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Interaction genotypes x environments of soybean sister lines through mixed models, GGE, and AMMI

Interação genótipos x ambientes de linhagens irmãs de soja por meio de modelos mistos, GGE e AMMI

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ABSTRACT

The objective of this work was to evaluate sister lines of soybean from the same family and determine a high degree of relationship through different concepts of adaptability and stability. The trials were conducted at 10 locations in 2018 and 2019 in Paraná and São Paulo, Brazil. Six soybean sister lines from the same family and two cultivars were used as controls. Grain yield data were subjected to stability and adaptability analysis using the mixed model restricted maximum likelihood and best unbiased linear predictor with the harmonic mean of the relative performance of genetic values (HMRPGV) method, additive main effects, and multiplicative interaction analysis (AMMI) methods, and genotype and genotype by environment interaction (GGE) biplot. Genotype classification differed between the AMMI and GGE methods. The GGE method using the biplot plot allowed the identification of the INT60.23 IPRO line as the closest to the ideal cultivar. Using the HMRPGV method, the positive estimates of the genotypic effects and genotypes and environment interaction demonstrated the superiority of the INT60.23 IPRO line.

Keywords: Adaptability and stability; Genetic breeding; *Glycine max* (L.) Merrill.

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RESUMO

O objetivo deste trabalho foi avaliar linhagens irmãs de soja de uma mesma família e determinar um alto grau de parentesco através de diferentes conceitos de adaptabilidade e estabilidade. Os testes foram realizados em 10 locais em 2018 e 2019 no Paraná e em São Paulo, Brasil. Seis linhagens irmãs de soja da mesma família e duas cultivares foram utilizadas como controle. Os dados de produtividade de grãos foram submetidos à análise de estabilidade e adaptabilidade utilizando o modelo misto de máxima verossimilhança restrita e melhor preditor linear imparcial com o método da média harmônica do desempenho relativo de valores genéticos (HMRPGV), efeitos principais aditivos e métodos de análise de interação multiplicativa (AMMI). e genótipo e biplot de interação genótipo por ambiente (GGE). A classificação genotípica diferiu entre os métodos AMMI e GGE. O método GGE utilizando o gráfico biplot permitiu identificar a linhagem INT60.23 IPRO como a mais próxima da cultivar ideal. Utilizando o método HMRPGV, as estimativas positivas dos efeitos genotípicos e da interação genótipos e ambiente demonstraram a superioridade da linhagem INT60.23 IPRO.

Palavras-chave: Adaptabilidade e estabilidade; Melhoramento genético; *Glycine max* (L.) Merrill.

INTRODUCTION

Soybean culture, owing to its production, productivity, and profitability, along with the availability of its adaptable, stable cultivars and globalized pricing, is of great economic importance to Brazil. Soybean breeding programs develop more productive, resistant, and stable cultivars adapted to different locations and regionalized by photoperiod because soybean plants are photosensitive (Borém et al. 2022). The interaction of genotypes and environment (G × E) is fundamental for the positioning of cultivars (Miranda et al., 2021). The challenge of predicting the performance of cultivars in the face of environmental variation between years is always a highly relevant and current topic for science and agricultural production (Miranda et al., 2021). It has recurrently aroused the interest of researchers and the cause of several publications, such as Mushoriwa et al. (2022) with soybean, Kouke et al. (2022) and Abdelrahman et al. (2022) with rice, Bakare et al. (2022a) with cassava, Khan et al. (2022) and Gela et al. (2022) with cowpea, Katsenios et al. (2021), Crevelari et al. (2022) and Castro et al. (2022) with corn.

G × E interactions can be classified as simple or complex (Bianchi et al. 2020). When the interaction is simple, the most productive cultivars maintain superiority in all environments owing to the difference in genotypes in the environments. In the complex interaction, cultivars have superior performance in one environment but not in another,

owing to the lack of correlation in the average performance of the genotypes between environments.

In the presence of the G × E interactions, adaptability and stability analyses were performed for cultivar positioning (Borém et al. 2021). While adaptability is the ability of a cultivar to take advantage of environmental variation, stability is its ability to remain constant during environmental variation.

Currently, multivariate and mixed-model methods have been applied more intensively to different species and traits under selection to interpret G × E interactions (Agahi et al., 2020; Evangelista et al. 2021; Katsenios et al. 2021; Abdelrahman et al. 2022). These methods allow additional inferences, such as the selection of specific cultivars for each location, stable cultivars in various locations, genotypes with wide adaptability, environmental improvement, and simultaneous selection for productivity, stability, and adaptability.

Among the multivariate and mixed model adaptability and stability methods, the main of them are: additive main effects and multiplicative interaction analysis (AMMI) (Gauch, 1988); genotype and genotypes by environments interaction (GGE) biplot (Yan et al. 2000); restricted maximum likelihood and best unbiased linear predictor (REML/BLUP) with the harmonic mean of relative performance of genetic values (HMRPGV) method (Resende 2004).

The AMMI analysis combines additive components for the main effects of genotypes and environments and multiplicative components for the interaction effect in a single model and the matrix of the effects of the G × A interaction (Olivoto et al. 2019).

The GGE analysis uses an analysis for the graphic interpretation of the G × E interaction based on the Sites Regression model (Yan et al. 2000).

The HMRPGV method evaluates the significance of the effects of the matrix model by deviance analysis. It has the advantage of considering the genotypic effects as random, thus providing values of genotypic and non-phenotypic stability and adaptability, unlike other methods that do not provide it (Resende 2007).

The objective of this work was to evaluate sister lines of soybeans from the same family and a high degree of relationship through different concepts of adaptability and stability.

MATERIAL E METHODS

The crop and use value trials of the INT Genetica breeding program were conducted in the states of Paraná and São Paulo (SP) in 10 municipalities, and two harvests (2017/2018 and 2018/2019) are mandatory by Brazilian legislation. The experiments were carried out in Ponta Grossa (PG18 or 1 and PG19 or 2), Luiziana (LZ18 or 3 and LZ19 or 4), Guarapuava (GP18, 5, GP19, or 6), Cruzália, SP (CZ18 or 7 and CZ19 or 8), Nova Tebas (NT19 or 9), Mandaguaçu (MD18 or 10 and MD19 or 11), Palotina (PL18 or 12 and PL19 or 13), Janiópolis (JN18 or 14 and JN19 or 15), Campo Mourão (CM18 or 16), and Forest (FL18 or 17 and FL19 or 18).

