

(Original Article)



The Interaction of Genotype × Environment for Seed Yield in Alfalfa (*Medicago sativa* L.)

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Abstract

The current work was studied the interaction of genotype environment for alfalfa seed yield. The split block design in three replications used for this study. Five sowing dates of 10th October (D₁), 10th November (D₂), 10th December (D₃), 20th March (D₄) and 20th April (D₅) were used. Seed yield was taken in the second year in the beginning of March, April and May for studied sowing dates. The genotype plus genotype by environment interaction (GGE) biplot analysis was done. The obtained results showed that the environments exhibited high contribution in the total sum of squares for seed yield/plant (SYP) and seed yield/m² (SYW) in values of 60.69 and 60.80%, respectively, which accounted ten times of genotypes contribution. PC1 and PC2 were significant factors as revealed from GGE-biplot analysis and explaining 40.23 and 21.36% for seed yield/ plant and 37.04 and 17.83% for seed yield/m² in total sum of squares, respectively. According to the GGE-biplot analysis, the genotype G9 was the highest seed yield and stable genotype across environments and the environment E₄ (sowing at 20th March) was yielded the highest seed yield/ plot which recorded 66.06 g.

Keywords: Alfalfa, *Medicago sativa*, Genotype environment interaction, Sowing dates, Seed yield

Introduction

Alfalfa or Lucerne (*Medicago sativa* L.) is a highly productive forage legume of global importance. Being a perennial, it's had been called "The king of the Forages". It is one of the most important forage species in many countries for high production, total area, economic value and energy efficiency. In Egypt, the total cultivated area of alfalfa was about 73321 feddan (one feddan = 4200 m²) with an estimated productivity of about 1953422 tons of green fodder (B.A.S, 2018). Because of alfalfa can fix nitrogen and synthesize protein, it is very useful to farmers, who have grown alfalfa as protein-rich fodder for cows, goats, sheep, chickens and others. Alfalfa is sown across the semi-arid and humid regions of the world.

The changes of climatic conditions at present time towards warming as in Egypt are expected to affect the crops productivity. Alfalfa is adapting with the changed sowing date to overcome the high or low temperature at the beginning of

season. Few workers practiced that matter. Seed yield of Alfalfa depends on many factors i.e., climatic condition and insect activity during the blooming period (Martiniello *et al.*, 1999).

Variation in climatic condition during development stages may affect the different response of alfalfa genotypes to environments. Because alfalfa genotypes are sown across a large range of conditions, i.e., type and fertility of soil, moisture, temperature and sowing date. All the variables encountered in producing alfalfa can be described collectively as the environment. Therefore, when the alfalfa genotypes are grown in the wide environments, they will be different in their performance. These changes of genotypes performance are interpreted to the interaction of genotype × environment. Such information could be used to design powerful improvement programs to develop new productive varieties or to improve the crop management i.e., forage and seed production of Alfalfa.

In Egypt, scarce information is available regarding to the effect of change in climatic conditions resulting from different planting dates and the interaction of genotype × environment on seed yield of Alfalfa.

Genotype by environment interaction (GEI) has been taking a big point among biologists and breeders since the early twentieth century. There are several statistical models employed to understand the complex GEI term (Yan and Kang 2003).

Recently, GGE biplot analysis is the most used model and a very potential tool for analyze multi-environmental trials (MET) data to interpret GEI (Yan 2001; Yan and Tinker 2006). Moreover, it detects the interaction view graphically besides identifying ‘which-won-where’ and delineation of mega-environments among different locations (Yan *et al.*, 2007). GGE biplot analysis depends on principal components analysis (PCA). Genotype main effect (G), environment main effect (E) and their interaction (GE) must be considered at the same time for evaluation any cultivar (Yan and Tinker 2006; Sabaghnia *et al.*, 2008). Consequently, GGE biplot is more powerful as compared to AMMI in detecting PC1 score, which represents genotypic effect rather than additive main effect (Yan 2002).

The objective of the current investigation was to study the interaction of genotype × environment for seed yield in ten alfalfa genotypes under five sowing dates.

Materials and Methods

The current work was carried out at the Experimental Farm of Agronomy Department, Assiut University, Egypt (27.19 N, 31.16 E; clay soil) during three years from 2017 to 2020 in two experiments.

The experimental soil characteristics are sand (25.9%), silt (24.7%), clay (49.4), soil pH (7.80), organic matter (1.62%), total nitrogen (0.09%) and CaCO₃ (1.2%).

