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Araştırma Makalesi/*Research Article (Original Paper)*

Estimating genotypic ranks by several nonparametric stability statistics in Barley (*Hordeum vulgare* L.)

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Abstract: Assessment of the yield stability of genotypes to various test environments is useful for recommending them for farmers and should be a requirement in plant breeding programs. Sixteen barley (*Hordeum vulgare* L.) genotypes were tested at four research locations for three years. The trials involved a randomized complete block design with four replications in which seven nonparametric stability statistics were used to analyze yield stability. The combined analysis of variance indicated the significance of the main effects of environments and genotypes as well as genotype by environment interaction. The overall mean grain yield for all the genotypes ranged from 3804.91 kg ha^{-1} for G1 to 3119.27 kg ha^{-1} for G13. The most stable genotypes based on the S1 and S2 nonparametric stability statistics, were G7, G10 and G11 while the most stable genotypes based on the S3, S4, S5 and S7 statistics, were G4, G5 and G10. Regarding mean yield, it could be grasped that genotype G10 (3560.91 kg ha^{-1}) was the most favorable genotype. In this study, none of the nonparametric stability statistics were positively associated with high mean yield, and instead characterized a static concept of stability. The results of factor analysis and correlation analysis of the nonparametric stability statistics and mean yield indicated that S1 and S2 would be useful for selecting for stability.

Keywords: Adaptability, Genotype by environment interaction, Multi-environment trials, Yield stability

Arpa'da Bazı Parametrik Olmayan Stabilite İstatistiği Kullanarak Genotipik Sıralamanın Tahmini

Özet: Farklı çevre koşullarında bir genotipin verim stabilitesine değerlendirilmesi, hem bu genotiplerin çiftçiler için tavsiyesinde yararlıdır hem de bitki ıslahı programlarında bir gereklilik olmalıdır. On-altı adet arpa (*Hordeum vulgare* L.) genotipi dört araştırma lokasyonunda üç yıl test edilmiştir. Denemeler, yedi adet parametrik olmayan stabilite istatistiğini, verim stabilitesini analiz etmek için dört tekrarlamalı tesadüf blokları deneme deseninde kurulmuştur. Birleşik varyans analizi çevre, genotip ve çevre-genotip interaksiyonunun temel etkilerininin önemini belirtmiştir. Tüm genotipler için genel ortalama tane verimi 3804,91 kg/ha (G1)'dan 3119,27 kg/ha (G13)'a kadar değişmiştir. S3, S4, S5 ve S7 istatistiklerine dayalı olarak en istikrarlı genotipler, G4, G5 ve G10 iken, S1 ve S2 parametrik olmayan stabilite istatistiklerine dayalı olarak en istikrarlı genotipler, G7, G10 ve G11 olarak bulunmuştur. Ortalama verim ile ilgili olarak, genotip G10 (3560,91 kg/ha), en uygun genotip olarak belirlenmiştir. Bu çalışmada, parametrik olmayan stabilite istatistiklerinden hiçbiri yüksek ortalama verim ile olumlu ilişkili bulunmamıştır ve bunun yerine stabilitenin statik bir kavramı olarak karakterize edilmiştir. Faktör analizi ve parametrik olmayan stabilite istatistiklerinin korelasyon analizi sonuçları, S1 ve S2'nin stabilite için seçilmelerinin için yararlı olacağını göstermiştir.

Anahtar kelimeler: Adaptasyon, Çevre-Genotip interaksiyonu, Çoklu-Lokasyon denemeleri, Verim stabilitesi

Introduction

Barley (*Hordeum vulgare* L.), as an ancient cereal grain, is one of the most widely cultivated crop in the world which upon domestication has evolved from a food grain to a feed and malting grain. Continuous efforts for developing of new cultivars indicating high mean yield, stability performance and better adaptation to several environmental growing conditions is essential to maintain barley competitiveness and increase economic returns comparatively to other cereal crops (Dehghani et al. 2006). Iran has had important barley improvement programs in recent years for increasing the genetic potential of yield. The improved barley genotypes are evaluated in multi-environment yield trials to test their performance across different environmental conditions. In most trials, genotype by environment (GE) interaction is observed and its interpretation can be aided by statistical modeling (Huehn 1996 and Sabaghnia et al. 2006).

