

GGE BIPLLOT ANALYSIS OF GENOTYPE × ENVIRONMENT INTERACTION AND FORAGE YIELD STABILITY IN GRASS PEA (*Lathyrus sativus*) GENOTYPES

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In crop breeding programs, biplot analysis is a well-known statistical method. This study aimed to survey the genotype × environment interaction (GEI) on grass pea genotypes in Iran. The experiment was conducted in twelve environments (four separate sites: Gachsaran, Kuhdasht, Mehran, and Shirvanchardavol) over three sequential years (2017, 2018, and 2019) with sixteen grass pea genotypes. The purpose of this research was to utilize the GGE biplot as a tool to identify the superior genotypes of grass peas. The results for the combined analysis of variance, genotypes, and the GEI revealed a significant impact ($p < 0.001$) on forage yield. Moreover, genotype × environment interaction responded differently under various climatic conditions. The interaction components evaluated by the biplots revealed the genotypes' predominant effect and the significant genotype × environment interactions (GEI). The first two principal components (PCs) interpreted up to 93.11% of the total variation in the GGE model (PC1 = 53.30%, PC2 = 37.80%). GGE biplot analysis categorized the studied environments

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into two mega-groups for forage yield. Genotype G11 (Russia) was superior in terms of mean forage yield (5.362 t/ha). The genotypes that performed best in each environment, were genotypes G11 (Russia) and G8 (Bangladesh-I). Among these genotypes, G11 (Russia) was the highest-yielding genotype in the field. The Kohdasht site was the most discerning and representative test environment for crop yield. The selected genotypes are recommended for breeding programs aimed to improve forage yield in the tested sites or similar agroecological areas.

Keywords: GGE biplot, multi-environment trial (MET), stability analysis

INTRODUCTION

Grass pea (*Lathyrus sativus*) as an annual and/or perennial legume belongs to the Fabaceae family (YADAV and MEHTA, 1995). The highlighted characteristic that differentiates grass pea from other legumes is its high-yielding potential, protein content, nitrogen fixation potential, and tolerant to diverse environmental stressors (VAZ PATTO *et al.*, 2006).

The genotype \times environment interaction is a crucial factor in breeding programs aimed at developing high-yielding, adaptable, and stable forage cultivars. By doing this, the idea is to breed crops with high and stable yields for various climatic conditions (YAN *et al.*, 2000). Genotype \times environment interactions assist in evaluating genotypes to select superior ones and help farmers cultivate a suitable cultivar in a given environment. Moreover, the breeders may introduce a cultivar with reliable and stable performance (FASAHAT *et al.*, 2015). Several environmental trials are conducted worldwide by different breeding agencies to develop cultivars suitable for various climatic conditions. The main idea is to identify the appropriate cultivars for the specific target area. The other idea was to characterize target areas in the various environments (YAN *et al.*, 2000). Forage yield is thought to be polygenic and, is highly influenced by the environmental cues (HILLOCKS and MARUTHI, 2012). The genotype \times environment interaction hugely influences the genotype and phenotype interactions. Moreover, the process of selecting the high-yielding genotypes is quite complicated. To justify the idea, genotype GGE biplot analysis lowers the environmental impact and focuses mainly on their interaction, which are the most relevant constituents in cultivar selection for a defined trait or environment (YAN and TINKER, 2005).

YAN *et al.* (2000) recommended a graphical method, GGE biplot to evaluate the multi-environment trials. The biplot concept was first proposed by GABRIEL (1971). The GGE biplot analysis has frequently been utilized for the graphical analysis of genotype and environment interactions. The methodology assists plant breeders in evaluating genotype and environment interactions (YAN *et al.*, 2001). The idea of genotype \times environment interactions accelerates the selection of suitable cultivars for a given environment (FASAHAT *et al.*, 2015). GGE biplot interprets the genotypes and environments interactions and interrelations (ASFAW *et al.*, 2009). The GGE comes from the analysis of METs of plant genotypes. The yield potential of a cultivar in a given environment is impacted by the genotype (G), the environment (E), and their interaction (GE). In common METs, E describes up to 80% of the total yield variation, while, G and GE interpret up to 10% of the total variance (YAN *et al.*, 2000). For cultivar assessment, only G and GE are the relevant factors (GAUCH and ZOBEL, 1996). Moreover, both G and GE have to be assessed in cultivar evaluation, under the term GGE (YAN *et al.*, 2000). The G and GE (GGE)

