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Phenotypic stability analysis of barley promising lines in the cold regions of Iran

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ABSTRACT

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Development of high-yielding new barley promising lines with wide adaptation across a wide range of diverse environments is a key goal of barley breeding program in the cold regions of Iran. The main objective of the current study was to use different stability analysis approaches to analyze phenotypic stability for selecting high-yielding with yield stability barley promising lines adapted to the cold regions of Iran as well as to investigate the relationships among different stability parameters and grain yield. Eighteen barley promising lines and two check cultivars; Bahman and Jolgeh were evaluated using randomized complete block design with three replications at six research stations during 2015–2017 cropping seasons. The AMMI analysis of variance indicated that the environment, genotypes and their interaction accounted for 53.60, 5.77 and 24.59% of the total variations, respectively. The first six interaction principal components (IPCA1 to IPCA6) were highly significant, revealing differential responses of the tested lines to different environments and the necessity of stability analysis. In total, 18 parametric and non-parametric statistics were used to analyze the data. According to PCA-based biplot and correlation heat-map, the stability statistics were classified into two main groups (CI and CII): CI comprised mean grain yield, θ_i, *TOP* and *bi*, which are referred to the dynamic concept of stability, and CII included S^1, S^2, S^3, S^6, NP^1, NP^2, NP^3, NP^4, CV, *ASV*, W_i^2, σ^2, $\theta_{(i)}$, S_{di}^2 and *KR*, which are referred to static concept of stability. In general, the parametric and non-parametric stability statistics indicated similar results, identifying the promising line G8 (Makouee/Jolge) as high-yielding with yield stability. Therefore, this promising line can be recommended for being grown and commercialized in the cold regions of Iran.

Key words: Barley, yield stability, stability parameters, grain yield, AMMI, heat-map

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth major cereal crop in the world following wheat, maize and rice (Poehlman, 1985; Langridge and Barr, 2003). Barley grain is consumed as food in human diets and animal fodder due to its dietary proteins, carbohydrates, fiber, vitamins, iron, calcium, zinc, fats and energy. About 85% of the world's barley production is estimated to be used to feed farm animals (e.g. cattle, sheep, goats, pigs, horses and poultry), while the remaining is used for malt production, human food, starch production, and seed (Fischbech, 2002).

Barley is widely adapted to unfavorable climatic conditions, particularly, its good performance in the poor soils and marginal lands, therefore, has been distributed in most parts of the world. It grows well under stress prone environments due to its better ability to avoid abiotic stresses such as high and low temperatures, drought and salinity (Vaezi *et al.,* 2017). Over the past decades, climate change has had significant impact on global barley production, hence its production reduced from 150.9 million tons in 2009 to 140.6 million tons in 2019 (STATISTA, 2019). Therefore, the development of new barley cultivars adapted to different climatic conditions is the main objective of any barley breeding program.

Barley is the second most important cereal grain in Iran. Based on the latest statistics in cropping season 2016-17, barley was grown on about 1.47 million hectares in Iran with produced about 2.97 million tons of grain (Anonymous, 2018). Low-temperature or cold stress is considered as one of the main abiotic stresses that limit barley production in the highland regions of Iran (Mahfouzi *et al*., 2008). In these regions, the average soil temperature can decrease to below 0 to -4 ˚C in winter and cold damage during this period can seriously damage seedlings. Screening barley germplasm for cold tolerance during the early stage of plant growth and development is an essential breeding strategy in highlands and cold regions of Iran. Another important criterion for assessing cold tolerance in barley is the evaluation of yield stability of the genotypes across different locations with freezing winter temperature.

Grain yield is a quantitative trait genetically controlled by multiple genes with minor effects. The expression of this trait is usually affected by genotype (G), environment (E), and G × E interaction (GEI). Understanding of G × E interaction is very important for plant breeders, because it reduces the association between genotypic and phenotypic values and therefore complicates the selection of superior lines (Ebdon and Gauch, 2002). To interpret this interaction effect, evaluation of genotypes in multi-environment trials (METs) is essential in any breeding program (Yan and Tinker, 2006). Indeed, this task helps to select genotype(s) with specific adaptation for specific environmental conditions and genotypes with yield stability and wide adaptation for being grown across different environments (Vaezi *et al.,* 2018).

Many statistical models and methods are available for estimating GEI and stability parameters to select genotypes with high grain yield and yield stability for a wide range of environments. Parametric and non-parametric statistics are two main approaches for dissecting GEI effects. The common parametric statistics consist of regression coefficient (Finlay and Wilkinson, 1963), deviation from regression slope (Eberhart and Russell, 1966), the mean variance component (Plaisted and Peterson, 1959), ecovalence (Wricke, 1962), GE variance component (Plaisted, 1960), stability variance (Shukla, 1972), coefficient of variation (Francis and Kannenberg, 1978), and AMMI-based stability parameters (Zhang *et al.,* 1998; Purchase *et al.,* 2000).

