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Determining the Idiotypes of Maize Genotypes across Several Organic and Mineral Fertilizer Combinations

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Abstract

This study aimed to develop both the idiotype and the simulated idiotype form utilizing environment-adjusted data. The idiotype was derived from the adjusted data after removing environmental effects, whereas the simulated idiotype was generated from the same regression framework by applying controlled deviations to represent realistic improvement potential. In this study, the term "idiotype" represents the ideal genotypic pattern, while the simulated idiotype represents its optimized, biologically plausible form.

The experiment was conducted as a split-plot arrangement within a randomized complete block design (RCBD) to test six organic and mineral fertilizer combinations in the main plots and six maize genotypes in subplots. The idiotype showed a high coefficient of determination (R^2=0.9548), confirming that the developed pattern effectively represents the true genetic behavior of the studied genotypes. The simulated idiotype, on the other hand, exhibited a 16.18% increase in grain yield relative to the developed idiotype. Integration with stability indices, including the Eberhart and Russell parameters, Lin and Binns (Pi) and Wricke (Wi^2), together with the GGE biplot showed that genotype G3 (5018) was found to be the most stable and high-yielding genotype, and environment E5 (320N+200P_2O_5+80K_2O kg ha^{-1} + 4 tons ha^{-1} of organic fertilizer) was found to be the optimal environment combining high representativeness and discriminating ability.

The results validate that the methodologies employed to derive both the idiotype and simulated idiotype, together with stability parameters and GGE biplot analysis, constitute a robust analytical framework for identifying stable, high-yielding maize genotypes across multiple environments, determining the most representative evaluation environment, and delineating idiotypes for breeding program enhancement.

Keywords: Maize; Genotypes; Fertilizer; Idiotype; GGE

1. Introduction

Maize is one of the most important grain crops in the world, contributing to food security and industries, and it is essential to focus on increasing the productivity of this crop due to its strategic importance.

The application of plant breeding concepts, particularly quantitative genetics, will contribute to the development of a desirable plant model. Phenotypic patterns result from the interaction of genetic makeup and environments, whereby the importance of genetic-environmental interaction can be understood from the role of modern genotypes and improved management practices in increasing yield. Consequently, evaluating the performance of genotypes in a single environment will provide less information compared with the comprehensive data obtained from multiple environments and better estimates of the components of variance. Hence, achieving an optimal crop genotype design

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requires the study of quantitative genetics concepts with particular attention to evaluating environmental genotypic interactions. (Al-Anbari, 2021). Environmental factors, including organic and mineral fertilizers, can mask the genuine true genetic potential of genotypes, leading to biased comparisons. Environment-adjusted models have therefore become critical for identifying genetically stable traits and for guiding breeding strategies toward sustainable yield improvement.

The results of recent studies that addressed the concepts of modeling and simulation in maize showed that the complete integration between these two technologies enhances grain yield prediction and increases the accuracy of breeding programs in crop improvement. (Fernandes et al., 2024; Shahhosseini et al., 2020). The predictive modelling applied in this study is consistent with the present direction of genomic prediction research, in which machine-learning algorithms such as regularized regression, ensemble methods, and deep learning are evaluated for their ability to improve the accuracy of genetic performance prediction. Lourenço et al. (2024) demonstrated that advanced machine-learning models can enhance the efficiency of genomic prediction by better capturing complex genetic patterns in both synthetic and empirical datasets. Yield stability across diverse environmental and management conditions continues to be a principal objective in maize breeding and agronomic enhancement. Genotype × environment (G×E) interaction plays a major role in determining the performance consistency of genotypes under varying fertilizer regimes and environmental conditions. The GGE biplot method provides an effective graphical tool for assessing both genotype performance and stability simultaneously, allowing the visualization of the ideal genotype and environment. Furthermore, stability indices such as Lin and Binns' Pi (Lin & Binns, 1988), Wricke's Ecovalence (Wi^2) (Wricke, 1962), and the regression method of Eberhart and Russell (1966) statistically assess the stability of genotypes across various environments.

The objective of this study was to identify the maize idiotype and its simulated form, followed by using stability indices and GGE biplot analysis to determine the most stable genotype and the optimal evaluation environment.

