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Identification of high-yielding and stable genotypes of barley for salinity-affected regions in the moderate climate of Iran

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ABSTRACT INFO ABSTRACT Salinity stress is one of the most important abiotic stresses in the arid and **Research Paper** semi-arid areas of the world. To identify superior genotypes of barley with high grain performance and stability under salinity stress conditions, 18 promising genotypes along with two local checks (Mehr cultivar and MBS-97-6 line) were investigated in three saline regions (Yazd, Isfahan, and Birjand) in the 2020-21 and 2021-22 cropping seasons. Based on the results of means comparison, genotypes G19, G17, G1 (Check 1), G20 (Check 2), G4 and G8 genotypes showed the highest grain yield compared to the other genotypes. The results of the GGE biplot analysis indicated that the first two principal components in total and in averaged data of two years, accounted for 49.29% and 21.6%, 60.42% and 25.8% of the total variation of grain yield, respectively. The vertex genotypes were identified as G1, G2, G10, G11, G17, G18, G19 and G20. Isfahan had representativeness ability and was identified as the ideal location for the selection of superior genotypes. On the basis of results, genotypes Rojo/3/LB.Iran/Una8271//Gloria"S"/Com"S"/4/Kavir (G4), Roho / Mazorka // Trompilo /3/ Lignee 527/Nk1272 // Jlb 70-63 (G20) and Manal / Alanda-01 // 1-BC-0152 /4/ Rojo /3/ LB. Iran / Una 8271 // Gloria "S" / Com "S" (G8) were recognized as the closest genotypes to the ideal genotype. Genotypes G4 and G20 were recognized as the best genotypes for cultivation in Yazd and G8 was recognized as the best genotype for cultivation in Isfahan and Birjand. Key words: GGE biplot, Promising genotypes, Saline Regions, Test locations. **Received: 26 Jun 2023 Accepted: 26 Jul 2023**

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INTRODUCTION

Soil salinity is one of the most important factors affecting plant production in arid and semi-arid areas of the world and in Iran. According to FAO's report, more than 397 million hectares of lands are affected by salinity stress. Iran has 6.8 million hectares of saline land and is identified as one of the most important countries threatened by salinity stress (Moameni, 2010). Some factors such as unsuitable irrigation and extra use of nutrients and chemical fertilizers can result in soil salinization. The plants growing in saline soils due to water sucked by salts are unable to absorb nutrients and water which results in water deficiency and dehydration. Salinity stress negatively changes the morphological and physiological traits. Moreover, this abiotic stress changes the ion homeostasis through increase or decrease sodium (Na^+) and potassium (K^+) concentrations (Khalily and Naghavi, 2020). This stress causes two types of stress on plants. The first, when salt accumulates around the roots, water availability decreases and osmotic stress occurs and transfer of water from the soil to the roots is reduced and it causes a decrease in shoot growth. The second phase, known as 'ionic stress' occurs when the contents of cytosolic chloride and sodium increase in developing leaves (Munns et al., 1995). Ionic homeostasis is one of the tolerance mechanisms disturbed in the case of salinity stress. Plants possess diverse protective mechanisms for maintaining ion homeostasis through $Na⁺$ exclusion (Muns and Tester, 2008). One of them is creating a balance between Na^+ and K^+ content in different tissues of plants. Various methods have been proposed to manage salinity stress, including the use of genetic diversity of crops and their pastures to identify cultivars that are tolerant to this stress (Ranjbar and Pirasteh-Anosheh, 2015; Shahmoradi et al., 2018). Cultivating the salt-tolerant cultivars is an efficient strategy to cope with negative effects of salinity. Although barley is one of the most tolerant crops to affected areas, salinity stress affects all growth stages salinity stress and it is widely cultivated in salinityof this crop and reduces its yield. There is a great variation among different barley cultivars in terms of salinity stress tolerance (Kharub *et al.*, 2013). This variation can be used to introduce to lerant cultivars to salinity stress. Currently, in Iran there are three salinity tolerant barley cultivars including Khatam (Ghazvini et al., 2016), Mehr (Nikkhah et al., 2018), and Golshan (Barati *et al.*, 2020), which were selected from the national barley breeding programs.

