Graphical analysis of forage yield stability under high and low potential circumstances in 16 grass pea (*Lathyrus sativus* L.) genotype

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Abstract: Introducing grass pea genotypes with wide adaptability across diverse environments is important. Dry forage yield of 16 grass pea genotypes, tested in a RCBD design with three replicates across 4 locations over 3 seasons in Iran. The GGE biplot method based on SREG model facilitated a visual evaluation of the best genotypes. The first two principal components accounted for 77 % of the GE interaction and revealed six winning genotypes and four mega-environments. The average location coordinate (ALC) was used to examine both yield performance and stability and indicated IFLA-1913, IFLA-1961, IFLA-1812, and IFLA-2025 were the best genotypes. Based on the ideal-genotype approach, genotype G5 was better than all other genotypes and showed both high forage yield and stability across locations. According to G + GE sources of variations, the genotypes (IFLA-1913, IFLA-1961, IFLA-1812, and IFLA-2025) were the most suitable varieties for the grass pea-producing regions in semi-arid and rain-fed conditions. An ideal location should be both discriminating of the genotypes and representative of the average location, but we could not find such location in this research. Results confirmed that G5 (IFLA-1961) has high stability and high yield performance (4.92 t ha⁻¹), and could introduce as favorable genotype for commercial variety release.

Key words: average location coordinate; biplot; GGE (Genotype+ Genotype Environment interaction)

Analiza stabilnosti pridelka krme 16 genotipov navadnega grahorja (*Lathyrus sativus* L.) v ugodnih in slabih okoljskih razmerah

Izvleček: Vzgoja genotipov navadnega grahorja z veliko prilagodljivostjo v različnih okoljih je zelo pomembna za pridelavo krme. Pridelek suhe krme 16 genotipov navadnega grahorja je bil preiskušen v popolnem naključnem bločnem poskusi s tremi ponovitvami na štirih lokacijah v treh rastnih sezonah v Iranu. Grafična analiza odnosov med genotipi in različnimi okolji je na osnovi SREG (Site Regression model) modela omogočila ovrednotenje najboljših genotipov. Prvi dve glavni komponenti sta razložili 77 % interakcij med genotipi in okoljem (GE) in odkrili šest zmagovalnih gentipov v štirih mega okoljih. Za preverjanje najboljših genotipov glede pridelka in njegove stabilnosti je bila uporabljena poprečna koordinata lokacije (ALC), ki je označila genotype kot so IFLA-1913, IFLA-1961, IFLA-1812, in IFLA-2025 kot najboljše. Na osnovi koncepta idealnega genotipa je bil genotip G5 boljši kot vsi ostali, saj je imel velik in stabilen pridelek krme na vseh preučevanih lokacijah. Glede na vire razlik v interakcijah med genotipi in genotipi in okoljem (G + GE) so bili genotipi IFLA-1913, IFLA-1961, IFLA-1812, in IFLA-2025 najprimernejše sorte navadnega grahorja za pridelavo krme v razmerah polsušnih in z dežjem namakanih območij. Idealna lokacija bi morala biti prepoznana po genotipu in reprezentativni poprečni lokaciji, a takšne v tej raziskavi niso našli. Rezultati so potrdili, da bi lahko bil genotip G5 (IFLA-1961) z veliko stabilnostjo in velikostjo pridelka (4,92 t ha⁻¹) lahko uveden kot priporočena komercialna sorta.

Ključne besede: povprečna koordinata lokacije; biplot; GGE (genotip + interakcije genotip-okolje)

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1 INTRODUCTION

Pulses from the Leguminosae family contains about 13,000 species which their seeds can be fractionated to obtain starch and protein concentrates as well as a by-product of the process, dietary fiber. The genus *Lathyrus* comprises approximately 160 species, primarily native to temperate regions of the world (Gcdt, 2007). Grass pea (*Lathyrus sativus* L.) is an important major pulse crop in some Asian countries where it is produced for feed and food. It was cultivated around 8000 BC due to archaeological investigations in the Middle East, and its seeds were observed among founded archaeological items (Lambein and Kuo-Genth, 1997). Grass pea (2n = 2x = 14) as an ancient crop, is found in Eurasia, North America, temperate South America and East Africa and perhaps one of the first crops to be domesticated, but its origin is not known; however, its presumed center of origin is Southwest and Central Asia (Smartt et al., 1994). It has been used as a pulse and its production as a forage crop has resulted in little evolutionary progress which result in its extensive dispersion worldwide while its excessive consumption may provoke the neurological form of lathyrism, so some efforts have been performed for selection and development of cultivars of low toxicity.

