



Assessment of genotype × environment interaction in popcorn genotypes using GGE biplot analysis

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Abstract

The study was conducted over three consecutive growing seasons (2022, 2023, and 2024) in Al-Amiriyah District, Al-Anbar Governorate, Iraq to evaluate and identify the most adaptable and stable genotype in terms of yield performance among five popcorn genotypes. The tested materials comprised three genotypes developed through reciprocal recurrent selection, one local variety, and one imported variety. The experiment was arranged in a randomized complete block design (RCBD) with three replications. Several agronomic traits were assessed, with particular emphasis on individual plant yield, which is a quantitative trait governed by multiple genes. A combined analysis across the three seasons was performed, followed by the application of the GGE biplot methodology to visualize genotype-by-environment interactions and to identify both the ideal testing environment and the superior genotype. The results revealed significant differences among the studied factors genotypes, environments, and their interactions as well as for the first principal component (PC1), which accounted for approximately 72% of the total variation. The local genotype (Safa) and the imported Argentine genotype were classified as high-yielding and genetically stable. They achieved the highest yields during the 2022 season, which was identified as the most stable and productive, and thus considered an ideal season for cultivating this crop.

Keywords: (G×E), Popcorn, GGE biplot, Grain yield, stability, adaptability

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Introduction

The maize crop (*Zea mays* L.) is one of the most important cereal crops worldwide, cultivated across extensive agricultural areas. It ranks third after wheat and rice in terms of economic importance and overall productivity. Popcorn (*Zea mays* L. everta) is an important plant with an increasing economic value because it is used in human nutrition, animal feed, and the food industry(2)(6). Popcorn is the most well-known subtype of corn used for making snacks. Consumers prefer popcorn with a high burst volume, a tasty product that is soft and has less crust residue. The types of popcorn grown in different regions depend on the variety, ecological conditions, and preferences of the consumers. It is grown mostly in hot and tropical climates. It matures at high temperatures. There are 3-5

ears on the plant.(1)(17). Yield and popcorn size are important agronomic traits of popcorn. Popcorn kernels are smaller and have a significantly lower ratio of soft starch to hard starch than flint corn. Corn can be grown under a wide range of environmental conditions. Popcorn has similar growth requirements to dent corn, but is less adaptable to extreme environmental conditions(3). The proportions of popcorn kernels depend on kernel quality. The specific starch content of popcorn can alter popcorn size due to differences in kernel growth rates. The starch content and structure of the kernels depend on the ability of the plant to meet water demands during the kernel attachment and kernel filling stages. These factors include air temperature, humidity, moisture, and soil nutrient content (23). Evaluating the existing genetic variation in each crop is essential for developing effective breeding strategies that

can be used to improve yield. Corn producers use a variety of analytical methods based on biostatistical concepts to evaluate the adaptability and stability of varieties. An ideal genotype would have both high yield and desirable agronomic traits. However, studying selective gain is challenging due to interactions between genotype and environment. Breeders should select genotypes that have both the highest yield and high yield stability.

Multi-location and multi-year trials facilitate the identification of superior and stable genotypes. They also support the identification of discriminatory environments (locations) with minimal replication by detecting differences between genotypes. Representative years are characterized by the superior performance of selected genotypes in the target environment (4)(1). This effect is known as the genotype-environment interaction (GEI). GEIs are often found in data from multi-environment trials (METs). Due to the nature of these data, they are usually presented in the form of two-way tables containing the means of genotypes in all studied environments(6).

Screening and identification of superior genotypes is difficult due to the genotype (G) $\times$ environment (E) interaction (GEI). However, assessing this interaction is important because it is the main factor affecting the variation in genotype performance under different environmental conditions (5). GEIs can weaken the association between maize phenotypic and genotypic values and lead to biased estimates of genotypic effects (18). Maize breeding programs have adopted a variety of methods to assess the adaptability and stability of varieties in target environments. Two main approaches have been used in various studies: additive main effects multiplicative interaction analysis (AMMI), and a modification of the traditional AMMI analysis, namely genotype (G) and genotype $\times$ environment interaction analysis (GE) (GGE biplot analysis) (6)(8). These methods estimate the sustainability and compatibility of genotypes by using quantitative analysis of singular value decomposition. The GGE biplot method has been shown to be more successful than the AMMI method in analyzing genotype data under different environmental conditions(12)(15).