Several statistical procedures have been introduced to evaluate genotype stability in a set of environments, each adopting different criteria to define and estimate these parameters (Flores et al. 1998). Exploring of yield stability allow for the identification of those which best respond in a predictable manner to different environment conditions. In spite of the availability of different stability methods, univariate, multivariate and nonparametric procedures, the criterion for selecting and releasing a cultivar are frequently based solely on the average of the yield in test environments (Sabaghnia et al. 2008 and Karimizadeh et al. 2012). However, generalized indication of genotypes for cultivation in good and poor locations may causes in wrong choices due to specific adaptation of genotypes to specific locations.

The most common approaches for yield stability analysis, parametric procedures, are based on statistical assumptions about the distribution of genotypic, environmental and GE interaction effects. These methods have good characteristics under certain statistical assumptions, based on the normal distribution of residuals and GE interaction effects, but may not perform well if these assumptions are violated by factors such as the presence of outliners (Huehn 1990a and Huehn 1996). In contrast, the nonparametric procedures make no specific modeling assumptions when relating environments and genotypes relative to soil and climatic factors. The nonparametric methods rank genotypes according to their mean performance in different environments. Several nonparametric procedures have been proposed based on comparing ranks of genotypes in each environment, with genotypes with similar ranking across test environments being considered most stable (Huehn 1979 and Kang, 1988; Thennarasu (1995).

The following seven nonparametric statistics have been proposed by Huehn (1979) and Huehn (1990b): S1, the genotype absolute rank difference mean; S2, the between-ranks variance over the test environments; S3, the sum of the absolute deviations of the squares of ranks for each genotype; S4, the root of the sum of the absolute deviations of the squares of ranks; S5, the sum of the squares of ranks for each genotype; S6, the sum of the squares of ranks for each genotype relative to the mean of ranks; and S7, the mean of squares of ranks for each genotype. Nonparametric stability methods have several benefits over parametric methods in that they are easy to use and interpret, no assumptions are needed regarding the distribution of the observed values, removal or addition of one or several genotypes cause little variation in the results and outlier bias is reduced (Huehn 1990a; Adugna and Labuschagne 2003).
The objectives of present investigation were to identify barley genotypes that have both high mean yield and stable performance across different environments for semiarid areas of Iran and study the relationships among seven nonparametric stability statistics.

Material and Methods

Field experiments

The plant material used in this investigation included 14 diverse new advance genotypes of barley with two checks (Izeh and Gachsaran local check). These elite lines of barley were drawn from Iran’s barley breeding program and their pedigrees are given in Table 1.

These genotypes, planted in randomized complete block design with four replications during growing seasons 2000-2003, were evaluated under four locations; Gachsaran, Gonbad, Khoramabad and Moghan. The third year experiment of Moghan was not in a good manner and so deleted from analysis. Different agro-geographic properties of these test locations are summarized in Table 2. Each genotype was grown in 6 rows of 7 m long plots with spacing of 17.5 cm between the rows. All trial plots in the all locations and years were fertilized with 60 kg of N ha^{-1} and 60 kg of P_2O_5 during sowing Grain yield (kg ha^{-1}) was determined according to of the harvested plot in all 11 environments and corrected to 12% moisture basis.

Table 1. The code, name and Pedigree of 16 barley genotypes

Code	Pedigree or Name
G1	Wi2291/Wi2269//ER/Apm ICB86-0629-0AP-2APH-0AP
G2	Pld10342//Cr.115/Por/3/Bahtim/4/Ds/Apro/5/wi2291/Wi2291/Wi2269/7/Wi2291/Wi2291/ Wi2291/Wi2269//Wi2291/Bgs ICB94-0402-0AP
G3	7028/2759/3/6982//Ds/Apro/4/H272//Wi2198/ID601810/5/Mazurka ICB95 –0437-0AP
G4	Zanbaca/3/H.Spont.21-3/Arar 84//Wi2291/Bgs ICB94-0314-0AP
G5	Hml/WI2291/4/Zanbaca/3/Er/Apm/Lignee131 ICB94 –0587-0AP
G6	Er/Apm//Cerise/3/lignee131/3/Er/Apm ICB83-1985-2AP-0AP
G7	Lignee 124/Hml 024 ICB 82-0757-10AP-0AP-23AP-0AP
G8	Alanda/Harma01/7/Gustoe/6/M6476/Bon//Jo/York/3/Ms/Colt/As46/4/Hy3480/Astrix/5/NK1272 ICB95 –0791-0AP-0AP
G9	IPA7//As46/Rhn-05 ICB95 –0162-0AP-0AP
G10	Weahll/Wi2291/Bgs/3/Er/Apm//Ac253 ICB 94 – 0707-0AP-0AP
G11	Roho Alger/Ceres 362 1-1/3/Kantara/4/Bowman ICB93 –0791-21AP-0AP
G12	Mari/Aths×2//Avt/Attiki/3/Aths/Lignee 686 ICB 91 –0368-3AP-0TR-3AP-0AP
G13	IPA 265/PA 7 ICB95 –0127-0AP
G14	Lignee 131/ArabiAbiad/3/Chiem/An57//Albert
G15	Izeh
G16	Gachsaran local check