Also, the cultivation of grass pea has an important role as a useful rotation crop in the soils adapted annual cereals and pulses crops and is presently considered as a model crop for sustainable agriculture with a great future because it is unique in that it can thrive under adverse environmental conditions such as drought and flooding. In the past decade's little effort has been performed towards genetic improvement of this crop as food, due to its successful utilization as a forage crop while it has potential as an alternative pulse in many cropping systems around the world as it is very tolerant of drought stress and is not affected by extreme rainfall (Croft et al., 1999). Despite its obvious advantages, relatively little effort has been done in the breeding of this hardy pulse crop while exception of its neurotoxin problem, is produced in significant quantities in many parts of the world and recently its breeding is now being performed in many countries through germplasm collection and evaluation, as well as breeding programs. Iran has grass pea breeding program in recent years with the forage yield as the most important objective due to the large demand for forage, supported by the national institute known as Dryland Agricultural Research Institute (DARI). Development of new grass pea cultivars is done to meet the requirements of consumers' demands (especially high forage yield), and breeders need consistent access to newly genetic improved plant materials.

In multi-environmental testing trials genetically, improved lines are evaluated in different locations and years before the final recommendation of cultivars. For forage yield, the relative performance of genotypes varies from an environment to another environment, i.e., there is a genotype by environment (GE) interaction which is the result of changes in the relative ranking of the genotypes or changes in the amounts of differences among genotypes from one environment to another, making it difficult to detect which genotypes should be chosen (Kang, 2002). The effectiveness of selection is also decreased by the large magnitudes of GE interaction, and various efforts have been performed to overcome the problems created by GE interaction. The estimates of GE interaction provide useful information on the existence and magnitude of GE interaction, but give no information of the response of individual genotypes with the test environment, and therefore no measurement of the stability of individual genotypes. Now, interest has been focused on the GGE biplot analysis, and approach proposed by Yan et al. (2000) which developed a graphical analysis of multi-environment trial data considering both the G (genotype effect) and GE interaction effect (G+GE), simultaneously via biplot presentation. The GGE biplot approach is a type of linear bilinear model suitable for grouping environments and genotypes which is known as the site regression (SREG) model and related biplots are drawn from graphing the first two principal components (PC1 and PC2). There is little information on the stability and yielding ability of grass pea under rainfed conditions and this study was designed to; (i) evaluate the forage production of genotypes; (ii) determine the GE interaction; and (iii) study the adaptation of genotypes using GGE biplot method for commercial recommendation as well as identification of mega-environments in target grass pea producing locations.

2 MATERIALS AND METHODS

2.1 EXPERIMENTS

Sixteen grass pea genotypes from diverse origins were used to examine GE interactions and forage yield stability analysis (Table 1). Seeds of these genotypes were supplied by the International Center for Agricultural Research in Dry Areas (ICARDA) and most of them were developed with the support of ICARDA. The genotypes were planted at four locations during three growing years (2017-2019) under rain-fed conditions. In each environment (year-location), genotypes were grown according to the randomized complete block design with three replicates. The plots were 4.5 m long and 1 m apart (four rows with spacing of 25 cm between rows) and the seeding

Table 1: The origin of 16 grass pea (*Lathyrus sativus* L.) genotypes evaluated in four locations across three years in Iran

Code	Name	Origin
G1	IFLA-1707	Morocco
G2	IFLA-1864	Bangladesh
G3	IFLA-1813	Pakistan
G4	IFLA-1913	Nepal
G5	IFLA-1961	Nepal
G6	IFLA-1553	Morocco
G7	IFLA-1857	Bangladesh
G8	IFLA-1812	Pakistan
G9	IFLA-1547	Morocco
G10	IFLA-2341	Bangladesh
G11	IFLA-2025	Bangladesh
G12	IFLA-2750	Bangladesh
G13	Naghadeh	Iran
G14	Sel.290	Iran
G15	Sel.449	Iran
G16	Sel.587	Iran

rate was 150 seeds per m² in all the environments. The fields were not supplied with irrigation and fertilizer and the weed control was carried out by hand during crop growth. In all environments, for excluding border effects, only the central two rows were harvested (0.5 ×4.0 m plots equal to 2.0 m²) for forage yield recording at 50 % flowering stage and then obtained data was converted to tons per hectare scale for statistical analysis.