The GGE biplot method can reliably estimate the effects of genotype and environmental conditions simultaneously(21), as recommended in(17)(18) and (22). The graphical GGE biplot method plays a crucial role in the selection of genotypes and their environmental

conditions (20). Therefore, the GGE biplot analysis is superior to other statistical methods because it can effectively interpret the sum of squares of GE and G + GE and provide higher prediction accuracy for maize genotypes. In addition, it is an effective method to determine the environmental conditions that can distinguish genotypes and can be used to identify promising maize genotypes(3).

Results from the(GGE-biplot) analysis and AMMI analysis the graph usually called biplot that is generated from genotypic and environmental scores are useful for breeders in the decision of the genotypes either for further evaluation or for cultivar recommendations. Studies conducted on various genotypes and across diverse environments have demonstrated that both GGE – biplot and AMMI analyses are robust effective models for evaluating genotype $\times$ environments interaction(GEI) across multiple environments(6)(7)(8) (11),(13), , and(16)

The aim of the present study was to select genotypes of Popcorn (*Zea mays* L. everta) through the GGE biplot analysis and to evaluate its adaptability and stability in different environments .

Materials and Methods

The experiments were conducted in the fields of a farmer in the Amiriya Fallujah area, Al-Anbar Governorate,Iraq. over three consecutive growing seasons: E1 = 2022, E2 = 2023, and E3 = 2024. The study involved five popcorn genotypes, as detailed in Table 1. The genotypes were cultivated during the fall season of each year, with sowing carried out on August 1st. All agricultural operations, including plowing, leveling, and soil preparation, were performed, and the land was fertilized with a compound fertilizer (N:P 27:27) at a rate of 300 kg ha⁻¹ during soil preparation. The experimental field was divided into plots, each consisting of four line , with a spacing of 0.70 meters between lines and 0.25 meters between plants, and each line being 5 meters long. Urea fertilizer (46% nitrogen) was applied at a rate of 300 kg ha⁻¹ in two batches : the first when plants reached a height of 30 cm, and the second before tasseling.

Weed control was carried out in all seasons after sowing and before emergence using atrazine (85% active ingredient) at a rate of 3.25 kg ha⁻¹. Standard crop management practices were applied, including irrigation, weeding, hoeing, and pest control. Corn stem borer (*Sesamia cretica*) was controlled using diazinon granules

(10% active ingredient) at a rate of 4 kg ha⁻¹, applied twice: the first at the 4-leaf stage, and the second twenty days after the first application, for all growing seasons.

Several traits were studied, plant yield per plant (g).

An evaluation of the plant yield per plant (g) trait was conducted to assess its variation among genotypes

Table 1. Genotypes pedigree

G1	Derived from Safa variety (A-S2-3)
G2	Derived from commercial Argentine (B-S1-2)
G3	Derived from commercial Argentine (B-S2-2)
G4	Local variety Safa
G5	Argentine variety (AGR-5)

Statistical analysis

. The experiment was carried out using a randomized complete block design (RCBD) with three replications.. Then, a comparison was made between the arithmetic means using the Least Significant Difference (LSD) at the 5% level.

The data were analyzed using the statistical program GenStat program.

General Equation of the GGE Biplot

The GGE Biplot is based on a model that combines the genotype main effect (G) and the genotype-by-environment interaction (GE), using singular value decomposition (SVD) or principal component analysis (PCA) to reduce dimensionality(21).

The general equation of the GGE Biplot is:

$$Y_{ij} - \mu - \beta_j = \sum_{k=1}^n \lambda_k \xi_{ik} \eta_{jk} + \epsilon_{ij} \quad Y_{ij} - \mu - \beta_j = \sum_{k=1}^n \lambda_k \xi_{ik} \eta_{jk} + \epsilon_{ij}$$

Where:

- Y_{ij} : Observed mean performance of genotype *i* in environment *j* (e.g., grain yield)
- μ : Grand mean across all genotypes and environments
- β_j : Main effect of environment *j*
- λ_k : Singular value (scaling factor) of the *k*th principal component (PC)
- ξ_{ik} : Score of genotype *i* for the *k*th PC (genotype eigenvector)
- η_{jk} : Score of environment *j* for the *k*th PC (environment eigenvector)
- ϵ_{ij} : Residual associated with genotype *i* in environment *j*

nnn: Number of principal components used (typically 2 for biplot visualization)

Results and Discussion

Table 2, presents the combined analysis of variance, indicates the presence of significant differences among genotypes, environments, and the genotype × environment interaction. The presence of a significant genotype × environment interaction suggests that environmental factors had a considerable influence on the stability of yield.