Six experimental inbred lines were used from the same family with high relationship to study adaptability and stability of them. Further, two controls were used because they were common to the two years of evaluation, requirements for adaptability, and stability methods that require data balancing, such as AMMI and GGE. The lines were Lineage 1 (INT60.23 IPRO), lineage 2 (INT60.33 IPRO), lineage 3 (INT60.34 IPRO), lineage 4 (INT60.43 IPRO), lineage 5 (INT60.45 IPRO), and lineage 6 (INT 60.57 IPRO), and checks were DM6563 IPRO (7) and M6210 IPRO (8).

The trials were conducted in randomized blocks with three replications. The plots consisted of 4 rows 0.5 m apart and 5 m long, with a sowing density of 12 seeds/m. The useful area consisted of two central lines, and the other two lines were embroidered and discarded during the mechanized harvest. Cultural treatments were based on those used in the region.

Individual analysis of variance (ANOVA) was performed for each trial, and the assumptions for joint analysis of variance considering genotype and environment random effects were evaluated.

After the significance of the interaction G × E in analysis of variance (ANOVA), the analysis of adaptability and stability was performed, mixed models REML/BLUP by the HMRPGV (Resende 2007), AMMI (Gauch 1988) and GGE biplot (Yan et al. 2000) using R software (R CORE Team 2021), Metan package (Olivoto and Lúcio 2020).

The AMMI analysis combines additive components for the main effects of genotypes and environments and multiplicative components for the interaction effect in a single model and the matrix of the effects of the G × E interaction was then displayed using Gabriel biplots (1971).

The average response of genotype i in environment j is given by

$y_{ij} = \mu + G_i + E_j + k \sum \lambda_k a_{ik} t_{jk} + \rho_{ij} + \varepsilon_{ij}$ e where:

μ is the constant inherent to all observations, being the adjusted general average;
G_i is the random effect of the adjusted genotype i (i = 1, 2, ..., g genotypes);
E_j is the random effect of environment j (j = 1, 2, ..., an environment);
λ_k is the singular value for the k-th axis of the principal component;
a_{ik} is the i-th element of the k-th eigenvector of genotypes;
t_{jk} is the j-th element of the k-th eigenvector of environments.
ρ_{ij} is an additional residual if all k-PCAs are not considered, where k = min (G−1;E−1).

Cross-validation procedures were performed for the AMMI analysis and estimated with different numbers of multiplicative terms and stability indices (Olivoto and Lúcio 2020b). The choice of the number of multiplicative terms in the AMMI analysis was based on "Post descriptive success" and "Predictive success" (Olivoto et al. 2019). By definition, "Predictive success" implies making a priori statement about what will happen in the future and "Postdiscritive success" implies making a statement about something that happened in the past. Cross-validation tests were used to assess the predictive success of the AMMI model members and the success of choosing the number of multiplicative terms in the AMMI analysis can be calculated (Olivoto et al. 2019).

The weighted average of absolute scores (WAAS) was used as a quantitative index of stability of cultivar in AMMI analysis (Olivoto et al. 2019). Other stability indices of cultivar were used such as AMMI stability value and additive main effects and multiplicative interaction stability value (ASV); the sum of the absolute values of the interaction principal component axis (IPCA) and sums of the absolute value of the IPCA scores (SPIC) scores was considered for the mean of the squared eigenvectors (SE) and the absolute value of the relative contribution of IPCAs to the interaction (ZA) (Olivoto et al. 2019).

The GGE analysis was performed according to Yan et al. (2000). To evaluate adaptability and productive stability using the GGE Biplot methodology, we calculated the genotypic means of the genotypes in each environment using the following model:

$$\bar{Y_{ij}} - \mu - E_j = G_i + GE_{ij}j \text{ so,}$$

$\bar{Yij}$: the genotypic value of genotype i in environment j;
μ: the overall mean of the observations;

Ej : the main effect of environment j;

Gi : the main effect of genotype i;

$GEij$: the effect of the interaction between genotype i and environment

In the GGE Biplot analysis, only the effects of genotypes and the G x A interactions were considered, and the environmental effect was removed. Then, the model sums the effects of G from G x E, holding them together in two multiplicative terms, according to:

$$Y_{ij} - \mu - \beta_j = g_{i1}e_{i1} + g_{i2}e_{i2} + \varepsilon_{ij}$$ where,

Yij: expected yield of genotype i in environment j;

μ: general average of observations;

β_j: main effect of environment j;

g_{i1} and e_{i1}: main scores of genotypes i and environment j, respectively;

g_{i2} and e_{i2}: secondary scores for genotype i and environment j, respectively;

ε_{ij}: residual not explained by both effects

The GGE Biplot plot occurs by the simple dispersion of gi1 and gi2 for genotypes and ei1 and ei2 for environments, by decomposing the singular value, according to the equation:

$$Y_{ij} - Y_j = \lambda_1\varepsilon_{i1}\rho_{j1} + \lambda_2\varepsilon_{i2}\rho_{j2} + \varepsilon_{ij}$$ were,

λ_1 and λ_2: the largest eigenvalues of the first (PCA1) and second (PCA2) principal components, respectively;

ε_{i1} and ε_{i2}: the eigenvalues of genotype i for PCA1 and PCA2, respectively;

ρ_{j1} e ρ_{j2}: the eigenvalues of environment j for PCA1 and PCA2, respectively.

The analysis of mixed models using the REML/BLUP was performed following the HMRPGV method (Resende 2007).

The statistical model used in the data analysis was 4.2.4 Design in Complete Blocks with Interaction in several places – HMRPGV Method: Model 54, whose statistical model in matrix form corresponds to:

$$y = Xr + Zg + Wi + e$$

y is the data vector,

r is the vector of (fixed) repetition effects added to the general average,

g is the vector of genotypic (random) effects,

i is the vector of the effects of the interaction between genotypes × locations

(random),

and e is the vector of errors or (random) residuals.

Capital letters in the equation represent the incidence matrices of the referred effects.