Non-parametric statistics comprise

Huehn's and Nassar and Huehn's non-parametric measures (Huehn, 1979; Nassar and Huehn, 1987), Thennarasu's non-parametric statistics (Thennarasu, 1995), Kang's rank-sum (Kang, 1988), and Fox's TOP-rank (Fox *et al.*, 1990). Since each of parametric and non-parametric stability statistics has its own merits and demerits for selection of enotypes with yiled stability as well as its specific concepts for addressing G × E interaction effects, most breeding programs combine statistics from both analytical approaches (van Eeuwijk *et al.,* 2001).

Parametric and non-parametric statistics are widely used for determining phenotypic stability and interpreting G × E interactions in plant breeding. These methods have been used to evaluate yield stability in many crops such as maize (Scapim *et al.,* 2010), barley (Vaezi *et al.,* 2019), wheat (Ahmadi *et al.,* 2012a), *Linum* (flax) (Adugna and Labuschangne, 2003), chickpea (Segherloo *et al.,* 2008), grass pea (Ahmadi *et al.,* 2015) and *Chenopodium* (Bhargava *et al.,* 2007). Barley breeding programs have mainly focused on improving grain yield in new promising genotypes possessing high grain yield and yield stability across variable environments. Therefore, the present study aimed to (i) interpret G × E interaction effect on grain yield and yield stability for 20 barley genotypes in six research stations in two successive cropping seasons using 18 parametric and non-parametric stability statistics, and (ii) investigate the associations among different stability parameters and grain yield.

MATERIALS AND METHODS

Plant materials and experimental setup

Eighteen barley promising lines and two check cultivars (Bahman and Jolgeh,) (Table 1), were evaluated in the elite barley yield trial (EBYT) in six filed stations in the cold regions of Iran in 2016–17 and 2017–18 growing seasons across (12 environments). The experimental stations included; Ardabil, Miandoab, Hamedan, Mashhad, Jolgeh-Rokh and Karaj. The experimental design was randomized complete block design with three replications.

Each plot included; six rows of 6-m long with 30-cm row spacing. Seeding was done using experimental Wintersteige plot seeders. Basal fertilizer of 32 kg N ha^{-1} and 100 kg P_2O_5 ha^{-1} were applied before planting in all experimental sites. Also, at the commencement of stem elongation stage 40 kg N ha^{-1} was applied as top dressing in each trial. Five times irrigation was applied during the growing season, considering crop requirement and environmental conditions, at Zadoks' growth scales of 00, 32, 51, 75 and 85 (Zadoks *et al*., 1974). After crop ripening, plots were harvested using Wintersteiger plot combine. Grain yield per plot were weighed, then data were converted to tonnes hectare^{-1} (t.ha^{-1}).

Data analysis

The additive main effect and multiplicative interaction (AMMI) model which combines standard analysis of variance (ANOVA) and principal component analysis (PCA) was used to estimate GEI using GenStat ver. 12 software (GENSTAT, 2008).

Parametric and non-parametric statistics

Eight parametric stability statistics including, the mean variance component (θi; Plaisted and Peterson (1959)), G × E variance component ($\theta_{(i)}$; Plaisted (1960)), Wricke's ecovalence (W_i^2;Wricke (1962)), regression coefficient (b_i; Finlay and Wilkinson (1963)), deviation from regression (S_{di}^2; Eberhart and Russell (1966)), Shukla's stability variance (σ_i^2; Shukla (1972)), coefficient of variation (*CV*; Francis and Kannenberg (1978), AMMI's stability values (*ASV*; Purchase (2000)) were calculated as following equations.

Table 1. Codes and pedigree/name of the 20 barley genotypes

Genotype code	Pedigree/Name
G1	Jolgeh (Check-1)
G2	L.131/Gerbel//Ager-Ceres/3/Scotial/Wa 1356-70//Wa 1245-68/Boyer.F7/4/Walfajre/Miraj 1
G3	L.131/Gerbel//Ager-Ceres/3/Scotial/Wa 1356-70//Wa 1245-68/Boyer.F7/4/Jolgeh
G4	Jolgeh/Bahman
G5	Mahtab/Makouee
G6	Monolit/Plaisant//Walfajre
G7	Bahman/Mahtab
G8	Makouee/Jolgeh
G9	SD729/Por-B/3/Apm/Aths-B//Gva/4/Ore/5/Bllu/6/Ciru/7/Khatam
G10	Fusion
G11	Jolgeh/Makouee
G12	Ashar/Victoria//CWB117-5-9-7/3/Sadik10
G13	CM67/IPA265//Gustoe/IPA8/3/Nik
G14	Germunknown1
G15	Germunknown3
G16	Germunknown4
G17	Monolit/Plaisant//Walfajre/Miraj 1's'
G18	Furat-3
G19	Bahman (Check-2)
G20	EBYT-C93-3