The analysis of variance also shows that the first interaction principal component axis (IPCA1) reached a of statistical significance, whereas the second principal component (IPCA2) did not reach the required significance. IPCA1 explained 72.32% of the total variation, while IPCA2 accounted for 23.75% of the total variation, as illustrated in Figure 1.

Table 3 presents the mean yield of each genotype across the environments in which they were grown. The results show that Genotype 5 recorded the highest average individual plant yield, reaching 91.87 g, followed by Genotype 4 with an average of 86.27 g per plant. The same table indicates that the highest average yield per plant was observed during the second season (2023), with a mean of 94.24 g per plant, while the lowest yield was recorded in the 2022 season, with an average of 77.65 g per plant.

Most genotypes outperformed during the second season (2023), whereas a general decline in yield was observed in the 2022 season. The observed variation in genotype performance across seasons can be attributed to the influence of environmental factors such as temperature, rainfall, soil moisture, and other abiotic stresses that vary from one growing season to another. The superior performance of most genotypes during the

2023 season suggests that the environmental conditions in that year were more favorable for popcorn growth and development. These conditions may have included optimal temperature ranges, adequate soil moisture, and reduced stress periods, all of which are critical for maximizing photosynthetic efficiency and grain filling.

The variation in yield performance among the genotypes further highlights their differential responses to changing environmental conditions, reinforcing the importance of genotype × environment interaction analysis. Genotypes 5 and 4 consistently performed well across environments, indicating not only high yield potential but also a degree of adaptability to variable environmental conditions (22),

This is further confirmed by the interpretation of the principal component analysis. Genotypes with positive values on the first principal component (IPCA1) were classified as high-yielding, specifically Genotypes 4 and 5. In contrast, genotypes with negative values on the second principal component (IPCA2) were identified as low-yielding or poorly adapted, as exemplified by Genotype 1 (Figure 1). Additionally, Genotype 2 was characterized as high-yielding but with low stability, indicating that its performance was more sensitive to environmental variation.

Table 2. Combined Analysis of Variance for the Yield of Five Genotypes Grown Across Three Seasons

Source	df	SS	MS	F	F_prob
Total	44	7396	168.1	*	*
Treatments	14	5926	423.3	12.83	0.00000**
Genotypes	4	1357	339.2	10.28	0.00005**
Environments	2	2589	1294.7	11.46	0.00032**
Block	6	678	113.0	3.42	0.01383**
Interactions	8	1980	247.5	7.50	0.00005**
IPCA1	5	1748	349.6	10.60	0.00002**
IPCA2	3	232	77.4	2.35	0.09802NS
Residuals	0	0	*	*	*
Error	24	792	33.0	*	*

*and ** significant at 0.05 and 0.01 NS non significant

Table 3. Average Plant Yield (g) of Five Popcorn Genotypes Grown Across Three Seasons

	E1	E2	E3	mean
G1	65.8	92.59	72.07	76.82
G2	82.25	100.48	70.54	84.42
G3	68.93	76.46	89.36	78.25
G4	84.67	95.31	78.83	86.27
G5	86.61	106.35	82.65	91.87
Mean	77.65	94.24	78.69	

Table 4. Ranking of Genotypes in Each Environment

E1 (2022)	E2 (2023)	E3 (2024)
G5	G5	G3
G4	G2	G5
G2	G4	G4
G3	G1	G1
G1	G3	G2

Figure 1 illustrates the interaction patterns among the tested environments, as estimated by the GGE Biplot analysis. Positive scores on the first principal component axis (IPCA1) represent environments with greater yield differentiation among genotypes, typically associated with both high productivity and high stability. In contrast, high scores on the second component (IPCA2) often reflect environments where yield is high but genotype performance is less stable.

According to this analysis, Genotypes 4 and 5 were identified as high-yielding and stable across environments, while Genotype 2 exhibited high yield but low stability. Genotype 1 showed both low yield and poor stability across the target environments. This pattern is likely due to crossover interaction, which frequently causes a significant shift in the ranking of genotypes across environments. Such interactions are critical in multi-environment trials (METs) as they contribute to a more accurate assessment of genotype adaptability and stability(19).

The GGE Biplot analysis enabled simultaneous visualization of the main effects of genotypes and their interaction with environments. Genotypes with IPCA1 values above zero were classified as high-yielding, while those below zero were considered low-yielding. In addition, genotypes with IPCA2 values close to zero were interpreted as stable (well-adapted), whereas those with large positive or negative IPCA2 values were regarded as unstable or poorly adapted(20).

