

## Original Research Article

### **Genotype by environment interaction effects on sugar beet crop using multivariate analysis**

**Comment [EY1]:** The effect of environment interaction on the genotypes of sugar beet crop using multivariate analysis

#### **ABSTRACT**

Evaluation of genotypes under Egyptian desert conditions comes in the first order for Plant Breeding and Conservation Program of Desert Research Center (DRC). The objective of this study was to analyze the effect of the genotype by environment interaction of sugar beet across various locations using multivariate models. Data for studied traits of sugar beet were obtained from experiments at three regions: Saint Catherine, South Sinai Governorate (E1); Baloza station, North Sinai Governorate (E2); and East El- Qantrastation, El-Ismailia Governorate (E3) in Egypt. All examined traits were significantly impacted ( $p < 0.05$  or  $0.01$ ) by environment (E), genotypes (G), and their interaction (GEI) using the AMMI model, with the exception of root length/plant by the environments as well as leaves weight/plant and total soluble solids percentage % traits by the genotypes. GEI is partitioned into two principal components (PCs), which were significant for all studied traits ( $P < 0.05$  and  $P < 0.01$ ). The highest variability from the total variance was recorded by environmental influences for leaves weight/plant and total soluble solids percentage % traits, as well as by genotype effects for the other studied traits. The environmental index showed that some environments were favorable and some environments were unfavorable for the two traits. The highest root weight/plant and most studied traits were noticed in the E2 environment. Based on the GGE model for root weight/plant, the test environments E1 and E2 are more representative and have the greatest ability to discriminate genotypes, thus favoring the selection of superior genotypes. The genotypes G2, G5, and G6 perform best in the E1 and E2 environments as well as are the most productive and stable compared with the other genotypes. According to PCA and cluster analysis, the genotypes G5 and G6 showed the best performance in response to environments and positive association with root weight/plant and most studied traits. Finally, and based on the results of statistical methods used in this study, G5 and G6 genotypes could be used in future sugar beet breeding efforts, and to improve productivity and sustainable production of sugar beet in Egypt.

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**Key words:** Sugar beet – GEI – environmental index – multivariate analysis.

#### **INTRODUCTION**

Sugar beet (*Beta vulgaris* L.) is considered one of the most important sugar crops in many countries all over the world and Egypt. It is the second sugar crop in the world after sugar cane (El Refaey et al. 2012; Al Jbawi et al. 2016). It ranks the first important sugar crop in Egypt and many countries all over the world (Sorour et al. 2020). Beet growing is becoming more and more important in sugar-producing, the secondary production, fodder and organic matter for the soil (Sorour et al. 2020) as well as in the production of biofuels (Anar et al. 2019). In the world, the total area harvested, yield and production of sugar beet were 4439073 ha, 569869 hg ha<sup>-1</sup> and 252968843 tons in 2020, respectively. While the total area harvested, yield and production of sugar beet were 263543 ha, 494933 hg ha<sup>-1</sup> and 13043612 tons in Egypt (FAOSTAT, 2022).

Locations, growth seasons, years, drought conditions, rainfall, the amount of precipitation received throughout each season, temperature, and other environmental factors (non-genetic factors) may have favorable or unfavorable effects on genotypes (El-Hashash et al. 2018). According to Falconer and Mackay (1996), the phenotypic expression of an individual is determined by both genotype and environmental effects such as locations and/or years, and the phenotypes can be observed, quantified, categorized, or counted. The term "genotype-environment interaction (GEI)" refers to the relationship between a genotype's phenotypic expression and the environment. Breeders assess genotypes in various environments (locations and/or years) to account for GEI effects in order to select those with high and stable performance and higher adaptation, taking GEI effects into account (Yan et al. 2000). Stable genotypes were identified with relatively high yield across environments (Björnsson, 2002), and with insignificant GEI (Ssemakula and Dixon 2007). With respect to sugar beet, the assessment of GEI would be highly beneficial in identifying traits promoting a better variety of performance in specific environments (Hoffman et al. 2009). Various statistical methods such as parametric, nonparametric statistics, and multivariate models are used for the investigation and interpretation of GEI and evaluation of different genotypes (Gauch et al. 2008). Among these methods, the additive main effect and multiplicative interaction (AMMI) model and genotype (G) main effect plus genotype-by-environment (GE) interaction (GGE) biplot.