The significance of the model's effects was estimated using deviance analysis, a statistic derived from the ratio between the likelihood of the complete model and the model without the effect to be tested (reduced model). Statistical significance was determined using the chi-square test with a degree of freedom of 1 and 5% probability, as recommended by Resende (2007).

Genotypic stability values were obtained using the harmonic mean of the genotypic values (HMGV), the relative performance of the genotypic values (RPGV) was used, and the simultaneous evaluation of the adaptability, stability, and productivity was used as the harmonic mean of the relative performance of genotypic values (HMRPGV) for all genotypes according to the following equations:

$$HMGV = 1 / \Sigma^{l}_{j=1} (1/VGj)$$

$$RPGV = (1/l)(\Sigma VGj / Mj)$$

$$HMRPGVi = 1 / \Sigma^{l}_{j=1} (1/RPGVj)$$

where l is the number of locations, VG is the genotypic value, and j is the genotype.

The analyses were performed with Software R (https://www.R-project.org/), Metan package (Olivoto and Lúcio 2020).

RESULTS

ANOVA showed a significant difference between the genotypes at 5% probability by the F test in 10 of the 18 environments, indicating that the cultivars presented a similar performance in these 10 environments. Further, ANOVA was not very efficient for the interpretation performance of cultivars in various locations, with only 10 of 18 valid experiments (56%) for cultivar differentiation. In turn, the joint ANOVA met the assumptions, and the G × E interaction was significant at 5% probability with a coefficient of variation of 8.1%, and none of them presented coefficients of variation below 12%.

The soybean genotypes presented an average of 4333 $kg.ha^{-1}$ with the highest yields of 2410 $kg.ha^{-1}$ of the INT60.57 IPRO line in Mandaguaçu 2019 (MD19) and 6370 $kg.ha^{-1}$ of the INT60.23 IPRO line in Ponta Grossa 2018 (PG18) (Table 1). The least productive environment was Mandaguaçu 2019, with 3322 $kg.ha^{-1}$, and the most

productive was Ponta Grossa 2018, with 6147 kg.ha^{-1} Only five of the 136 yields in Paraná were below 3000 kg.ha^{-1}, demonstrating the state's edaphoclimatic aptitude for soybean.

The INT60.23 IPRO line was significantly the most productive, with an average of 4657 kg.ha^{-1} at 5% by Tukey's test in 10 environments with significant differences between them (Table 1). The INT60.33 IPRO and INT60.45 IPRO lines were statistically similar to the INT60.23 IPRO lines in eight of the 10 environments that differentiated the lines by Tukey's test at 5%. Regarding the controls, the INT60.23 IPRO line was superior to DM6563 IPRO and M6210IPRO in five environments out of 10 with significant genotypes by the 5% F test. These results showed the superiority of the INT60.23 IPRO lines over the others and commercial controls.

Table 1. Averages of grain yield (kg. ha^{-1}) of the six lines and two checks (T) in the 18 environments in the 2017/2018 and 2018/2019 harvests.

	INT60.23 IPRO	INT60.33 IPRO	INT60.34 IPRO	INT60.43 IPRO	INT60.45 IPRO	INT60.57 IPRO	DM6563 IPRO (T)	M6210 IPRO (T)
PG18*	6370	5057	4655	4473	4767	4585	4586	4372
PG1*	4905	6283	5724	6213	6240	6211	6083	6051
LU18	5734b	5699ab	5998a	5081bc	5861ab	6027a	4722c	5311ac
LU19*	4717	4541	4758	4205	4511	4651	4457	4982
GP18	4837b	5090a	4710ab	4752ab	4847ab	3987bc	4936a	3801c
GP19	4425a	4403a	4086ab	3487bc	4033ab	2812c	3987ab	4408a
CZ18*	3948	3562	3376	3300	3466	4016	3500	3512
CZ19	4426a	2895e	3427ce	4084abc	4369ab	3778abd	3519bcde	2904de
NT18	3438b	3643a	3376ab	2618b	3255ab	3597a	3438ab	3669a
MD18	5047a	5276a	5294a	4621ab	3845bc	3820bc	3725c	4968a

MD19	3547a	3764a	2986ab	3381[a]	3487[a]	2410b	3282ab	3717a
PL18*	4025	3558	3765	3483	3398	3416	3975	3653
PL19*	4705	4683	4561	4604	5332	4794	4754	4393
JN18	4540ab	4715a	4800a	4574[a]	3686bcd	3089d	3537cd	4090ac
JN19*	4327	4356	4135	3834	4258	4196	3706	4260
CM18	5522a	4863ab	5246ab	5021ab	5168ab	5335ab	4752ab	4580b
FL18	5107a	3447b	3627b	5073[a]	4879[a]	3971b	3694b	3555b
FL19*	3785	4292	4162	3834	4361	3849	3827	4152
Mean	4634	4451	4371	4258	4431	4141	4138	4243

* Experiment without significance difference by F-Test at 5%
+ Ponta Grossa (PG18 and PG19), Luiziana (LZ18 and LZ19), Guarapuava (GP18 and GP19), Cruzália, SP (CZ18 and CZ19), Nova Tebas (NT19), Mandaguaçu (MD18 and MD19), Palotina (PL18 and PL19), Janiópolis (JN18 and JN19), Campo Mourão (CM18) and Floresta (FL18 and FL19).

The AMMI method combines the benefits of factor analysis in bringing them together without the need for a prior understanding of the data with the orthogonal decomposition of ANOVA in a single method to study cultivar stability (Olivoto and Lúcio 2020). This method uses additive ANOVA to the main factors (genotype and environment) and decomposition by singular values to the residual of the additive model, that is, the effect of the G × E interaction added to the experimental error (Olivoto and Lúcio 2020).

The AMMI analysis showed the significance of environmental effects, genotypes, and the G × E interaction, and all the main components were significant, with the first having 43.4% of the variation, the second 24.6%, and the third 14.3 %. The other four main components were also significant, with 17.8% of the variations. The different AMMI models were in agreement with the number of multiplicative terms considered and could be used to predict the productivity of genotype i in environment j. In the AMMI0 model, only the additive effects are considered; in the AMMI1 model, the first

multiplicative term is considered, and so on, until the AMMIF model with seven terms and other analyses need to be applied, not showing the complete data adequacy by AMMI analysis. Thus, the choice of the number of multiplicative terms to be used was based on "Postdiscritive success" and "Predictive success" (Olivoto et al. 2019).