2.2 DATA ANALYSIS

The GGE biplot approach was performed considering the simplified model for the first two principal components as:

$$Y_{ij} - y_{.j} = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

where: Y_{ij} is the mean dry forage yield of genotype *i* in environment *j*; $y_{.j}$ is the mean of environment *j*; $\lambda_1 \xi_{i1} \eta_{j1}$ is the first principal component (PC1); $\lambda_2 \xi_{i2} \eta_{j2}$ is the second principal component (PC2); λ_1 and λ_2 are the eigenvalues related to the PC1 and PC2, respectively; ξ_{i1} and ξ_{i2} are scores of the PC1 and PC2 axes for G effects; η_{j1} and η_{j2} are the scores of the PC1 and PC2 for E effects; and ε_{ij} is the error term or residual of the model. The GGE biplot was constructed by first subjecting the GGE matrix (the environment-centered data) to singular-value decomposition (SVD). The used symmetric scaling method

has the advantage that PC1 and PC2 have the same unit (square root of original unit t ha⁻¹ in terms of dry forage yield). Burgueno et al (2003) developed a SAS program for obtaining the SREG analysis while to facilitate the use of GGE biplot method Yan (2001) developed a Windows application called GGE biplot and both packages were used for performing all statistical analyses in the present investigation.

3 RESULTS AND DISCUSSION

3.1 ANALYSIS OF VARIANCE

Mean forage yield for grass pea genotypes across each environment (year-location) were comprised via LSD test (Table 2). The summary of the yearly combined analysis of variance (Table 3) showed that all sources of variations were significant by the F test and these results demonstrate the existence of locational heterogeneity and also indicate significant differences among the genotypes since their responses were not coincident in the test locations. Variance components for L, G, and GL interaction based on the yearly data showed their relative magnitudes as the L was always the most important source of variation (relative to G and GL interaction) accounting for 94.6, 86.9 and 84.6 % of the total variance (G + L + GL). When the SREG model was fitted, the first two PCs explained about 77 % (PC1 = 44.3 % and PC2 = 32.5 %) of G + GE variation for grass pea multi-environmental trails (Table 4). In this research, F-test Gollob (1968) was applied to test the significance of PCs for the SREG model which indicated two significant PCs. The amount of GE interaction for the dry forage yield of 16 grass pea genotypes tested across four locations was larger than that of G effect, but smaller than that of E effect (Table 3). The genotypes indicated both crossover and additive types of GE interaction which led to differential rankings of genotypes across locations, thereby making selection difficult under the rain-fed circumstances. The relative contributions of G and GE interaction effects to the total variation for dry forage yield observed in this research are similar to those found in other crop GE interaction investigations in rain-fed climates (Berteroa et al., 2004; Sabaghnia et al., 2013). This founding proposes that it would be difficult to gain an indirect response to selection over all of the grass pea target plant materials of locations from selection in a few locations as well as environments, ignoring the GE interaction. In other words, GE interaction makes it difficult to select the most favorable (high yield and most stable) and so it must be considered in breeding programs because it reduces the selection promotion (Yau, 1995). Finally, results of yearly

Table 2: Mean forage yield for grass pea multi-environmental trials, 2017 to 2019