2. Materials and methods

On March 18, 2022, a field experiment was conducted at the Ibn Al-Bitar Preparatory Vocational School's experimental field in the Al-Hussainiya area of Kerbala Governorate. The experiment was conducted with two factors utilizing a split-plot arrangement within a randomized complete block design (RCBD), and included three replications. The main plots had six environments (six fertilizer levels). These were fertilizer combinations consisting of mineral and organic fertilizers, as follows. The E1 was 160N+100P_2O_5+40K_2O kg $ha^{-1,}$ E2 was 160N+100P_2O_5+40K_2O +4 tons ha^{-1} of organic fertilizer, E3 was 160N+100P_2O_5+40K_2O kg ha^{-1} + 8 tons ha^{-1} of organic fertilizer, E4 was 320N+200P_2O_5+80K_2O kg ha^{-1}, E5 was 320N+200P_2O_5+80K_2O kg ha^{-1} + 4 tons ha^{-1} of organic fertilizer and E6 was 320N+200P_2O_5+80K_2O kg ha^{-1} + 8 tons ha^{-1} of organic fertilizer. Six genotypes(synthetic varieties) of maize were grown in the subplots: G1(Fajr 1),G2(Maha), G3(5018), G4(Sumer), G5(Sarah), and G6(Baghdad 3). The cultivation was done in experimental units with an area of 3 x 3 m^2 for each experimental unit. The distance was 75 cm apart and 25 cm between hills, having a distance of 1.5 m between main plots. We used cow and poultry waste as organic fertilizer in a 3:1 ratio.

The traits used included: Days to 75% silking, days to physiological maturity, plant height (cm), leaf area (cm^2), nitrogen uptake (kg ha^{-1}), phosphorus uptake (kg ha^{-1}), potassium uptake (kg ha^{-1}), seed oil (%), the number of ears per plant, the number of rows per ear, grains per row, grains per ear, the weight of 500 grains (g), the biological yield (kg ha^{-1}), the harvest index (%), and grain yield (kg ha^{-1}). The first fifteen traits served as independent variables, while grain yield represented the dependent variable in the environment-adjusted multiple regression model.

Environment-adjusted values were computed to minimize the effect of environmental variation according to the adjusted-mean concept described by Gomez and Gomez (1984). This adjustment was conceptually aligned with the general principle of ecocentrism, which is commonly applied in multi-environment trial (MET) analyses to account for environmental variation (Alesso and Martin, 2021; Wei et al., 2025; Ward et al., 2019).

The adjusted value for each genotype in each environment was calculated using the following formula:

$$\text{Adjusted Value} = \text{Observed Value} - \text{Environment Mean} + \text{Grand Mean}.$$

This procedure ensures that subsequent analyses reflect mainly genotypic rather than environmental differences. The modified dataset was used to create both the idiotype and its simulated form, representing the environment-independent genetic pattern and its optimized simulated counterpart.

After making this adjustment for all attributes, the dataset (genotype × environment × replication) was ranked based on the grain yield values that had been corrected for the environment. The top-performing genotypes were then selected to represent the environment-adjusted ideal genetic pattern, as they combined the highest productive efficiency with superior genetic stability after the removal of environmental effects. No new regression model was estimated at this stage, since the main objective was to identify the best-performing genotypes that were actually realized after environmental adjustment. For interpretative verification, the adjusted grain yield values were evaluated against the predicted trend derived from the general model established for the adjusted dataset (interpretative R^2). This is to confirm the consistency of the identified pattern with the overall predictive structure of the data after excluding environmental influences.

