Parametric and Non-parametric Measures for Evaluating Yield Stability and Adaptability in Barley Doubled Haploid Lines

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ABSTRACT

Multi-environment trials have a significant role in selecting the best cultivars to be used at different locations. The objectives of the present study were to evaluate GE interactions for grain yield in barley doubled haploid lines, to determine their stability and general adaptability and to compare different parametric and nonparametric stability and adaptability measures. For these purposes, 40 doubled haploid lines as well as two parental cultivars (Morex and Steptoe) were evaluated across eight variable environments (combinations of location-years-water regime) during the 2012-2013 and 2013-2014 growing seasons in Iran. The Additive Main effect and Multiplicative Interaction (AMMI) analysis revealed that environments, genotypes, and GE interaction as well as the first four Interaction Principal Component Axes (IPCA1 to 4) were significant, indicating differential responses of the lines to the environments and the need for stability and general adaptability analysis. The stability parameters $S_i^{(3)}$, $S_i^{(6)}$, NP2, NP3, NP4 as well as Fox-rank (Top) were positively and significantly correlated with mean yield, suggesting these statistics can be used interchangeably as suitable parameters for selecting stable lines. The results of Principal Components Analysis (PCA) showed that the first two PCAs explained 92% of total variation for ranks of mean grain yield and parameters, and also clustered stability parameters on the basis of static and dynamic concepts of stability. In general, the parametric and non-parametric stability measures revealed that among tested doubled haploid lines at different environments, the line DH-30 followed by DH-29 and DH-3 were identified as lines with high grain yields as well as the most stable for variable environments of semi-arid regions of Iran.

Keywords: Dynamic and static stability, GE interaction, Principal Components Analysis (PCA).

INTRODUCTION

One of the great challenges facing economies and societies over the next decades is feeding the population, and providing water resources to produce food for a world that experiences a rapid population growth in the time of global climate change (Dorostkar *et al.,* 2015). Barley (*Hordeum vulgare* L.) is a major crop ranked fourth in the worldwide production of cereals. This crop is considered as a primary staple food in the semi-arid tropics of Asia, Africa, and South America. The grains of barley are usually used as food and animal fodder, and moreover it has also been applied as raw material for the production of beer (Pour-Aboughadareh *et al.,* 2013).

The development of cultivars, which can be adapted to a wide range of diversified environments (widely adapted), is the final objective of plant breeders in a crop improvement program. Cultivars showing

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wide adaptation have to be stable for yield in dynamic sense across a range of environments [to exhibit small variation of Genotype by Environment (GE) interaction effects] and also their mean performance (yield potential) has to be relatively high. Then, the major goal of plant breeding programs is to improve wide adaptation of cultivars through increasing both their yield potential and stability (Segherloo *et al.,* 2008). Several statistical measures (parameters) have been proposed for stability analysis of yield (or other plant productivity traits), with the aim of describing the information contained in the GE interaction effects. These measures are parametric to non-parametric in statistical sense. Although most of the offered measures are suitable for describing stability of cultivars in dynamic sense (for stability analysis of cultivars), only those measures describing wide adaptation (suitable for general adaptability of cultivars) are useful to identify cultivars exhibiting high degree of wide adaptation. Cultivars identified to be stable in dynamic sense can have wide adaptation if they show simultaneously high yield potential (high mean yield across environments), or they do not show this agronomic attribute if they do not have high yield potential. Then, in order to identify wide adapted cultivars, a breeder or researcher should use jointly cultivar stability measures and cultivar means of yield across environments, or general adaptability measures which integrate information both on variation of GE interaction effects regarding a given cultivar and its mean yield.

Shukla (1972) developed a method of cultivar stability in dynamic sense which partitions the GE sum of squares into components attributable to individual genotypes. Wricke (1962) defined the concept of ecovalence as the contribution of each genotype to the GE sum of squares. Francis and Kannenberg (1978) used the coefficient of variation and the genotypic variances across environments for each genotype as a static stability parameter.

Additionally, regression coefficient (b_i) was proposed by Finlay and Wilkinson (1963) as a stability parameter in dynamic sense, and according to this method the cultivars with $b= 1$ and small deviations from regression are stable. All mentioned methods are parametric approaches. In contrast, the non-parametric methods rank genotypes according to their similarity of response to a range of environments (Lin *et al.,* 1986). Additionally, these methods do not require any assumptions about the normality and independence of observation as well as homogeneity of error variances. To define and interpret the responses of genotypes to environmental variation, biometricians have expanded several non-parametric statistics. Huehn (1979) and Nassar and Huehn (1987) suggested four non-parametric statistics, namely, $Si^{(1)}$, $Si^{(2)}$, $Si^{(3)}$ and $Si^{(6)}$ based on the ranking of the genotypes in each environment, and described stable genotypes as those whose position in relation to the others remained unaltered in the set of environments assessed. Kang (1988) proposed a general adaptability measure integrating cultivar mean and Shukla's stability variance (Shukla, 1972) for selecting high yielding and stable cultivars, i.e. those showing wide adaptation. Fox *et al.* (1990) using the ranking of the cultivars suggested another non-parametric measure for general adaptability. In this measure, also, integration of cultivar stability of yield performance with mean yield is used for selecting high-yielding, stable genotypes. Thennarasu (1995) developed four Non-Parametric stability statistics (NP1, NP2, NP3 and NP4) based on ranks of adjusted means of the genotypes in each environment, and described stable genotypes as those whose position in relation to the others remained unaltered in the set of environments assessed. Therefore, the objectives of the present study were to: (i) Evaluate GE interactions for grain yield in barley doubled haploid lines across different environments for semi-arid regions of Iran; (ii) Determine their stability in dynamic sense and general adaptability, and (iii)

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Compare different parametric and nonparametric stability and adaptability measures.