Ten genotypes of Alfalfa, nine from Egypt i.e., Ismailia-1, Nubaria-1, Ramah-1, Populations from F.R.S., Kharja, El-Dahlia, Farafra, Aswan and Balady, beside one genotype (Cuf 101) introduced from U.S.A.

Two experiments were carried out as experiment I (2017-2019) and experiment II (2018-2020). Each experiment included three autumn sowing dates, i.e., 10th of October (D₁), 10th of November (D₂) and 10th of December (D₃) and three spring sowing dates, i.e., 20th of March (D₄), 20th of April (D₅) and 20th of May (D₆). The sowing date of 20th May (D₆) in both experiments did not germinate under Assiut condition consequently, the rest five sowing dates improved across the two experiments.

A split block arranged in a randomized complete block design (RCBD) and three replications were used in both experiments. Sowing dates were arranged in vertical strips and the genotypes in horizontal strips. Plot size was one meter square (1-meter-long × 1 meter apart). Alfalfa seeds were broadcasted in rate of five g/m² (plot). All cultural practices were arranged using optimum level for maximum alfalfa productivity. In second year for each sowing date, the plants were left until flowering and seed production in the two experiments.

At seed maturity stage the seed yield/plant (SYP) was recorded as average of 10 plants randomly harvested from the center of each plot and for each sowing dates. As well as seed yield/plot, g (SYW) was recorded for each plot.

Table 1. Mean of daily temperature (°C) during the period of alfalfa growth from 2017 to 2020 years

Month	Average temperature (°C)								
	2017/2018			2018/2019			2019/2020		
	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
Oct.	31.93	16.90	24.42	32.13	18.13	25.13	33.20	19.53	26.37
Nov.	24.70	11.37	18.03	26.20	12.90	19.55	28.13	14.00	21.07
Dec.	22.53	9.17	15.85	20.53	8.30	14.42	21.07	8.47	14.77
Jan.	19.50	6.90	13.20	18.83	6.07	12.45	18.17	5.93	12.05
Feb.	25.57	11.87	18.72	21.33	7.83	14.58	23.37	7.70	15.53
Mar.	30.10	14.57	22.33	24.33	10.00	17.17	25.77	11.53	18.65
Apr.	32.07	16.30	24.18	29.57	14.13	21.85	29.77	15.17	22.47
May	37.33	22.20	29.77	37.23	22.33	29.78	34.77	19.53	27.15
Jun.	38.23	22.80	30.52	38.53	25.10	31.82	38.13	23.07	30.60
Jul.	37.63	25.07	31.35	38.57	24.80	31.68	38.27	24.13	31.20
Aug.	36.80	25.10	30.95	35.03	25.57	30.30	38.07	23.67	30.87
Sept.	35.17	22.13	28.65	34.73	21.73	28.23	37.23	25.96	31.60

Climatic data during growing seasons are presented in Table1. The total growing degree days (GDD) (base=7) were calculated for each sowing date according to Saeed and Francis (1984) as follows (Table 2):

"Total growing degree days (GDD)

$$= \Sigma[(Maximum + Minimum\ temperature)/2] - 7"$$

Where, 7= Zero growth point.

Table 2. Total growing degree days (GDD) for each sowing date

Sowing date	Seed yield at second years until seed maturity	
	2019	2020
10 th of October	1650	1592
10 th of November	2007	1917
10 th of December	2264	2215
20 th of March	1990	1954
20 th of April	2376	1917

Stability analysis

The data of seed yield for all genotypes recorded in five planting dates across two years (represent 10 environments i.e., E₁ = first sowing date in the first year, ... and E₁₀ = five sowing date in the second year) were arranged for the combined analysis of variance (ANOVA) to detect the effects of each of environment, genotype and GEI using the Statistical Analysis System SAS (SAS Institute, ver. 9.2 2008), PROC GLM procedure. Bartlett's test used to test the homogeneity of variances among all environments. The GGE biplot analysis was done according to Yan *et al.* (2000) formula:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \epsilon_{ij}$$

Where, Y_{ij} : mean for the i^{th} genotype in the j^{th} environment, μ : grand mean, β_j : main effect of environment j , λ_1 and λ_2 : singular values of the 1st and 2nd principal components, ξ_{i1} and ξ_{i2} : PC1 and PC2 scores, respectively, for genotype i^{th} , η_{j1} and η_{j2} : eigenvectors for the j^{th} environment for PC1 and PC2 and ϵ_{ij} : residual error term. The GGE biplot analysis applied using Genstat ver. 15th Edition (Payne *et al.*, 2012).