Cross-validation tests were used to assess the predictive success of the AMMI model members (Olivoto et al. 2019). The AMMI3 model with three multiplicative terms was the most accurate because it presented the lowest mean root mean square of the prediction difference and was used to estimate the grain yield.

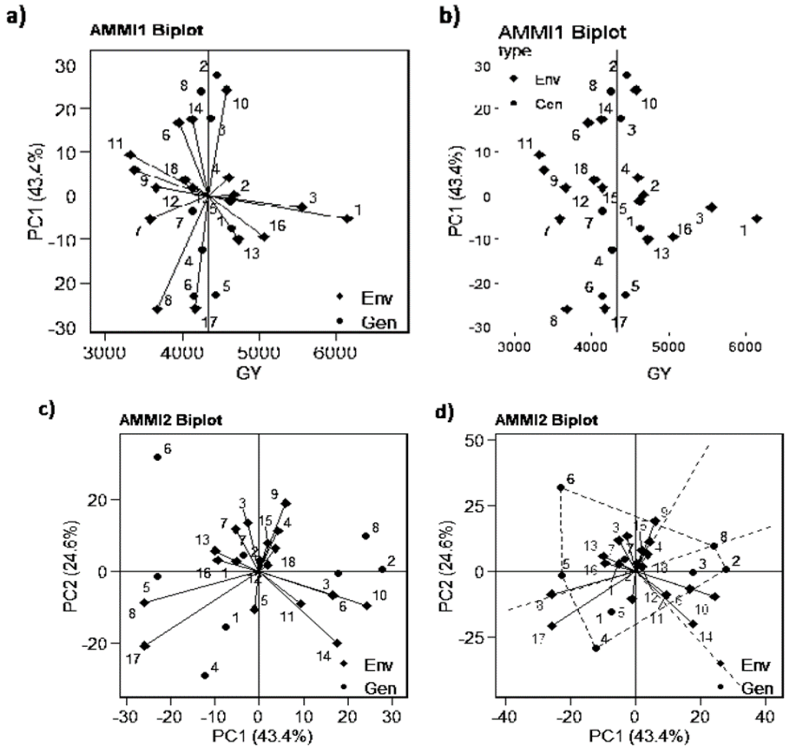
The average superiority of the INT60.23 IPRO line of 6.93% with the overall average of the experiments is represented by the highest absolute values in seven environments based on the averages predicted by the AMMI3 model. The INT60.33 IPRO line was superior in six other environments, the INT60.57 IPRO lines in two environments, and the INT60.43 IPRO and INT60.45 IPRO lines in two environments (data not shown).

The different stability indices of the AMMI analysis, ASV, IPCA, SPIC, SE, ZA, and WAAS classified the INT60.23 IPRO line as the first or second cultivar with greater stability. The INT60.33 IPRO and M 6210 IPRO lines were classified as the most unstable, and the other lines were classified as intermediate to extreme conditions.

The PC1 × GY biplot plot (first principal component × productivity) was used to identify both the stability and productivity of genotypes, such as the INT60.23 IPRO (1) and the DM6563 IPRO (7) control, with scores of PC1 close to zero, that were considered the most stable. The genotypes further to the right of the vertical line have higher productivity than the general average, such as the lines INT60.23 IPRO (1) and INT60.33 IPRO (2) (Figure 1, graphs "a" and "b"). Thus, the INT60.33 IPRO (2) and INT60.45 IPRO (5) lines were productive but not stable because of their greater distance from the origin of the graph. The PC1 x PC2 AMMI biplot plots represent the first two PCAs from the singular value decomposition of the interaction effects matrix (Figure 1, plots "c" and "d"). The first two PCAs explained 68.1% of the sum of squares of the genotype × environment interaction. Markers close to zero, that is, those with low scores, are typical of genotypes or environments that contribute little to the G × E interaction, characterizing themselves as stable. Thus, DM6563 IPRO (7) and INT60.23 IPRO (1) were the most stable, and the environments that least differentiated the cultivars were Floresta (18),

Luiziana (4), Janiópolis (15), Ponta Grossa (2), and Guarapuava (5), according to the AMMI methodology by interpreting the biplot graph (Figure 1, graphs “c” and “d”).

Figure 1. Graphs of PC1 x GY (“a” and “b”) and principal components (“c” and “d”) of the AMMI analysis representing: (a) genotype stability; (b) productivity in kg.ha-1 (GY) of genotypes (Gen) and environments (Env); c) Environment vectors in the main components PC1 x PC2; d) Mega environments formed by the dashed lines. The round markers represent the cultivars INT60.23 IPRO (1), INT60.33 IPRO (2), INT60.34 IPRO (3), INT60.43 IPRO (4), INT60.45 IPRO (5), INT 60.57 IPRO (6), DM6563 IPRO (7) e M6210 IPRO (8). The diamond markers represent the environments: Ponta Grossa (1 and 2), Luiziana (3 and 4), Guarapuava (5 and 6), Cruzália, SP (7 and 8), Nova Tebas (9), Mandaguaçu (10 and 11), Palotina (12 and 13), Janiópolis (14 and 15), Campo Mourão (16) and Floresta (17 e 18).



Fonte: Reginaldo Rosa (2022).