The multiple regression model was developed based on the environmentally adjusted values, which determined the contribution of each trait to grain yield. The regression equation was then used to generate the simulated ideotype. The simulation of true genetic variation is done by the use of the calculated regression coefficient resulted in new performance values that were generated for each genotype by adding realistic random deviations (approximately ±2-8%) around the expected means. The ±2–8% range used to create the simulated values for each genotype showed minor and realistic changes in genetics and the environment. This range corresponds to the classification of low experimental variability as described by Jamieson et al. (1991) and Beah et al., (2021), who suggest that high precision and low variability in agricultural experiments need a coefficient of variation (CV) of less than 10%. To determine the strength and direction of the relationship before developing the multiple regression model, the Correlation coefficients were calculated for all traits with grain yield (Napier & Des Marais, 2023). Furthermore, the multiple regression model was established using environment-adjusted values to confirm and estimate the contribution of each trait to grain yield (Tolhurst et al., 2022). The idiotype was derived from the adjusted data to represent true genotypic performance, while the simulated idiotype was produced from the regression model with realistic deviations to reflect genetic variability (Fernandes et al., 2024). Stability indices were calculated using the original data, non-environment-adjusted grain yield data, since these indices are designed to measure the genotypic response to environmental variation (G×E interaction). The Pi of Lin and Binns (1988) was calculated as the distance mean square between each genotype's response and the maximum response across environments. The Wricke's ecovalence stability index (Wi^2) of Wricke (1962) indicates the contribution of each genotype (i) to the G×E interaction sum of squares. The regression-based parameters of Eberhart and Russell (1966), namely the regression coefficient (b_i) and deviation from regression (S^2di), and their standard deviation (Sd), were also computed to evaluate genotype adaptability and predictability. In addition, Shukla's stability variance (Shukla, 1972) was used to further assess genotypic stability across environments was also computed to evaluate genotype adaptability and predictability. The studied indices describe the consistency, responsiveness, and environmental sensitivity of each genotype across the tested environments. In addition to the stability indices, a GGE biplot was constructed using the original yield data to assess genotype performance and stability across environments. The study was performed with scaling set to 0, centering at 2, and SVP at 2, in accordance with the methodologies outlined by Yan and Tinker (2006). Principal component analysis (PCA) was included in the GGE framework to segregate genotype and environmental effects into PC1 and PC2 axes, thereby demonstrating the patterns of genotype × environment interaction and identifying the most suitable genotypes for specific environments. The stability indices were analyzed using the GEA-R software (Pacheco et al., 2016), developed by the Biometrics and Statistics Unit of CIMMYT. Subsequent analyses, including the construction of the ideal genotype pattern (Idiotype) and its simulated version (Simulated Idiotype), were performed using R Studio environment within R software version 4.0.3 (R Core Team, 2020). In addition, graphical interpretations, such as GGE biplots and environment ranking diagrams, were created in R Studio to visualize genotype adaptability and environmental representativeness.

3. Results and discussion

3.1. Performance of the idiotype and simulated idiotype

As shown in Table 1 that although the coefficient of determination (R^2) had a slight decrease in the simulated pattern, this reduction represents a shift toward biological realism rather than model weakness. The interpretations proposed in this paper align with previous studies that emphasized the importance of incorporating biological realism in prediction models, where a slight reduction in R^2 is expected when models account for genetic variability and environmental complexity (Shahhosseini et al., 2020; Messina et al., 2015; Van Eeuwijk et al., 2019). Tables 2 and 3 show that the simulated pattern in this study exhibited a 16.18% increase in average grain yield relative to the developed idiotype after environmental adjustment, confirming that genetic improvement remains achievable even when environmental effects are controlled. Similar yield gains have been reported in previous simulation-based studies. Tao and Zhang (2010) studied and evaluated adaptation strategies to confront climate change to assess maize productivity in the North China Plain and found that suitable adaptation strategies led to comparable yield improvements (10–20%). Comparable outcomes were also observed when crop simulation modelling was used to guide

idiotype design for future cereal cultivars (Rötter et al., 2015). Together, these studies confirm that environment-adjusted modelling and simulation effectively isolate genetic potential and support the development of high-yield, stable idiotypes under variable fertilizer and climatic conditions. The idiotype (Table 2) represents true genetic values after removing environmental effects, showing a balanced combination of vegetative and reproductive traits with efficient nutrient uptake. Comparison with the simulated idiotype (Table 3) revealed substantial improvement potential in key traits, including leaf area, nutrient uptake, rows and kernels per ear, and 500-kernel weight, resulting in an increase in the harvest index from 42.5% to 46.0% and a 16.18% rise in grain yield. These improvements were the outcome of the use of improved photosynthetic capacity and better assimilate distribution, which align with the principles of optimal maize breeding. The enhancements in the crop characteristics were represented by an increase in leaf area, dry-matter accumulation, kernel number, and kernel weight which were major factors effecting yield potential (Fernández et al., 2022), Field evaluations of maize genotypes further confirm that increases in leaf area, ear fertility traits, and kernel weight directly translate into higher grain yield, reflecting the strong genetic contribution of these traits to yield performance (Sharif & Al-Rawi, 2019). These are the traits most sensitive to genetic variation and the primary driver of increased productivity. Therefore, Tables 2 and 3 present two complementary models: the first represents actual genetic performance, while the second represents future potential achievable through improvement programs. These results demonstrate that organic-mineral fertilization systems, when combined with environmentally modified value modeling, provide an effective framework for guiding selection towards optimal, high-yielding patterns in maize.