$$(1)\ \theta_i = \frac{p}{2(p-1)(q-1)} \sum_{j=1}^{q} (x_{ij} - \bar{x}_{i.} - \bar{x}_{.j} + \bar{x}_{..})^2 + \frac{\sum\sum (x_{ij} - \bar{x}_{i.} - \bar{x}_{.j} + \bar{x}_{..})^2}{2(p-2)(q-1)}$$

$$(2)\ \theta_{(i)} = \frac{-p}{(p-1)(p-2)(q-1)} \sum_{j=1}^{q} (x_{ij} - \bar{x}_{i.} - \bar{x}_{.j} + \bar{x}_{..})^2 + \frac{SSGE}{(p-2)(q-1)}$$

$$(3)\ W_i^2 = \sum (X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..})^2$$

$$(4)\ b_i = 1 + \frac{\sum_i (X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..})(\bar{x}_{.j} - \bar{x}_{..})}{\sum_j (\bar{X}_{.j} - \bar{X}_{..})^2}$$

$$(5)\ S_{di}^2 = \frac{1}{N-2}\left[\sum_i (X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..}) - (b_i - 2)^2 \sum_j (\bar{X}_{.j} + \bar{X}_{..})^2\right]$$

$$(6)\ \sigma_i^2 = \left[\frac{p}{(p-2)(q-1)}\right] W_i^2 - \frac{\sum W_i^2}{(p-1)(p-2)(q-1)}$$

$$(7)\ CV_i = \frac{SD_x}{\bar{X}} \times 100$$

$$(8)\ ASV = \sqrt{\frac{SS_{IPCA1}}{SS_{IPCA2}}(IPCA1)^2 + (IPCA2)^2}$$

In equations 1–7, p, q, N, X_{ij}, $\bar{X}_{i.}$, $\bar{X}_{.j}$, $\bar{X}_{..}$, W_i^2 and SD_x are the number of lines, number of environments, the grain yield of line *i* in environment *j*, the mean grain yield of line *i*, the mean of grain yield of the environment *j*, grand mean, Wricks's ecovalance statistic and the standard deviation of the mean yield for each genotype over different environments, respectively. In equation 8, SS_{IPCA1} and SS_{IPCA2} are the sum of square of interaction for two first principal components, respectively.

Ten non-parametric statistics including; Nassar and Huehn's stability statistics ($S^{(3,6)}$; Nassar and Huehn (1987)), Huehn's statistics ($S^{(1,2)}$; Huehn (1990)), Thennarasu's stability parameters ($NP^{(1-4)}$; Thennarasu (1995)), Kang's sum of ranks (*KR*; Kang (1988)) and Fox's TOP-rank (*TOP*; Fox *et al.*(1990)) were also calculated using following equations:

$$(9)\quad S_i^{(1)} = 2\sum_{j}^{n-1} \frac{\sum_{j'=j+1}^{n} \left| r_{ij} - r'_{ij} \right|}{\left[N(N-1) \right]}$$

$$(10)\quad S_i^{(2)} = \frac{\sum_{j=1}^{n} (r_{ij} - \bar{r}_{i.})^2}{(N-1)}$$

$$(11)\quad S_i^{(3)} = \frac{\sum_{j=1}^{n} (r_{ij} - \bar{r}_{i.})^2}{\bar{r}_{i.}}$$

$$(12)\quad S_i^{(6)} = \frac{\sum_{j=1}^{n} \left| r_{ij} - \bar{r}_{i.} \right|}{\bar{r}_{i.}}$$

$$(13)\quad NP^{(1)} = \frac{1}{N} \sum_{j=1}^{n} \left| r^*_{ij} - M^*_{di} \right|$$

$$(14)\quad NP^{(2)} = \frac{1}{N} \left[\sum_{j=1}^{n} \left| r^*_{ij} - M^*_{di} \right| / M_{di} \right]$$

$$(15)\quad NP^{(3)} = \frac{\sqrt{\frac{\sum (r^*_{ij} - \bar{r}^*_{i.})^2}{N}}}{\bar{r}_{i.}}$$

$$(16)\quad NP^{(4)} = \frac{2}{N(N-1)} \left[\sum_{j=1}^{n-1} \sum_{[j'=j+1]}^{n} \left| r^*_{ij} - r^*_{ij'} \right| / \bar{r}_{i.} \right]$$

(17) KR = the grain yield rank of each line + the stability variance rank of each line

(18) *TOP* = this statistic consists of scoring the number of environments in which each line ranked in the top third of trial entries.

In equations 9–16, r_{ij}, $\bar{r}_{ij}$ and N are the rank of the *i*th genotype in *j*th environment, the mean rank in all environments for each genotype, and number of environments, respectively. Also, r^*_{ij}, $\bar{r}^*_{ij}$, M^*_{di} and M_{di} are the rank of *i*th genotype in the *j*th environment based on adjusted data, the mean ranks for adjusted data, the median ranks for adjusted data and the median ranks for unadjusted data, respectively.