Results and Discussion

Stability analysis for seed yield

Analysis of variance and means

Mean squares and contribution percentages of environments (E), genotypes (G), and their interaction (G by E) to the total sum of squares for the seed yield/ plant (SYP) and seed yield/ plot (SYW) are shown in Table 3. Since highly significant differences were observed for all sources of variance. Moreover, the environments exhibited the highest percentage from the total sum of squares for SYP and SYW and explained 60.69 and 60.86%, respectively. Also, this contribution of the environments was approximately ten times of genotypes contribution which explained 6.87 and 5.15% for both traits, respectively. The interaction of genotypes × environments accounted for 29.89 and 23.58% of the total variance for both traits, respectively. The high significance of all sources of

variation indicates the different responses of the genotypes to the planting dates for seed yield

Table 3. The combined analysis of variance for seed yield per plant (SYP) and seed yield per plot (SYW) for the ten investigated genotypes across tested environments (planting dates)

S.O.V	d.f.	M.S		Contr. %	
		SYP	SYW	SYP	SYW
Env.	9	0.53**	5870.46**	60.69	60.86
Geno.	9	0.06**	497.13**	6.87	5.15
G×E	81	0.029**	252.70**	29.89	23.58
Error	200	0.001	45.18	-	-

** Significant at 0.01 level of probability., S.O.V.: Sours of variance , d.f.: Degree of freedom and M.S.: means square .

Table 4. Least squares mean of seed yield per plant and seed yield per plot for genotypes across environments (planting dates) and environments across genotypes

Seed yield per plant				Seed yield per plot			
Genotype		Environments		Genotype		Environments	
G1	0.234 ^F	E ₁	0.203 ^F	G1	40.76 ^F	E ₁	55.00 ^C
G2	0.247 ^F	E ₂	0.289 ^D	G2	40.09 ^F	E ₂	42.23 ^E
G3	0.250 ^F	E ₃	0.146 ^H	G3	42.04 ^{EF}	E ₃	49.12 ^D
G4	0.275 ^E	E ₄	0.277 ^D	G4	45.74 ^{DC}	E ₄	66.06 ^A
G5	0.352 ^{AB}	E ₅	0.312 ^C	G5	49.74 ^{AB}	E ₅	50.92 ^D
G6	0.307 ^D	E ₆	0.505 ^B	G6	44.21 ^{DE}	E ₆	49.75 ^D
G7	0.276 ^E	E ₇	0.546 ^A	G7	44.46 ^{DE}	E ₇	26.72 ^{FG}
G8	0.333 ^{BC}	E ₈	0.276 ^D	G8	45.78 ^{DC}	E ₈	30.00 ^F
G9	0.359 ^A	E ₉	0.170 ^G	G9	52.82 ^A	E ₉	59.13 ^B
G10	0.322 ^{DC}	E ₁₀	0.230 ^E	G10	48.83 ^{AC}	E ₁₀	25.53 ^G
Average	0.296			Average	45.45		
LSD _{0.05}	0.022			LSD _{0.05}	3.42		

Means with the same letter are not significantly different, the different letter is significantly. E₁: first sowing date in first year,, E₁₀: five sowing date in second year.

Table 4 shows the means of seed yield/ plant and seed yield/ plot for all genotypes across planting dates and environments across genotypes. It could be observed that the highest seed yield/ plant was produced by the genotype G9 which recorded 0.359 g followed by the genotypes G5 and G8 which registered 0.352 and 0.333 g, respectively. The lowest genotype across planting dates was G1 which gave 0.234 g. In addition, the analysis revealed that the highest seed yield/ plant was produced by environment E₇ which recorded 0.546 g followed by environment E₆ which recorded 0.505 g. The lowest environment across genotypes was E₃ which gave 0.146 g. The highest seed yield/ plot was yielded by the genotype G9 which recorded 52.82 g followed by the genotypes G5 and G10 which registered

49.74 and 48.83 g, respectively (Table 4). The lowest genotype across planting dates was G2 which gave 40.09 g. Also, environment 4 (E₄) was yielded the highest seed yield/ plot which recorded 66.06 g followed by environment E₉ which recorded 59.13 g, while the lowest environment across genotypes was E₁₀ which gave 25.53 g.