These results indicate that maize (*Zea mays* L.) has considerable genetic potential to increase yields. Identifying idiotype and its simulated form after environmental adjustment enables the selection of stable, high-yielding maize genotypes across a wide range of growing conditions. This confirms the suitability of maize as a model crop for testing environment-adjusted simulation approaches and optimizing breeding strategies under environmental variability.

Table 1 Multiple linear regression

Grain yield = -4156.118 - 2.8018(Days to 75% silking) - 10.4623(Days to physiological maturity) - 3.5423(Plant height) + 0.0155(Leaf area) + 3.7378(N uptake) - 0.2088(P uptake) - 0.9225(K uptake) + 44.5448(Seed oil %) - 4.9964(Ears per plant) + 14.4238(Rows per ear) - 29.5383(Grains per row) + 2.5297(Grains per ear) + 9.1822(500-grain weight) + 0.2239(Biological yield) + 157.2616(Harvest index).
R^2 (Original Idiotype) = 0.9548 R^2 (Simulated Idiotype) = 0.7759

Table 2 Maize idiotype

Trait	**Mean**	**Range**
Days to 75% silking	71.78	[69.77 – 73.16]
Days to physiological maturity	103.73	[102.28 – 105.56]
Plant height	200.81	[189.77 – 206.74]
Leaf area	5952.69	[5545.05 – 6290.90]
N uptake	305.97	[292.84 – 313.29]
P uptake	59.01	[53.12 – 65.34]
K uptake	192.80	[191.32 – 195.91]
Seed oil (%)	4.29	[4.10 – 4.43]
Ears per plant	1.30	[1.21 – 1.38]
Rows per ear	16.65	[15.86 – 17.13]
Grains per row	39.53	[38.51 – 40.21]
Grains per ear	688.70	[677.29 – 698.19]
500-grain weight	140.91	[138.46 – 142.91]

Biological yield	19105.80	[18915.00 – 19252.56]
Harvest index	42.55	[42.21 – 42.80]
Grain yield	8279.27	[8235.13 – 8386.52]

Table 3 Simulated maize idiotype (Trait-based improvement)

Trait	**Mean**	**Range**
Days to 75% silking	71.62	[67.97 – 75.08]
Days to physiological maturity	104.03	[99.01 – 109.17]
Plant height	224.16	[217.92 – 230.43]
Leaf area	6604.94	[6423.42 – 6793.95]
N uptake	342.57	[333.29 – 352.82]
P uptake	70.12	[68.02 – 71.93]
K uptake	211.21	[205.32 – 217.32]
Seed oil (%)	4.29	[4.09 – 4.51]
Ears per plant	1.18	[1.12 – 1.24]
Rows per ear	17.98	[17.48 – 18.49]
Grains per row	42.18	[41.02 – 43.41]
Grains per ear	738.77	[718.21 – 760.36]
500-grain weight	154.41	[150.46 – 159.20]
Biological yield	20292.64	[19650.92 – 20792.21]
Harvest index	46.03	[44.83 – 47.41]
Grain yield	9619.35	[9223.44 – 10016.30]

3.2. Evaluation of Maize (*Zea mays* L.) Genotypes and Organic Fertilizer Using GGE Biplot and Stability Indices

While the idiotype and simulated idiotype were developed to represent the true genetic pattern and its potential enhancement, the stability indices and GGE biplot were applied for genotypes and environments to assess performance across the environments that are represented by fertilizer conditions. These complementary analyses help identify which real genotype is most aligned with the derived idiotype and which environment provides the most representative and discriminating conditions for evaluation.