The online program, STABILITYSOFT (Pour-Aboughadareh *et al.,* 2019) was used to calculate all parametric and non-parametric statistics (except *ASV* and *TOP*). After calculating parametric and nonparametric stability statistics, a heat map was rendered for investigating relationships among different stability parameters and grain yield of genotypes using the '*gplot*' package of R software (Warne *et al.,* 2014). Principal component analysis (PCA) was performed using XLSTAT package (XLSTAT, 2017) and a biplot based on the first two components (PC1 and PC2) was performed.

RESULTS AND DISCUSSION

AMMI analysis of variance

The results of METs combined analysis of variance revealed the impact of environment and genotypes on the grain yield of barley genotypes. The results of AMMI analysis of variance indicated that main effects of the E, G and G × E interaction effects were highly significant and accounted for 53.60, 5.77 and 24.59% of the total variations, respectively (Table 2). This result indicated that the test environments were very diverse, refelcting the differences among the environments in climate, soil and geographical coordinates and elevation, etc.

The highest variation for grain yield is usually assigned to environmental factors (Jamshidi-Moghaddam and Pourdad, 2013). The results indicated that the mean grain yield across environments varied between 2.77 t. ha^{-1} at Jolgeh-Rokh and 7.15 at Ardabil for cropping season 2016–2017. The first six interaction principal components (IPCA1 to IPCA6) were highly significant and accounted jointly for 91% of the total G × E variation, while 9% of the variation was assigned to residual effect.

Table 2. The AMMI analysis of variance for grain yield of 20 barley genotypes across 12 environments

Source of variation	df	SS	MS	*F*-value	% (G + E + G × E)	% G × E
Total	**719**	**1950.7**	**2.71**			
Treatments	**239**	**1637.7**	**6.85**	**11*****	**83.95**	
Genotypes	**19**	**112.6**	**5.92**	**9.51*****	**5.77**	
Environments	**11**	**1045.6**	**95.05**	**78.74*****	**53.60**	
Block	**24**	**29.0**	**1.21**	**1.94****		
Interaction	**209**	**479.6**	**2.29**	**3.68*****	**25**	
IPCA1	**29**	**172.2**	**5.93**	**9.54*****		**36**
IPCA2	**27**	**135.5**	**5.02**	**8.06*****		**28**
IPCA3	**25**	**49.4**	**1.97**	**3.18*****		**10**
IPCA4	**23**	**31.0**	**1.34**	**2.16****		**6**
IPCA5	**21**	**26.4**	**1.25**	**2.02****		**6**
IPCA6	**19**	**22.3**	**1.17**	**1.89***		**5**
Residuals	**65**	**42.7**	**0.65**	**1.06**		**10**
Error	**456**	**284**	**0.62**			

***, ** and ***: Significant at the 5%, 1% and 0.1% probability levels, respectively.**

The two first IPCAs explained a total of 64 % of the G × E (IPCA1 = 36% and IPCA2 = 28%) (Table 2).

Results also indicated that AMMI analysis was could extract a large portion of the G × E interaction effects. This agreed with results of previous studies which revealed the high efficiency of AMMI method in analyzing G × E interaction in different crops such as grass pea (Ahmadi *et al*., 2012b), wheat (Tesemma *et al.,* 1998; Ahmadi *et al.,* 2012a), safflower (Jamshidi-Moghaddam and Pourdad, 2013) and barley (Taheripourfard *et al.,* 2017).

Parametric stability statistics

Phenotypic stability values for genotypes based on grain yield and the eight parametric statistics are presented in Table 3. Mean grain yield of genotypes varied from 5.23 to 7.07 $t.ha^{-1}$ across environments, with an average of 6.14 $t.ha^{-1}$. Three genotypes G15 with 7.07 $t.ha^{-1}$ had highest mean grain yield, while genotypes G9 with 5.56 $t.ha^{-1}$ had the lowest mean grain yield (Table 3). According to Eberhart and Russell (1996) lines with higher grain yield, regression slope equal one (bi = 1) and least deviation from regression slope (S_{di}^2) can be considered as genotypes with the most grain yield stability.

G8 and G1 (Jolgeh) genotypes possessed high grain yield with the regression coefficient of 1.03 and 0.95 and deviation from the regression of 0.51 and 0.36, respectively, had high grain yield stability combined with very good adaptation across environments. G15 and G20 genotypes with acceptable yield level and $b_i > 1$ had specific adaptation in the high-yielding environments (Table 3). Genotype G10 with acceptable mean grain yield and $bi < 1$ had greater specific adaptability in low-yielding environments.

Statistics W_i^2 (Wricke, 1962), σ^2 (Shukla, 1972) and $\theta_{(i)}$ (Plaisted, 1960) demonstrated similar patterns for identification of genotypes with yield stability, thus G1 (Jolgeh), G8 and G19 (Bahman) were identified as genotypes with high grain yield stability using these stability statistics. However, G13, G14 and G18 were recognized for low grain yield stability (Table 3). Likewise, Vaezi *et al.* (2019) reported a similar pattern when they used these three statistics for studying yield stability of barley genotypes.

