

Adaptability and stability of soybean [*Glycine max* (L.) Merrill] strains in Central-West Brazil

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Received: March 09, 2024

DOI: 10.14295/bjs.v3i7.594

Accepted: May 28, 2024

URL: <https://doi.org/10.14295/bjs.v3i7.594>

Abstract

Soybean (*Glycine max* (L.) Merril) is one of the most important seed legumes in the world due to its high protein and vegetable oil content, being widely used in the food industry and animal feed. However, environmental changes, especially those related to rising global temperatures due to CO₂ emissions, are impacting plant productivity, including soybeans. In this context, genetic improvement programs have been fundamental to develop cultivars that are more resistant to abiotic stresses, such as droughts and intense rains. To evaluate the adaptability and stability of soybean genotypes in different environments, statistical methods such as AMMI (Multiplicative Model of Interpretation and Intersection) and BLUP (Best Linear Unbased Prediction) have been widely used. The AMMI model is used to analyze the interaction between genotype and environment, while the BULP considers random genetic effects, providing a more accurate estimate of genetic value. Furthermore, the weighting between stability (WAASBY) (Weighted Average WAASB) index has been used to identify stable, high-performance genotypes, combining stability and yield characteristics. In the research carried out, eleven improved soybean lines were evaluated in five municipalities in the central-western region of Brazil. Data were analyzed using statistical techniques such as linear mixed model, GGE biplot and AMMI and BLUP models. The results indicated that soybean lines showed significant variations in productivity in different environments, highlighting specific genotypes for each location. The productivity prediction analysis showed that the BLUP model was more accurate compared to the AMMI model. Furthermore, the GGE biplot identified the most suitable genotypes for each environment, considering both average performance and stability. Finally, the combination of characteristics from the AMMI and BLUP techniques, using the WAASBY index, made it possible to identify genotypes with high potential for yield and stability. In summary, the results of this research contribute to the development of soybean cultivars more adapted to variable environmental conditions, providing valuable information for genetic improvement programs and agricultural practices in the central-western region of Brazil. The integration of different statistical methods and evaluation indices has been fundamental to improving the selection of genotypes with high performance and stability, contributing to food security and the sustainability of agricultural production.

Keywords: productivity, genotypes, environments, selection.

Adaptabilidade e estabilidade de linhagens de soja [*Glycine max* (L.) Merrill] no

Centro-Oeste do Brasil

Resumo

A soja (*Glycine max* (L.) Merrill) é uma das leguminosas de semente mais importantes do mundo devido ao seu alto teor de proteína e óleo vegetal, sendo amplamente utilizada na indústria alimentícia e na alimentação animal. No entanto, as mudanças ambientais, especialmente as relacionadas ao aumento das temperaturas globais devido à emissão de CO₂, estão impactando a produtividade das plantas, incluindo a soja. Nesse contexto, programas de melhoramento genético têm sido fundamentais para desenvolver cultivares mais resistentes a estresses abióticos, como secas e chuvas intensas. Para avaliar a adaptabilidade e a estabilidade de genótipos de soja em diferentes ambientes, métodos estatísticos como *AMMI* (Modelo Multiplicativo de Interpretação e Interseção) e *BLUP* (Melhores Predições Lineares Não Viesadas) têm sido amplamente empregados. O modelo *AMMI* é usado para analisar a interação entre genótipo e ambiente, enquanto o *BLUP* considera os efeitos genéticos aleatórios, proporcionando uma estimativa mais precisa do valor genético. Além disso, o índice de ponderação entre estabilidade (*WAASBY*) (*Weighted Average WAASB*) tem sido utilizado para identificar genótipos estáveis e de alto desempenho, combinando características de estabilidade e rendimento. Na pesquisa realizada, foram avaliadas onze linhagens melhoradas de soja em cinco municípios da região centro-oeste do Brasil. Os dados foram analisados utilizando técnicas estatísticas como modelo linear misto, biplot *GGE* e modelos *AMMI* e *BLUP*. Os resultados indicaram que as linhagens de soja apresentaram variações significativas na produtividade em diferentes ambientes, destacando-se genótipos específicos para cada localidade. A análise de predição de produtividade mostrou que o modelo *BLUP* foi mais preciso em comparação com o modelo *AMMI*. Além disso, o biplot *GGE* identificou os genótipos mais adequados para cada ambiente, considerando tanto o desempenho médio quanto a estabilidade. Por fim, a combinação de características das técnicas *AMMI* e *BLUP*, utilizando o índice *WAASBY*, permitiu identificar genótipos com alto potencial de rendimento e estabilidade. Em resumo, os resultados desta pesquisa contribuem para o desenvolvimento de cultivares de soja mais adaptadas às condições ambientais variáveis, fornecendo informações valiosas para programas de melhoramento genético e práticas agrícolas na região centro-oeste do Brasil. A integração de diferentes métodos estatísticos e índices de avaliação tem sido fundamental para aprimorar a seleção de genótipos com alto desempenho e estabilidade, contribuindo para a segurança alimentar e a sustentabilidade da produção agrícola.