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The additive main effect and multiplicative interaction (AMMI) model established by Zobel et al. (1988) is the most well-known of the multivariate methods used for the interpretation of GEI data. The AMMI model characterizes genotype and environment main effects using analysis of variance as an additive model, and their interactions using principal components analysis as a multiplicative model (IPCA). The visual method GGE biplot (Genotype and Genotype-Environment Interaction) represents a modification of the AMMI model and gives an extensive explanation of GEI effect (Yan et al. 2000). The method is used for visual analysis of multi-environment trial data and it is based on two important concepts: the biplot concept (Gabriel, 1971) and GGE concept (Yan et al. 2000). This method uses a biplot to describe two factors, genotype, and interaction between genotype and environment (G and GEI), thus the name GGE, which can be very useful in the evaluation of the performance of genotypes in different environments (Yan and Tinker 2006). Sugar beet breeders all over the world have been using AMMI analysis of variance and the GGE model to investigate GEI in environmental trials (Curcic et al. 2017; Curcic et al. 2018; Hassani et al. 2018; Studnicki et al. 2019; Bocianowski et al. 2022a).

To visualize the outcomes of sugar beet trials, principal component analysis (PCA) and cluster analysis are required, where many researchers have used the PCA to assess the relationship and diversity between several sugar beet genotypes, in addition to knowing the relationships between yield, its components, and other traits, such as Škrbićet al. (2010), Hu et al. (2019), Alami et al. (2021), Islam et al. (2022), Kleuker and Hoffmann (2022) and Majumdar et al. (2022). The aim of this work was to study the effect of environment on genotypes of sugar beet under various regions in Egypt, and evaluate the magnitude of genotype x environment interaction using the multivariate methods, thus discovering the most stable sugar beet genotype and determining associations for the studied traits in ten sugar beet genotypes under three different environments.

## MATERIALS AND METHODS

### *Plant Material and Field Trials:*

The three field experiments were conducted 1) at a local community farm, Saint Catherine (E1), South Sinai, Governorate; 2) at Baloza station (E2), DRC, North Sinai Governorate; and 3) at East El-Qantra station (E3) of DRC, El-Ismailia Governorate in Egypt. Ten sugar beet genotypes were used to evaluate under three locations (environments) during the 2021/2022 growing season. The genotypes used in this study are SK58-4-S4 (G1), SK48-C (G2), SK70 (G3), SK90 (G4), SK44-1-4R2 (G5), SK27-38 (G6), SK27-32 (G7), SK Fc723 (G8), SK44-1-9C1 (G9) and SK17-2-7W (G10). Healthy seeds of sugar beet genotypes were obtained from the Plant Breeding and Conservation Program of Desert Research Center (DRC), Egypt. Sugar beet genotypes were evaluated in Randomized Complete Block Design (RCBD) with three replicates across the three environments. Each replicate included ten plots of genotypes. Each experimental plot has five rows, and the genotypes were planted using standard agronomic practices and proper plant geometry with a row length of 5 m and comprised 25 hills. Under each plot, the row x row and plant x plant distances were 50 and 20 cm, respectively. The crop was sown in one day, and all the recommended cultural practices of sugar beet production in the area were done as needed, under uniform field conditions to minimize environmental variations to the maximum possible extent.

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In Table 1, the meteorological data for the three environments is shown as monthly averages for precipitation (mm), average temperature (°C), and relative humidity (%) during the experimental period (from October to May) during the 2021/2022 growing season. Under the three studied environments, the highest temperature typically occurs in October and May months, while the lowest temperature was recorded in January month. The maximum monthly precipitation rates were recorded at Saint Catherine and Baloza Stations in January, and at East El-Qantra Station in February. The highest values of relative humidity were observed in October, January, and November months at Baloza Station, Saint Catherine, and East El-Qantra Station, respectively. Generally based on the grand mean, Saint Catherine had the lowest average temperature during the study period, followed by Baloza and East El-Qantra Stations. While the greatest values of precipitation and relative humidity were observed in Baloza Station compared with Saint Catherine, and East El-Qantra Station.

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Table 1. Monthly climatic data for the studied environments during 2020/2022 sugar beet growing season.

Environments	Climatic data	October	November	December	January	February	March	April	May
Saint Catherine (E1)	Temperature (°C)	18.9	13.4	9.2	7.4	9.4	12.9	17.1	21.1
	Precipitation (mm)	8.0	4.0	3.0	8.0	6.0	6.0	4.0	4.0
	Relative Humidity %	34.0	37.0	38.0	39.0	32.0	27.0	22.0	21.0
Baloza Station (E2)	Temperature (°C)	22.4	18.0	13.7	11.9	13.0	15.6	18.5	21.8
	Precipitation (mm)	7.7	11.7	19.0	26.0	21.0	13.3	5.0	2.3
	Relative Humidity %	68.0	63.7	64.7	66.0	62.7	58.3	55.7	57.3
East El-Qantra Station (E3)	Temperature (°C)	23.5	19.1	15.0	13.3	14.5	17.3	20.5	24.2
	Precipitation (mm)	2.0	2.7	3.3	6.0	6.3	4.0	2.0	0.7
	Relative Humidity %	58.0	58.3	58.0	57.0	52.0	48.3	44.3	43.7

Source: <https://en.climate-data.org>.