The AMMI's stability values (*ASV*) is a suitable statistic in situations when the two first interactions of principal components explain significant G × E interaction. Using this statistic G2, G5 and G19 (Bahman) were found to have high grain yield stability, while G10, G14 and G16 had low grain yield stability (Table 3). Based on *CV* statistic (Francis and Kannenberg, 1978), three genotypes; G1 (Jolgeh), G8 and G12 were identified with high grain yield stability, and G2, G9 and G18 with low grain yield stability. The θ_i (Plaisted and

Table 3. Mean grain yield, parametric and non-parametric stability statistics for 20 barley genotypes across 12 environments in 2016-17 and 2017-18 cropping seasons

Genotype code	Grain yield (t ha^{-1})	$S^{(1)}$	$S^{(2)}$	$S^{(3)}$	$S^{(6)}$	$NP^{(1)}$	$NP^{(2)}$	$NP^{(3)}$	$NP^{(4)}$	W_i^2	σ^2_i	$S^2_{d_i}$	b_i	CV	$\theta_{(i)}$	θ_i	ASV	KR	Top
G1	6.300	4.59	14.99	15.11	3.57	2.92	0.30	0.32	0.42	2.56	0.22	0.36	0.95	20.55	0.79	0.53	0.50	9.00	33.33
G2	6.110	6.29	28.08	34.64	5.94	4.33	0.55	0.59	0.71	5.56	0.52	0.68	1.22	27.24	0.78	0.67	0.14	22.00	16.67
G3	6.040	6.45	29.24	35.09	6.11	5.00	0.49	0.61	0.70	4.43	0.41	0.59	1.13	25.70	0.78	0.62	0.51	21.00	8.33
G4	6.100	6.33	28.09	32.53	5.68	4.17	0.47	0.53	0.67	6.13	0.58	0.82	1.15	26.47	0.77	0.70	0.45	25.00	8.33
G5	6.140	5.88	24.97	25.75	4.44	4.00	0.39	0.46	0.55	4.35	0.40	0.61	1.07	24.13	0.78	0.61	0.17	17.00	25.00
G6	6.280	6.95	36.63	33.81	5.30	5.17	0.36	0.51	0.58	5.16	0.48	0.67	1.16	25.53	0.78	0.65	1.18	17.00	33.33
G7	6.460	7.39	41.64	35.23	5.38	6.58	0.41	0.53	0.57	8.55	0.82	1.22	1.00	23.71	0.76	0.81	1.26	16.00	50.00
G8	6.470	5.70	25.00	22.00	3.76	3.75	0.35	0.35	0.46	3.59	0.32	0.51	1.03	21.80	0.79	0.58	0.34	5.00	25.00
G9	5.560	6.15	28.33	38.16	6.12	5.08	0.88	0.72	0.75	13.20	1.29	1.84	0.86	27.47	0.74	1.04	0.59	35.00	16.67
G10	6.390	7.62	42.93	43.93	6.42	6.50	0.61	0.66	0.71	13.14	1.28	1.79	0.81	23.15	0.74	1.03	1.97	20.00	33.33
G11	5.920	7.29	39.36	45.17	6.78	5.42	0.64	0.62	0.76	8.13	0.78	1.13	0.89	23.71	0.76	0.79	0.81	28.00	16.67
G12	6.290	6.80	33.72	32.02	4.72	4.75	0.38	0.50	0.59	5.82	0.55	0.48	0.62	15.23	0.78	0.68	0.37	18.00	16.67
G13	5.860	7.88	43.79	47.38	6.33	6.17	0.60	0.69	0.77	14.48	1.42	1.98	0.81	25.83	0.73	1.10	0.98	36.00	16.67
G14	5.410	6.27	30.24	52.53	8.53	5.17	1.36	0.98	0.99	13.60	1.33	1.76	0.73	25.91	0.73	1.05	1.74	37.00	8.33
G15	7.070	5.85	28.09	19.94	3.23	5.25	0.39	0.40	0.38	11.94	1.16	1.62	1.18	25.49	0.74	0.97	1.38	16.00	50.00
G16	6.330	7.80	42.81	43.14	5.97	5.50	0.50	0.59	0.71	8.66	0.83	1.23	1.07	25.41	0.76	0.82	1.40	20.00	25.00
G17	6.280	6.12	26.61	27.44	4.88	4.25	0.38	0.46	0.57	4.01	0.36	0.49	1.18	25.31	0.79	0.60	0.40	13.00	8.33
G18	5.230	7.56	48.57	79.15	10.96	6.08	2.64	1.01	1.12	19.60	1.94	2.68	0.78	31.29	0.70	1.34	0.54	40.00	16.67
G19	6.190	4.33	14.82	15.52	3.14	3.17	0.25	0.37	0.41	1.43	0.10	0.17	1.12	23.45	0.80	0.47	0.03	12.00	8.33
G20	6.330	6.14	26.75	25.04	4.47	4.42	0.41	0.42	0.52	5.46	0.51	0.64	1.24	26.57	0.78	0.66	0.63	13.00	16.67

$S^{(1-6)}$: Nassar and Huehn's and Huehn's stability statistics, $NP^{(1-4)}$: Thennarasu's stability statistics, W_i^2: Wricke's ecovalence; σ^2_i: Shukla's stability variance, b_i: regression coefficient, $S^2_{d_i}$: deviation from regression, *CV*: coefficient of variance, $\theta_{(i)}$: GE variance component, θ_i: mean variance component, *ASV*: AMMI's stability values, *KR:* Kang's sum of ranks, *TOP*: Fox's TOP-rank.