	2017				2018				2019				Mean
	Gac.	Kho.	Meh.	Shi.	Gac.	Kho.	Meh.	Shi.	Gac.	Kho.	Meh.	Shi.	
G1	1.79	3.66	1.00	10.10	2.11	5.22	1.94	8.80	7.21	3.69	6.80	3.73	4.67
G2	1.19	4.12	0.94	8.54	2.32	6.25	1.56	8.52	7.62	3.33	8.27	3.62	4.69
G3	0.94	3.42	1.22	8.06	1.74	5.78	1.96	6.24	8.86	3.39	12.00	4.49	4.84
G4	1.39	1.59	1.05	7.74	2.05	4.26	2.01	7.60	4.51	2.49	14.00	5.11	4.48
G5	1.61	2.27	1.14	9.48	2.55	3.13	2.19	8.20	7.46	2.88	13.87	4.22	4.92
G6	1.45	3.10	0.98	8.16	1.93	5.10	2.00	8.06	5.17	3.42	11.47	5.03	4.66
G7	1.86	2.83	1.11	8.56	2.46	4.09	2.00	6.67	7.71	4.08	12.40	4.94	4.89
G8	1.10	2.94	1.10	9.63	1.41	4.70	2.04	8.11	5.94	2.21	12.27	4.71	4.68
G9	1.01	3.10	0.90	7.82	2.53	5.32	2.01	7.16	6.41	4.02	13.07	4.54	4.82
G10	1.25	2.82	0.97	8.30	1.82	5.96	1.64	6.12	7.78	3.12	12.13	3.59	4.62
G11	1.67	2.46	1.58	8.54	2.27	5.77	2.84	6.74	9.71	3.59	13.73	5.27	5.35
G12	1.41	2.89	0.74	7.87	1.52	4.90	1.26	5.43	4.83	2.65	10.27	4.32	4.01
G13	1.70	2.46	1.10	6.24	1.84	8.04	2.06	6.90	6.76	2.90	13.60	3.82	4.78
G14	1.79	2.70	0.98	6.94	1.52	5.76	2.08	5.73	6.29	3.00	8.00	2.15	3.91
G15	2.27	3.09	1.18	5.91	2.10	5.48	2.09	5.01	7.24	2.74	11.20	1.77	4.17
G16	1.50	2.96	1.09	6.73	1.45	6.17	1.93	5.12	5.40	3.09	13.87	2.49	4.32
LSD†	0.70	0.80	0.24	2.35	1.24	1.96	0.16	2.21	2.46	1.69	4.08	2.16	0.69

Locations are: Gac., Gachsaran; Kho., Khoramabad; Meh., Mehran and Shi., Shirvan
† LSD (0.05) = 0.69 t ha^{-1} for comparison of mean dry forage yield within an environment (location–year)

analysis of variance indicated the large variation due to location which is irrelevant to genotype assessment as well as mega-environment identification (Gauch et al., 2008), legitimatized the use of SREG (Yan et al., 2000) as a proper method for analyzing the multi-environmental trials data of the current study.

3.2 MEGA-ENVIRONMENTS

The establishment of six mega-environments, that is, environments defined by winner genotypes which are the farthest from the biplot origin, is indicated in Fig. 1. The winner genotypes (G1, G5, G11, G14, G15 and G16)

Table 3: Genotype (G), location (L), and genotype × location (GL) variance terms for grass pea multi-environmental trials, 2017 to 2019

		2017		2018		2019	
Sources of Variation	DF†	SS‡	% of §	SS	% of §	SS	% of §
Location (L)	3	1479.6**	94.6	886.2**	86.9	2130.1**	84.6
Replication within L	8	18.7		27.8		205.6	
Genotype (G)	15	19.9*	1.3	27.8*	2.7	126.2**	5.0
GL	45	63.9**	4.1	106.0**	10.4	261.9**	10.4
Error	120	72.4		111.0		325.8	

** and * Significant at the 0.01 and 0.05 level, respectively.
† DF is degrees of freedom.
‡ SS is sum of squares.
§ % of L+G+GL

Table 4: Site regression (SREG) analysis of variance for dry yield of 16 grass pea genotypes

Sources of Variation	DF†	SS‡	MS§	% of GE
Environment (E)	3	320.89	106.96**	
Genotype (G)	15	32.11	2.14**	
GE	45	105.36	2.34**	
SREG Model				
PC1	17	60.97	3.59**	44.3
PC2	15	44.72	2.98**	32.5
Residual SREG	24	31.79	1.32ns	23.1