biplots are confident multi-environment data to assess the genotypic potential of cultivars in different environments (YAN *et al.*, 2000; YAN *et al.*, 2007). The GGE biplot has commonly been used to evaluate the localities' potential for the reliable production of soybean crops (YAN and RAJCAN, 2002). GGEs are employed to plot the PC1 and PC2 values for the diverse genotypes and environments. The environmental cues address the variation span in a given environment and may represent its 'discriminatory power' (YAN *et al.*, 2007). In the cultivar assessment, the GGE biplot eliminates the environment-dependant predominant effects and relies mainly on the genotype plus GGE components (YAN, 2002). The other major objective of GGE biplot analysis is to assess whether the defined cropping areas are similar or can be logically divided into diverse mega-environments (YAN *et al.*, 2000).

The current study aimed to assess the intensity of genotype-environment interaction (GEI) on forage yield and to determine the stability of 16 grass pea genotypes in the test environments in Iran. GGE biplot analysis was utilized to depict graphs for the following purposes: (i) which-won-where pattern, (ii) mean yield and stability analysis, and (iii) ranking discrimination and representativeness of test locations for the grass bean genotypes for the forage yield and yield components.

MATERIALS AND METHODS

Study site specifications

The experiment was planned in four various agro-ecological zones; Mehran 46°36'E 33°47'N, Kohdasht 48°28'E 34°08'N, Gachsaran 50°50'E 30°17'N, Shirvanchardavol 57.54'E 37°24'N at 2017, 2018 and 2019 planting seasons. The description of the study sites is represented in Table 1.

Table 1. The description of study sites

Site	Longitude	Latitude	Average Rainfall (mm)	Elevation AMSL (m)
Mehran (Ilam)	46°36'E	33°47'N	350.0	975
Shirvanchardavol (Ilam)	47°31'E	33°05'N	345.0	1100
Kohdasht (Lorestan)	48°28'E	34°08'N	373.8	1125
Gachsaran (Kohgiluyeh and Boyer-Ahmad)	50°50'E	30°17'N	302.3	710

Plant materials and collection of yield data

The plant material comprised of a check and fifteen grass pea genotypes provided by the International Center for Agricultural Research in the Dry Areas (ICARDA). The origin of these genotypes is given in Table 2.

The genotypes were evaluated using a randomized complete block design with three replications. The seeds of each genotype were planted during the three planting seasons of 2017, 2018, and 2019. Each experimental plot consisted of four planting rows with a length of 4.5

meters and a row spacing of 25 cm. The planting operation was done using a seeder with a density of 150 seeds per m². At harvest time, the dried forage was weighed to obtain the forage yield per plot, which was then converted to kg ha⁻¹.

Table 2. Description of 16 grass pea genotypes used in the study

Genotype number	Origin
1	Greece (Greece-I)
2	Greece (Greece-II)
3	---
4	Morocco
5	Ethiopia
6	Hungary
7	India
8	Bangladesh (Bangladesh-I)
9	Bangladesh (Bangladesh-II)
10	Greece (Greece-III)
11	Russia
12	Bangladesh (Bangladesh-III)
13	Bangladesh (Bangladesh-IV)
14	Greece (Greece-IV)
15	Bangladesh (Bangladesh-V)
16	Naghadeh (Local Check)

Statistical analysis

Before ANOVA, forage yield-related data were tested for normality with Kolmogorov-Smirnov. Genotypes were considered as fixed effects; and the replications, years, and sites as the random effects. Under the significant difference in genotype \times environment interaction; the GGE biplot analyzed and assessed the interactions and yield stability. Two-way data were displayed by GGE biplot. The GGE analysis also studied the genotype \times environment interaction on forage yield. To generate a GGE biplot, the data were transferred by a scaling and data-centering method. A biplot was depicted via the PC1 scores against the PC2 scores. GGE using PBSTAT-GE (a web-based statistical analysis software for plant breeding) was used to distinguish the stable and high-yielding grass pea genotypes across the experimental sites.