Peterson, 1959) revealed a different pattern of yield stability for genotypes compared to the above mentioned statistics. Based on θ_i, G13, G14 and G18 showed high grain yield stability than other genotypes, whereas genotypes G1 (Jolgeh), G8 and G19 (Bahman) were identified with low grain yield stability (Table 3).

Non-parametric stability statistics

Several non-parametric stability statistics including Nassar and Huehn's ($S^{(1-6)}$), Thennarsu's ($NP^{(1-4)}$), Fox's-Top rank (*TOP*) and Kang's rank-sum (*KR*) were used for further evaluation the grain yield stability of barley genotypes (Table 3). These statistics have been widely used in previous studies to identify genotypes with grain yield stability in different crops (Adugna *et al.,* 2003; Ahmadi *et al.,* 2015; Abdipour *et al.,* 2017; Vaezi *et al.,* 2018). Based on $S^{(1)}$ and $S^{(2)}$ statistics, G1 (Jolgeh), G5, G8 and G19 (Bahman) with the lowest values were identified with high grain yield stability. Two statistics $S^{(3)}$ and $S^{(6)}$ indicated almost a similar pattern for identification of yield stability in genotypes. Therefore, using these statistics G1 (Jolgeh), G8, G15 and G19 (Bahman) had high grain yield stability. The only observed difference between these two was G15 in the latter instead of G5 in the former.

Similar to the other non-parametric statistics using $NP^{(1)}$ and $NP^{(2)}$ could identify G1 (Jolgeh), G8 and G19 (Bahman), with the lowest values, with high grain yield stability. $NP^{(3)}$ and $NP^{(4)}$ recognized G1 (Jolgeh), G8, G15 and G19 (Bahman) genotypes with high grain yield stability. Using the *KR* statistic, genotypes G8 followed by G19 (Bahman), G18 and G20 had the lowest values, and therefore were identified to have high grain yield stability. However, using *TOP* statistics G1 (Jolgeh), G6, G7 and G15 were identified with high grain yield stability genotypes (Table 3).

Relationship among stability statistics

The results of Pearson's correlation coefficients are presented in Fig. 1. The relationship between mean grain yield and $\theta_{(i)}$, b and *TOP* was significant (Fig. 1). The relationship between *ASV* and $S_i^{(1)}$, $S_i^{(2)}$, $NP^{(1)}$, S_{di}^2, W_i^2, σ^2, $\theta_{(i)}$ and θ_i were positive and significant. The $\theta_{(i)}$ was only positively correlated with b_i. Furthermore, relationship among $S^{(i)}$ and $NP^{(i)}$ statistics were positive and significant. The *bi* statistics positively and significantly correlated with *CV*, θi and *KR*. These results indicate that these correlated statistics can be interchangeably used as useful parameters for selecting genotypes with grain yield stability. However, all parametric and non-parametric statistics except b_i, θ_i and *TOP* were negatively and significantly correlated with mean grain yield (Fig. 1). All parameters, regardless of their relationships with grain yield, were used in this study for identifying barley genotypes with high grain yiled stability.

Stability statistics can be categorized into two concepts (Becker and Leon, 1988): (1) static stability which is comparable to the biological concept of homeostasis, and refers to a genotype that tends to maintain constant yields across environments; and (2) dynamic stability which indicates the mean yield of a genotype in each environment is always similar to the mean grain yield of all tested genotypes.

To better understand the associations among stability statistics and to classify them into distinct groups, a principal component analysis (PCA) was performed. The first four principal components (PC1–4) accounted for 94% of the total variation among estimated statistics. The first two PCs indicated the highest values of eigenvalues (12.45 and 2.93, respectively) and variability (65.53% and 15.44%, respectively). A PCA-based biplot of different statistics (Fig. 2) was performed to evaluate the relationship between different parameters. Using this biplot, mean grain yield along with 18 parametric and