**, * and ns significant at the 0.01 and 0.05 level, and nonsignificant, respectively.
† DF is degrees of freedom.
‡ SS is sum of squares
§ MS is mean squares.

are located on the borders of the polygons, and the mega-environments are separated by lines perpendicular to the polygon but only the genotypes G1, G5, G11 and G14 determined the mega-environments I (Shirvan), II (Gachsaran), III (Mehran) and IV (Khoramabad), respectively. In other words, these genotypes are recommended for locations included within each mega-environment but genotypes G15 and G16 did not give the highest yield in any of the locations. The mega-environment III, Mehran location, was distinct from the mega-environments due to high PC2 values which is demonstrating that Mehran location contributed most to the GE interaction, and was therefore recommended for future investigation of

adaptability. In a more detailed trial analysis, locations with the same GE response pattern (located in the same mega-environment) can be discarded but in this study, our locations felled in different mega-environments and none of them can be discarded. In other words, this result demonstrates the benefits of the GGE biplot method since locations with different patterns of genotype response and unlike patterns of GE interaction are maintained. Therefore, the polygon view of GGE biplot method suggests that there exist four possible grass pea mega-environments in Iran but this pattern requires validation through other multi-environment trials. As discussed by Sabaghnia et al. (2012), the above inferences
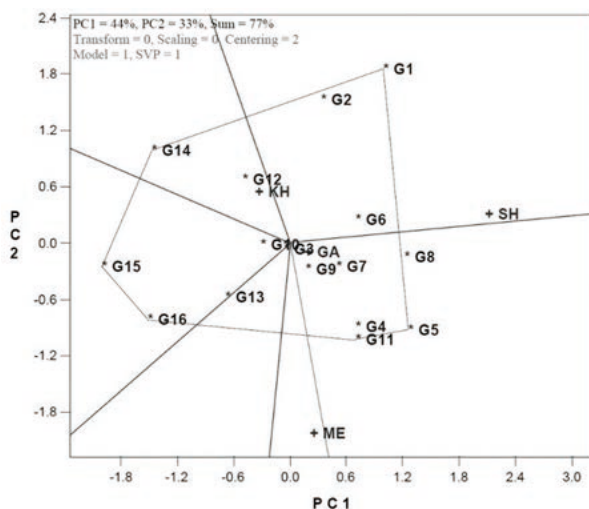

Figure 1: Site regression (SREG) biplot identification of winning genotypes and their mega-environments. Sixteen grass pea genotypes grown in four locations: GA, Gachsaran; KH, Khoramabad; ME, Mehran; and SH, Shirvan. Commonly PC1 indicates the additive effects and the PC2 shows the interaction effects
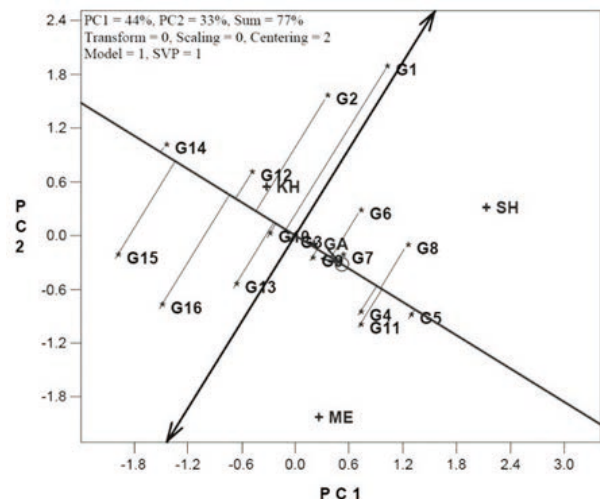

Figure 2: Site regression (SREG) biplot of mean and stability of sixteen grass pea genotypes for dry yield and specific genotype × environment interactions. Four locations are: GA, Gachsaran; KH, Khoramabad; ME, Mehran; and SH, Shirvan. Commonly PC1 indicates the additive effects and the PC2 shows the interaction effects

about observed patterns are mostly verified from the original data because GGE model is fitted to the original data incompletely, and the model consequence is valuable for recommendation purposes since, as reported by Yan (2002) and applied to GE modelling by Gauch et al. (2008).