RESULTS AND DISCUSSION

Combined analysis of variance

The Kolmogorov-Smirnov test revealed the normal distribution for forage yield. Considering the nonsignificant experimental error variances for the various experiments based on Bartlett's test and to evaluate genotype \times environment interaction, a combined analysis was carried out assuming the random effects of years and sites, and the fixed effect of genotypes. Duncan's multiple range test evaluated the mean comparisons (Table 4).

To quantify the GEI variations, a combined analysis of variance was conducted (Table 3). Finding the most adaptable genotypes for improving forage yield is challenging due to the significant impact of genotype-environment interaction (GEI) on forage yield (FASAHAT *et al.*, 2015). For successful breeding methods, especially for complex and highly quantitative traits such as forage yield, breeders must quantify genotype-environment interaction (GEI). Therefore, the selection procedure should evaluate both genotype and environmental factors (YAN *et al.*, 2001). The combined analysis (Table 3) revealed the significant effects of year, site, and the interaction of year × site ($p < 0.001$). This indicates that factors such as precipitation, minimum and maximum temperature, soil properties, latitude, longitude, elevation, and other variables have influenced the evaluated traits in various years and locations. The interaction effect of year × site was significant, indicating that the year's effect varied across different locations.

The genotypes had a significant influence on forage yield ($p < 0.001$). The genotypes showed different yield potential for different years and environments.

Table 3. The combined analysis of variance for yield data obtained from trials conducted in four sites in 2017-2019

Source	df	SS	MS	F
Year (Y)	2	750.9	375.4	283.1**
Site (S)	3	180.5	60.2	45.4**
Y × S	6	2202.3	367.0	276.8**
Rep / (Y × S)	24	146.8	6.1	4.6**
Genotype (G)	15	62.5	4.2	3.1**
Y × G	30	121.5	4.1	3.1**
S × G	45	147.8	3.3	2.5**
Y × S × G	90	328.8	3.7	2.8**
Pooled Error	360	477.4	1.3	-

** is significant at 0.01 level.

The interaction effect of genotype × year was significant, indicating that genotypes had varying yield potential from one year to another. However, the interaction of genotype × site was significant, indicating that the diverse genotypes differently responded to the environments. In other words, genotypes exhibit specific adaptability, and within a particular site, a specific genotype may have greater yield potential than others. The trice interaction of year × site × genotype was significant. This indicates that there were fluctuations in the order of genotypes in the environments, and the genotypes exhibited varying yield components in the tested environments.

The observed differences confirm the presence of consistent variability in grass pea genotypes suitable for crop breeding programs. Trait variability aids in the trait-assisted selection of the best genotypes for breeding (MORADI *et al.*, 2019; DEWI *et al.*, 2020). The genotypes do not show stable yields in the studied environments. Our findings align with those of AHMADI *et al.* (2012), who reported high interaction between site and species for forage yield. Therefore, to identify the stable genotypes, the stability determination method of the GGE biplot will be used.