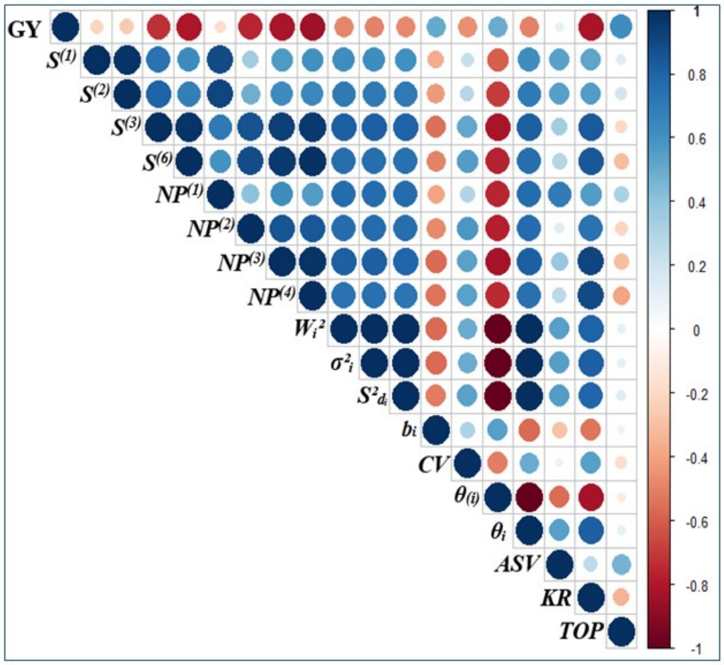

Fig. 1. Correlation heatmap between grain yield and different parametric and non-parametric stability statistics. GY: grain yield, $S^{(1-6)}$: Nassar and Huehn's and Huehn's stability statistics, $NP^{(1-4)}$: Thennarasu's stability statistics, W_i^2: Wricke's ecovalence, σ^2_i: Shukla's stability variance, b_i: regression coefficient, S^2_{di}: deviation from regression, *CV*: coefficient of variance, $\theta_{(i)}$: GE variance component, θ_i: mean variance component, *ASV*: AMMI's stability values, *KR*: Kang's sum of ranks, *TOP:* Fox's TOP-rank.

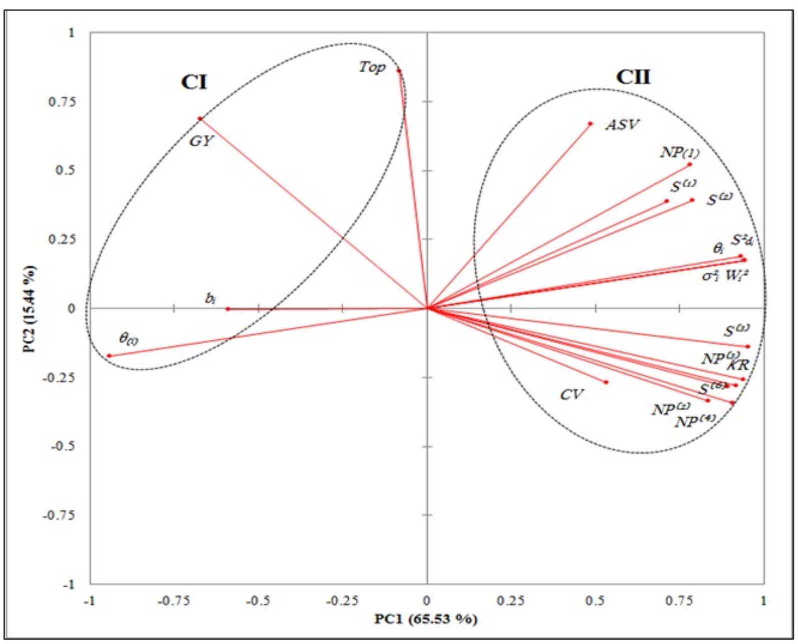

Fig. 2. PCA-based biplot for interpreting the associations between grain yield and different stability statistics in 20 barley genotypes. GY: grain yield, $S^{(1-6)}$: Nassar and Huehn's and Huehn's stability statistics, $NP^{(1-4)}$: Thennarasu's stability statistics, W_i^2: Wricke's ecovalence, σ^2_i: Shukla's stability variance, b_i: regression coefficient, S^2_{di}: deviation from regression, *CV*: coefficient of variance, $\theta_{(i)}$: GE variance component, θ_i: mean variance component, *ASV*: AMMI's stability values, *KR*: Kang's sum of ranks, *TOP:* Fox's TOP-rank.

non-parametric statistics grouped into two main clusters: (1) CI, which comprises mean grain yield, θ_i, *bi* and *TOP*; and (2) CII, which consists of all S^1, S^2, S^3, S^6, NP^1, NP^2, NP^3, NP^4,*CV*, *ASV*, W_i^2, σ^2, $\theta_{(i)}$, S_{di}^2 and *KR*.