The mean forage yield performance and stability property of the genotypes were examined by defining an average location coordinate (ALC) and an average location is indicated virtually by a circle and indicates the positive end of the ALC x axis (Fig. 2). The average yield performance of the genotypes is approximated by the projections of their markers on the ALC axis. According to Yan et al. (2000), in the GGE biplot method, the PC1 shows the genotype adaptability due to the high association of adaptability and high yielding while the PC2 shows the stability (genotypes low PC2 would be stable). In this research, the length of the average location vector was adequate to detect genotypes based on forage yield mean performances and some superior genotypes with above-average means (such as: G4, G5, G8 and G11) were selected, whereas the others were discarded while genotype G5 was the most stable genotype as well as high yielding (Fig. 2). Conversely, G1 was the least stable genotype (variable performance) but yielded with average means while genotype G14 was the least yielding. Our results confirmed that genotype G5 (IFLA-1961) has high stability and high yield performance (4.92 t ha^{-1}), therefore is introduced as the most favorable genotype. The need for the application of SREG model based GGE biplot for the determination of the premier genotypes is
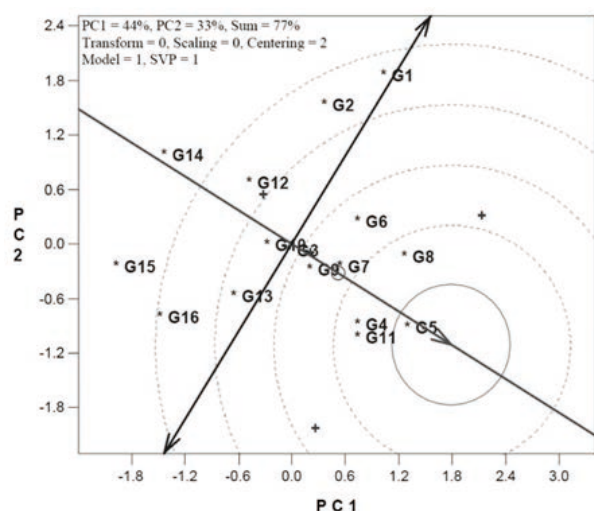
to facilitate the identification of such genotypes (Yan et al., 2007). This research has clearly that the SREG model can analyze GE interaction patterns plus G main effect and reveal the associations of genotypes and locations successfully as well as prepare a worthwhile prediction. Although, according to Sabaghnia (2010), the multivariate methods such as SREG model are too sophisticated to prepare a simple measure of yield stability but using graphical facilities of biplot presentation and their integration via GGE biplot method can eliminate such sophistication and provide a simple method for interpretation of GE interaction.

3.3 IDEAL GENOTYPE

An ideal genotype is one that has both high yield performance as well as high stability and the center of concentric circles (Fig. 3) indicates its position which is defined by a projection onto the mean-environment axis that equals the longest vector of the genotypes that had above-average mean yields and by a zero projection onto the perpendicular line as an index of minimum variation across all locations. The closer a genotype to this position is the more valuable it is and such an ideal genotype may not exist in reality, it only can be applied as an index for comparison (Yan and Tinker, 2006). In the biplot of ideal genotype, the ranking of genotypes is performed based on the genotype-focused scaling which assumes that both stability and yield are equally important thus,
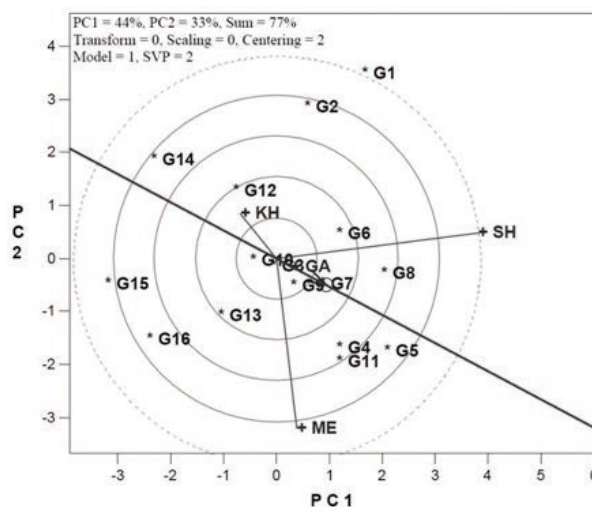