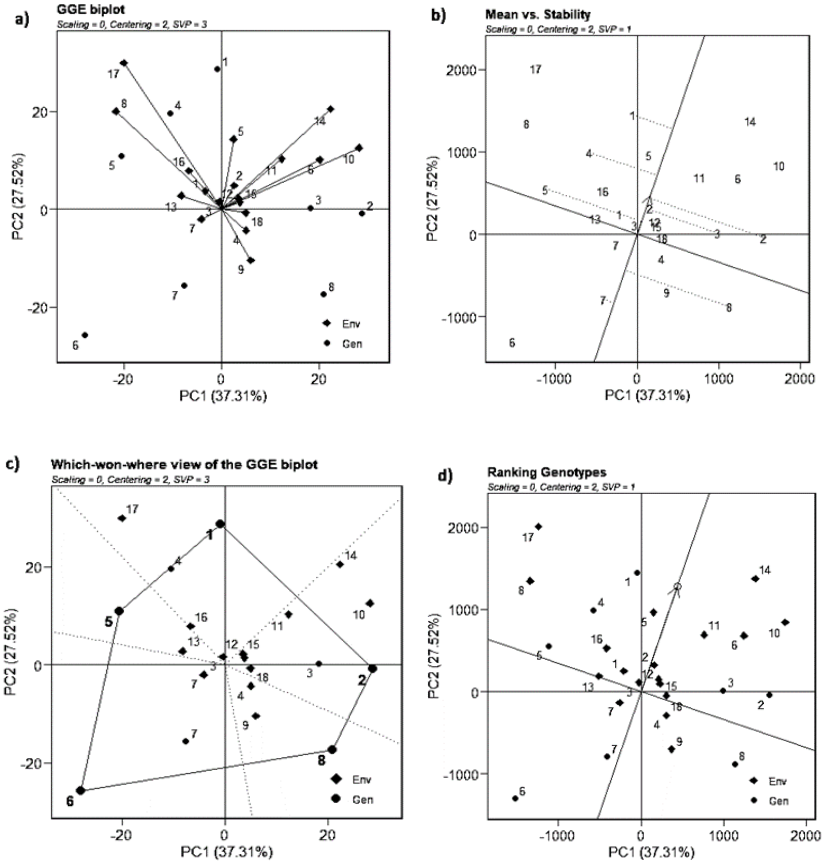
The vectors of the environments are drawn from the graph origin to the endpoints determined by their scores (Figure 1, graph “c”). The cosine of the angle between environmental vectors indicates the approximate linear correlation between them (Olivoto et al. 2019). Thus, an angle of 0º (collinear markers) indicated a +1 correlation, suggesting a high correlation for the Guarapuava (6) and Mandaguaçu (10) environments. An angle of 90° or -90° (perpendicular) indicates zero correlation, indicating independence between them as Nova Tebas (9) with Mandaguaçu (10), Guarapuava (6), Floresta (17), and Janiópolis (14). The 180° angle between the vectors indicates a correlation of -1 with Palotina (13) and Mandaguaçu (10). Angles between the vectors

less than 90° and greater than 270° indicate the existence of a positive response of the genotype to these environments, such as the Guarapuava (6) and Mandaguaçu (10) environments with Mandaguaçu (11) and Janiópolis (14). Environments with negative responses are indicated by angles between 90º and 270º, such as Mandaguaçu (10) with Floresta (17) and Cruzália, SP (8).

The length of a marker vector or the distance from the origin is related to the variance associated with it and, therefore, the environments with the highest variances were Cruzália, SP (8), Floresta (17), Janiópolis (14), and Mandaguaçu (10), as well as the INT 60.57 IPRO (6), INT60.43 IPRO (4), and INT60.33 IPRO (2) lines (Figure 1, graph "c"). The genotypes closest to the origin of the axes showed the smallest variances, with DM6563 IPRO (7) and INT60.23 IPRO (1), being the most stable ones. Between them, indicating similarity and dissimilarity between genotypes or between environments, consisting of graphically visualized environmental stratification (Figure 1, graph "c and d"). Thus, Cruzália, SP (8), and Floresta (17) were more similar and dissimilar in relation to Janiópolis (14), Mandaguaçu (10), and INT 60.57 IPRO (6), which are more dissimilar in relation to the other lines (Figure 2).

For each pair of genotypes and environments in the same quadrant of the graph, the signs of the scores of the same sign interact positively, evidencing an adaptive synergism to be used in the positioning of the cultivar as INT60.23 IPRO (1) and INT60.43 IPRO (4) and Cruzália, SP (8) and Floresta (17) (Figure 1 "c" and "d"). The inverse is also true, that is, those pairs of genotypes and environments with opposite signs interact negatively, suggesting a certain degree of antagonism, an unfavorable combination of genotype and environment as the genotypes INT60.33 IPRO (2) and M6210 IPRO (8) and the Cruzália, SP (8) and Floresta (17) environments (Figure 1 "c" and "d").

Figure 2. Biplot graphs a) GGE PC1 x PC2 with the distribution of genotypes (gen) and environments (env) that contributed most to each main component; b) GGE performance x stability with the projection of genotypes in the medium environment; c) GGE of the most responsive genotypes and some environments represented in each ME; d) GGE with the positioning of genotypes and environments in relation to the ideal genotype (arrowhead).



Fonte: Reginaldo Rosa (2002).

If the markers for genotypes and environments are arranged along the bisector line of the odd quadrants, that is, 45º with the abscissa axis, the use of a linear regression model is recommended, for example, that of Eberhart and Russell (1966). However, as happened here, when the points are dispersed in the biplot, no simplified model would conveniently adjust the behavior of the genotypes; therefore, the AMMI analysis proves to be appropriate (Gauch 1988).

In the AMMI analysis, a mega environment (ME) is represented in the graph by polygons delimited by dotted lines, and the environments that compose it are within this limit (Figure 1, graph “d”):

ME1 (PC1 positive): Janiópolis (14), Mandaguaçu (11), Guarapuava (6), and Mandaguaçu (10);
ME2 (PC1 positive): Luiziana (4) and Floresta (18);
ME3 (PC1 negative): Campo Mourão (16), Ponta Grossa (1), Palotina (13), Cruzália, SP (7), Luiziana (3), Ponta Grossa (2), Janiópolis (15), Cruzália, SP (8), and New Thebes (9);
ME4 (PC1 negative): Pallottine (12);
ME5 (PC1 negative): Guarapuava (5) and Floresta (17);

The MEs formed by the AMMI analysis did not show a relationship with the locations and years in which the experiments were conducted. In each ME, it is possible to identify the most responsive genotypes that are located in the vortexes of the polygons for ME1: the INT60.33 IPRO line; for ME2: the check M6210 IPRO; for ME3: the INT60.57 IPRO line where Ponta Grossa (2), Luiziana (3), Campo Mourão (16), Ponta Grossa (1), and Palotina (13), more productive environments were included; for ME4: the INT60.45 IPRO line; and for ME5: the INT60.43 IPRO line.