**Data recording:**

The data on an individual plant basis of the ten genotypes were recorded. Ten guarded plants randomly were collected from each genotype from each replication to

evaluate the following traits: leaves weight/plant(g; LW/P), root length/plant (cm; RL/P), root diameter/plant (cm; RD/P), root weight/plant (g; RW/P) and total soluble solids percentage (%; TSS). TSS% was determined by using Hand Refractometer and expressed as a percentage of the juice.

**Statistical Analysis:**

The analysis of variance by the AMMI model was performed to determine the main and interaction effects of environments and genotypes on evaluated traits. The least significant differences (LSD) at 0.05 and 0.01 levels of probability were also used for means separation and comparison after significance (Steel and Torrie, 1980). After determining the significance of the GEI, adaptation ability and phenotypic stability analyses for genotypes studied were performed graphically using the GGE-biplot model (Yan et al., 2000). The AMMI model, GGE-biplot, cluster analysis and PCA were done using computer software programs PBSTAT, PAST version 4.03 and OriginPro 2018 version b9.5.0.193.

**RESULTS AND DISCUSSION**

**AMMI Analysis of Variance**

Table 2 displays the results of the AMMI analysis of variance of the environments, genotypes, and GEI effects on investigated traits in sugar beet. The mean squares due to environments, genotypes, and GEI were significant at 0.05 or 0.01 probability levels for all investigated traits, with the exception of RL/P trait by environments, as well as LW/P and TSS % traits by genotypes, which had insignificant. A considerable part of the overall variation was formed by the environments for LW/P and TSS% traits, as well as by the genotypes for other studied traits. Curcic et al. (2018), Hassani et al. (2018) and Bocianowski et al. 2022a) have previously revealed significant between genotypes, environments, and their interactions for all considered traits of sugar beet using AMMI model. Significant variations in the response of genotypes to the effect of environments show the right choice of experimental sites for GEI assessment (Hassani et al. 2018).

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Table 2. Combined ANOVA with AMMI analysis for studied traits of sugar beet genotypes evaluated across different environments.

S.O.V	D.F.	LW/P	RL/P	RD/P	RW/P	TSS %
Environment (E)	2	19584.43**	0.17 <sup>NS</sup>	1.25*	95721.29**	144.63**
Replication/E	6	31.89 <sup>NS</sup>	0.34 <sup>NS</sup>	0.12 <sup>NS</sup>	2914.65 <sup>NS</sup>	0.66 <sup>NS</sup>
Genotype (G)	9	1118.38 <sup>NS</sup>	17.60**	8.06*	137451.45*	6.05 <sup>NS</sup>
G x E	18	582.60**	2.78**	2.80**	40296.15**	2.92**
PC1	10	752.02**	3.11**	3.09**	42828.81**	3.65**
PC2	8	370.83*	2.36*	2.43**	37130.33**	2.00**
Residuals	54	149.12	1.06	0.30	7373.78	0.51
Contribution to the sums of squares % of total variance explained						
% due to E		57.63	0.13	1.75	7.45	67.65
% due to G		14.81	59.11	51.04	48.14	12.74
% due to G x E		15.43	18.66	35.40	28.23	12.28
PCs variance % of the total variance of variables						
PC1		71.71	62.21	61.34	59.05	69.60
PC2		28.29	37.79	38.66	40.95	30.40
CV%		12.88	6.98	6.60	12.77	3.84

Statistically significant differences at \* $p \leq 0.05$  and \*\* $p \leq 0.01$ ; ns: indicate the non-significant difference. LW/P: leaves weight/plant; RL/P: root length/plant; RD/P: root diameter/plant; RW/P: root weight/plant; TSS%: total soluble solids percentage %.