It is widely accepted that a high-yielding genotype in an environment might not be the best one for other

environments due to different responses of genes or their different emergency in different environments (Perkins and inks, 1968; Falconer, 1981). The interaction between genotype and environment (GEI) is one of the most complicated challenges in any plant breeding program for identifying the high-yielding and stable genotypes. Thus, knowledge of the nature and extent of GEI is useful for the accurate evaluation of genotypes in multi-environment trials (METs). In the evaluation of genotypes in different environments, the sum of the main effect of genotype and GEI is very important for selecting stable genotypes, thus these effects should be investigated simultaneously. In this regard, Yan et al. (2000) combined the main effect of genotype and GEI and suggested the GGE biplot model (Genotype+Genotype×Environment).

The GGE biplot method is one of the parametric methods to determine the yield stability. This method has been used for visual evaluation and graphical analysis of data in METs (Yan et al., 2007). The GGE biplot is based on principal components analysis and it is widely used in different cops plants such as wheat (Askari *Golestani et al., 2018*), rice (Hosseini Chaleshtori et al., 2021) and canola (Amiri Oghan et al., 2021). This method has been used in barley by various researchers *(Jalata, 2011; Mortazavi et al., 2014; Solonechnvi et* al., 2015; Kendel, 2016; Taheripourfard, 2017). The main objective of the present study was to identify the superior barley genotype(s) with the high yield performance and stability across several salt-affected areas in the moderate climate of Iran. The selected genotypes can be candidates for introducing as new salt-tolerant varieties or using in crossing blocks for future breeding programs.

MATERIALS AND METHODS

A set of promising lines of barley along with a local cultivar (cv. Mehr) and pure line $(MBS-97-6)$ (as two experimental checks) were investigated using a randomized complete block design with three replicates in three salinity-affected regions including Yazd (EC_{water}: 10 ds/m, EC_{soil}: 10 - 12 ds/m), Isfahan $(EC_{water}: 14$ ds/m and $EC_{soi}: 10$ ds/m), and Birjand $\overline{\text{EC}}_{\text{water}}^{202}$ 10 ds/m and $\overline{\text{EC}}_{\text{soil}}$: 14 ds/m) in the 2020-21 and 2021-22 cropping seasons (Tables 1-3). Each genotype was planted in six rows with 6 meters in length and a distance between rows of 20 cm. Seed density in each plot was determined as 450 seeds per square meter. The date of planting was in November in all research stations. During the tillering stage, Granstar and Pumasuper herbicides were used to control wide-

Genotypes	Pedigree
G1	Mehr (Check-1)
G ₂	Karoon/Kavir/3/Rhodes'S'//Tb/Chzo/4/Gloria'S'/5/Nik
G ₃	Bda/Rhn-03//ICB-107766/3/Rhn-03//L.527/NK1272
G4	Rojo/3/LB.Iran/Una8271//Gloria"S"/Com"S"/4/Kavir
G ₅	Legia//Rhn/Lignee 527/3/Kavir
G6	Legia//Rhn/Lignee 527/3/Kavir
G7	Legia//Rhn/Lignee 527/3/Rhn03
G8	Manal/Alanda-01//1-BC-0152/4/Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"
G9	Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"/4/Kavir
G ₁₀	Legia//Rhn/Lignee 527/3/Yousef
G11	Sahra/4/ Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"
G ₁₂	Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"/4/Anoidium/Arbayan-1/3/Lignee527/NK1272//JLB
G ₁₃	Alanda//Lignee527/Arar/3/Aths/4/Briges
G14	Dasht//EBC(a)/Badia/3/Torsh/4/Sahra
G ₁₅	Bgs/Dajia//L.1242/3/(L.B.IRAN/Una8271//Gloria'S'/3/Alm/Una80)/4/ Torsh/5/Fajr30
G ₁₆	Beecher/Kavir/4/Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"
G17	Bereke-54/3/MAKOUEE//ZARJOW/80-5151
G ₁₈	26216/4/Arar/3/Mari/Aths*2//M-Att-73-337-1/5/Nik
G ₁₉	26216/4/Arar/3/Mari/Aths*2//M-Att-73-337-1/5/Nosrat
G ₂₀	MBS 97-6 (Check-2)

Table 1. Pedigree of evaluated promising barley lines under salinity stress condition during 2020-22 cropping seasons.