Table 2. Geographical properties of four test locations.

Location	Longitude Latitude	Altitude (m)	Soil Texture	Soil Type¶	Rainfall (mm)
Gachsaran	50° 50 E 30° 20 N	710	Silty Clay Loam	Regosols	430.8
Gonbad	55° 12 E 37° 16 N	45	Silty Clay Loam	Regosols	367.5
Khoramabad	23° 26 E 48° 17 N	1148	Silt-Loam	Regosols	523.1
Moghan	48° 03 E 39° 01 N	1100	Sandy-loam	Cambisols	271.2

Stability statistics

Huehn (1979) and Huehn (1990b) proposed seven nonparametric methods for estimating GE interaction and yield stability analysis. For a two-way dataset with *k* genotypes and *n* environments, we denote the phenotypic value of *i*th genotype in *j*th environment as x_{ij}, where $i = 1,2,...,k$, $j = 1,2,...,n$, r_{ij} as the rank of the *i*th genotype in the *j*th environment, and $\overline{r_{ij}}$ as the mean rank across all environments for the *i*th genotype. The statistics based on yield ranks of genotypes in each environment are expressed as follows:

$$S_i^{(1)} = 2\sum_{j}^{n-1}\sum_{j'=j+1}^{n}\left|r_{ij} - r_{ij'}\right| / [n(n-1)]$$

$$S_i^{(2)} = \sum_{j=1}^{n}(r_{ij} - \bar{r}_{i.})^2 / \sum_{j=1}^{n}|r_{ij} - \bar{r}_{i.}|$$

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

$$S_i^{(4)} = \sqrt{\frac{\sum_{j=1}^{n}(r_{ij} - \bar{r}_{i.})^2}{n}}$$

$$S_i^{(5)} = \frac{\sum_{j=1}^{n}|r_{ij} - \bar{r}_{i.}|}{n}$$

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

$$S_i^{(7)} = \sum_{j=1}^{n}(r_{ij} - \bar{r}_{i.})^2 / (n-1)$$

The seven mentioned nonparametric measures of phenotypic stability were calculated according to original dataset. The SAS software (SAS Institute, 1996) was used to analyze the results of the nonparametric stability analysis based on the Lu (1995), program for computing the S1 and S2 statistics and Hussein et al. (2000), program (SASG × ESTAB) for computing the S3 and S6 statistics. The nonparametric stability statistics were compared using Spearman's rank correlation and graphic presentation of first two components of factor analysis.

Results and Discussion

The combined analysis of variance (Table 3) indicated the significance of the main effects of environments as well as genotypes. The environments accounted more than 94% of total variation due to GE+E+G sources while the genotypes accounted only about 2% of total variation due to GE+E+G sources. The differences in the classification of the genotypes in the different test environments indicated the presence of GE interaction (Table 3). The significant GE interaction indicated that the responses of the genotypes changed depending on environmental conditions. The GE interaction accounted 4% of total variation due to GE+E+G sources. According to Yan and Rajcan (2003), although, the measured yield of each genotype in each test environment is a result of the effects of genotype, environment and GE interaction, but environment variation is said to cause more than 80% of yield variation. Accordingly in this investigation, both genotype and GE interaction effects accounted only 8% of yield variations while these effects are relevant to cultivar evaluation (Yan, 2002; Yan and Tinker, 2005).

Table 3. Combined analysis of variance for barley performance trial yield data.