The obtained results revealed different responses for studied genotypes across different environments. The same view of results could be found for different environments along with the genotypes.

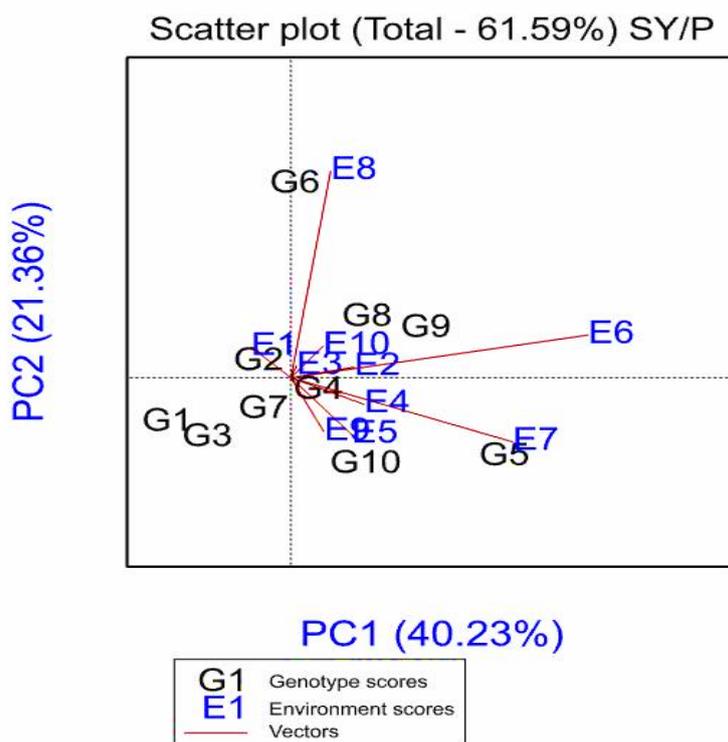


Figure 1. GGE biplot based on environment focused scaling for correlation among environments.

Stability parameter for seed yield/plant

Figure 1 shows the relationship among studied environments. Environments E₆, E₇, and E₈ were the most discriminating environments because they have the longest distance from the origin of the biplot. Environments that have smaller vector angles are closely related, and vice versa; larger vector angles are not correlated or negative correlation.

Figure 2 shows the pattern of seed yield/ plant. The GGE biplot explained which genotypes performed best in which environment as represented from the polygon diagram. The polygon diagram of the GGE biplot is the best method for the detection of winning genotypes by visualizing the patterns of genotype-environment interaction (Yan and Kang, 2003). MET data analysis is helpful to estimate the possible existence of different mega-environments (Yan and Tinker, 2006). In this biplot, polygon vertices were the genotype signs located farthest away from the origin of the biplot in various directions, these genotype signs were

contained within the resulting polygon. Consequently, five genotypes were identified as the signs farthest away from the biplot origin and the others remaining five genotypes lied within this polygon.

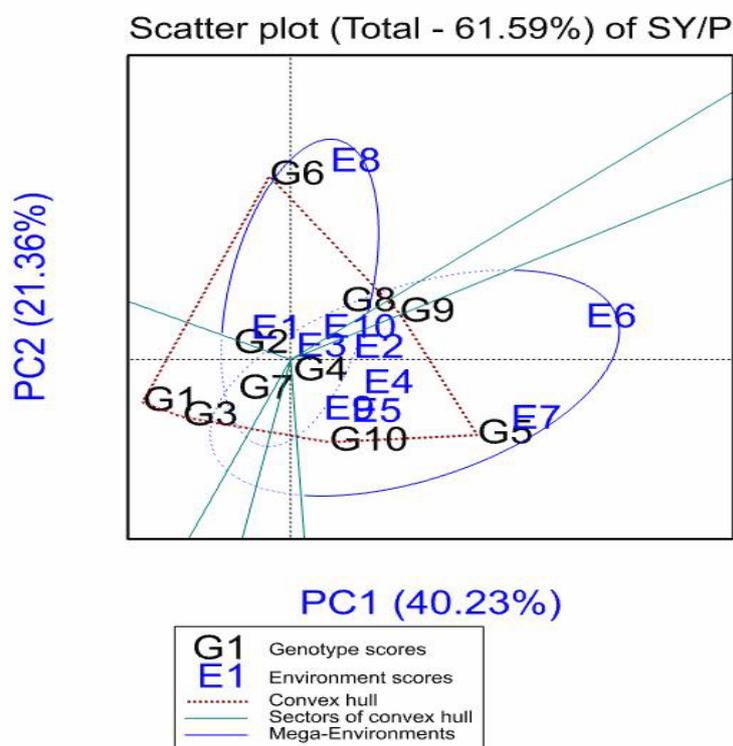


Figure 2. Polygon views of GGE biplot based on environmental scaling for the 'which-won-where' pattern of genotypes and environments.