Table 2. Geographical location of the test locations.

Station	Latitude	Longitude	Altitude (m)
Isfahan	$32^\circ 30'$ N	51°16'E	1541
Yazd	$31^\circ 54'$ N	$54^{\circ}16'E$	1237
Birjand	$32^{\circ}52^{\prime}N$	$58^\circ 59'E$	1491

leaved and thin-leaved weeds, respectively. After seed planting, one and four times irrigation were applied in the autumn (planting) and spring (tillering, stemming, flowering and grain filling), respectively. At the harvest time after removing the border effect, all plots were harvested and the grain yield of the investigated genotypes was estimated based on t/h. After collecting experimental data, a combined analysis of variance was computed using SAS ver.9.1 software. Duncan's multiple range test (DMRT) was used for means comparison. To investigate the grain yield stability the GGE biplot analysis was performed using GenStat ver.12 software in two ways: Total data of two years (6 environments including the combination of location and environment) and averaged data of each location in two cropping seasons (3 environments). (Genestat, 2008). The GGE biplot methodology is composed of the biplot (Gabriel, 1971) and the GGE (Yan et al., 2000) concept.

RESULTS AND DISCUSSIONS

The results of combined analysis of variance

indicated that the interaction effects of year×location, genotype×year and genotype×year×location were significant (Table 4).

The average grain yield of the evaluated promising genotypes of barley genotypes ranged from 2.640 t/h (corresponding to genotype G2 at Yazd station in the second year) to 8.444 t/h (corresponding to Mehr cultivar $(G1)$ at the Yazd station in the first year) across six environments (Table 5). Due to the significant effect of the genotype \times year \times location interaction, the results of the combined ANOVA method could not efficiently determine the stable genotypes with high grain yield. Hence, it is necessary to investigate grain yield stability of the evaluated genotypes, using multivariate analyses. The means comparison analysis using the Duncan's multiple range test (DMRT) revealed that genotypes $G19, G17, G1, G20, G4$ and $G8$ with the highest grain yield compared to other genotypes can be considered as superior genotypes. Considering the significance of the triple interaction effect, the grain yield data were subjected to stability analysis.

The results of GGE biplot analysis in 2020-2021 and $2021-2022$, showed that PC1 and PC2 accounted for 49.29% and 21.6% of the total variation, respectively. These components display the main and interaction $effects of genotype and genotype\times environment,$ respectively. The biplot based on the averaged data of two years showed that the two first PCs accounted for 86.22% of the total variation (PC1=60.42% and

Table 3. Monthly meteorological data in cropping seasons of 2020-2021 and 2021-2022 in the test locations.

Table 4. Combined ANOVA for grain yield data of barley promising barley genotypes in salinity regions of Iran during 2020-21 and 2021-22 cropping seasons.

Source of variation	df	SS	
Year (Y)	1	0.003	0.003 ^{ns}
Location (L)	2	2.00	1.00 ^{ns}
Y×L	2	351.17	69.8**
Replication (Y×L)	12	30.17	5.19
Genotype (G)	19	22.69	1.50 _{ns}
G×L	38	31.18	1.03 _{ns}
G×Y	19	30.46	$2.63**$
G×Y×L	38	30.23	$1.64*$
E2	228	110.50	

ns, * and **: non-significant, significant at 5% and 1% probability levels, respectively.

$PC2 = 25.80\%$) (Figure 1B).