SOV†	DF‡	Mean Squares	% of GE+E+G†
Environment (E)	10	35222228.3**	94.2
Replication/E	33	702331.2	
Genotype (G)	15	1489296.7**	4.0
GE	150	676638.1**	1.8
Error	495	153069.3	

† Sources of variation
‡ Degrees of freedom
** Significant at the 0.01 probability level

The overall mean grain yield for all the genotypes ranged from 3804.91 kg ha^{-1} for G1 to 3119.27 kg ha^{-1} for G13 (Table 4). The most stable genotypes based on the first two nonparametric stability statistics of Huehn (1990b), S1 and S2, were G7, G10 and G11 while the most unstable genotypes were G1, G13 and G16 (Table 4). Regarding mean yield, it could be grasped that genotypes G10 (3560.91 kg ha^{-1}) and G11 (3549.27 kg ha^{-1}) were the most favorable genotypes. The most stable genotypes based on the S3, S4, S5 and S7 nonparametric stability statistics, were G4, G5 and G10 while the most unstable genotypes were G8 and G15 (Table 4). Only genotype G10 was the most favorable genotype based on both mean yield and these nonparametric stability statistics. The most stable genotypes based on the S6 nonparametric stability statistic, were G5, G10 and G13 while the most unstable genotypes were G1, G6 and G8 (Table 4). Only genotype G10 was the most favorable genotype based on both mean yield and S6 nonparametric stability statistic.

Table 4. Mean yields in kg/ha and nonparametric stability estimates for barley yields of 16 genotypes tested in 11 environments.

	Mean	S1	S2	S3	S4	S5	S6	S7
G1	3804.91	6.44	29.47	36.98	3.87	3.31	8.16	16
G2	3689.64	5.16	19.42	25.76	3.78	3.19	5.76	16
G3	3474.00	5.85	24.82	29.78	4.71	3.80	5.11	24
G4	3392.82	5.24	19.42	15.33	3.57	3.15	3.79	14
G5	3165.73	5.67	22.56	11.14	3.48	2.79	2.56	13
G6	3591.36	5.89	25.02	35.95	4.66	3.90	6.47	24
G7	3367.09	4.84	16.82	20.60	4.21	3.69	4.29	19
G8	3482.73	6.04	27.16	32.79	5.10	4.66	5.88	29
G9	3347.18	5.78	23.42	21.33	4.11	3.70	4.67	19
G10	3560.91	3.85	10.67	12.19	2.96	2.26	3.15	10
G11	3549.27	4.95	18.22	20.65	3.71	3.17	4.76	15
G12	3439.91	5.05	18.85	22.53	4.24	3.43	4.30	20
G13	3119.27	6.80	32.96	16.12	4.21	3.17	2.89	19
G14	3488.18	5.20	19.02	30.49	4.52	3.92	5.85	22
G15	3487.91	6.25	27.82	30.60	4.85	4.41	5.74	26
G16	3191.73	6.51	31.27	19.59	4.37	3.57	3.66	21

Two main contrasting concepts of yield stability are identified: static and dynamic (Becker and Leon, 1988). In static stability concept, the best genotype tends to maintain a constant yield across diffirent environments while from dynamic stability concept; it implies that for a stable genotype a yield response in each environment that is always parallel to be mean response of the tested high yield stability genotypes (Annicchiarico 2002). According to Flores et al. (1998), S1 and S2; based on Sabaghnia et al. (2006), S3 and S6; and according to Karimizadeh et al. (2012), S4, S5 and S7, have static concept of yield stability and usually detect low mean yielding genotypes as the most stable genotypes. An ideal genotype should have a high mean yield as well as a low degree of fluctuation when this genotype is grown over diverse test environments. However, analysis of GE interaction of a particular genotype can reduce the errors in the breeding process, as the selection in one environment cannot provide benefits in other different environments.

Each one of the nonparametric stability statistics produced a unique genotype ranking (Table 5). The Spearman's rank correlations between each pair of nonparametric stability statistics were calculated (Table 6) and demonstrate a highly significant negative rank correlation between mean yield and both S3 and S6. Similarly, Sabaghnia et al. (2006) reported significant negative rank association between mean yield and both S3 and S6 nonparametric stability statistics while Kang and Pham (1991) found that these nonparametric stability statistics are related with high yield performance, and therefore define stability with dynamic concept. There was highly significant positive rank correlation between S1 and S2 nonparametric stability statistics. Flores et al. (1998) in faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.), Scapim et al. (2000) in maize (*Zea mays* L.), Sabaghnia et al. (2006) in lentil (*Lens culinaris* Medik.) found significantly positive correlations between these nonparametric stability statistics.