The genotypes close to the origin of the axes of the graph show wide adaptation to environments, such as the INT60.23 IPRO lines (Figure 1, graph “d”). The INT 60.57 IPRO (6), M6210 IPRO (8), and INT60.43 IPRO (4) lines had the lowest yields, as demonstrated by their positions far from the environmental markers, reflecting their low performance in all environments.

In the search of a productive and stable genotype in environments that facilitate this identification, it was found that the Mandaguaçu (10) and Guarapuava (6) environments were the most suitable, as they had a considerable score for PC1 and low score for PC2 among the other environments (Figure 1, graph “c”). For genotypes, the INT60.33 IPRO (2) line had the best position with high PC1 score and close to zero PC2.

Based on the estimated values, the lines with the highest mean in most environments were INT60.23 IPRO (1) and INT60.33 IPRO (2). The INT60.23 IPRO line presented the highest productivity averages for the Ponta Grossa (1 and 2), Luiziana (3), Guarapuava (5), Campo Mourão (16), and Floresta (17) environments, and the INT60.33 IPRO lines for the Guarapuava (6), Mandaguaçu (10 and 11), Palotina (12), Janiópolis (14 and 15), and Floresta (18) environments. The pairs of environments in Ponta Grossa (1 and 2), Mandaguaçu (10 and 11), and Janiópolis (14 and 15) were the only places in the 2 years in which the same line was superior.

In the GGE biplot plot, the environments and genotypes with the most positive scores for principal components 1 or 2 were those that contributed most to the variation capitalized by them (64.83%) (Figure 2, “a”). Therefore, the INT60.33 IPRO line (2) and the Mandaguaçu (10), Janiópolis (14), Guarapuava (6), and Mandaguaçu (11) environments contributed the most to PC1, and the INT60.23 IPRO line (1) and the Floresta (17) and Cruzália, SP (8) environments contributed the most to PC2, agreeing with their highest averages in 13 of the 18 environments.

In the performance x stability biplot of the genotypes, the visualization of the mean and the stability of the genotypes were obtained with a coordinate representing the average environment identified (AEI) by the arrow on the graph in the biplot next to the Ponta Grossa (2) marker (Figure 2 “b”) (Yan et al. 2007). The line that passes through the origin of the biplot and the AEI is the abscissa and corresponds to the mean of the genotypes. Projections of genotypic markers on this axis approximated the average productivity of the genotypes. Thus, the line INT60.23 IPRO (1) with 4531 kg ha^{-1} was the most productive, followed by INT60.43 IPRO (4) with 4443 kg ha^{-1}, and INT60.33 IPRO (2) with 4401 kg ha^{-1} (Figure 2 “b”).

The AEI coordinate is the line that passes through the biplot origin and is perpendicular to the AEI abscissa. The AEI coordinate should approximate the G × E interaction associated with each genotype, which is a measure of variability or instability of genotypes (Yan et al. 2007). Smaller the projection on the AEI coordinate means greater stability regardless of the direction. DM6563 IPRO (7) and INT60.23 IPRO (1) were the most stable, while INT60.33 IPRO (2), INT60.45 IPRO (5), M6210 (8), and INT 60.57 IPRO (6) lines were the most stable and the others were the most unstable (Figure 2 “b”).

By the graphical analysis of the biplot, the MEs are represented by the environments that are in the area delimited by the dotted lines, and the genotype that is at the end of the polygon for each ME is the most responsive (Figure 2 “c”).

Thus, MEs are formed in the following environments:

ME1: Ponta Grossa (1 and 2), Luiziana (3), Guarapuava (5), Floresta (17), and Campo Mourão (16);

E2: Guarapuava (6), Janiópolis (14), Mandaguaçu (10 and 11), Palotina (12), Janiópolis (15), and Floresta (18);

ME3: Cruzália, SP (8), and Palotina (13);

ME4: Cruzália, SP (7);

ME5: Louisiana (4) and Nova Tebas (9).

The INT60.23 IPRO line was the most responsive to ME1, the INT60.33 IPRO line (2) to ME2, the INT60.45 IPRO line (5) to ME3, the INT60.57 IPRO line (6) to only Cruzália, and SP (7) and M6210 (8) to ME5.

All genotypes were compared simultaneously using the GGE biplot (Figure 2 "d"). In this biplot, all genotypes are compared with the ideal genotype that is defined as having the highest productivity in all environments. It is represented by the small circle with an arrow pointing to its coordinate in the graph. Thus, genotypes can be classified based on their distance from an ideal genotype (Yan et al. 2007). Therefore, the INT60.23 IPRO (1) line was the closest to the ideal, followed by the INT60.43 IPRO (4) line. The INT60.57 IPRO (6) line was farthest from the ideal genotype.

The deviance analysis of the mixed model REML/BLUP showed a significant effect of genotypes and G x E interaction by the likelihood ratio test (LRT) on grain yield (LRTχ^2<0.001), indicating that the genotypes have genetic variability and there is an interaction of G x E.

The estimate of the genotypic variance was 19.91, variance of the GxA interaction was 125.54, residual variance was 123.09, and individual phenotypic variance was 268.54. The estimate of genetic variance is much lower than the estimates of the variance of the interaction G x E and environment, which provided heritability of the average of the genotypes (h^2mg) of 68% and the individual heritability in the broad sense (h^2g) of 7%. These percentages characterize that the cultivars with the highest yields are explained by their genotypes.

Accuracy is defined as the correlation between the genotypic values predicted from experimental data, and the true genotypic values are considered one of the most relevant parameters for evaluating the quality of experiments (Resende 2007). The value of genotype selection accuracy was 83%, indicating that genetic gain may occur.

In the joint analysis of environments, the mixed model methodology considers the standard deviation of the genotypes in each environment, penalizing genotypes whose values are high and generating high reliability in the methodology (Resende 2007).

The harmonic mean of genotypic values (HMGV) allows for selection based on stability and productivity. HMGV values are estimates of productivity discounted from instability, representing the most productive and stable genotypes (Resende 2007). The

results of genotypic stability (HMGV) for grain yield showed that the superior lines were INT60.23 IPRO (1) and INT60.33 IPRO (2), owing to their higher values than other cultivars (Table 2).