After removing sums of squares (SS%) due to error and replication, the greatest contribution to the SS% of the total variance was due to genotypes for root traits including RL/P, RD/P and RW/P, followed by GEI. While, the environments source had recorded the highest contribution to the SS% of the total variance for LW/P and TSS% traits, followed by GEI for LW/P, and followed by genotypes for TSS%. These results indicated more than 50% of the total variance was observed for LW/P and TSS % traits due to environmental influences, and for RL/P and RD/P traits due to the genotype effects. Curcic et al. (2017) reported that the environment had the greatest effect on the root yield of sugar beet. On the other hand, the variance due to GEI ranged from 12% for TSS % to 35 for RD/P. The production of sugar beet crop is based on root and its quantitative traits, which are estimated, which is the main cause of the low degree of interaction in this crop (Curcic et al. 2017). Contrary to other field crops, the sugar beet root grows throughout the vegetative stage of development and does not pass through delicate phases like bolting, flowering, pollination, and seed filling (Hoffmann et al., 2009). According to Basford and Cooper (1998), the breeders may be able to create more stable genotypes if they have a better grasp of the relative contributions of genotypes, environments, and GEI as sources of variation.

Differences in the variances of the phenotypes produced by the various genotypes can be used to detect the GEI (Mather and Jinks, 1971). According to the AMMI analysis, GEI was partitioned into two principal components (PC1 and PC2). The PC1 and PC2 showed significant ( $P < 0.05$  or  $P < 0.01$ ) for all examined traits. More than 59% of the total SS from GEI were explained by the PC1 for all studied traits. In contrast, PC2 contribution ranged from 28% for the LW/P trait to 40% for the RW/P trait. The fact that there was a significant GEI for yield shows that some genotypes were stable while others were unstable (El-Hashash and Agwa, 2018). The values of coefficients of variation (CV%) ranged from 3.84% for TSS% trait to 12.88% for LW/P trait. The values of CV% indicate that the genotypes had exploitable genetic variability during selection, also the low CV% proved the accuracy of the sugar beet experiments in three studied environments. Our results of CV% are in accordance with earlier findings on sugar beet crop by Bayomi et al. (2019).

***Main effects of environments, genotypes, and their interaction on sugar beet traits:***

Table 3 includes the mean performances of the main effects of environments, genotypes, and their interaction on studied traits as well as the environmental indicator (EI) and coefficient of variation (CV%). The average comparisons of the analyzed traits showed that the evaluated genotypes in each environment differed significantly from one another. Based on the grand mean, the E2 location had the highest mean performances for all studied traits with compared to the other environments, except RL/P trait. The highest mean performances of genotypes over three environments were observed by G9 genotype for LW/P (116.52 g), by G2 genotype for RL/P (16.29 cm) and TSS% (20.11%), and by G5 genotype for RD/P (10.00 cm) and RW/P (886.49 g). With respect to GE interaction, the highest values of mean performance were recorded by the G9 and G4 genotypes for LW/P (163.50 g) and TSS% (23.33%), respectively in E2, as well as by the G2 genotype for RL/P (17.47 cm) and by G5 genotype for RD/P (12.23 cm) and RW/P (1078.67 g) in

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E2. The differential yield ranking of genotypes across environments is proof that the GEI effect was of the crossover type (Yan and Hunt 2001). According to Thillainathan & Fernandez (2002), consistent performance across many environments (locations and/or years) may be the cause of yield stability. LW/P and RW/P traits showed a high CV% in E2 and moderate CV% in E3 and E1, respectively. Opposite, the other studied traits had low CV% values during the studied environments. The estimates of CV% show that the genotypes exhibited genetic variability that may be used during the selection of sugar beet yield under various environmental conditions.

Table 3. Mean values of studied traits of ten sugar beet genotypes grown under different environments.