MATERIALS AND METHODS

Plant Materials, Design, and Experimental Sites

Data for this study was obtained from sets of barley yield trials conducted for two consecutive years (2012-2013 and 2013-2014) under two water regimes at two different research stations in northwest of Iran. In each environment (combination of year×location×water regime), 40 doubled haploid lines as well as parental cultivars were tested. These doubled haploid lines were developed in barley breeding program at Oregon University (North American Barley Genome Mapping Project) (Kleinhofs *et al.,* 1993). Field experiments were conducted at two research stations, Miandoab Agricultural Research Station

(36.58° N latitude, 46.09° E longitude, AT at altitude 1,314 m above sea level) and Research Station of Mahabad Payame-Noor University (36.01° N latitude, 46.43° E longitude, altitude 1,371 m above sea level), in West Azerbaijan Province (northwest of Iran). Based on De-Martonne index climatic classification (1925), these experiment sites are classified as semi- arid regions of Iran (detailed description of these test research stations is shown in Table 1). In each of the environments, 40 doubled haploid lines as well as parental cultivars were arranged in a 7×6 rectangular lattice design with two replications and grown under two separate water regimes. Sowing was done by hand in November in all experiments. The experimental plots consisted of four rows of 2.5 m length. The plant materials were grown under two moisture regimes of irrigation i.e. after 90 and 190 mm evaporation from a Class-A pan for normal and drought-stress conditions, respectively. The drought-stress treatments were applied from the booting stage till physiological maturity. Crop management practices such

Table 1. Agro-climatic characteristics of environments and mean yield of barley lines tested in 8 environments.

Station	Environment	Rainfall (mm)[a]	Soil texture	Grain yield (kg h^{-1})		
				Mean	Max	Min
Mahabad 2012-13	E1	326.20	silt-clay-loam[b]	5218.57	6340	3740
Mahabad 2013-14	E2	397.60	silt-clay-loam	5848.45	7660	4400
Miandoab 2012-13	E3	243.50	silt-clay-loam	5318.21	7160	3900
Miandoab 2013-14	E4	283.00	silt-clay-loam	5639.88	7410	4250
Mahabad 2012-13	E5	326.20	silt-clay-loam	4009.52	4980	2935
Mahabad 2013-14	E6	397.60	silt-clay-loam	4558.57	5590	3255
Miandoab 2012-13	E7	243.50	silt-clay-loam	4003.21	5170	3000
Miandoab 2013-14	E8	283.00	silt-clay-loam	4367.74	5535	3055

[a] Total seasonal rainfall

[b] Soil texture at Mahabad station is composed of 30% clay, 54% silt and 16% sand, and at Miandoab station is composed of 30% clay, 52% silt and 18% sand. E1, E2, E3 and E4 indicate non-stressed environments. E5, E6, E7 and E8 indicate drought stressed environments.

as pest and weed control were practiced as needed during the growing season. At harvest time, grain yield was determined for each line at each test environments.

Statistical Analysis

A combined AMMI analysis was performed to determine the effects of Genotype (G), Environment (E), and GE interaction effects using IRRISTAT version 5 software (IRRISTAT, 2005). Several parametric and nonparametric stability statistics including the regression coefficient (b_i) and deviation from regression (S_{di}^2), Wricks's ecovalance (W_i^2), Shukla's stability variance (σ_i^2), Francis and Kannenberg's Coefficient of Variability (CV_i), and AMMI Stability Value (ASV_i), Nassar and Huehn's ($S^{(i)}$), Kang's rank-sum, Fox-rank, and Thennarasu (NP_i) were calculated using the formulas suggested by Eberhart and Russell (1966), Wricke (1962), Shukla (1972), Francis and Kannenberg (1978), Purchase *et al*. (2000), Nassar and Huehn (1987), Huehn (1990), Kang (1988), Fox *et al*. (1990), and Thennarasu (1995), respectively. All statistical approaches of stability parameters were performed by C# code. Spearman's rank correlation was calculated to measure the relationships among the statistics using SAS software (SAS, 1987). To better understand the relationships among the parametric and non-parametric statistics, a Principal Component Analysis (PCA) based on ranks of stability parameters were performed by STATISTICA software (STATISTICA, 2007). For clustering of lines, a hierarchical cluster analysis based on mean yield and stability measures was performed. The Euclidean distance was used as a dissimilarity measure required in Ward's clustering method (Ward, 1963), and the discriminant analysis test was used to estimate the optimal number of clusters.

RESULTS

Combined AMMI Analysis of Variance and Partitioning of the GE Interactions

The AMMI analysis of variance on grain yield showed that main effects due to Environment (E), Genotype (G), and GE interaction were found to be highly significant. The environments explained 51% of the total variation, followed by G and GE interaction, which justified 34% and 13%, respectively. These results were predictable because the tested environments were very similar. The linear regression explained 41% of GE interaction variation, whereas the residual of the variation around regression slope clarified 58% of variation. Large contribution of GE interaction was due to a non-linear component that can be regarded as an important parameter for selection of stable genotypes. A segregation of the GE interaction into the first four IPCAs (IPCA1 to IPCA4) demonstrates that the GE sum of square was spread in decreasing order of magnitude of 46.91%, 35.83, 10.63, and 4.39%, respectively, of the GE sum of square (Table 2). Mean grain yield of eight environments is shown in Table 1. The mean yields of environments ranged from 4,003.21 kg ha^{-1} at Miandoab in 2012-2013 under drought-stressed environment to 5,848.45 kg ha^{-1} at Mahabad in 2013-14 under non-stressed environment. Also, the highest grain yield (7,660 kg ha^{-1}) was produced by doubled haploid number 35 (DH-35) at Mahabad in 2012-2013 under non-stressed environment and the lowest (2,935 kg ha^{-1}) was produced by doubled haploid number 28 (DH-28) at Mahabad 2012-2013 under stressed environment. Also, as shown in Figure 1, grain yield under non-stressed Environments (E1, E2, E3 and E4) was positively correlated with grain yield under drought stressed Environments (E5, E6, E7 and E8), however, a high potential yield under optimum condition does not necessarily

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Table 2. AMMI analysis of grain yield of barley lines grown at 8 environments.[a]

Source of variation	df	MS	%TTS	%GE
Line	41	2563146[**]	34.76	
Environment	7	22284571[**]	51.60	
GE interaction	287	143551.90[**]	13.62	
Regression	41	417561[**]		41.55
Deviation	246	97883.30[**]		58.45
IPC 1	47	411238.29[**]		46.91
IPC 2	45	328033.30[**]		35.83
IPC 3	43	101868.40[**]		10.63
IPC 4	41	44145.80[**]		4.39
GE residual	111	8281.93		2.23
Total	335	902331.30		