Figure 3 illustrates the interrelationships among the tested environments based on GGE Biplot analysis. The angle between the vectors representing Environments 1 and 2 was less than 90°, indicating a positive correlation between them with respect to genotype performance. In contrast, the angle between Environment 3 and the other two environments exceeded 90°, suggesting a negative correlation. These vector angles are a direct reflection of

the genetic correlation among environments: acute angles (<90°) imply a strong positive correlation, while obtuse angles (>90°) indicate a negative correlation (10).

Based on these results, indirect selection for yield can be effectively conducted between Environments 1 and 2 (2022 and 2023 seasons), due to their high positive correlation. Genotypes that perform well in one of these environments are likely to maintain similar performance in the other. On the other hand, Environment 3 demonstrated a divergent genotype response, making it less suitable for indirect selection when compared to the first two environments.

Furthermore, Environments 1 and 2 were closely aligned with the average environment axis (AEC), suggesting that they are more representative of the overall testing environments. They also displayed relatively long vectors, indicating high discriminating power. These two environments thus combine both representativeness and discrimination, making them ideal for genotype evaluation and selection. In breeding programs, such environments are particularly valuable, as they allow for the identification of both high-performing and stable genotypes across multiple environments.(14)

The results of the Genotype $\times$ Environment interaction analysis using the GGE Biplot method, specifically through the Average Environment Coordination (AEC) view (Figures 2 and 3), show that the average environment is determined by calculating the mean of the first and second principal component scores across all environments. A horizontal axis is then drawn through this average point and the origin, referred to as the "environmental axis" or the AEC abscissa. A perpendicular line is drawn from the origin, which represents the AEC ordinate (vertical axis).

In Figure 2, an arrow extending diagonally from the origin represents the direction of maximum genotype effect (i.e., yield potential). The projection of each genotype onto the AEC abscissa reflects its mean

performance across environments. Genotypes located to the right of the origin along this axis are considered high-yielding, whereas those on the left are low-yielding. Based on this, Genotypes 4 and 5 showed higher-than-average performance, while the remaining genotypes yielded below the overall mean.

The AEC ordinate, which is perpendicular to the environmental axis, reflects genotype stability. The length of a genotype's vector from the AEC abscissa represents the degree of interaction with the environment. Genotypes located closer to the AEC axis (i.e., with shorter vectors) are considered more stable, while those further away exhibit greater genotype × environment interaction and thus lower stability. Accordingly, Genotypes 4 and 5 demonstrated high stability, as evidenced by their shorter projections onto the AEC ordinate.

One of the primary goals of plant breeders is to identify an ideal genotype that combines both high yield and high stability across the tested environments. Such a genotype typically has a genotype-by-environment interaction (GEI) value close to zero and is easily identified using the GGE Biplot technique. In this method, the ideal genotype is represented by a point located at the center of the concentric circles along the AEC (Average Environment Coordination) abscissa, indicating the highest mean performance and the greatest stability, as it has the shortest vector from the origin.

As shown in Figure 2, Genotype 5 is positioned closest to the center of the biplot, making it the most "ideal" genotype among those evaluated. In contrast, Genotype 1 is the furthest from the ideal point, reflecting both lower yield and reduced stability. Although a truly ideal genotype may not exist in practice, its theoretical position on the biplot can serve as a reference to evaluate and compare the desirability of other genotypes. A

genotype is considered desirable when it is located near the ideal point or near the center of the concentric circles.

Furthermore, Genotype 5 is considered desirable because it combines a high positive value on the first principal component (PC1), which represents yield, and a low absolute value on the second principal component (PC2), which represents stability. Similarly, an environment is deemed ideal if it is located at or near the center of the concentric circles on the AEC abscissa, indicating both strong discriminatory power and representativeness.

Figure 4 demonstrates that Environment 1, corresponding to the 2022 growing season, is positioned closest to the center of the concentric circles on the GGE Biplot, identifying it as the most ideal environment for evaluating the tested genotypes. Its proximity to the center suggests that it is both highly representative of the average conditions across all environments and exhibits moderate stress levels, allowing for consistent expression of genetic potential. This makes Environment 1 optimal for reliable genotype selection and breeding decisions.

In contrast, Environment 3 (the 2024 growing season) is located farthest from the center, indicating it is the least ideal environment. Such a position often reflects harsher or more variable environmental conditions, which can intensify genotype × environment interactions and reduce the stability of genotype performance. While Environment 3 may not be suitable for general selection, it can be valuable for identifying genotypes with specific stress tolerance traits.