Traits	Env.	Genotypes										Mean	LSD at		CV%	EI
		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10		0.05	0.01		
LW/P	E1	65.57	69.27	72.97	58.67	99.37	107.13	104.17	79.33	95.00	69.00	82.05	6.79	9.04	5.84	-12.76
	E2	107.43	140.00	127.60	117.93	133.33	123.70	102.87	122.33	163.50	103.50	124.22	26.42	NS	15.02	29.42
	E3	65.20	81.53	88.47	78.60	77.73	84.00	63.23	65.67	91.07	85.93	78.14	12.35	NS	11.17	-16.66
	Mean	79.40	96.93	96.34	85.07	103.48	104.94	90.09	89.11	116.52	86.14	94.80	NS	NS	12.88	
RL/P	E1	15.90	17.47	12.20	14.70	14.43	16.83	15.53	16.90	12.03	12.33	14.83	1.36	1.82	6.49	0.08
	E2	16.70	15.07	11.03	14.67	15.37	15.27	14.97	15.60	14.50	13.67	14.68	1.80	2.40	8.66	-0.07
	E3	15.63	16.33	14.03	15.07	14.60	15.17	15.00	15.30	14.07	12.27	14.75	1.12	1.50	5.38	-0.01
	Mean	16.08	16.29	12.42	14.81	14.80	15.76	15.17	15.93	13.53	12.76	14.75	0.81	1.08	6.98	
RD/P	E1	8.33	8.33	7.60	6.83	12.23	8.67	7.37	6.93	10.23	7.03	8.36	0.95	1.26	8.03	0.05
	E2	9.27	8.67	7.63	9.00	9.13	8.60	7.93	8.03	9.07	7.40	8.47	0.80	1.07	6.70	0.17
	E3	7.43	7.83	9.87	7.33	8.63	8.17	7.10	7.13	9.97	7.30	8.08	0.49	0.66	4.30	-0.23
	Mean	8.34	8.28	8.37	7.72	10.00	8.48	7.47	7.37	9.76	7.24	8.30	0.43	0.57	6.60	
RW/P	E1	460.17	762.03	582.10	781.00	1078.67	1001.27	691.67	591.67	571.00	453.33	697.29	105.23	140.14	10.66	24.90
	E2	781.23	708.33	513.33	670.00	845.33	856.30	690.20	706.67	738.33	611.67	712.14	166.96	NS	16.56	39.75
	E3	427.67	595.33	684.20	670.33	735.47	757.17	423.70	510.00	752.77	520.63	607.73	73.46	97.82	8.54	-64.66
	Mean	556.36	688.57	593.21	707.11	886.49	871.58	601.86	602.78	687.37	528.54	672.39	67.75	90.22	12.77	
TSS%	E1	16.33	19.33	16.67	16.00	15.33	17.67	16.00	17.67	16.00	16.67	16.77	0.96	1.27	4.03	-1.83
	E2	21.00	22.33	19.00	23.33	21.33	21.00	20.67	22.00	20.33	19.33	21.03	1.19	1.59	4.00	2.43
	E3	16.67	18.67	17.33	17.33	18.33	18.67	16.67	19.33	18.67	18.33	18.00	0.85	1.13	3.33	-0.60
	Mean	18.00	20.11	17.67	18.89	18.33	19.11	17.78	19.67	18.33	18.11	18.60	NS	NS	3.84	

E1, E2 and E3: Saint Catherine, Baloza and East El-Qantra, respectively. EI: Environmental index. The traits key names can be found in Table 2.

Estimates of the environmental index under the tested environments ranged from -64.66 to 39.75, demonstrating significant differences across these environments for studied traits. The maximum values of the environmental index were recorded for LW/P (29.42), RD/P (0.17), RW/P (39.75) and TSS% (2043) traits in E2, followed by RD/P (0.05) and RW/P (24.90) in E1, indicating these environments were favorable for these studied traits. While other environments were unfavorable for the other studied traits. It is evident from the environmental index that an environment that was good for a trait was poor for another (El-Hashash and El-Absy, 2013).

### GGE Biplot Model:

#### 1. Discriminateness vs. Representativeness:

The GEI is partitioned into two components (PC1 and PC2) by GGE biplot. The GGE biplot of PC1 and PC2 contributed 77.50% and 15.60%, and collectively they explained 93.10% of the total G+GE variation for root weight/plant (Fig. 1). After partitioning GEI by GGE biplot, the PC1 contribution in the GEI was greater than the PC2 under the various environments, suggesting that the GGE biplot effectively partitioned the variability in root weight trait. A similar trend has been

reported in sugar beet by Curcic et al. (2017) and Studnicki et al. (2019). Finding the most suitable (ideal) test environment through the test-environment evaluation is crucial for a successful breeding technique in the selection of superior genotypes for a variety of environments (Yan et al., 2007). The idealness of the tested environments is defined by two characteristics: a) discriminating ability (the ability of an environment to differentiate genotype in terms of main genotype effects), which has a high PC1 score, and b) representativeness (the ability of an environment to represent all other evaluated environments), which has a zero score for PC2. As a report by Yan and Tinker (2006), the length vectors and the cosine of the angle between the two environments determine their similarity (covariance) of them. Therefore, the environments for RW/P were divided into two distinct groups by the ray's lines. The first group included the E3 environment in a sector of its own and had two suitable varieties (G3 and G9), while E1 and E2 environments formed the second group where varieties G5 and G6 had the highest RW/P. As a report by Yan et al. (2007), due to having the smallest angles with average environment coordination (AEC), the test environments E1 and E2 for RW/P are more representative of other test environments (Fig. 1). These results indicate that these environments are idyllic and have the greatest ability to discriminate genotypes, thus favoring the selection of superior genotypes. Yan et al. (2000) and Yan and Rajcan (2002) reported that the most genotypes desirable is the one closest to the graph of the ideal environment. Thus, the genotypes G2, G5 and G6 are the most productive and stable for RW/P. While the test environment E3 had a larger angle with AEC for RW/P, indicating that it was the least discriminating and representative in both irrigation conditions. Non-discriminating test environments provide minimal information about genotypes and must not be used as test environments (Yan and Tinker, 2006). According to Yan and Kang (2002), strong positive correlation was observed among E1 and E2 (the acute angle), while E3 had positively correlated with E1 (slight). GGE biplot model indicated the most representative testing environments with the discriminating ability for root yield trait tested (Hassani et al. 2018).