Considering the stability concepts, CI can be referred to dynamic stability concept and it might be used to recommend genotypes adapted to favorite environments under high-input conditions. Our results are in agreement with findings of Khalili and Pour-Aboughadareh (2016) who also stated that *bi* and *TOP* statistics had a dynamic stability concept when evaluating stability parameters in a barley MTEs analysis. Furthermore, several researchers have shown positive correlation between these statistics and grain yield (Mut *et al.,* 2009; Ahmadi *et al.,* 2015; Abdipour *et al.,* 2017; Vaezi *et al.,* 2019). On the other hand, CII implied the static concept of stability; hence, these group of stability statistics can be identically used to classify genotypes with grain stability in a similar approach. Lin *et al.* (1986) and Kang *et al.* (1987) also found positive correlation among several parametric statistics such as CV, W_i^2, σ^2 and $\theta_{(i)}$ and grouped them based on the static stability concept.

Since the selection of high-yielding with yield stability genotypes based on a single stability statistic is confusing, an average sum of ranks (ASR) for all parametric and non-parametric statistics was used to select genotypes with minimum ASR values (Table 4). The results revealed that, in general, the seven parametric statistics (CV, W_i^2, σ^2, S_{di}^2, $\theta_{(i)}$, θi and *ASV*) identified genotypes G1 (Jolge), G5, G8, G17 and G19 (Bahman) as genotypes with high grain yield stability. Average sum of ranks for ten non-parametric statistics ($S^{(1–6)}$, $NP^{(1–4)}$, *KR* and *TOP*) indicated that G1 (Jolge) followed by G19 (Bahman), G8, G15 and G5 had high grain yield stability. By compiling results of both ASR values of parametric and non-parametric statistics, three genotypes G1 (Jolge), G8 and G19 (Bahman) showed the highest grain yield and yield stability across different environments.

Generally, analysis of GEI for grain yield in barley METs data resulted in a successful evaluation of barley genotypes with high grain yield and yield stability that could be used in future studies. Excluding two commercial checks (cv. Jolgeh and cv. Bahman), the promising line G8 with the pedigree of "Makouee/Jolgeh" represented an acceptable grain yield and yield stability pattern across different environments. It can be noticed that pedigree of Jolgeh is "Makoee//Zarjow/80-5151" in which cultivar 'Makouee' contributed as the female parent. 'Makouee' is the first improved barley cultivar introduced for the cold regions of Iran, and released in 1990 (Yousefi and Ghazvini, 2002). Due to its great adaptability, 'Makouee' has been widely grown in cold regions during the last three decades and despite the release of new cultivars such as 'Bahman', 'Jolgeh' and 'Mahtab', this cultivar is still favored by some farmers. Line G8 with a high proportion of genetic background inherited from 'Makouee' is expected to perform successfully in target regions and adopted by farmers as its progenitor.

Plant breeders always encounter GEIs, when genotypes are tested across different locations with diverse environments. Hence, METs are useful for the selection of desirable genotypes with ideal performance and high yield stability. In the present study, both parametric and non-parametric statistics presented similar results for identification of genotypes G1 (Jolge), G8 and G19 (Bahman) as the suitable genotypes for being grown in cold regions of Iran. These results indicate that commercial barley cultivar such as 'Bahman' and 'Jolgeh' have still retained their good yield level and adaptation in the cold regions. Line G8 in this study as well

Table 4. Ranking of 20 barley genotypes based on grain yield and different stability statistics

Genotype code	Grain yield (t ha^{-1})	Grain yield's rank	[a]PSR	[b]NSR	[c]TSR	[d]ASR
G1	6.300	7	20	37	64	3.76
G2	6.110	13	110	69	192	11.29
G3	6.040	15	126	61	202	11.88
G4	6.100	14	108	77	199	11.71
G5	6.140	12	58	49	119	7.00
G6	6.280	10	92	71	173	10.18
G7	6.460	3	108	82	193	11.35
G8	6.470	2	34	39	75	4.41
G9	5.560	18	136	103	257	15.12
G10	6.390	4	148	94	246	14.47
G11	5.920	16	154	77	247	14.53
G12	6.290	8	93	50	151	8.88
G13	5.860	17	168	106	291	17.12
G14	5.410	19	163	107	289	17.00
G15	7.070	1	50	94	145	8.53
G16	6.330	6	137	91	234	13.76
G17	6.280	9	71	48	128	7.53
G18	5.230	20	183	111	314	18.47
G19	6.190	11	32	30	73	4.29
G20	6.330	5	65	74	144	8.47

[a]Sum of ranks for the group of parametric statistics (W_i^2, σ^2_i, S^2_d, CV, $\theta_{(i)}$, θ_i and ASV).
[b]Sum of ranks for the group of non-parametric statistics ($S^{(1)}$, $S^{(2)}$, $S^{(3)}$, $S^{(6)}$, $NP^{(1)}$, $NP^{(2)}$, $NP^{(3)}$, $NP^{(4)}$, *KR* and *TOP*).
[c]Sum of ranks for grain yield and all parametric and non-parametric statistics.
[d]Average of sum of ranks for grain yield and all parametric and non-parametric statistics.

as some other barley promising lines identified through previous METs can herald the hope that desirable barley cultivars with acceptable grain yield and yield stability are in pipeline and will be released in the near future.

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