Table 2. Genotypic values of grain yield (Y), stability of breeding values (HMGV), adaptability of breeding values (RPGV and RPGV_Y), and adaptability and stability (HMRPGV and HMRPGV_Y) by REM/BLUP.

Genotypes	Y kg ha^{-1}	HMGV kg ha^{-1}	RPGV	RPGV_Y kg ha^{-1}	HMRPGV	HMRPGV_Y kg ha^{-1}
INT60.23 IPRO (1)	4634	4500	1.07	4621	1.06	4611
INT60.33 IPRO (2)	4451	4302	1.03	4442	1.02	4418
INT60.34 IPRO (3)	4371	4226	1.01	4361	1.00	4348
INT60.43 IPRO (4)	4258	4118	0.98	4259	0.98	4236
INT60.45 IPRO (5)	4431	4290	1.02	4423	1.02	4405
INT 60.57 IPRO (6)	4141	3973	0.96	4138	0.95	4097
DM6563 IPRO (7)	4138	4050	0.96	4161	0.96	4148
M6210 IPRO (8)	4250	4140	0.98	4262	0.98	4238

Fonte: Reginaldo Rosa (2022).

The relative performance of the genotypic values (RPGV) demonstrates the adaptability of the genotypes, which is the response capacity of each genotype to the improvement of the environment and indicates the superiority of the genotype in relation to the average environment in which it is evaluated. Thus, the superiority of the INT60.23 IPRO (1) line was observed, followed by the INT60.33 IPRO (2) and INT60.45 IPRO (5) lines, with the highest estimates and their corresponding RPGV_Y transformed into kg ha^{-1}.

The HMRPGV method has the advantage of providing results on the scale and measurement of the evaluated character, which can be interpreted directly as genetic values. In other words, the HMRPGV estimate multiplied by the general average provides the predicted average of the genotype when planted in another environment considering

the instability and capitalized by adaptability (Resende 2007). It provides a simultaneous selection for adaptability, stability, and productivity. The line with the highest estimate for HMRPGV was INT60.23 IPRO (1), followed by INT60.33 IPRO (2) and INT60.45 IPRO (5).

LIIC and LSIC values refer to the lower and upper limits of the confidence interval for genotypic effects, respectively (Table 3). It is necessary to observe the population size and the number of genotypes of the selected breeding program to select the genotypes using LSIC and LIIC (Resende 2007).

Table 3. Values of genotypic effects (BLUPg), predicted genotypic values (VG= u + g) and their lower (LIIC) and upper (LSIC) confidence intervals, values of G x A interaction effects (BLUPge), genotypic values (Y = u + g + ge) for grain yield in kg ha-1 for the eight genotypes in 18 environments.

Genotypes	BLUPg	VG u+g	VG LIIC	VG LSIC	BLUP ge	Y u+g+ge
INT60.23 IPRO (1)	205	4538	4351	4726	72	4610
INT60.33 IPRO (2)	81	4414	4227	4601	28	4442
INT60.34 IPRO (3)	26	4359	4172	4547	9	4368
INT60.43 IPRO (4)	-52	4282	4095	4469	-18	4263
INT60.45 IPRO (5)	67	4400	4213	4587	23	4423
INT 60.57 IPRO (6)	-131	4202	4015	4390	-46	4156
DM6563 IPRO (7)	-134	4200	4013	4387	-47	4153
M6210 IPRO (8)	-62	4272	4085	4459	-22	4250

Fonte: Reginaldo Rosa (2022).

The positive estimates of the values of the genotypic effects and of the genotype × environment interaction demonstrated the superiority of the INT60.23 IPRO (1), INT60.33 IPRO (2), INT60.34 IPRO (3), and INT60.45 IPRO (5) lines (Table 3). However, the other lines and controls presented negative estimates for the estimated effects, demonstrating their inferiority compared to the other four.

DISCUSSION

ANOVA differentiated the cultivars in only 56% of the experiments. The average test revealed that the four lines with the highest productivity averages were different only in two environments. This demonstrates the limitation of ANOVA and testing of means

in positioning elite genotypes when they are similar in a set of experiments in multiple locations, as is common in breeding programs.

In the AMMI multivariate analysis, the two main components captured 68% of the variance, a value commonly found by other authors working with different species and experimental sets (Katsenios et al. 2021).

The grouping of environments between the AMMI and GGE analyses was partial, with only the ME1 of AMMI being more similar to the ME2 of GGE, whereas the rest were organized differently. Despite having similar methodological properties, the results differed between the AMMI and GGE analyses in most cases. The AMMI and GGE analyses were advantageous in explaining a considerable portion of the sum of squares of the G × E interaction, allowing THE easy graphical interpretation of statistical analysis results. The GGE analysis, as it incorporates the genotype effect and, in most cases, is highly correlated with the scores of the first principal component, has the advantage of allowing a direct graphical evaluation of the genotype effect. Neisse et al. (2018) highlighted that AMMI and GGE, despite their different approaches, are complementary in their results and that interpretation is limited when the first two principal components do not capture enough variation, as occurred in this study.

No practical relationship between the environments was observed in terms of location or year. The AMMI and GGE analyses do not characterize the productive potential of the environments, so the information obtained from the MEs can be applied to the following years for the positioning of the cultivars since the information on the interactions is only valid for the years under study.

The HMRPGV by REML/BLUP provided simultaneous selection for adaptability, stability, and productivity, simplifying the interpretation of the analysis results. The line identified as superior was INT60.23 IPRO, which presented the highest genotypic value and also the one that most capitalized on the genotype × environment interaction in the average genotypic value. The balanced data of the experimental set showed similar results with the other AMMI and GGE methods.

Among the advantages of HMRPGV, selection for adaptability and genotypic stability is applicable to unbalanced data and the heterogeneity of variances, providing results in the very magnitude of the character and allowing its use for any number of environments (Resende 2007). However, the HMRPGV does not have any statistics on

the environment, although these environments do not have a relationship between years; thus, like the other analyses, we carried out a posteriori to obtain the data.

The positioning of the genotypes in the environments was partially coincident between the methods, not allowing a common direction when applying the four analyses to the lines interacting with different environments (Table 4).