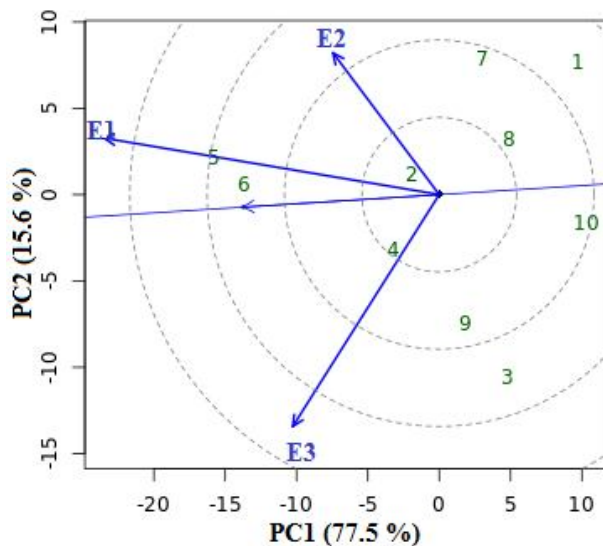


Fig. 1. GGE biplot of discriminativeness vs. representativeness for root weight/plant traits with ten sugar beet genotypes (green color) and three environments (blue color). E1, E2 and E3: Saint Catherine, Balozza and East El-Qantra, respectively; 1: SK58-4-S4; 2: SK48-C; 3: SK70; 4: SK90; 5: SK44-1-4R2; 6: SK27-38; 7: SK27-32; 8: SK Fc723; 9: SK44-1-9C1; 10: SK17-2-7W.

## 2. Mean vs. stability analysis:

The AEC method based on genotype-focused singular value partitioning (SVP) was utilized to assess genotype yield stability using average PCAs in all environments. If  $SVP = 1$ , the AEC line with a single arrow passes through the biplot's origin (Yan, 2002), the arrow points to a higher mean yield. The mean of PC1 and PC2 of the environmental scores is defined, as a report by Yan and Rajcan (2002). The 'Mean vs. stability' view is frequently referred to as AEC with  $SVP = 1$  which helps to simplify the genotype assessment based on the mean performance and stability across environments within a multi-environment (Fig. 2). The GGE biplot was created by plotting the PC1 and PC2 produced from subjecting data of environment-centered yield to singular value decomposition (Yan et al., 2000). The genotypes are grouped according to their average root weight, as indicated by the arrow sign on the AEC. The genotypes with above-average means were G2, G4, G5, and G6, while the other genotypes were below-average means. The highest values of RW/P were observed by the genotypes G5, and G6 in the E1 and E2 environments, and the genotypes G6 and G9 in the E3 environment. While G3, G7, and G10 genotypes had the least mean root weight in E2, E3, and E1, respectively. GGE biplot model revealed that some environments were the best test environments where the best genotypes could easily be identified with respect to the root trait of sugar beet (Hassani et al. 2018).

The most stable genotypes were  $G6 > G2 > G5 > G4$ , which were practically on the AEC abscissa and had a close to zero projection onto the AEC ordinate. This demonstrates that these genotypes' ranking was remarkably constant across environments, according to Yan et al. (2007). In addition to good sugar beet root weight, genotype stability is more important, in terms of Yan (2001) established an "ideal" genotype based on average performance as well as stability. Thus, the genotypes G6 and G5 are more stable with better mean sugar beet yield other than the other genotypes. Opposite, the genotypes G1 and G3 are more variable and highly unstable with below and above-average mean performance in both conditions, respectively. The use of GGE biplots helped to indicate the mega-environments and sugar beet genotypes that yield the best in each of them (Studnicki et al. 2019). Some genotypes of sugar beet had specific adaptability to the best environment identified for root yield in the GGE biplot model (Hassani et al. 2018).