It is concluded that mentioned two main components in each biplots can be used for justifying the grain yield of genotypes. The polygon view of GGE biplot displays the genotype main effect plus GEI effects (Figure 1). In these biplots, polygons are formed by connecting the vertex genotypes with straight lines and the rest of the genotypes placed within the polygon. The length of each environment vector represents its discriminating ability to distinguish genotypes in the environment. In the present study, the vertex genotypes of 2020- 2021 and $2021-2022$ cropping seasons were G1, G2, $G11, G18, G17, and G19.$ and the vertex genotypes of averaged data of two years were G20, G17. G18 and G10. Indeed, these genotypes are defined by having the greatest vector length in their respective directions, which shows they are more interactive. However, other genotypes were less interactive in response to the environmental changes. The genotypes close to the origin of biplot (G8, G10, G7 and G5, G9, G15, G19) in two years and averaged data of two years, respectively) showed more stability than the others (Figure 1).

The vertex genotypes were the best or the worst genotypes with respect to the grain yield in some or all of the test locations due to their distance from the origin of the biplot (Table 5). For example in two years (6 locations) the genotypes $G17$ and $G18$ (vertex genotypes) had the highest and lowest grain yield in the first year of Isfahan and Yazd, respectively. Which Won Where/What polygons plot showed that genotypes G4 and G20 were suitable for Yazd. The rank means of these genotypes in two cropping seasons of Yazd are 3.5 and 5, respectively (Tale 5). Genotype $G8$ was distinguished as desirable genotype for Birjand and .Isfahan

Table 5. The mean and rank of grain yield in each test location and total mean comparison of evaluated promising barley genotypes using Duncan's multiple range test . Seasons cropping 2020-21 and 2020-21 and 2020-21 and 2021-22 $\rho_{\rm s}$ are seasons and $\rho_{\rm s}$ T**able 5**. The mean and rank of grain yield in each test location and total mean comparison of evaluated promising barley genotypes using Duncan's multiple range test

Common letters indicates non-significant.

Common letters indicates non-significant.

Figure 1. Graphical display for barley genotypes adapted to the test locations (A: two cropping seasons, B: averaged data).

This genotype is placed in the section where Birjand and Isfahan are located. Maniruzzaman et al. (2019) analyzed performance of seven barley genotypes across three different environmental conditions of Bangladesh. Considering yield stability, genotypes "E3", "E4" and "E1" were found to be more stable, whereas genotype "E2" was the most unstable over all locations. Genotypes " $E7$ " and " $E3$ " were found to be close to the ideal genotype position. Taherian et *al.* (2022) studied nineteen promising barley lines for quantifying genotype×environment interaction effect on grain yield and grain yield stability in temperate regions of Iran (Karaj, Varamin, Birjand, Neishabour, Mashhad, Zarghan, Isfahan and Yazd) in 2016 -17 and 2017 -18 cropping cycles. Among environments, Karaj was identified as the closest environment to the ideal environment. Comparison of barley promising lines with ideal genotype identified G7 as the closest genotype to ideal genotype. Also the closest genotypes to G7 were G5, G13, G20 and G4. These barley promising lines had high grain yield, wide adaptation and grain yield stability in temperate regions of Iran. Kendal (2016) investigated four barley genotypes (Kendal, Alnikat, Samili and Sahin 91) in 8 environments. Kendal and Alnikat showed a general concept of adaptation to four test environments. Whereas, varieties Samili and Sahin91 showed a specific concept of adaptation to two test environments and each was placed in two different mega-environments.

The visualization of the mean of grain yield and stability of investigated genotypes is achieved by drawing an average environment (AEC) in the

genotype based biplot (Ahakpaz and Ahakpaz, 2014). The ranking of the genotypes based on the grain yield means and the degree of stability in the test locations (two and averaged data of two cropping seasons) is shown in Figure 2. An average environment is defined in this figure, which is shown with a small circle and is defined using the mean PC1 and PC2 scores of the locations (Figure 2). The line that passes through the origin of the bi-plot and the average environment $(horizontal axis)$ can be considered as the axis of the average environment. Any genotype that is closer to the horizontal axis is more stable and the farther from the arrowhead, it has the higher performance. (Yan et al., 2000). The vertical axis that passes through the origin of the biplot and is perpendicular to the horizontal axis, indicates the mean grain yield of all genotypes. The vertical axis is an estimate of the GEI for each genotype. Indeed, this estimate is a criterion to test yield stability for genotypes. By using this biplot (Figure 2A), it was determined that genotypes $G4, G20$, $G8, G7, G13, G14, G11, and G12$ are perpendicular to the horizontal axis with a shorter line length and have more stability than others. Moreover, genotypes t G12, G11, G14, G13, and G7 with lower yield than the mean grain yield and less distance from the horizontal axis are among the genotypes with stable yield. The genotype $G1$ (check-1) showed high grain yield with a considerable distance from the horizontal axis and hence it was recognized as the less stable genotype but G20 (check-2) with lower performance than the mean grain yield was recognized as more stable due to low distance from the horizontal axis. On the basis of averaged data (Figure 2B), it was determined that