[a] TSS and GE indicate Total Sum of Squares and Genotype by Environment interaction, respectively. ** Significant at $P \leq 0.01$.
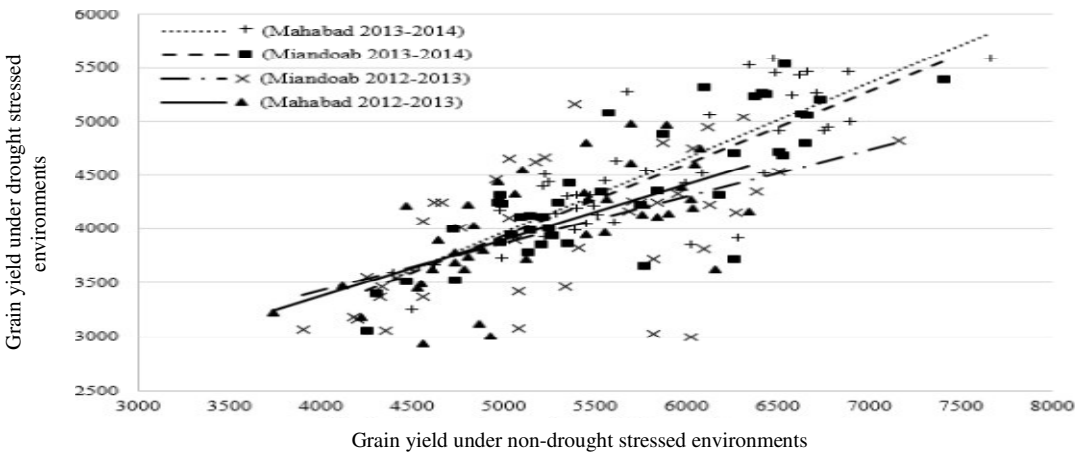

Figure 1. Association between mean grain yield (kg ha^{-1}) of non-stressed and drought stressed environments.

result in enhanced yield under stress conditions.

Parametric Measures of Stability

The doubled haploid lines showed significant differences in grain yield. Taking mean yield as a first parameter for assessing the lines, DH-35, DH-30, DH-3, DH-29 and DH-24 gave the highest grain yield; whereas, DH-9, DH-28, DH-34, DH-36 and DH-37 had the lowest yield performance across environments. Doubled haploid lines DH-11, DH-32, DH-33, DH-35 and Steptoe cultivar (parental cultivar) with regression coefficients (bi) higher than one had the highest mean yield and were adapted to favorable environments. In contrast, DH-5, DH-8, DH-16, DH-1 and Morex cultivar (parental cultivar) with $bi < 1$ and lowest average yields were poorly adapted across environments and might have specific adaptation to unfavorable conditions. Among the latter ones, lines DH-11, DH-30 and DH-31 were more suitable, because these lines had the best yield performance, bi close to 1 and low S_{di}^2 (Mohammadi and Amri, 2008). Wricke's (1962) ecovalance (W_i^2) and Shukla (1972) stability variance (σ_i^2) statistics revealed that lines DH-27, DH-28, DH-30, DH-31 and DH-40 had the

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lowest values and identified as stable lines. Although lines DH-1, DH-5, DH-8 and DH-16 along with Morex cultivar had low yield performance (except DH-8); based on Coefficient of Variation stability statistic (CV_i), these lines were considered to be desirable and stable lines. The *ASV* statistic, which uses two IPC scores to produce a balanced measurement between them, can be useful two the first IPCs counted considerable amount of genotype by environment interactions. According to this method, DH-3, DH-7, DH-27, DH-28 and DH-30 were found to be stable lines.

Non-Parametric Measures of Stability

Nassar and Huehn's (1987) and Thennarasu's (1995), Fox-rank (Fox *et al.,* 1990), and Kang's rank-sum (Kang, 1988) non-parametric statistics of stability for grain yield of 40 doubled haploid lines along with parental cultivars are presented in Table 3. According to the $S_i^{(1)}$ (varied from 2 to 18.1) and $S_i^{(2)}$ (varied from 3 to 234.8) (Nassar and Huehn, 1987), DH-12, DH-20, DH-28, DH-31 and DH-34 with the lowest value were identified as desirable. Also, based on $S_i^{(3)}$ (varied from 1.3 to 85.4), the lines DH-3, DH-12, DH-19, DH-30, DH-31 and DH-35 were recognized as stable lines. $S_i^{(6)}$ ranged from 0.4 to 7.5 and, according to this parameter, DH-3, DH-29, DH-30, DH-31 and DH-35 had the lowest value and DH-4, DH-5, DH-9 and DH-36 had relatively higher values of this statistic, indicating higher and lower stability, respectively. According to Thennarasu's (1995) stability statistics (NP1, NP2, NP3 and NP4), lines with minimum values are considered more stable. *NP1* ranged from 3.4 to 19.3, and the lines DH-27, DH-28, DH-29, DH-30 and DH-40 with lower values were identified more stable than the other lines. According to the values of *NP2* (ranged from 0.1 to 4.6) and *NP3* (ranged from 0.1 to 2.9), DH-3, DH-29, DH-30 and DH-31 and DH-39 had the lowest value compared to other lines. *NP4* varied from 0.1 to 1.2, and the lines

DH-3, DH-29, DH-30, DH-31 and DH-35 had the lowest values. Therefore, these lines were the most stable lines. The highest value of Fox-rank (Fox *et al.,* 1990) was shown by DH-3, DH-21, DH-24, DH-29, DH-30 and DH-35. These lines were adapted, because they ranked in the top third of lines in most of the environments (TOP= 87.50% and TOP= 100%, respectively). Kang's rank-sum (Kang, 1988) stability statistic (ranged from 11 to 80) also indicated that lines DH-3, DH-29, DH-30, DH-31 and DH-39 with lowest value were stable lines.