Moreover, the present findings are similar to those of AHMADI (2012) and RUBIALES *et al.* (2020), who reported a meaningful interaction between genotype and environment for grass pea forage yield. Finding the most adaptable genotypes for yield improvement is challenging due to the significant impact of genotype-environment interaction (GEI) on yield. The successful breeding methodologies for complex quantitative traits, such as forage yield in grass peas, require further studies, and plant breeders must quantify GEI effect. Thus, the selection method has to consider both genotype and environmental factors. According to AHMAD *et al.* (2012), grass pea forage yield was significantly responsive to the environment (E), genotype (G), and their interactions (G×E). This verifies that the genotypes do not show high performance in the tested environments. Our study is supported by previous reports that have shown the Gene-Environment Interaction (GEI) effect on several crops such as common bean (PHILIPO *et al.*, 2021), grass pea (AHMADI, 2012), faba bean (FLORES *et al.*, 2013) and grass pea (RUBIALES *et al.*, 2020). YAN and KANG (2003) reported that the number of genotypes and environments distinguishes the extent of environmental variation. According to AREMU *et al.* (2019), the environment is consistently the primary source of variation and should be given priority in plant breeding.

Table 4. Mean forage yield data (kg ha⁻¹) of 16 grass pea genotypes tested in 4 locations of Iran.

Genotype		Forage Yield	Genotype		Forage Yield
		Mean			Mean
1	Greece (Greece-I)	5.14ab	9	Bangladesh	5.10ab
2	Greece (Greece-II)	4.15d	10	Greece (Greece-III)	4.71bcd
3	---	4.96abc	11	Russia	5.36a
4	Morocco	4.368 cd	12	Bangladesh	4.69bcd
5	Ethiopia	4.99abc	13	Bangladesh	4.67bcd
6	Hungary	4.97abc	14	Greece (Greece-IV)	4.39cd
7	India	4.87abc	15	Bangladesh	5.14ab
8	Bangladesh	5.27ab	16	Naghadeh (Local)	4.73abcd

GGE biplot Analysis

The GGE analysis introduced the right genotypes for each environment. The biplot explained 93.11% of the total variation, with 53.30% belonging to the first principal component (PC1) and 37.80% to the second principal component (PC2) (Figure 1). Overall, the GGE-biplot explained up to 91.10% of the variations. Ahmadi *et al.* (2012) noted that the first two principal components explained up to 92.10% of the variations in the GGE biplot for grass pea genotypes. G plus GE of a MET facilitates cultivar evaluation and mega-environment categorization.

The polygon nature of the "which-won-where" biplot describes the GEI, mega-environments, and specific adaptation patterns (YAN and TINKER, 2005). The GGE biplot of "Which-won-where" is depicted in Figure 1.

The genotypes at the polygon vertices in any environment are the selected cultivars suitable to the environment (YAN *et al.*, 2001). The 16 genotypes were distributed across five sectors. The yield potential can be compared using the GGE biplot under different environments.

Generally, there were gene-environment interactions that restricted genotype selection for planting in a specific environment. Some geo-climatological criteria may create mega-environments. The genotypes G11 (Russia), G12 (Bangladesh-III), G4 (Morocco), G9 (Bangladesh-II), and G2 (Greece-II) were all positioned at the vertices of the polygon (Figure 1), confirming their yield extremes in those environments. In some environments, the cultivars respond differentially because they are the farthest from plot center. Genotypes G2 (Greece-II), G4 (Morocco), and G12 (Bangladesh-III) did not show significant performance in any environment. Genotype G11 (Russia) exhibited the highest yield potential in the Kohdasht and Mehran areas. Genotype G9 (Bangladesh-II) showed the lowest recorded yield in the Shirvanchardavol and Gachsaran regions. The environments mentioned above can be regarded as a mega-environment. The genotypes at the vertices of the polygon in a "which-won where" scenario are the appropriate genotypes in that environment. Some genotypes, such as G5 from Ethiopia, were positioned near the center of the GGE biplot. This envy that the mentioned genotypes yielded stably in the various tested environments (YAN and TINKER, 2005). In this study, the GGE biplots for forage yield demonstrated their potential usefulness in evaluating genotypes across multiple environments. Considering the GGE biplots can be employed by grass pea breeders to identify high-yielding genotypes by simultaneously taking into account both G and GE interaction factors (YAN and KANG, 2003). The reliable genotypes with high and low values of PC1 and PC2, can also be easily characterized using GGE biplot. The best environment for forage yield can be visually characterized on a GGE biplot by its large PC1 values compared to the low PC2 values.