According to Figure 2, the vertex genotypes were G1, G5, G6, G9, and G10. These genotypes showed the behavior of the best or worst in some or all environments because they are farthest from the biplot origin (Yan and Kang, 2003), (Mostafa, 2020) and were more responsive to environmental changes and recorded as specifically adapted genotypes. G6 was the highest yielding at E₈, while G5 was the best at E₇. The vertex genotype G1 was the poorest genotype in most tested environments since it had the longest distance from the biplot origin on the opposite side of the environments. Also, the GGE biplot analysis showed two mega environments, since the environments are in the same area. These environments are highly correlated, closer together in this biplot. The first mega environment contained sowing dates or environments E₁, E₃, E₈, and E₁₀ in one area, indicating identical conditions of these planting dates. The second mega environment consisted of the other sowing dates. The genotype G9 was the best in the second mega-environment.

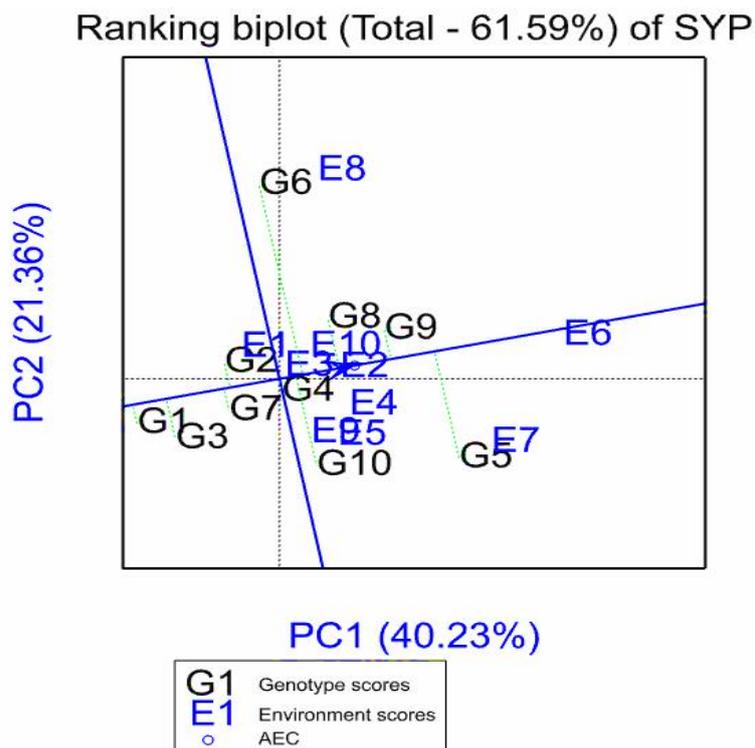


Figure 3. Average environment coordination (AEC) views of the GGE biplot based on genotype focused scaling for the means performance ranking and stability of genotypes.