Figure 3: Site regression (SREG) biplot of ideal genotype and comparison of the sixteen grass pea genotypes with the ideal genotype. Commonly PC1 indicates the additive effects and the PC2 shows the interaction effects

Figure 4: Site regression (SREG) biplot of discrimitiveness versus representativeness of testers. Four locations are: GA, Gachsaran; KH, Khoramabad; ME, Mehran; and SH, Shirvan. Commonly PC1 indicates the additive effects and the PC2 shows the interaction effects

G5 which is close to the center of concentric circles, was an ideal genotype in terms of yield potential and stability compared with the other grass pea genotypes. Following G5, genotypes G4, G8 and G11 located in the next concentric circle were also considered as superior genotypes regarding both yield and stability (Fig. 3). The PC1 and PC2 scores of SREG model indicates the yield and stability, respectively as they are comparable to the G main effect or yield performance and adaptability index (line slope coefficient) of the joint linear regression model in the method of Eberhart and Russel (1966). The relative contributions of stability and yield performance to the detection of the most favorable genotypes found in this research by ideal genotype view of GGE biplot method are similar to those found in other crop stability investigations in bean (Kang et al., 2006), lentil (Karimizadeh et al., 2013), and field pea (Yihunie and Gesesse, 2018).

3.4 DISCRIMITIVENESS AND REPRESENTATIVE-NESS

The discriminative and representativeness properties of the test locations for grass pea dataset were explored by GGE biplot method and results are shown in Fig. 4 and similar to Fig. 2, the average location coordinate (ALC) passes through the average location and the biplot origin relative to genotype mean yield performance and the small circle is the average location, and the arrow pointing to it is used show the direction of the ALC. The locations that have shorter vectors are less informative in contrast to those with longer vectors, thus the locations Shirvan and Mehran were the most discriminating locations based on their vector length which are mostly in the warm zone which is characterized by low rainfall (Table 2). However, these discriminating locations can distinguish among tested genotypes and results of genotypes' comparison is more reliable. Also, the angle between a location vector and the ALC represents the representativeness of the location; the large angle causes less representativeness of location, thus locations Gachsaran and Khoramabad were most representative whereas Shirvan

and Mehran were the least representative (Fig. 4). The most representative locations (Gachsaran and Khoramabad) are the dry zone and are characterized by high seasonal precipitation and a high risk of drought (Table 5). However, these representative locations can be regarded as the best agent among tested locations and can show characteristics of warm and dry areas. An ideal location should be both discriminating of the genotypes and representative of the average location, but we could not find such location in this research. To make breeding progress testing locations should be a combination of high and low yielding locations and in most cases testing and selection of genotypes are performed under high potential circumstances and selected genotypes usually exhibit poorly under low potential circumstances in contrast to genotypes that are selected under both circumstances (Setimela et al., 2007). To make a breeding gain, selection should be done under both low and high potential locations which permits the plant breeder to identify genotypes that will improve yield performance for both circumstances.

4 CONCLUSIONS

We identified the mega-environments I (Shirvan), II (Gachsaran), III (Mehran) and IV (Khoramabad) for grass pea production. The G5 (IFLA-1961) was an ideal genotype in terms of yield potential and stability compared with the other grass pea genotypes. According to the discriminativeness and representativeness properties of the test locations for grass pea, Shirvan and Mehran were the most discriminating locations. Finally, we found G5 (IFLA-1961) as high stable and high yield (4.92 t ha^{-1}) genotype for commercial variety release.

5 ACKNOWLEDGMENT

Table 5: Agro-climatic characteristics of test locations in multi-environmental trails of grass pea

Location	Longitude	Latitude	Altitude (m)	Rainfall (mm)	AYT†	Max	Min
Gachsaran	30°18′N	50°59′E	668	443	15.7	23.6	7.9
Khoramabad	33°39′N	48°28′E	1125	520	17.9	26.0	9.9
Mehran	33°07′N	46°09′E	136	275	18.0	24.6	11.3
Shirvan	37°27′N	57°55′E	1091	227	14.8	21.6	8.0

†AYT, average yearly temperature

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