These findings underscore the importance of carefully selecting test environments in multi-environment trials to ensure both representativeness and discrimination, thereby facilitating effective genotype evaluation and breeding progress(6)

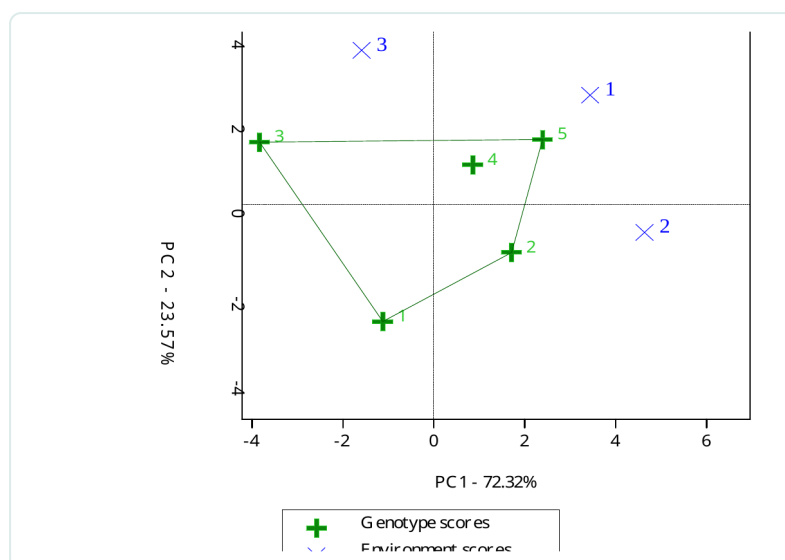


Figure 1. Preferred Genotypes for Each Environment

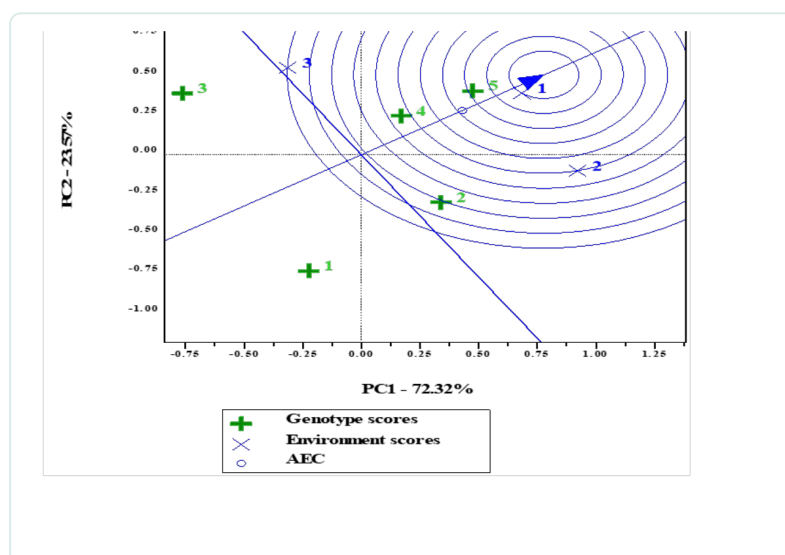


Figure 2. Center of the Circle Representing the Ideal Genotype

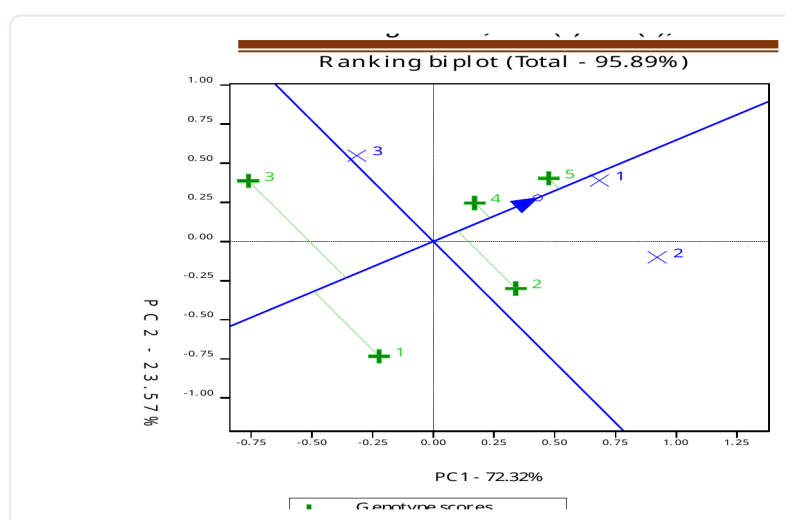


Figure 3. Stability of Genotypes

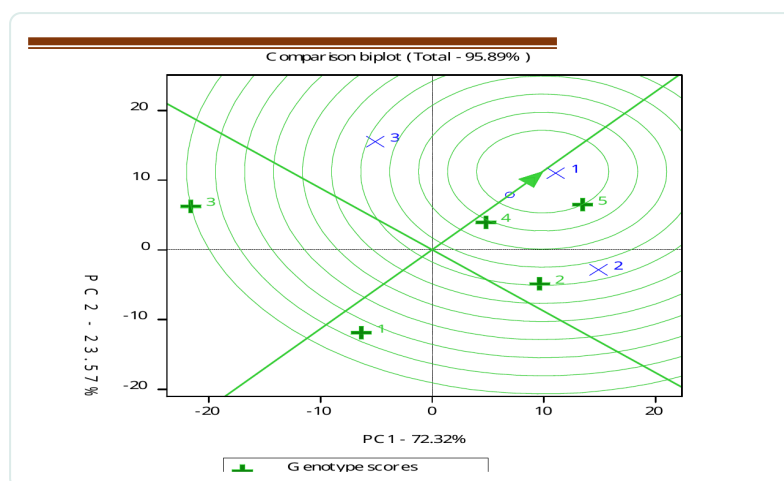


Figure 4. Center of the Circle Representing the Ideal Environment

Conclusions

the cultivation and evaluation of multiple popcorn genotypes across diverse environments revealed significant variation in their performance, as evidenced by the significant genotype $\times$ environment interaction. Among the tested genotypes, Genotype 5 exhibited the highest stability and yield, aligning with the primary goal of plant breeders and producers to select genotypes that demonstrate consistent and superior performance across varying environments. These environments may differ by season, location, or other abiotic and biotic factors, underscoring the importance of selecting genotypes that are both high-yielding and stable under diverse environmental conditions. Future research should expand multi-environment trials to include a broader range of agro-ecological zones and stress factors, facilitating the identification of genotypes with broad adaptability as well as those with specific tolerance traits. Integrating molecular tools with phenotypic selection may further accelerate the development of superior popcorn varieties.

These strategic approaches will enhance crop resilience and productivity, contributing to sustainable popcorn production and food security.