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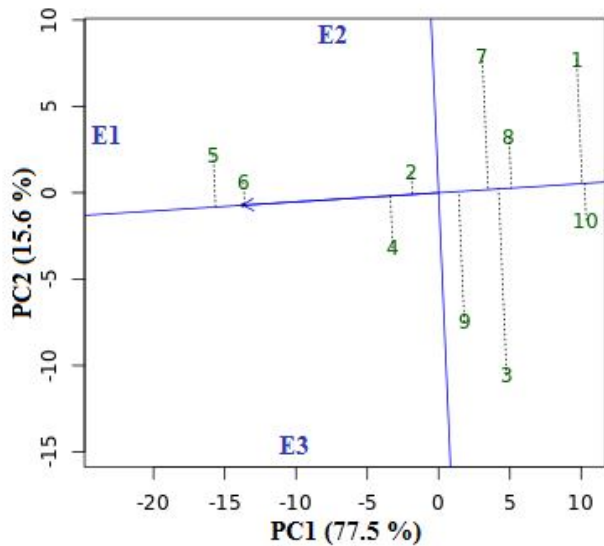


Fig. 2. GGE biplot of mean vs. stability for and root weight/plant traits with ten sugar beet genotypes (green color) and three environments (blue color). The genotypes and environment key names can be found in Fig. 1.

### 3. Which-won-where pattern:

The polygon view of the GGE biplot pattern of root weight of sugar beet yield was constructed to show which genotypes with the best performance best in which environment and groups of environments (Yan et al. 2000), as well as to demonstrate the presence of crossover GEI, mega-environment differentiation, and specific adaptation (Yan and Tinker, 2006). Four genotypes including G2, G4, G8 and G9 have situated within the polygon. While other genotypes are located away from the biplot origin in all directions and which formed the polygon vertices (Fig. 3). A line perpendicular to each polygon side was drawn starting from the biplot origin. The biplot is divided into sectors by these lines. The rays are perpendicular lines to the sides of the polygon or their expansion (Yan, 2002). Thus, the three environments are divided into different apparent groups. The genotype at the vertices of each sector is the nominal highest yielder for the environments or mega-environments that fell into it. Accordingly, G5 produced maximum root weight in the E1 environment. Also, the genotypes G5, G6, and G2 perform best in the E1 and E2 environments and gave the highest root weight, due to being located in the sector of these environments. While the genotype G4 occurred in the sector of the E3 environment and gave the highest root weight after genotypes G5 and G6. The genotypes G1, G3, G7, and G10 were the poorest across the environments, due to no environment falling into the sectors of these genotypes. GGE biplot analysis suggested that optimal locations were identified for each genotype, which can be useful when recommending sugar beet varieties for certain growing areas of this field crop (Curcic et al. 2017). Winner genotypes were also identified based on the results of the GGE biplot model (Hassani et al. 2018).

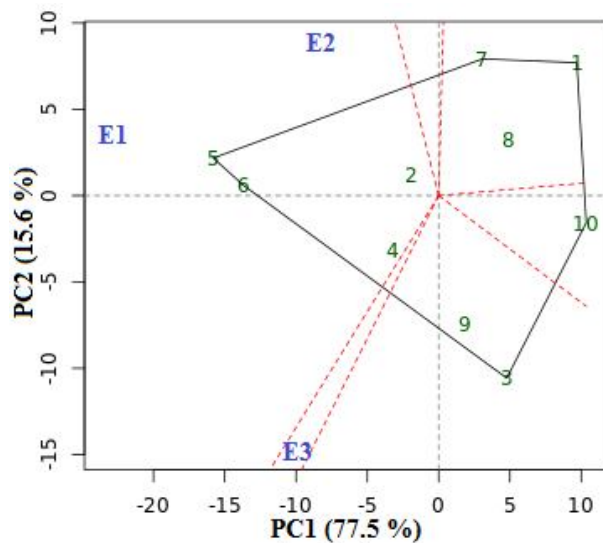


Fig. 3. GGE biplot polygon of "which-won-where" for root weight/plant with ten sugar genotypes (green color) and three environments (blue color). The genotypes and environment key names can be found in Fig. 1.

**Cluster analysis and Principle Component Analysis (PCA):**

Based on the mean performances of all studied traits under this study, the hierarchical cluster analysis method was performed to classify the ten sugar beet genotypes into four clusters (Fig. 4). Each cluster contained genotypes that were highly similar. The first and fourth clusters comprised two genotypes including G5 and G6, as well as G1 and G10, respectively. While both second and third clusters enclosed three genotypes including G2, G9 and G4, as well as G3, G7 and G8, respectively. The genotypes in the first cluster had recorded the highest root weight/plant, followed by the genotypes in the second and the third clusters, opposite is true for the genotypes in the fourth cluster. According to Hu et al. (2019), cluster analysis classifies sugar beet genotypes into four clusters.