The S2 nonparametric stability statistic had significant native rank association with S3 and had not any significant native or positive rank correlations with the other remained nonparametric stability statistics (Table 6). Ebadi-Segherloo et al. (2008) and Karimizadeh et al. (2012) reported similar properties for S2 statistic in comparison to the other nonparametric stability statistics. All of the five nonparametric stability statistics (S3, S4, S5, S6 and S7) were related positively with each other. Sabaghnia et al. (2006) and Scapim et al. (2000) reported high positive rank association between S3 and S6, Dehghani (2008) found high positive rank correlation between S4 and S5, and Karimizadeh et al. (2012) reported high positive rank association among all of the S3, S4, S5, S6 and S7 nonparametric stability statistics.

Table 5. Ranks of barley genotypes based on mean yield and nonparametric stability estimates.

	Mean	S1	S2	S3	S4	S5	S6	S7
G1	1	14	14	16	6	7	16	5.5
G2	2	5	6.5	10	5	6	12	5.5
G3	9	10	10	11	14	12	10	13.5
G4	11	7	6.5	3	3	3	5	3
G5	15	8	8	1	2	2	1	2
G6	3	11	11	15	13	13	15	13.5
G7	12	2	2	6	8.5	10	6	8
G8	8	12	12	14	16	16	14	16
G9	13	9	9	8	7	11	8	8
G10	4	1	1	2	1	1	3	1
G11	5	3	3	7	4	4.5	9	4
G12	10	4	4	9	10	8	7	10
G13	16	16	16	4	8.5	4.5	2	8
G14	6	6	5	12	12	14	13	12
G15	7	13	13	13	15	15	11	15
G16	14	15	15	5	11	9	4	11

Table 6. Spearman's correlation coefficients among ranks of nonparametric stability statistics for 16 barley genotypes at 11 environments

	Mean	S1	S2	S3	S4	S5	S6
S1	0.23^{ns}						
S2	0.20^{ns}	0.99^{**}					
S3	-0.62^{*}	0.33^{ns}	0.34^{ns}				
S4	0.00^{ns}	0.49^{ns}	0.47^{ns}	0.67^{**}			
S5	-0.13^{ns}	0.34^{ns}	0.32^{ns}	0.76^{**}	0.92^{**}		
S6	-0.74^{**}	0.17^{ns}	0.18^{ns}	0.96^{**}	0.51^{*}	0.66^{**}	
S7	-0.01^{ns}	0.48^{ns}	0.46^{ns}	0.68^{**}	0.99^{**}	0.94^{**}	0.53^{*}

To better understand the relationships among the nonparametric stability statistics, a factor analysis through principal component analysis and based on the rank correlation matrix (Table 6) was performed. Table 7 shows the original and varimax rotated loadings of the first two components of ranks of different nonparametric stability statistics. When applying the factor analysis, the two first components explained 84.5 and 70.7% for the un-rotated and rotated conditions, respectively (Table 7). The relationships among the different nonparametric stability statistics are graphically displayed in a plot of factor 1 versus factor 2 (Fig. 1). In this plot, the factor 1 axis mainly did not distinguish the nonparametric stability statistics and mean yield from each other. Also, the factor 2 axis distinguishes mean yield from nonparametric stability statistics. Therefore, it seems that most of the nonparametric stability statistics tend to detect static stability concept. Many authors reported similar properties for nonparametric stability statistics of Huehn (1979) and Huehn (1990b) and emphasized that these stability statistics could be useful for detection of static or biologic stability concept in multi-environment trials (Yue et al., 1997; Knezović and Gunjača 2002; Kaya and Taner 2003; Scapim et al. 2000; Dehghani 2008; Balalić et al. 2011).

The nonparametric stability statistics do not need any special assumptions about the normality of the data distribution and variance homogeneity. The GE interaction concepts of the classification they represent are related to that of selection in which plant breeders are interested (Huehn 1996; Akcura and Kaya 2008). In other words, whether the best genotype in one test environment is the best in other test environments. The nonparametric stability statistics could be used by agronomists and plant breeders (Balalić et al. 2011). These statistics are easy to use and interpret and could contribute to supplementary information on the performance of genotypes and enable their recommendation to farmers. In conclusion, the nonparametric stability statistics seem to be useful alternatives to conventional parametric statistics (Yue et al. 1997), although they do not supply information about genotype adaptability.