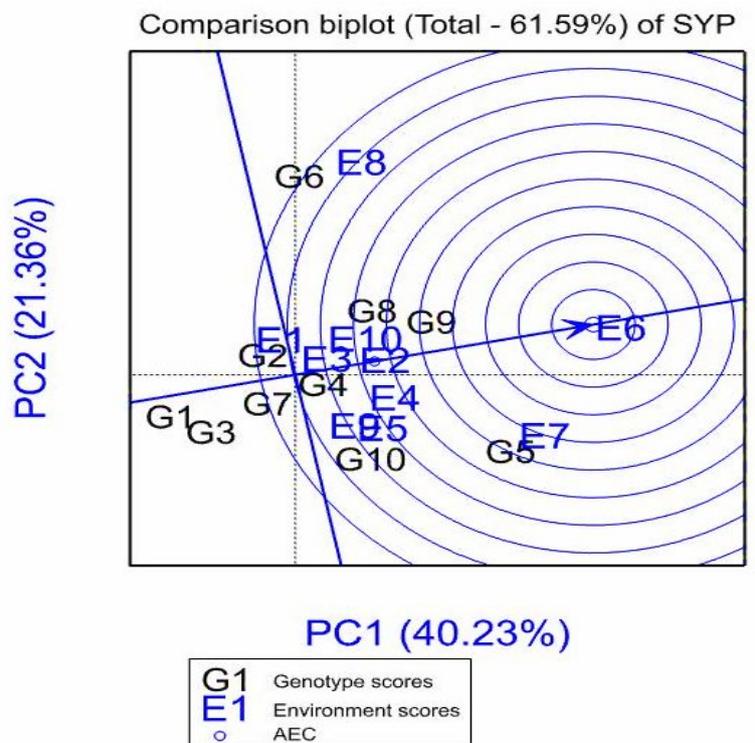


Figure 4. GGE biplot based on environment focused scaling for comparison of the environments with the best test environment.

GGE biplot analysis in Figure 3 revealed that the genotypes G5, G6, and G10 having mean scores greater than the average environment coordinate point and are considered unstable genotypes for seed yield/plant. G2, G4, G8, and G9 exhibited to be the most stable genotypes as they were subtended by relatively low PC2 score. According to this, genotype G9 is a high-yielding and stable genotype across studied environments.

Moreover, GGE biplot based on environment gave scaling for environments comparison by ideal test environment (Figure 4). The best environments were E6 and E7 because they have high average seed yield per plant among the others and the most suitable genotypes to these environments were the genotypes G5 and G9

Stability parameters for seed yield/m²

The GGE-biplot analysis for both genotype and interaction of genotype-environment presented that PC1 and PC2 were significant, and accounted for 37.04 and 17.83% of the total sum of squares, respectively (Figure 5).

Figure 5 represents the relationship among the environments for seed yield/m². Environments E1, E2, E4, E6, and E9 were the most distinguishing environments because they have the longest distance from the biplot origin. The closely related environments recorded smaller vectors angles, whereas; with larger vector angles represent no relationship or negative correlation.

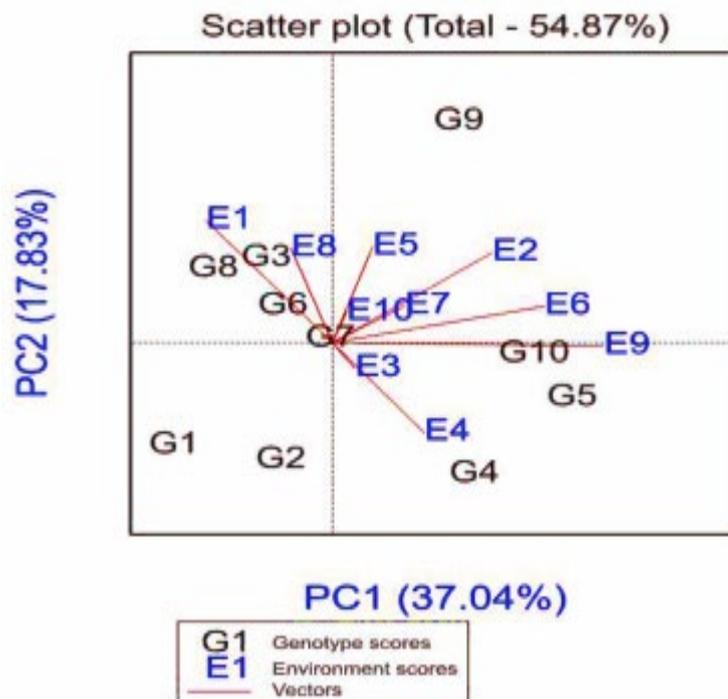


Figure 5. GGE biplot based on environment focused scaling for correlation among environments.

Figure 6 shows the "Which Won Where Pattern of seed yield/m²". Since the polygon profile of the GGE biplot show which genotypes performed best in which environment. In this biplot, the polygon vertices were the genotype signs located

farthest away from the biplot origin in different directions. These all genotype signs were contained within the polygon. Consequently, six genotypes identified with signs farthest away from the biplot origin, but the remaining four genotypes located within the polygon.