Interrelationship among Parametric and Non-parametric Methods

The results of Spearman's rank correlation coefficients between mean yield and the parametric and non-parametric stability statistics are shown in Table 4. The mean yield as well as Fox-rank (Top) (Fox *et al.,* 1990) positively and significantly correlated with $S_i^{(3)}$, $S_i^{(6)}$, *NP2*, *NP3* and *NP4*. Also, these statistics showed a significant negative relation with Kang's rank-sum (Kang, 1988) and regression coefficients (bi). Kang's rank-sum significantly and positively correlated with $S_i^{(1)}$, $S_i^{(1)}$ and regression coefficients and it had significantly negative correlation with other stability parameters, except *CVi* parameter. The stability statistics $S_i^{(1)}$ and $S_i^{(2)}$ positively and significantly correlated with each other and showed a negative correlation with other stability statistics. Also, $S_i^{(3)}$ and $S_i^{(6)}$ positively and significantly correlated with each other and with *NP2*, *NP3*, *NP4*, W_i^2 and σ_i^2. Wricke's ecovalance (W_i^2) and Shukla stability variance (σ_i^2) negatively associated with *NP1*, *NP2*, *NP3* and *NP4*. The Coefficient of Variation stability parameter (CVi) only correlated with regression coefficient (bi). Variance in regression deviation (S_{di}^2) had positive and significant correlation with non-

Table 3. Mean grain yield (kg ha⁻¹) and estimates of stability parameters for barley lines tested in 8 environments. [a]

Code	GY	W_i^2	σ_i^2	b_i	CV_i	Sd_i^2	ASV_i	$S^{(1)}$	$S^{(2)}$	$S^{(3)}$	$S^{(6)}$	$NP1$	$NP2$	$NP3$	$NP4$	R-sum	Top
DH-1	4528.8	778803.8	113225.7	0.6**	10.0	25802.3	14.2	8.8	53.1	26.1	3.4	10.8	0.8	0.8	0.6	46.0	0.0
DH-2	4891.3	1295980.6	190802.2	1.0	17.1	215031.6	17.9	11.1	86.6	28.3	3.0	14.1	0.6	0.7	0.5	52.0	25.0
DH-3	5648.8	289727.6	39864.3	1.0	13.7	48196.3	3.2	4.6	15.4	3.0	0.7	6.3	0.2	0.2	0.1	11.0	87.5
DH-4	4291.9	770696.4	112009.6	0.9	17.8	126957.2	14.2	7.2	41.7	25.7	4.1	9.9	0.7	1.0	0.6	54.0	0.0
DH-5	4397.5	1299677.4	191356.7	0.4**	7.8	18965.2	18.2	9.6	66.5	39.6	4.4	12.0	1.1	1.1	0.8	67.0	0.0
DH-6	5269.4	935260.1	136694.1	0.8	12.9	133056.5	12.4	7.1	40.0	9.2	1.3	11.9	0.4	0.4	0.2	37.0	50.0
DH-7	4427.5	296752.4	40918.0	0.9	16.0	46409.0	4.7	4.5	16.0	8.4	2.1	5.6	0.4	0.5	0.3	42.0	0.0
DH-8	4650.0	909491.0	132828.8	0.5**	9.0	22359.9	14.4	8.7	56.9	22.7	2.6	10.1	0.6	0.7	0.5	46.0	12.5
DH-9	4081.3	972941.3	142346.3	0.7	13.9	93825.5	15.5	6.4	35.6	34.4	5.3	9.6	2.4	1.6	0.9	66.0	0.0
DH-10	5415.6	1784449.1	264072.5	0.9	14.7	283354.5	21.7	10.0	71.3	15.5	1.6	13.1	0.4	0.5	0.3	49.0	75.0
DH-11	5456.9	788071.4	114615.8	1.4*	19.3	9089.8	15.1	7.1	36.3	7.8	1.3	12.4	0.4	0.4	0.2	28.0	75.0
DH-12	4254.4	214067.2	28515.2	0.9	15.0	22786.9	6.2	2.0	3.0	2.6	1.4	4.9	0.7	0.8	0.3	43.0	0.0
DH-13	5439.4	1397731.5	206064.8	0.6	10.4	140465.8	19.4	9.8	67.1	14.5	1.8	12.8	0.4	0.4	0.3	43.0	62.5
DH-14	5525.0	963388.7	140913.4	1.3	18.3	93033.6	13.7	8.6	50.9	10.8	1.4	11.5	0.3	0.4	0.3	31.0	75.0
DH-15	5122.5	1293986.9	190503.1	1.0	16.9	213633.9	16.7	7.3	43.7	10.9	1.4	10.6	0.4	0.4	0.3	48.0	37.5
DH-16	5073.8	1700066.9	251415.2	0.5**	10.1	138998.5	20.6	10.4	74.6	18.9	2.0	13.1	0.5	0.6	0.4	55.0	37.5
DH-17	4440.0	602294.6	86749.3	1.1	19.5	90110.4	7.2	7.9	43.7	24.7	3.2	8.1	0.7	0.8	0.6	47.0	0.0
DH-18	5423.8	672919.3	97343.0	1.3	17.6	69282.8	12.1	7.2	34.7	7.8	1.3	11.0	0.4	0.4	0.2	27.0	62.5
DH-19	4684.4	1144402.9	168065.6	0.6	11.4	98653.6	15.2	9.3	61.6	23.6	2.9	11.4	0.5	0.5	0.5	52.0	0.0
DH-20	4581.9	242117.8	32722.8	0.8*	12.9	15002.8	6.1	3.5	9.1	4.1	1.3	4.8	0.3	0.4	0.2	32.0	0.0
DH-21	5425.6	769668.1	111855.3	1.3*	18.4	58962.4	12.7	6.5	29.1	6.3	1.0	9.3	0.3	0.4	0.2	27.0	87.5
DH-22	5130.6	807011.0	117456.8	1.0	16.0	135038.7	14.3	8.4	54.1	14.1	1.7	10.6	0.4	0.4	0.3	37.0	50.0
DH-23	4805.6	1431001.5	211055.3	1.1	19.6	225584.7	19.8	10.6	77.7	27.2	2.6	11.3	0.6	0.7	0.5	57.0	12.5
DH-24	5535.6	967922.9	141593.5	1.4*	19.2	47373.1	15.4	7.5	42.0	8.7	1.2	11.5	0.4	0.4	0.2	31.0	87.5
DH-25	4556.9	540147.6	77427.2	1.1	18.0	87585.7	10.7	6.5	29.7	14.2	2.3	7.8	0.7	0.7	0.4	39.0	0.0
DH-26	4351.3	319815.1	44377.4	0.9	15.3	43473.9	9.0	5.1	17.6	11.5	2.5	6.5	0.8	0.8	0.5	45.0	0.0
DH-27	4465.6	210736.0	28015.5	1.0	15.3	29803.9	2.9	4.7	16.5	9.1	1.8	4.4	0.3	0.5	0.4	35.0	0.0
DH-28	3983.8	103375.3	11911.4	1.1	19.6	14448.2	1.3	2.4	4.0	6.2	2.7	3.6	0.8	1.1	0.5	43.0	0.0
DH-29	5640.0	353620.8	49448.2	0.9	12.1	50694.5	7.4	5.4	25.7	5.0	0.8	4.6	0.1	0.2	0.2	15.0	87.5
DH-30	5659.4	32424.4	1268.8	1.0	12.7	5321.2	1.1	3.1	6.8	1.3	0.4	2.4	0.1	0.1	0.1	3.0	100.0
DH-31	5278.8	201225.3	26588.9	1.2	16.5	11091.0	7.0	2.9	5.8	1.4	0.5	5.4	0.2	0.2	0.1	17.0	62.5
DH-32	4509.4	2295151.8	340677.9	1.7**	28.2	60902.6	25.7	13.2	129.1	65.7	6.0	16.5	1.4	1.2	1.0	69.0	0.0
DH-33	5196.3	1113239.0	163391.0	1.4*	20.8	62383.4	17.8	8.3	48.3	12.2	1.6	12.6	0.5	0.5	0.3	43.0	50.0
DH-34	3832.5	568567.8	81690.3	0.6**	12.4	13060.4	11.6	2.3	4.7	10.5	4.3	6.6	3.3	2.9	0.7	56.0	0.0
DH-35	6049.4	2183226.8	323889.1	1.6**	20.3	117374.7	23.6	3.4	9.9	1.7	0.5	13.3	0.3	0.4	0.1	40.0	100.0
DH-36	4007.5	2216844.0	328931.7	0.9	21.3	362645.0	23.7	9.7	73.3	65.1	7.5	16.1	4.6	2.2	1.2	80.0	0.0
DH-37	4092.5	961678.5	140656.9	0.9	18.7	156279.6	15.6	5.2	18.8	17.9	3.9	10.3	1.2	1.6	0.7	62.0	0.0
DH-38	4444.4	1879312.5	278302.0	0.8	17.7	294566.7	11.8	10.6	82.5	36.7	3.7	10.5	0.6	0.9	0.7	69.0	0.0
DH-39	5497.5	536177.4	76831.7	1.2	16.3	68751.9	8.9	5.9	25.1	5.4	1.0	8.6	0.3	0.3	0.2	19.0	75.0
DH-40	4509.4	200883.9	26537.7	1.1	17.9	29204.8	4.8	4.8	18.6	9.0	2.1	4.6	0.4	0.4	0.3	31.0	0.0
Morex	5114.4	1293582.7	190442.5	0.5	8.2	55795.9	18.5	8.6	57.4	14.2	2.0	14.3	0.5	0.5	0.3	48.0	50.0
Steptoe	4871.9	3930896.4	586039.6	1.9**	29.2	177865.3	32.8	18.1	234.8	85.4	5.7	19.3	1.0	1.0	0.9	63.0	37.5