Figure 2. Genotype ranking biplots based on the mean yield and stability (A: two cropping seasons, B: averaged data).

genotypes $G2$, $G5$, $G8$, $G9$, $G13$ and $G15$ had more stability than others.

According to the mentioned cases, simultaneous selection for high performance and stability is not possible, so genotypes with moderate yield and stability should be selected. For this purpose, we used an ideal genotype biplot. As shown in Figure 2, there is a small circle on the horizontal axis which is indicated by an arrow. The genotype that is placed in circle will be identified as an ideal genotype. The hypothetical ideal genotype is defined based on the most productive genotype and the most stable yield and is used as a reference for evaluating the studied genotypes. On the other hand, the distance of other genotypes from the *ideal genotype determines their desirability (Yan et al.,* 2000). Based on Figure 3A, genotypes G4 and G20 with the smallest distance from the hypothetical ideal genotype were recognized as the closest genotypes to the ideal genotype. On the basis of averaged data, genotypes G4 and G8 were closest to the ideal genotype. According to Figure 4, it is clear that there is a high similarity between the locations of the first year of Yazd and the first year of Birjand, as well as between the locations of the first year of Isfahan and the second year of Yazd. According to the angle between these two locations groups, it can be concluded that these locations have a different behavior in the ranking of genotypes (Figure 4A). Multi-environment experiments can also be used to evaluate test environments (Yan et al., 2000). The main characteristics of ideal environments are: (i) a high ability to separate and differentiate between genotypes, and (ii) being

representative of other tested environments (Yan and Tinker, 2006). The ability to isolate and differentiate the environment means that an environment is capable of displaying maximum diversity among genotypes. The ability to represent an environment shows that an environment is representative of the conditions of other environments in the desired experiment. An ideal environment should have a combination of the two mentioned characteristics for the development of adaptable genetic material. In the GGE biplot model, these features are described as "the ability to separate environments versus their representativeness" (Yan and Tinker, 2006). The ability to distinguish is one of the characteristics of any environment, and environments without the ability to distinguish are not only weak in providing useful information about the cultivars but may also be misleading by providing incorrect information. This feature has been used for the identification of ideal environments in different crops such as barley (Koocheki et al., 2012) and durum wheat (Mohammadi et al., 2010). To identify a sample location in biplot, an average location is defined and used as a reference or control. This point is considered as the ideal virtual location. Environment with longer length of the environmental vector and low distance to the horizontal axis is closer to the desired environment $Yan, 2001$). A longer environmental vector shows the high ability of that environment to separate genotypes from each other. In terms of representativeness power, the environments that have the smallest angle with the arrowed horizontal axis are better representatives for other environments. According to Yan's (2001) theory, an environment with the most discriminating

Figure 3. Comparison of barley promising lines with ideal genotype based on yield and its stability (A: two cropping seasons, **B**: averaged data).

Figure 4. Biplots of comparison of locations with the target location based on the discriminating and representativeness ability of the target location (A: two cropping seasons, B: averaged data).

and representative powers can be considered as an ideal target environment for evaluating new varieties for their full yield potential. The average environment is shown in Figure 4 by a small circle. The line that passes through the origin of the biplot and the average environment is AEC. As shown in this figure, it is clear that the second year of Isfahan had a smaller angle with the arrowed horizontal axis and it is concluded that the mentioned environment had a more ability in terms of representativeness of other environments for barley genotypes. By using averaged data, it is

also revealed that Isfahan with a small angle with the arrowed horizontal axis can be representative of other environments (Figure 4B). A smaller environmental vector length indicates that the environment has a less ability to separate and differentiate among genotypes. Among the test locations, the first years of Yazd and Isfahan were more capable of separating and creating diversity among genotypes. Hence, these locations are the closest locations to the ideal environment and are more favorable than the rest (Figure 4A). Based on averaged data, it was found that Isfahan is also the closest location to the ideal environment (Figure 4B).