The genotypes in any sector were very alike to one another. For example, genotypes G8 (Bangladesh-I) and G3 were similar to the superior genotype G11 (Russia) and exhibited suitable adaptability in the mega-environment of Kohdasht and Mehran. However, their yield was not comparable to the genotype located at the polygon vertex. Even though the genotypes G2, G4, and G12 were located at the polygon vertex; their yield related data was not comparable to the superior genotypes. The mega-environment consisted of genotypes G11 (Russia), G8 (Bangladesh-I), G3 (unknown), G5 (Ethiopia), and G1 (Greece-I). Genotype G3 constantly yielded a relatively high data.

Genotype 14 (Greece-IV) was found in the sector where genotypes 2 (Greece-II) and 4 (Morocco) intersect at the vertex. In the sector where genotype 11 is located, genotypes 3, 8, 5, and 1 are also present. Therefore, the mentioned genotypes are suitable for Kohdasht and Mehran. In addition to identifying the genotypes compatible with any environment, this type of biplot is utilized to identify genotypes with high yield stability as they are located near the origin of the biplot. Consequently, genotype 5 exhibits the lowest distance.

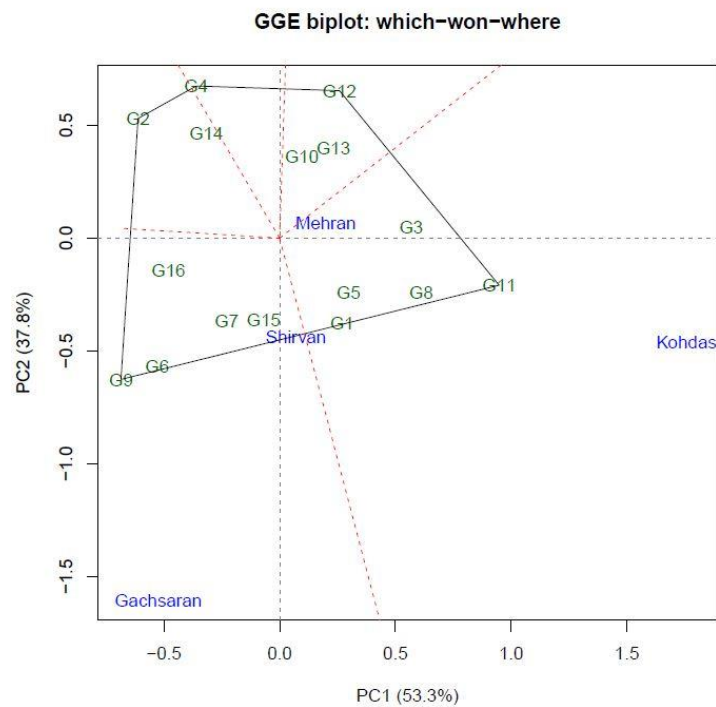


Figure 1. GGE scatterplot for the “which-won-where” pattern of 16 grass pea genotypes evaluated in four locations in Iran.

In the selection programs, both the yield and stability criteria should be considered. Those traits were evaluated in grass pea genotypes using the average environment coordination (AEC) method. The AEC represented the GGE biplot based on environmental assessment of the mean value and genotype stability. A horizontal axis with a circle and an arrow indicates the stability rate, and any genotype near this axis can be considered more stable. The small circle located on the horizontal axis, indicated by an arrow, represents the ideal genotype with the highest yield in the studied environments. This genotype is completely stable in a given environment as it is positioned on the horizontal axis. Those genotypes hold minimal role in the interaction between genotype and environment. The vertical axis also displays the yield of the genotypes, with those on the left side of this axis showing a lower yield than the average (Figure 2). The genotypes G11 (Russia), G3, G6 (Hungary), and G9 (Bangladesh-II) exhibited high-yield data but low stability. The genotypes G8 (Bangladesh-I), G15 (Bangladesh-V), and G7 (India) achieved high yields with medium stability. Eventually, genotypes G1 (Greece-I) and G5 (Ethiopia), which exhibited significantly high yield and stability potential, were selected as the superior genotypes suitable for future breeding programs (Figure 2).