[a] $LSD_{0.05}$= 1170.26, $LSD_{0.01}$= 1565.70. GY; W_i^2; σ_i^2; b_i; CV_i; Sd_i^2; ASV_i; $S^{(1)}$- $S^{(6)}$; $NP1$–$NP4$; R-sum, and Top indicate: Mean Grain Yield; Wricks's ecovalance; Shukla's stability variance; regression coefficient of Eberhart and Russell; Francis and Kannenberg's Coefficient of Variability; deviation from regression (Eberhart and Russell); AMMI Stability Value of Purchase *et al.*; Nassar and Huehn's non-parametric stability statistics; Thennarasu's Non-Parametric stability statistics; Kang's rank-sum, and Fox-rank, respectively.

Table 4. Spearman's rank correlation coefficients between mean yield and stability parameters in barley lines in semi-arid regions of Iran.[a]

	GY	$S^{(1)}$	$S^{(2)}$	$S^{(3)}$	$S^{(6)}$	NP1	NP2	NP3	NP4	R-sum	Top	W_i^2	σ_i^2	b_i	CV_i	Sd_i^2	ASV_i
GY (Nonparametric)																	
$S^{(1)}$	0.05																
$S^{(2)}$	0.03	0.99**															
$S^{(3)}$	0.46**	-0.81**	-0.83**														
$S^{(6)}$	0.80**	-0.45**	-0.47**	0.86**													
NP1	-0.19	-0.78**	-0.79**	0.57**	0.27												
NP2	0.80**	-0.36*	-0.38*	0.76**	0.92**	0.30											
NP3	0.85**	-0.30	-0.32*	0.74**	0.93**	0.23	0.96**										
NP4	0.80**	-0.47**	-0.48**	0.86**	0.99**	0.25	0.92**	0.93**									
R-sum	-0.65**	0.56**	0.60**	-0.86**	-0.87**	-0.49**	-0.87**	-0.89**	-0.88**								
Top	0.94**	-0.01	-0.03	0.49**	0.78**	-0.19	0.73**	0.78**	0.77**	-0.61**							
W_i^2 (Parametric)	-0.05	-0.81**	-0.84**	0.70**	0.40**	0.91**	0.38*	0.37*	0.41**	-0.68**	-0.06						
σ_i^2	-0.05	-0.82**	-0.83**	0.72**	0.41**	0.90**	0.38*	0.37*	0.40**	-0.68**	-0.06	1.00**					
b_i	-0.37*	0.07	0.09	-0.24	-0.29	0.07	-0.29	-0.34*	-0.27	0.34*	-0.37*	-0.02	-0.02				
CV_i	0.03	-0.08	-0.07	0.08	0.11	0.19	0.11	0.10	0.13	-0.08	-0.01	0.19	0.19	0.84**			
Sd_i^2	-0.03	-0.63**	-0.65**	0.55**	0.28	0.57**	0.23	0.23	0.31*	-0.49**	-0.02	0.72**	0.71**	0.03	0.28		
ASV_i	-0.07	-0.74**	-0.76**	0.64**	0.37*	0.92**	0.40**	0.35*	0.36*	-0.62**	-0.10	0.94**	0.93**	0.01	0.18	0.61*	

