant positive association with grain yield in drought conditions suggesting its usefulness for identifying superior genotypes under drought-stressed conditions.

The PCA-biplot results confirmed the results obtained from drought tolerance indices and correlation matrix analyses which is in accordance with findings of other researchers (Mwadingeni *et al.*, 2016; Ayed *et al.*, 2021). Previous studies (Sio-Se Mardeh *et al.*, 2006; Nouri *et al.*, 2011; Mohammadi *et al.*, 2010) have also demonstrated that genotypes that having lower SSI and TOL or higher YSI can also be selected as tolerant genotypes, as they can perform well under drought stress conditions than other genotypes. Based on the results of the present study, genotypes; G14, G13, G11, G22, G10, G9 and G1 with the highest STI, GMP and MP were more tolerant and identified as suitable for both drought-stressed and supplemental irrigation conditions. Genotypes; G18, G19, G16, G23, G15, G12 and G5 confirmed to be more susceptible to drought stress due to high grain yield reduction under drought stress conditions. The genotypes G2, G17, G3 had the highest YSI and lowest SSI and TOL and were characterized as tolerant



genotypes. G2 and G3 stand for Hashrood and Sadra improved cultivars that have been released by DARI, Iran, in the past decade for cultivation in cold and moderate cold regions of Iran; while G17 is a new promising line (Sorkhtokhm/Desconciod-7) that is developed by the national rainfed winter bread wheat breeding program at DARI.

In this study, the rainfed winter bread wheat genotypes were evaluated under different moisture-regimes and climate conditions to identify the new promising lines for terminal drought stress conditions which usually constrains crop performance in the rainfed conditions. Drought stress tolerance is a key component and in some cases the major determinant trait in improving yield of crops (Tollenaar and Wu, 1999; Poch, 2006). Drought stress indices were efficient to discriminate genotypes with different levels of tolerance to drought stress under different weather and moisture-regime conditions.

The PCA-biplots constructed based on drought tolerance indices found to be efficient tools for visual comparison among genotypes on the basis of drought tolerance screening criteria as they displayed the variability patterns of the studied genotypes based on drought selection indices. Relationships among drought selection indices allowed the identification of superior genotypes. Previous studies have also demonstrated the usefulness of drought tolerance indices for identifying genotypes adapted to each moisture-regime and weather conditions (Mohammadi *et al.*, 2010; Nouri *et al.*, 2011; Singh *et al.*, 2016).

G × E interaction analysis using GGE biplot method showed that genotypes which had similar grain yield across environments clustered together. However, four environment groups could be distinguished that each shared similar characteristics. G14 was the best performer in the environment group contained MI21, MR21, MI20 (Maragheh), KI20 and KI19 (Kermanshah), while G13 showed that it was specifically adapted to environment group comprised of KR20, KR21, KR19, KI21 (Kermanshah) and MR19 (Maragheh) in second environment group, and G19 was specifically adapted to the drought-stressed environment (MR20) and, in contrast, G20 well performed in MI19 (corresponding to environment with supplemental irrigation in

Maragheh). The three breeding lines; G11, G13 and G14 were identified as high-yielding genotypes among which G11 had the highest yield stability across environments, and G13 and G14 positively interacted with specific targeted environments (Fig. 6).

Graphical comparison of GGE biplot analyses based on G × E for grain yield data and the PCA biplot analyses based on drought tolerance indices can be useful for understanding of and the relationships between adaptation of genotypes to different environments and their tolerance to drought stress. According to the GGE biplot (Fig. 6), G11 was a superior genotype with high mean grain yield and yield stability, and G13 and G14 with the highest grain yield had the highest adaptation to moderate cold and cold environments, respectively. This shows that genotypes that had high level of tolerance to drought stress also had both high grain yield and yield stability/adaptability. In contrast, G7, G15, G18, G12, G5, and G19 which had moderate and low level of grain yield were highly susceptible to drought stress.

However, if the crop breeding strategy is to improve grain yield and yield stability under drought stressed and non-stressed environments, it might be rational to focus on specific adaptation to increase genetic gains from direct selection in the target environment (Atlin and Frey 1990; Hohls, 2001). However, selection should be based on the drought selection indices calculated from grain yield under both stress and optimum conditions, when the breeder is looking for the genotypes adapted for a wide range of environments or a location with unpredictable conditions (Mohammadi *et al.*, 2011).

STI, GMP and MP indices can be suggested for selection of drought tolerant genotypes with high grain yield under drought stress conditions, particularly for drought-prone environments such as western parts of Iran where drought conditions is predominant over years and favorable years are infrequent (Mohammadi, 2016; Mohammadi *et al.*, 2011). SSI, TOL and YSI can also be used as useful indicators for distinction between susceptible and tolerant genotypes. In addition, GGE biplot method applied to drought and supplemental irrigation grain yield data provided useful information on

yield stability and adaptability of genotypes. Integrating drought tolerance indices and methods underlying  $G \times E$  interaction analysis such as GGE biplot model would help breeders to select and recommend genotypes that combine both drought tolerance and adaptation.

### CONCLUSION

In the present study, most of the observed variation were explained by location followed by location  $\times$  year interaction and moisture-regime reflecting much wider range of environment main effects than genotype main effect. Drought tolerance assessment in multi-location and multi-year trials demonstrated that it is crucial to identify suitable genotypes possessing high yield and good adaptation to a wide range of environments. Using GGE biplot analysis, genotypes were classified into groups according to their performance. The results revealed that drought tolerance indices and genotype ranks served as helpful tools to screen drought-tolerant genotypes under a range of environments. STI, GMP and MP were significantly correlated with grain yield under drought and supplemental irrigation conditions showing the efficiency of these indices for selection of high-yielding genotypes in both drought-stressed and supplemental irrigation environments.

Genotypes; G11, G13, G14, and G22 demonstrated as the most drought-tolerant genotypes with high grain yield in both conditions. In contrast, G18, G19, G15, G23 and G16 which were drought-susceptible genotypes were only suitable for supplemental irrigation conditions. Comparison of genotypes based on drought tolerance indices and the GGE biplot analysis revealed that drought-tolerant genotypes had high-mean grain yield and yield stability across environments, showing strong relationship between drought tolerance and mean grain yield and yield stability of rainfed winter bread wheat genotypes. The genotypic variation for drought tolerance and yield stability in this study should be further explored in the national rainfed winter wheat breeding programs for improving drought tolerance and yield stability in breeding materials for variable dryland environments in west and northwest of Iran.

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