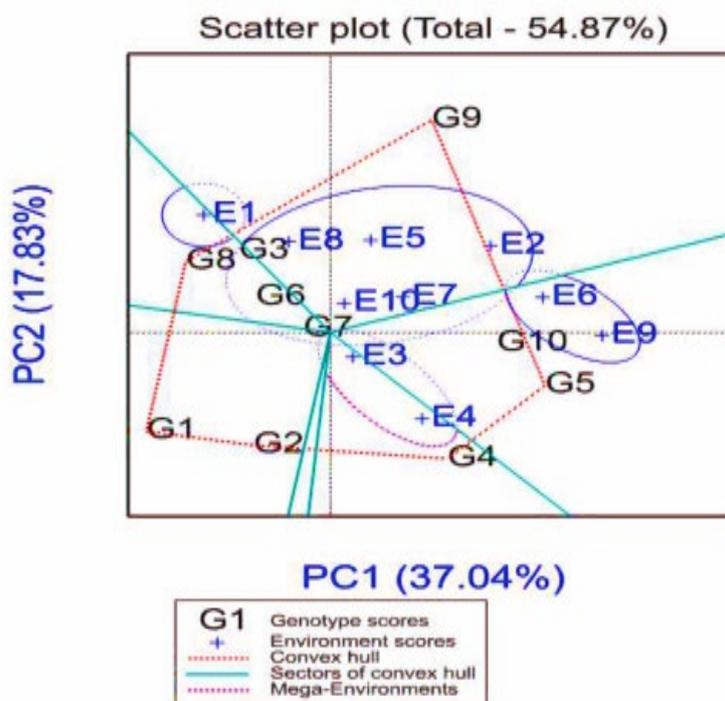


Figure 6. Polygon views of GGE biplot based on environmental scaling for the ‘which-won-where’ pattern of genotypes and environments.

Also Figure 6 released that the vertex genotypes were G1, G2, G4, G5, G8, and G9. The performance of these genotypes was the best or worst across the environments due to their farthest from the biplot origin (Yan and Kang, 2003). Also, these genotypes are more responsive to environmental change and considered as specifically adapted genotypes. G5 and G10 gave the highest yield at E₉ and E₆, respectively. The vertex genotype G1 was the poorest across most of the environments since it had the longest distance from the biplot origin of the biplot on the adverse side of the environments. Also, the GGE biplot analysis revealed four mega environments, since they located in the same area. These environments are highly correlated; they are closer together in this biplot. The first mega environment contained only the sowing date or environment E₁. The second mega-environment consisted of E₂, E₅, E₇, E₈, and E₁₀ in one area. The third mega environment consisted sowing dates or E₃ and E₄. The fourth mega-environment consisted of sowing dates or E₆ and E₉ in one area.

GGE biplot analysis in Figure 7, revealed that the genotypes G10, G5, G4, G8 and G3 having mean scores greater than the average environment coordinate point and considered as unstable genotypes for seed yield/plant. Genotypes G1, G7 G2 and G9 were the most stable genotypes as they were subtended by relatively

low PC2 score. According to this, genotype G9 possessed high yield and stable genotype across environments.

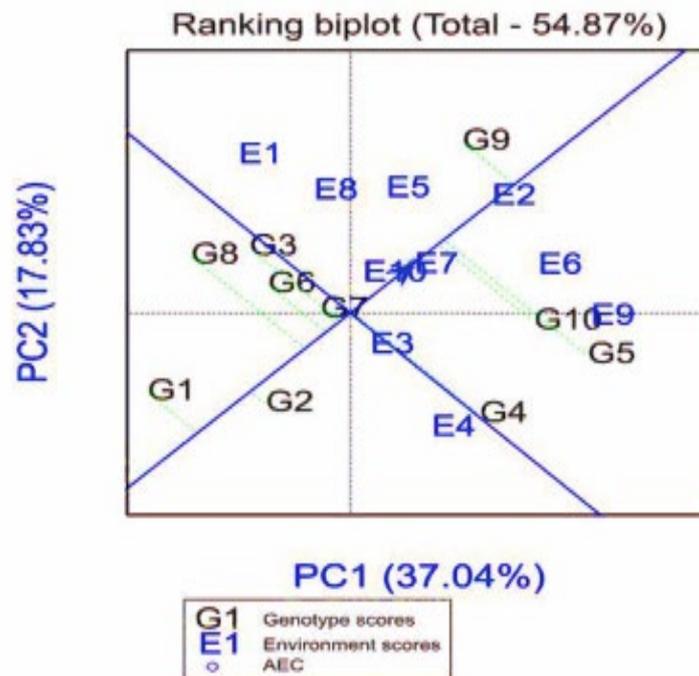


Figure 7. Average environment coordination (AEC) views of the GGE biplot based on genotype focused scaling for the means performance ranking and stability of genotypes.