[a] GY; W_i^2; σ_i^2; b_i; CV_i; Sd_i^2; ASV_i; $S^{(1)}$– $S^{(6)}$; NP1–NP4; R-sum, and Top indicate: Mean Grain Yield;Wricks's ecovalance; Shukla's stability variance; regression coefficient of Eberhart and Russell; Francis and Kannenberg's Coefficient of Variability; deviation from regression (Eberhart and Russell); AMMI Stability Value of Purchase *et al.*; Nassar and Huehn's non-parametric stability statistics; Thennarasu's Non-Parametric stability statistics; Kang's rank-sum, and Fox-rank, respectively. * and ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

parametric statistics of $S_i^{(3)}$, *NP1*, *NP4*, W_i^2, σ_i^2 and ASV. Correlation between *ASV* with non-parametric statistics $S_i^{(3)}$, $S_i^{(6)}$, *NP1*, *NP2*, *NP2* and *NP4*, as well as W_i^2, σ_i^2 and deviation from regression (S_{di}^2) were positive and significant.

Studies of Relationships among Stability Parameters and Grouping Lines

In order to obtain information on the relationships, differences, and similarities among the parametric and non-parametric statistics, Principal Component Analysis (PCA) based on the rank correlation matrix was performed. The first two PCAs explained 66.58 and 26.09% of total variation for ranks of mean grain yield and stability parameters, respectively. The PC1 versus PC2 were used to produce the biplot illustrated in Figure 2. According to the biplot, mean yield and Fox-rank (Fox *et al.*, 1990) were placed in group I. The grouping of the stability parameter (Fox-rank) related to the concept of dynamic stability and relate

to genotypic mean yield. Group II was intermediate between group I and III, and it consisted of $S_i^{(3)}$, $S_i^{(6)}$, *NP2*, *NP3* and *NP4* statistics. The parameters in this group significantly associated with group I and II. The statistics of *NP1*, W_i^2, σ_i^2, *ASV* and S_{di}^2 were classified in group III, and this group provided a measure of stability in the static concept and did not relate to genotypic mean yield. Also, group IV was intermediate between static and dynamic concept and it included *CVi* and *bi* parameters. The remaining stability parameters such as $S_i^{(2)}$, $S_i^{(2)}$ and Kang's rank-sum (Kang, 1988) were put in group V, so that these statistics had a static concept of stability. To group the lines tested in terms of high yielding and stability, cluster analysis was performed and the resultant dendrogram is shown in Figure 3. Group I comprised two sub-groups so that the firs sub-group included the low yielding lines DH-4, DH-7, DH-12, DH-17, DH-20, DH-24, DH-25, DH-26, DH-27, DH-28 and DH-40. However, these lines were identified suitable by Thennarasu's (1995) *NP1* statistic, Wricke's ecovalance (W_i^2), Shukla
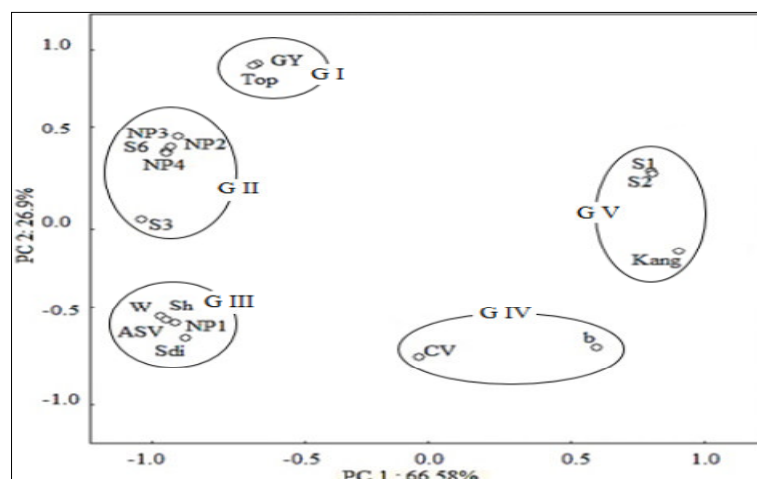