The GGE biplot (Figure 1) explained 93.96% of the total variation along PC1 and 3.6% along PC2, indicating that most of the genotype × environment interaction was captured in the first two principal components. Environments located near the average-environment coordinate were more representative, whereas those farther from the origin exhibited strong discriminating ability. Genotypes located near the origin were generally more stable, although their yield performance varied among them. In Figure 1, G3 appeared very close to the biplot origin and aligned with the AEC axis, indicating minimal G×E interaction and confirming its superior stability. Figure 2 illustrates the ranking of environments using concentric circles, where the environment closest to the ideal point (E5) is identified as the optimal environment for genotype testing. Based on the GGE-biplot analysis (Figure 1) and the stability indices (Table 4), the stability parameters (Shukla, Wi^2, b_i, S^2di, and Sd) differed markedly among maize genotypes under fertilizer environments. Genotype G3 had the best stability profile, with the lowest Shukla (3857.55), Wi^2 (30541.07), and S^2di (2624.04) values with $b_i = 1.03$. This shows the genetic stability and adaptability to a wide range of conditions. The low Sd (0.001) for G3 confirms its consistent performance across organic fertilizers, as Sd represents the standard deviation of deviations from regression, a clear indicator of how predictably a genotype responds to diverse environments Although G6 achieved the highest mean grain yield (8141.3 kg ha^{-1}), its higher S^2di, Wi^2, and b_i (> 1) values compared with the most stable genotype (G3) reflected stronger responsiveness to high-input conditions rather than overall

stability, indicating a pronounced G×E interaction and consequently lower predictability. Conversely, higher Sd values, such as 0.01 in G4 show decreased stability by increased fluctuations. Genotype G1 (b_i = 1.00) exhibited wide adaptation but only moderate stability, with Shukla and Wi^2 values higher than those of G3, although still lower than G4 and G5. In contrast, G4 (b_i = 0.96) and G5 (b_i = 0.90) were better adapted to low input environments but exhibited relatively higher instability. These findings are in agreement with the interpretations of Eberhart and Russell (1966), who posited that genotypes exhibiting b_i = 1 and low or non-significant S^2di (or very small Sd) are considered stable and broadly adapted, whereas b_i > 1 and b_i < 1 indicate adaptation to favorable and stressful environments, respectively. Farshadfar et al. (2012), Beah et al. (2021), and Yan and Kang (2020) all found similar results, which show that breeders are able to reliably find high-yielding and stable genotypes by looking at b_i, S^2di, Shukla's variance, and Sd together with GGE biplot analysis. G3 was recognized as the most promising, high-yielding, and widely adaptable maize genotype under the tested fertilizer environments, while environment 5 (E5) was identified as the most representative and discriminating site for future evaluation (Yan & Tinker, 2006).

Table 4 Stability indices of maize genotypes across environments

Genotype	Mean overall	Shukla	Wi^2	Eberhart and Russell			Adaptation
				b_i	S^2di	sd	
G1	7520.222	7520.00	42749.24	1.00	9323.85	0.003	Wide adaptation
G2	7629.222	7852.57	43857.80	1.01	8694.29	0.003	High-input adaptation
G3	7912.500	3857.55	30541.07	1.03	2624.04	0.001	Wide adaptation (most stable)
G4	7251.222	39129.28	148113.50	0.96	32455.31	0.010	Low-input adaptation (unstable)
G5	7208.000	45581.95	169622.40	0.90	8179.51	0.003	Low-input adaptation
G6	8141.277	23373.09	95592.87	1.07	2927.32	0.001	High-input adaptation

Note: Pi = Lin and Binns' superiority index; Wi^2 = Wricke's Ecovalence; Shukla = Shukla's stability variance; b_i = regression coefficient indicating environmental responsiveness; S^2di = deviation from regression representing consistency; Sd = standard deviation of deviations from regression.