Previous GEI analyses on several traits have revealed that barley is sensitive to environmental changes similar to other crops. According to Ahakpaz *et al.* (2021), Hilmarsson *et al.* (2021), Ghazvini *et al.* (2022) and Pour-Aboughadareh et al. (2023) changes in environmental conditions have been indicated to affect grain yields in barley. This implies that dissection of GEI plays a crucial role in genotype selection, cultivar release, and identification of suitable target production of environments for optimum yield performance. Many regions of Iran such as parts of Isfahan, South Khorasan, Yazd, Kerman, Khuzestan, Razavi Khorasan, Alborz, Tehran, Fars, and etc. are affected by the salinity stress, tolerant cultivars for these regions. It is notable that thus it is necessary to improve and develop salinitymost salt-tolerant lines delivered so far to farmers' fields have come from yield-based selection (Melino and Tester, 2023). Barati et al. (2019) investigated a set of elite barley lines in Yazd, Isfahan, and Birjand stations during the 2012-14 cropping seasons and reported significant effects on genotype, environment, and their interaction. Their result showed that genotype G8 with a pedigree of "L.527/Nk1272//Jlb70-63/3/1-BC-80320" was selected based on optimal grain yield and general adaptability and therefore, it was introduced as the Golshan cultivar which is currently *et* eultivated in areas affected by salinity stress (Barati et *al.*, 2020). To select the best genotypes, both grain yield and stability factors should be considered. The studied lines in this experiment were selected in different barley breeding stages (observational, preliminary, advanced, and adaptation) in these regions and this has reduced the diversity especially in terms of grain yield and as a result, the difference between genotypes in terms of grain yield is not significant (Table 4). The recommendation of a new cultivar in one region will help stability and maintain stable production by increasing diversity, even if it is not superior to the check cultivar in terms of grain yield. In this study, genotypes $G19, G17, G1, G20, G4$ and $G8$ had the highest yield, respectively (Table 3). Of these, genotypes G20, G4 and G8 have no significant difference in grain yield with the first three genotypes but they had more yield stability than those genotypes. In conclusion, genotypes Rojo/3/LB.Iran/Una8271//Gloria"S"/Com"S"/4/Kavir (G4) and Roho / Mazorka // Trompilo $/3$ / Lignee 527 $\frac{1}{\text{Nk1272}}$ // Jlb 70 -63 (G20) are introduced as the selected lines of this study for commercial release in Yazd and G8 (Manal/Alanda-01//1-BC-0152/4/Rojo/3/ LB.IRAN/Una8271//Gloria"S"/Com"S") for Isfahan and Birjand areas affected by salinity stress in Iran.

These three genotypes obtained from Iran international irrigated barley breeding program. They are selected from genotypes which are evaluated in salinity-
affected-environments. The initial crosses of these lines was carried out in order to identify salinity tolerant genotypes. Due to the nature of salinity experiments, it was necessary to consider other traits which are effective in increasing salinity tolerance such as stay greening time, stem thickness and flag leaf area. Thus the final selection of superior genotypes was carried out on the basis of analysis results and the mentioned .characters

Considering the extent and dispersion of the areas affected by salinity stress in Iran, it is difficult to identify high-yielding and stable genotypes with general adaptability for all salinity affected areas. According to the results of this study, specific adaptation is suggested for identifying salinity resistant genotypes because of the different responses of the studied genotypes to test locations. Paying attention to specific adaptability in barley breeding programs in Iran has also been mentioned by other breeders (Taheripourfard *et al.*, 2017; Barati *et al.*, 2020). yielding genotypes with general adaptability can be However, it seems that the identification of highachieved using the evaluation of salinity-tolerant lines in multi-environment experiments.

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