Figure 2. Biplot of PCA1 versus PCA2 for different parametric and non-parametric measures of stability. GY; W_i^2; σ_i^2; *bi*; *CVi*; Sd_i^2; *ASVi*; $S^{(1)}$- $S^{(6)}$; *NP1–NP4*; *R*-sum, and Top indicate: Mean Grain Yield; Wricks's ecovalance; Shukla's stability variance; regression coefficient of Eberhart and Russell; Francis and Kannenberg's Coefficient of Variability; deviation from regression (Eberhart and Russell); AMMI Stability Value of Purchase *et al.;* Nassar and Huehn's non-parametric stability statistics; Thennarasu's Non-Parametric stability statistics; Kang's rank-sum, and Fox-rank, respectively.
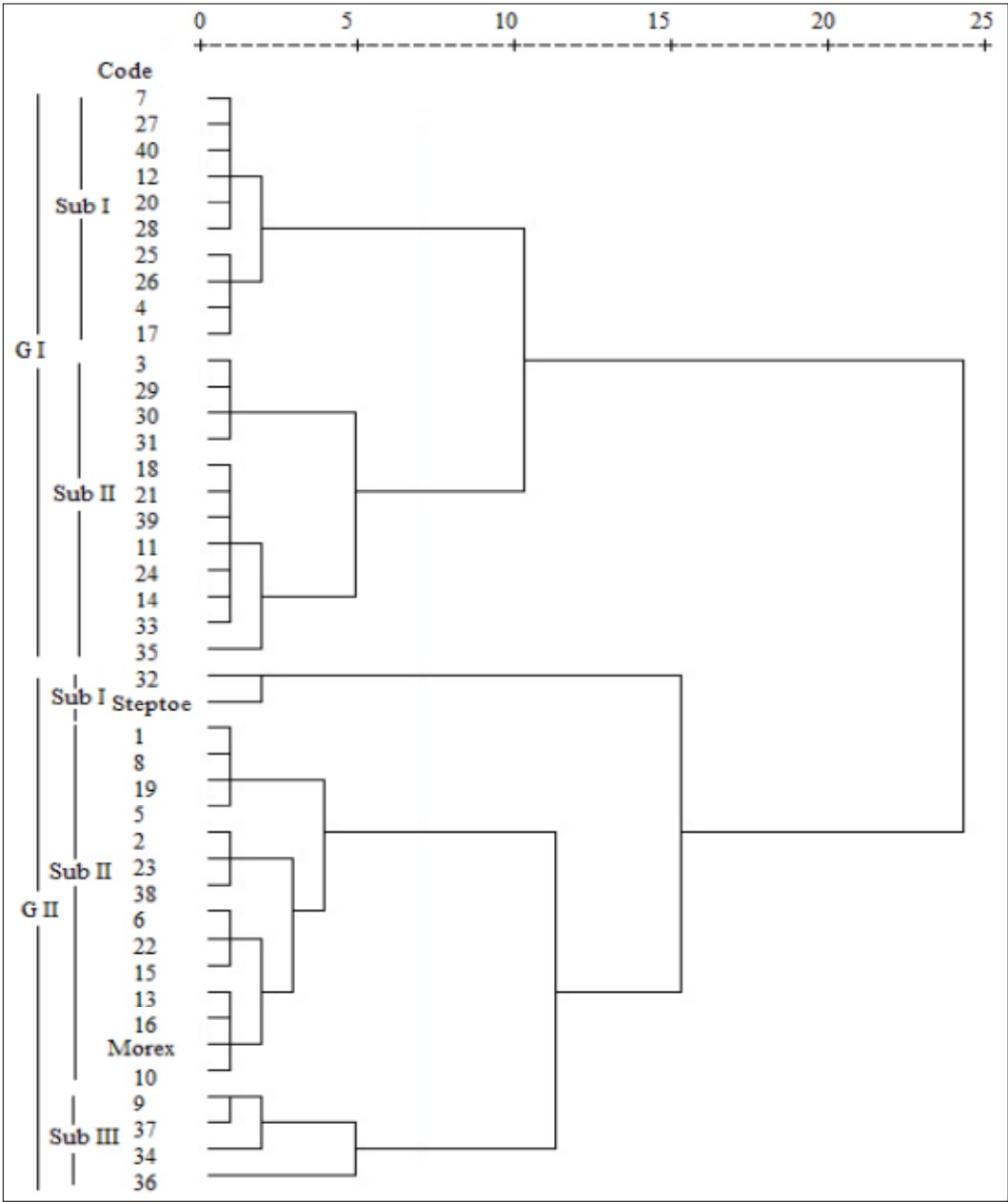
Figure 3. Dendrogram generated for 40 barley doubled haploid lines along with parental cultivars based on mean yield and parametric and non-parametric measures of stability.

stability variance (σ_i^2) and *ASV* stability parameters. In contrast, sub-group II included the high yielding lines DH-3, DH-11, DH-14, DH-18, DH-21, DH-24, DH-29, DH-30, DH-31, DH-33, DH-35 and DH-39. Among them, DH-3, DH-29, DH-30, DH-35 and DH-39 were identified as remarkable

and stable lines by $S_i^{(3)}$, $S_i^{(6)}$, *NP2*, *NP3* and *NP4*, Fox-rank and Kang's rank-sum as well as parametric statistics such as W_i^2 and σ_i^2. Main group II with three sub-groups consisted of lines that had moderate yields, among which lines DH-5, DH-8 and DH-34

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were classified as stable lines by CVi, S_{di}^2 and b_i.

DISCUSSION

Several statistical methods have been proposed to consider GE interaction. These methods ranged from univariate non-parametric/parametric to multivariate models. Among these methods, the Additive Main effect and the Multiplicative Interaction (AMMI) (Zobel *et al.,* 1988) analysis are the most well-known and appealing methods for analyzing GE interaction data. Differences in genotype stability in the environments can be qualitatively assessed using the biplot graphical representation that scatters the genotypes according to their Principal Component (PCA) scores. In general, a significant GE interaction effect often prevents researcher's ability to select high yielding and stable genotypes in breeding programs (Kang and Pham, 1991). In this study, AMMI analysis of variance showed that the main effects due to Environment (E), Genotype (G) and GE interaction were highly significant (Table 2). These results indicated that lines' performance changed across variable environments (Sio-Se Mardeh *et al.,* 2006). Furthermore, the positive correlation between grain yield under non-stressed (E1, E2, E3 and E4) and stressed (E5, E6, E7 and E8) environments suggests that indirect selection for a drought-prone environment based on the results of optimum condition will be efficient (Mohammadi *et al.,* 2011). The AMMI analysis shows to be able to extract a large portion of the GE interaction and is more efficient in analyzing GE interaction pattern in different crops such as lentil (Dehghani *et al.,* 2008), grass pea (Ahmadi *et al.,* 2012b), Wheat (Tesemma *et al.,* 1998; Mohammadi and Amri, 2008; Ahmadi *et al.,* 2012a) and safflower (Jamshidmoghaddam and Pourdad, 2013). In this study, we found that the two nonparametric statistics of Nassar and Huehn (1987) ($S_i^{(3)}$ and $S_i^{(6)}$) and

the three statistics of Thennarasu (1995) (NP2, NP3 and NP4) clustered together as the same class statistics. These recognized lines as stable or unstable in a similar fashion. The stability parameters $S_i^{(3)}$, $S_i^{(6)}$, *NP2*, *NP3*, *NP4* as well as Fox-rank were positively and significantly correlated, indicating that these statistics can be used interchangeably as parameters for selecting stable lines. In line with our results, Mohammadi *et al.* (2007) reported high correlations between $S_i^{(3)}$, $S_i^{(6)}$, *NP2*, *NP3* and *NP4* in durum wheat. The Kang's rank-sum and regression coefficient (b_i), Wricke's ecovalance (W_i^2) and Shukla stability variance (σ_i^2), deviation from regression (S_{di}^2) and AMMI Stability Value (ASV_i) statistics were negatively correlated with mean yield and, thus, are not recommended for use in line selection (Table 4). Also, the highly positive significant correlation between Fox-rank and mean yield indicated that this parameter was the best method to identify high yielding lines. Similarly, Segherloo *et al.* (2008) found a highly significant correlation between mean yield and Fox-rank.