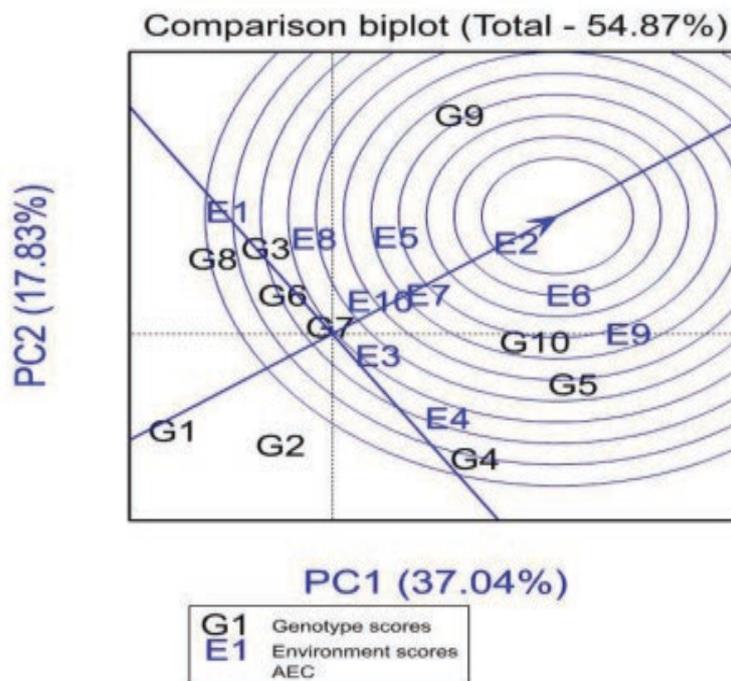


Figure 8. GGE biplot based on environment focused scaling for comparison of the environments with the best test environment.

In Figure 8, GGE biplot focused scaling for environments comparison. The environments E₂, E₆ and E₉ were the best because they have high average seed yield per plant among the others and the most suitable genotypes to these environments were the genotypes G5, G9 and G10.

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التفاعل بين التراكيب الوراثية والبيئة لمحصول البذور في البرسيم الحجازي

باهي راغب بخيت، المهدي عبد المطلب طعمية، فتحي محمد فتحي، أسماء على محمد

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الملخص

اجري هذا البحث في مزرعة قسم المحاصيل، كلية الزراعة، جامعة أسيوط لدراسة تأثير درجات الحرارة الناتجة عن اختلاف مواعيد الزراعة خلال النمو الخضري ونضج البذور لبعض التراكيب الوراثية والتفاعل بينهما على المحصول البذري ومكوناته في عشرة تراكيب وراثية من البرسيم الحجازي. تم زراعة هذه التراكيب الوراثية في خمس مواعيد زراعية ثلاثة خريفي وهي (10 اكتوبر , 10 نوفمبر , 10 ديسمبر) واثنين ربيعي (20 مارس , 20 ابريل). في تصميم الشرائح الكاملة العشوائية باستخدام ثلاث مكررات. كما استخدم تحليل المحاور الثنائية للتراكيب الوراثية وتفاعل التراكيب الوراثية مع البيئة وكانت أهم النتائج اظهر تحليل المحاور الثنائية للتراكيب الوراثية وتفاعل التراكيب الوراثية مع البيئة (مواعيد الزراعة) مساهمة عالية بالنسبة لمجموع مربعات الانحراف الكلية لصفتي محصول البذور للنبات ومحصول البذور في المتر المربع حيث بلغت 60.69%، 60.80% على التوالي وذلك بزيادة عشرة اضعاف عن مساهمة التراكيب الوراثية. كما أشار تحليل المحاور الثنائية للتراكيب الوراثية وتفاعل التراكيب الوراثية مع البيئة إن المكونين الرئيسية الأول PC1 والثاني PC2 كان معنويين حيث ساهما بنسبة 40.23، 21.36% من التباين الكلي بالنسبة لصفة محصول البذور للنبات 37.04%، 17.83% بالنسبة لمحصول البذور في المتر المربع على التوالي. كما أشار التحليل أيضاً أن التركيب الوراثي G9 كان الأعلى محصول والأكثر ثباتاً عبر كل البيئات (مواعيد الزراعة) وأيضاً البيئة الرابعة E4 (الزراعة في 20 مارس) أعطت أعلى محصولاً وسجلت 66.06 جم.