Table 4. Classification of the superiority of the lines in the environments by ANOVA, AMMI, GGE, and HMRPGV methods

	Anova	AMMI	GGE	MHPRVG
	Environments			
INT60.23 IPRO	3* 5 6 8 9 10 11 14 16 17	All	1, 5, 12, 16, 17	1, 6, 7, 8, 12, 15, 16, 17
INT60.33 IPRO	3 5 6 9 10 11 14 16	6, 10, 11, 14	2, 6, 10, 11, 14, 15, 18	2, 5, 9, 11, 14
INT60.34 IPRO	3 5 6 9 10 11 14 16	-	-	3, 10
INT60.43 IPRO	8 10 11 14 16 17	5, 17;	-	-
INT60.45 IPRO	3 5 6 8 9 11 16 17	12	8, 13	13,18
INT60.57 IPRO	3 9 16	1, 2, 3, 7, 8, 9, 13, 15, 16	3, 7	-
DM 6563 IPRO	5 6 9 11 16			
M 6210 IPRO	3 6 9 10 11 14	4, 18	4, 9	4

*Ponta Grossa (1 and 2), Luiziana (3 and 4), Guarapuava (5 and 6), Cruzália, SP (7 and 8), Nova Tebas (9), Mandaguaçu (10 and 11), Palotina (12 and 13), Janiópolis (14 and 15), Campo Mourão (16) and Floresta (17 e 18).

The positioning of the cultivars can only be better explained by applying the concepts of adaptability and stability. The concepts of adaptability by the AMMI and GGE methods use the formation of MEs, which comprise groups of environments. However, the joint analysis of the tests carried out in the same place in different years did not allow a practical conclusion on the environmental stratification of the North, West, and Center-South micro-regions of Paraná and Paranapanema, São Paulo, which were carried out in soybean macro-regions 201 and 103 delimited by the Brazilian Ministry of Agriculture, Livestock, and Supply.

The environmental classification of the AMMI and GGE methods does not have any relationship with soil and climate zoning, as it is based on the productivity of the cultivars, which is dependent on the genotype, environment, and their interaction. However, it is also possible to verify that macroregions can be subdivided in the State of Paraná.

The classification of genotypes was different between the AMMI and GGE methods because they considered different concepts of adaptability. Both methods have two-dimensional biplots of the first two principal components; however, other components were also significant and disregarded in the preparation of the plots, which can alter the visual analyses of the positions of genotypes and environments that are fundamental for interpretation. This demonstrates the importance of installing trials continuously in all years and the significant interaction between genotypes × years rather than genotypes × locations.

The GGE method classified the genotypes in a more coincidental manner with ANOVA and HMRPGV because it considers the G × E interaction in the analysis of adaptability and stability, which was important in the variation of the data. The AMMI method presents an adaptability classification that differs from the other methods, as it includes only the main and multiplicative effects. Several authors have used AMMI and GGE methodologies to assist in cultivar positioning.

The INT60.23 IPRO line presented the highest genotypic value. It utilized most of the genotype × environment interaction by HMRPGV and was also considered stable by AMMI. Using the GGE method, INT60.23 IPRO was considered the closest to the ideal genotype and adapted to 10 environments in ANOVA (Table 4). The INT60.23 IPRO line was identified as suitable for Ponta Grossa (1), Palotina (12), Campo Mourão (16), and Floresta (17) using both the GGE and HMRPGV methods.

All environments of the MEs of the INT60.33 IPRO line from AMMI were included for the same line in the GGE, in addition to the environments Janiópolis (15), Ponta Grossa (2), and Floresta (18), three of which were also the most productive in ANOVA (Table 4).

The INT60.43 IPRO line was responsive to the AMMI for Guarapuava (5) and Floresta (17) and in several environments by ANOVA and was not classified by the GGE and HMRPGV for any environment.

The INT60.45 IPRO line was the most responsive in the ME of Palotina (12) of the AMMI, in Cruzália (8) and Palotina (13) of the GGE environments, in Palotina (13) and Floresta (18) of the HMRPGV, and different environments for ANOVA (Table 4).

The INT60.57 IPRO line was responsive to Luiziana (3) by ANOVA, AMMI, and GGE, as well as to Cruzália (7), and several other environments using the AMMI method (Table 4).

The check DM6563 only stood out for ANOVA and M6210 for Luiziana (4) in the three methods and different environments for ANOVA (Table 4).

Santos et al. (2019), working with a common bean, obtained the environmental classification by the GGE method to differentiate the cultivars and concluded that the selection of genotypes was consistent with the GGE and HMRPGV methods and that the understanding of the genotype by environment interaction allowed them to identify possible cultivars for release.

The best positioning of the cultivar will be rather than to identify the best cultivar and the environment should be better characterized for practical generalizations, regardless of the interpretation method of the G × E interaction. For example, Resende et al. (2021) worked with simulated data showing that environments can be optimized to improve productivity and positioning of cultivars, especially in the current scenario of dynamic climate change applying a new class of environmental markers, such as geographic and edaphoclimatic positioning information which are obtained at low cost, are increasingly available, and extrapolated between crops.

The HMRPGV method classifies the genotypes, based on adaptability and stability in the same unit of the variable under study, as productivity, thereby facilitating easier interpretation. In addition, it allows the use of unbalanced data that occur in breeding programs by selecting superior genotypes from one year to another. Owing to the limitations of AMMI and GGE analyses, one can cite the requirement of balanced data, the explanation of only a small portion of the total sum of squares G or G + G × E, respectively, and the loss of the uncertainty measure, as a particular hypothesis cannot be calculated.

For future studies, analysis methods based on edaphoclimatic data will be able to add information to the traditional analyzes of the genotype x environment interaction with practical application.

CONCLUSIONS

The INT60.23 IPRO line stands out from the others regarding productivity, adaptability, and stability. The environmental classification of the AMMI and GGE methods did not have any relationship with edaphoclimatic zoning. The HMRPGV method considers environments more uniformly in identifying genotypes with broad stability and shows results similar to those of GGE and AMMI methods.

The adaptability and stability analysis methods should also be chosen to interpret environments to approach a practical classification for cultivar positioning.

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