The relationships among the different stability statistics are graphically exhibited in a biplot of PCA1 and PCA2 (Figure 2) allowing five groups to be distinguished: Group I included the mean yield and Fox-rank statistics. Accordingly, selection based on these two parameters is favored, and is related to the dynamic concept of stability and relate to genotypic mean yield, respectively. Group II included two nonparametric statistics of Nassar and Huehn ($S_i^{(3)}$ and $S_i^{(6)}$) and the three statistics of Thennarasu (NP2, NP3 and NP4). These parameters were significantly correlated with mean yield. The statistics *NP1*, W_i^2, σ_i^2, *ASV* and S_{di}^2 were classified in-group III, which provided a measure of stability in the static concept and did not relate to genotypic mean yield. Also, group IV was intermediate between static and dynamic

concept and it included *CVi* and *bi* parameters. Group V with statistic concept of stability included $S_i^{(1)}$, $S_i^{(2)}$ and Kang's rank-sum . The latter group was not significantly correlated with mean yield, thus, it seems that these methods allow the identification of genotype adapted to environments with unfavorable growing conditions. Additionally, Mohammadi and Amri (2008) found the static concept of stability for the *NP1*, W_i^2, σ_i^2, *ASV* and S_{di}^2 parameters in durum wheat MET. Nassar and Huehn (1987) also revealed that the $S_i^{(1)}$ and $S_i^{(2)}$ were correlated with the static concept of stability. Likewise, Becker and Leon (1988) indicated the static concept for the regression coefficient (bi) and Francis and Kannenberg's (1978) coefficient of variability.

The stability approaches used in our study, however, did not seem to provide an overall picture of the individual line responses to environment. Some lines showed stability using some parameters and instability for others. This is a problem that has been identified in GE interaction studies (Lin *et al.,* 1986). The multivariate approaches provide further information on the real multivariate response of genotypes to environments (Becker and Leon, 1988). One method of getting over this problem is to allocate genotypes into qualitatively homogeneous stability subsets through cluster analysis (Lin *et al.,* 1986). In the present study, cluster analysis separated 40 doubled haploid lines into two main groups, so that group I included the high yielding lines and among them DH-3, DH-24, DH-29, DH-30, DH-35 and DH-39 were identified as stable lines through many of the parametric and nonparametric statistics (Figure 3).

In general, both yield and stability of performance should be considered simultaneously to take advantage of the useful effect of GE interaction and to make a selection of the lines more precise and refined. Several stability parameters and approaches that have been employed in the

present study determined stability of barley doubled haploid lines with respect to yield, stability, and both of them. In conclusion, according to the present study, among the various stability parameters, statistics such as $Si^{(3)}$, $Si^{(6)}$, *NP2*, *NP3* and *NP4* can be used as the suitable parameters for screening desirable lines. Furthermore, our results revealed that, among the tested doubled haploid lines at different environments, the doubled haploid line DH-30 followed by DH-29 and DH-3 were the lines with high grain yield and highest stability for variable environments of semi-warm areas.

ACKNOWLEDGEMENTS

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تجزیه پارامتری و ناپارامتری پایداری برای ارزیابی پایداری عملکرد دانه و سازگاری در لاین‌های دابل هاپلوئید جو

م. خلیلی، و ع. پورابوقداره

چکیده

آزمایش‌های چند محیطی نقش مهمی را در انتخاب بهترین ارقام جهت استفاده در مناطق متفاوت نشان می‌دهند. هدف از این مطالعه ارزیابی اثر متقابل ژنوتیپ × محیط، تعیین پایداری و سازگاری عمومی عملکرد دانه و مقایسه معیارهای پایداری پارامتری و ناپارامتری بود. بدین منظور ۴۰ لاین دابل هاپلوئید جو به همراه دو ژنوتیپ والدی (Morex و Steptoe) در هشت شرایط محیطی متفاوت در طول سال-های زراعی ۱۳۹۳-۱۳۹۱ مورد ارزیابی قرار گرفتند. نتایج حاصل از تجزیه AMMI اثرات معنی‌داری را برای محیط، ژنوتیپ، اثر متقابل ژنوتیپ × محیط و همچنین چهار مؤلفه نخست نشان داد، که بیانگر پاسخ متفاوت لاین‌های دابل هاپلوئید به شرایط محیطی و ضرورت انجام تجزیه پایداری می‌باشد. معیارهای پایداری $Si^{(3)}$، $Si^{(6)}$، $NP2$، $NP3$ و $NP4$ و همچنین آماره پایداری رتبه Fox با عملکرد دانه همبستگی مثبت و معنی‌داری نشان دادند بنابراین، این معیارهای پایداری می‌توانند به عنوان مناسب-ترین پارامترها در شناسایی لاین‌های پایدار مورد استفاده قرار گیرند. تجزیه به مؤلفه‌های اصلی (PCA) نشان داد که دو مؤلفه نخست در مجموع ۹۲ درصد از تغییرات میانگین عملکرد دانه و معیارهای پایداری

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را توجیه نمودند و مطابق آن معیارهای پایداری بر اساس مفهوم "استاتیک" و "دینامیک" گروه‌بندی شدند. به طور کلی بر اساس نتایج حاصل از معیارهای پایداری پارامتری و ناپارامتری در بین لاین‌های مورد مطالعه، لاین دابل هاپلوئید شماره ۳۰ و به دنبال آن لاین‌های شماره ۲۹ و ۳ به عنوان مناسب‌ترین لاین‌های پایدار و با عملکرد بالا در شرایط محیطی متغیر در نواحی نیمه خشک ایران شناسایی شدند.