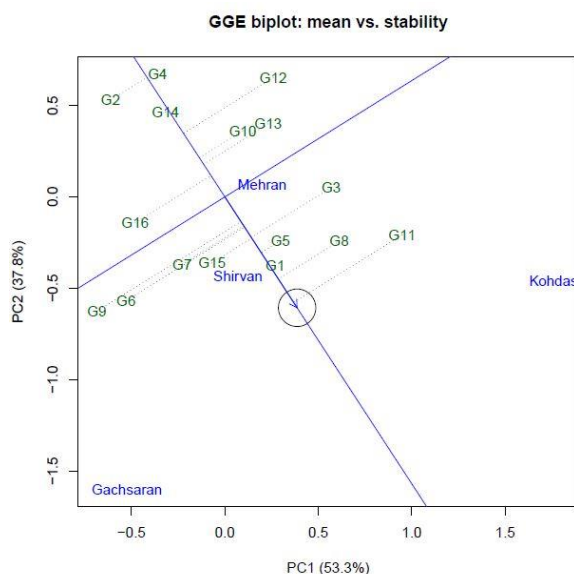


Figure 2. A GGE ranking biplot depicting the mean yield and stability of 16 grass pea genotypes in four locations of Iran.

The vertical dimension, which is perpendicular to the horizontal dimension of ATC, approximates the genotype × environment interaction. Therefore, it can be viewed as an indicator of the diversity or instability of genotypes. Genotypes away from the ATC line are more dependent on the environment, resulting in less stability.

The discriminativeness power and representativeness of experimental sites on grass pea genotypes forage yield

The GGE biplot differentiates growing locations by the discriminativeness and representativeness ability of the GGE view (DEGHANI *et al.*, 2006). The GGE biplot (Figure 3) illustrates the yield of the grass pea genotypes based on those criteria. In a biplot, sites with longer vectors from the origin had greater discriminatory ability for high-yield genotypes, whereas those with shorter vectors had lower discrimination power. The vector of the Gachsaran site had a larger angle than the AEC, making it the least representative site among the experimental sites. A test location must be homogeneous across several years in ranking genotypes for various traits to be considered a suitable location for cultivating and producing a desired cultivar or genotype (YAN *et al.*, 2011).

The selectee environments and genotypes are positioned near or at the center of the concentric circle (see Figure 3). Based on the GGE biplot analysis, genotypes G1 (Greece-I), G5 (Ethiopia), G7 (India), and G15 (Bangladesh-V) were located at the heart of the concentric circles, closer to the ideal genotype, which is characterized as the most stable and high-yielding

grass pea genotype. The other stable and high-yielding grass pea genotypes were located in the next concentric circles, including genotypes G3 and G8 (Bangladesh-I), followed by G11 (Russia) (Figure 3). Genotypes G2 (Greece-II), G4 (Morocco), G14 (Greece-IV), G12 (Bangladesh-III), G13 (Bangladesh-IV), and G16 (Local Check) were identified as the poorest performing genotypes since those are far from the middle of the concentric circle. Moreover, the Mehran can be considered as the ideal environment.

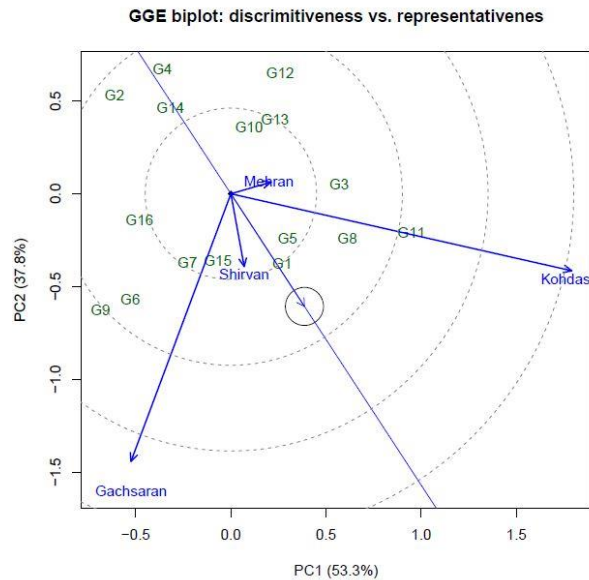


Figure 3. Biplot for environment comparison based on the discriminativeness and representativeness ability of the target environment.