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No Supplementary Materials.

Author Contributions:

conceived and designed the experiment, supervised the research process, and revised the manuscript.

collected the data, and performed the statistical analysis.

contributed to data interpretation and prepared the first draft of the manuscript.

discussed the results, reviewed, and approved the final version of the manuscript.

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The authors declare no conflict of interest.

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مقالة أصيلة

تقييم التداخل الوراثي البيئي لتراكيب وراثية من ذرة الفشار باستعمال تحليل GGE biplot

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الخلاصة

نفذت هذه الدراسة خلال ثلاث مواسم زراعية 2022 و2023 و2024 في محافظة الأنبار قضاء العامرية. العراق، لمعرفة التركيب الوراثي الأكثر تكيفا والمستقر في حاصله لخمسة تراكيب وراثية من ذرة الفشار ثلاثة منها مستنبطة خلال الانتخاب التكراري المتبادل وصنف محلي وآخر مستورد نفذت التجربة باستعمال تصميم القطاعات العشوائية الكامل بثلاث مكررات. درست العديد من الصفات بضمنها صفة حاصل النبات الفردي الذي يعد من الصفات التي يتحكم في تورثها العديد من الجينات. اجري تحليل التجميعي للمواسم الثلاثة ثم استعمل تحليل GGE biplot لغرض عرض البيانات بشكل رسوم بيانية توضح البيئة المثالية مع التركيب الأمثل. اظهرت النتائج وجود فروق معنوية بين عوامل الدراسة التراكيب الوراثية والبيئات والتداخل فيما بينهما وكذلك المكون الرئيسي الاول PC1. استطاع المكون الرئيس الاول ان يفسر ما يقارب 72% من التباير الكلي. صنف التراكيب الوراثية المحلي صفا والارجنتيني على انها تراكيب عالية الحاصل ومستقرة وراثيا وذات حاصل مرتفع ضمن الموسم الاول 2022 الذي يعد أكثر المواسم ثباتا وانتاجا لذلك عد الموسم موسم مثالي لزراعة المحصول

الكلمات المفتاحية: التداخل الوراثي البيئي، ذرة الفشار، حاصل النبات، الثبات، التكيف