G3	-0.49	-2.03	0.34	-0.10	-0.13
G4	-0.64	0.60	-0.07	-0.67	-0.35
G5	2.41	-0.41	-0.85	-0.29	-0.38
G6	1.67	0.98	-0.02	-0.65	0.45
G7	-1.19	-0.29	-0.55	-0.06	0.76
G8	-0.95	1.63	0.60	0.22	0.02
G9	1.98	-1.47	0.61	0.76	0.16
G10	-1.91	-1.19	0.64	-0.36	-0.17
Eigenvalues	2.35	1.75	0.50	0.27	0.13
Variance %	47.03	34.98	9.97	5.40	2.62
Cumulative%	47.03	82.01	91.98	97.38	100.00

The traits and genotypes key names can be found in Table 2 and Fig. 1, respectively.

Based on their data from the three researched environments, the PC1 and PC2 were used to draw a biplot for the studied attributes and genotypes (Fig. 5). Using PCA, a sharp angle among LW/P, RD/P and RW/P traits, as well as among RL/P, RW/P and TSS% traits in this study was found, indicating the positive correlation between these variables, but they differed in their degree and consistency in quantity. Generally, RW/P positively correlated with the other studied traits. This means that selection based on these traits will result in an increasing sugar beet yield in all environments (Bayomi et al. 2019). Evaluation of the genotypes according to their locations on the basis of the studied variables by using the PCA model might be a feasible approach (Hu et al. 2019). The PC1 and PC2 mainly distributed and distinguished the studied traits and genotypes into two groups. The first group was related to PC1 and includes all studied traits, which are strongly positively associated with the G2, G5, G6 and G9 under the three environments (the first and fourth quarters). While the second group is related to PC2, which includes the other genotypes (the second and third quarters). The PCA with the AMMI model enables clustering of genotypes based on similarity of response characteristics and identifying potential trends in environments (Bocianowski et al. 2022b). Generally, the G5 and G6 genotypes were located near the most studied traits across the three environments. The results of PCA analysis were in accord with the results of cluster analysis with respect to genotypes delineation with higher root weight and most studied traits. Based on our results, the G5 and G6 genotypes across the three environments have the potential to improve plant growth and increase the sustainable productivity of sugar beet in Egypt.

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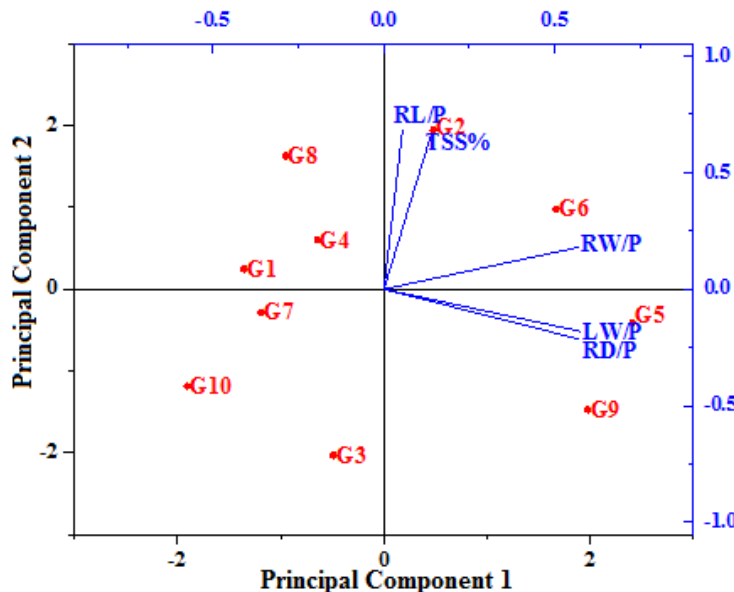


Fig. 5. A biplot diagram based on the first two PCs shows the relationships among the measured traits across ten sugar beet genotypes under various environments. The traits (blue color) and genotypes (red color) key names can be found in Table 2 and Fig. 1, respectively.

### CONCLUSIONS

The results of AMMI analysis reflect the divergent climatic conditions of three environments, resulting in a high level of genetic variability among ten genotypes for sugar beet crop under these environments. GGE biplot performed well in the study of the GEI, and provide a clear idea of genotype stability behavior in the different environments. PCA and cluster analysis could be used as suitable methods to identify the best genotypes in the examined environments and to clearly understand the association between the studied traits. The multivariate models were useful in identifying the G5 and G6 as the most stable genotypes with the greatest potential for high-yielding across various environments. Thus, these genotypes can be used in future breeding programs to higher root yield of sugar beet across different environments in Egypt.

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**Comment [EY25]:** Let the year of publication be after the authors for the sake of Consistency. Do same for the others

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