Bi-plot via linking the environments to the origin of the bi-plot through lines, helps to understand the relationships between the environments. Accordingly, some of the environmental vectors including Gachsaran and Kohdasht are very long with a high differentiation ability and have the potential to estimate the relative efficiency of genotypes. Therefore, environments with small vectors cannot be used as reference test environments for the instability experiments.

CONCLUSIONS

The results emphasize the highlight impact of genotype, environment, and their interaction on the yield potential of grass peas in the tested environments. Although some yield-stable genotypes were identified in this study, consistency was not recorded with all high-yielding genotypes. Therefore, grass pea breeders interpret the relative genotypic potentials in various environments. This study evaluated grass pea genotypes through GGE biplot analysis. Evaluation by the GGE biplot revealed five sectors and two mega-environments. Genotypes G1

(Greece-I) and G5 (Ethiopia) were selected for their stability and high yield potential. The identified grass pea genotypes can be further tested in additional growing areas, and the results would provide valuable insights for extension services and pioneering farmers. Moreover, the identified high- and low-yielding grass pea genotypes are important resources in the study of QTLs and other breeding programs.

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**GGE BIPLLOT ANALIZA GENOTIPA × INTERAKCIJA ŽIVOTNE SREDINE
I STABILNOST PRINOSA KRME KOD GENOTIPOVA GRAŠKA**
(*Lathyrus sativus*)

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Izvod

U programima oplemenjivanja useva, biplot analiza je dobro poznata statistička metoda. Ova studija je imala za cilj da istraži efekte interakcije genotip × okruženje (GEI) na proizvodnju graška trave u Iranu. Eksperiment je sproveden u dvanaest sredina (četiri odvojena mesta: Gachsaran, Kuhdasht, Mehran i Shirvanchardavol) tokom tri uzastopne godine (2017, 2018 i 2019) sa šesnaest genotipova trave graška. Svrha ovog istraživanja je bila da se koristi GGE biplot kao alat za identifikaciju superiornih genotipova graška. Prema kombinovanoj analizi varijanse, genotipovi i interakcija genotip-sredina (GEI) imali su značajan uticaj ($p < 0,001$) na prinos krme. Štaviše, interakcija genotip × okruženje je različito reagovala u različitim uslovima testiranja. Komponente interakcije su analizirane putem biplota koji prikazuju glavni efekat genotipa i interakciju genotip × okruženje (GEI). Prve dve glavne komponente (PC) objasnile su 93,11% ukupne varijacije u GGE modelu (tj. G + GE) (PC1 = 53,30%, PC2 = 37,80%). GGE biplot analiza je grupisala testne lokacije u dva mega okruženja za prinos krme. Genotip G11 (Rusija) bio je superiorniji u pogledu srednjeg prinosa krme (5,362 t/ha). Genotipovi koji su se najbolje pokazali u svakoj sredini, kao što je naznačeno poligonom „ko-pobedilo-gde”, bili su genotipovi G11 (Rusija) i G8 (Bangladeš-I). Među ovim genotipovima, G11 (Rusija) je bio genotip sa najvećim prinosom na terenu. Lokacija Kohdasht je bila najzahtjevnije i najreprezentativnije okruženje za testiranje prinosa useva. Odabrani genotipovi se preporučuju za oplemenjivačke programe koji imaju za cilj poboljšanje prinosa krme na ispitivanim lokalitetima ili sličnim agroekološkim područjima.

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