Regarding the choice among the nonparametric stability statistics, Nassar and Huehn (1987) and Huehn (1990a) suggest that the S1 statistics should be used in any case in which a genotype indicates unusual fluctuations between different environments. Kang and Pham (1991) suggested that S3 and S6 are easier to apply and interpret than other the nonparametric stability statistics while Miranda (1993) suggested that S1 and S2 are easier to apply and interpret than S3 statistic. Sabaghnia et al. (2006) suggested that S2 is useful to application than other the nonparametric stability statistics while Dehghani (2008) suggested that S4 is easier to apply and interpret than S2, S3 and S5 statistics. However it seems that both S1 and S2 nonparametric stability statistics are good candidates for stability analysis. It could be verified from Fig. 1.

Table 7. First two components loadings of ranks obtained from seven nonparametric methods used to analyze GE interaction of barley genotype yields.

Nonparametric statistics	Original		Rotated	
	Factor 1	Factor 2	Factor 1	Factor 2
Mean	-0.274	0.849	0.089	-0.949
S1	0.588	0.668	0.238	-0.020
S2	0.581	0.653	0.214	0.002
S3	0.888	-0.395	0.563	0.779
S4	0.910	0.167	0.941	0.116
S5	0.908	-0.043	0.939	0.261
S6	0.773	-0.569	0.429	0.881
S7	0.915	0.151	0.947	0.128
% of variance	57.9	26.6	40.9	29.8

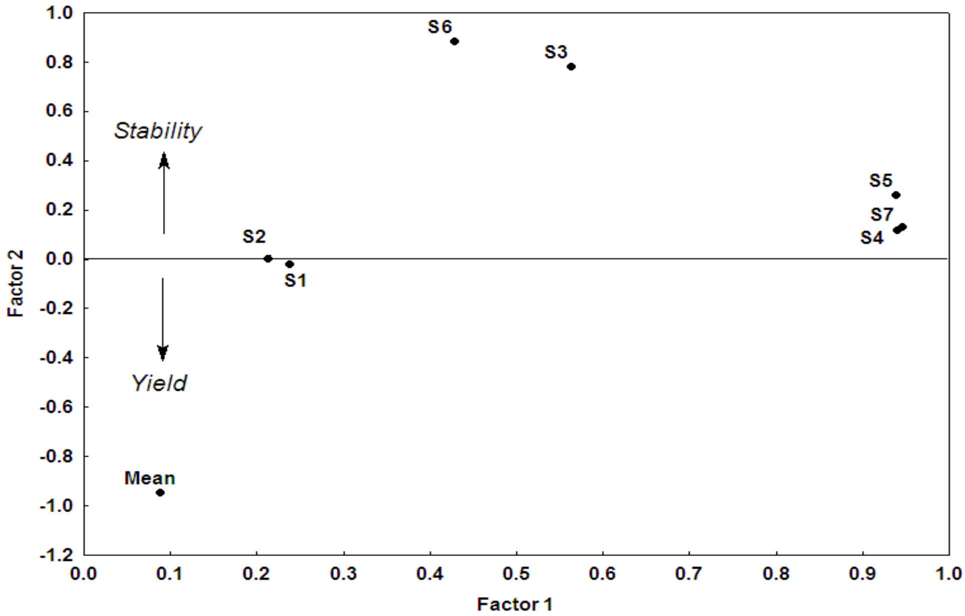

Fig. 1. Factor analysis plot of ranks of stability of yield, estimated by seven methods using yield data from 16 barley genotypes grown in 11 environments and showing interrelationships among these statistics

Conclusion

The results from the investigation suggested that a significant GE interaction existed among 16 barley genotypes grown in 11 environments for grain yield. The presence of GE interaction suggests high yielding barley genotypes which are stable in different environments. Significant differences in rank stability among 16 barley genotypes grown in 11 environments were found. According to the most of nonparametric stability statistics and regarding high mean yield, genotype G10 (3560.91 kg ha^{-1}) was the most stable and favorable in all environments. The use of S1 and S2 nonparametric stability statistics were recommented in stability analysis as an alternative strategy beside conventional parametric models.

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