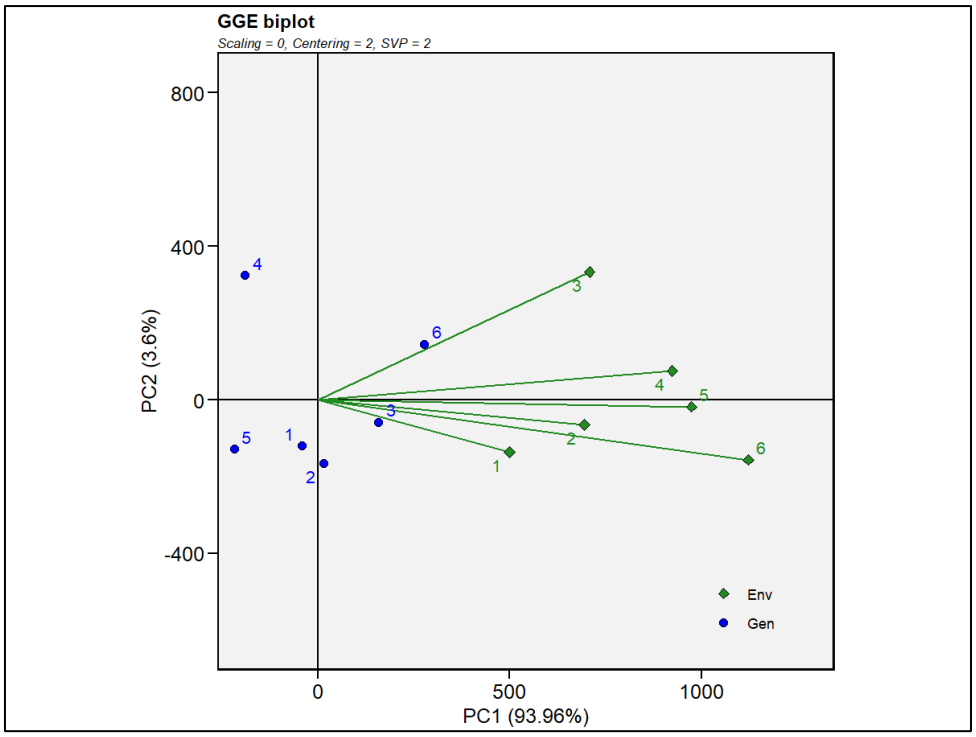

Figure 1 GGE Biplot showing genotype and environment interactions

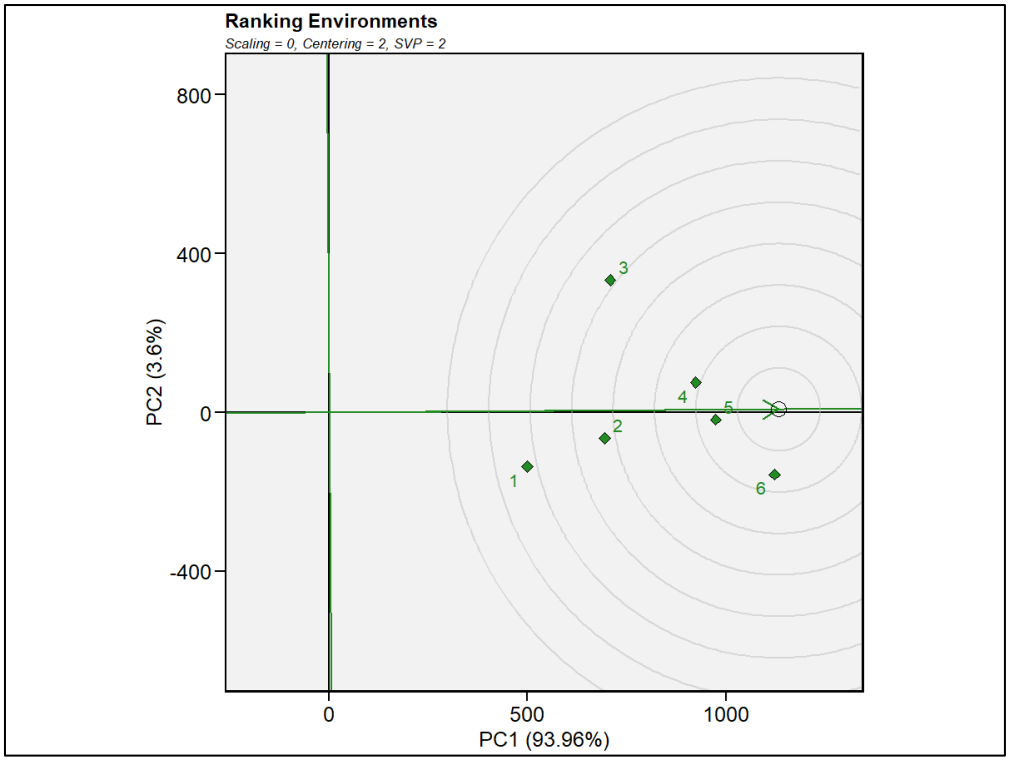

Figure 2 Ranking environments based on representativeness and discriminating ability.

4. Conclusion and Recommendations

The idiotype of maize represents a real model that can be accessed in the field, while the simulation model represents a goal for plant breeders to achieve through breeding and improvement programs. Integrating GGE biplot and stability indices confirmed genotype G3(5018) as the most stable across fertilizer environments, with environment E5 (320N + 200 P_2O_5+80K_2O kg ha^{-1} + 4 tons ha^{-1} of organic fertilizer) identified as the most suitable for achieving consistent and superior yield performance.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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