Palavras-chave: produtividade, genótipos, ambientes, seleção.

1. Introduction

Soybean (*Glycine max* (L.) Merrill) is the most important seed legume in the world, occupying a prominent place among modern agricultural commodities (Maranna et al., 2021), due to its viable source and high protein and vegetable oil (Abdelghany et al., 2021). It is an important raw material for the food industry, contributing around 25% to the production of edible oil, in addition, it makes up around two-thirds of the global protein concentrate used to feed livestock, poultry and fish (Maranna et al., 2021).

However, with abrupt changes in environmental conditions, impacts on plant productivity are progressing at great intensity due to the direct and indirect effects of abiotic stresses (Raza et al., 2019). CO₂ emissions are the main contributing factor to the greenhouse effect and the increase in global average temperatures, which affect agricultural productivity (Vaughan et al., 2018).

Genetic improvement programs have been an important tool for improving soybean performance in regions affected by droughts and intense rains (Maranna et al., 2021). The main objective of this technology is to develop high-yielding cultivars in a wide range of environmental conditions (Alencar et al., 2018). For plant improvement, most agricultural crops depend on available genetic variability and diversity (Shilpashree, et al., 2021).

It is known that important complex traits are controlled by a large number of genomic regions with small effects whose magnitude and direction are modulated by environmental factors (Alencar et al., 2018). When dealing with quantitative and complex traits such as grain yield, the effects of G (Genotype) × E (Environment) interactions are considered for genotypic assessment and varietal selection (Maranna et al., 2021). Stable genotypic performance is highly desirable in improved cultivars, yield stability demonstrates the ability of a genotype to avoid significant fluctuations under a range of conditions (AlBallat et al., 2019).

The Multiplicative Model of Interpretation and Intersection (*AMMI*) model is often used in conjunction with the *GGE* biplot graphical model to identify Experimental Environments (*EEs*) and also winning genotypes in each

MEs (Teleghani et al., 2023). This graphical model was proposed based on principal component analysis. This method helps breeders to simply evaluate genotype stability and the combination of stability with the yield of genotypes and different environments through the graphical representation of Genotype-Environment Interference (GEI) (Hailemariam Habtegebriel et al., 2022; Milioli et al., 2018). The unique feature of the GGE biplot is that, based on the graphs, it is possible to decide which genotype has the greatest potential in which environment or subgroup. In addition to the methods mentioned above, Best Linear Unbiased Predictions (BLUP) has also been used to analyze Metabolic Equivalents (METs). This method estimates the average yield of genotypes in mixed models with high efficiency (Shojaei et al., 2023; Yan; Tinker, 2005).

To take advantage of both MMII and BLUP, an index called Weighted Average Absolute Scores of BLUPs (WAASB) was introduced, which is actually the integration of MMII and BLUP (Ruabiale et al., 2022; Teleghani et al., 2023). As, when identifying and introducing new cultivars, breeders simultaneously consider stability and yield characteristics, in addition to reducing GEI, genotypes with high yield potential are selected; Therefore, considering the WAASB index and yield (Y), the WAASBY index was introduced, in which both stability and yield characteristics are taken into account at the same time (Lee et al., 2023; Pour-Aboughadareh et al., 2022).

In summary, the objective of this research was to identify stable and high-performance soybean genotypes, using AMMI and BLUP methods, in addition to using the WAASBY index to determine the genotypes with the best average performance and stability for complementary studies aimed at cultivation and use in the region Central-West of Brazil.

2. Materials and Methods

2.1 Plant material and field experiment

The different soybean lines (Table 1) were supplied by “Caraíba Genética”. The phenology of these cultivars varies between 28 and 47 days from sowing to flowering (Average of 34 days) and 96 and 128 days from sowing to maturity (Average of 111 days). Eleven improved soybean lines were evaluated in five municipalities allocated to the soybean macroregion 3 (Microregion 301 and 302) and macroregion 4 (Microregion 402, 401 and 403) in the 2021/2022 harvest (Table 2). According to the National Institute of Meteorology (INMET), the predominant climate in the regions is semi-humid tropical with average annual precipitation of 1200 to 1800 mm.

Table 1. Identification of lineages.

GEN	Lineage
1	BMXBonusIPRO
2	CG141794R59ip
3	CG141890R5144rrM
4	CG141998R632ipM
5	CG142000r345rr
6	CG142022R970ip
7	CG152169R585rr
8	CG152173R630rrM
9	CG152173R656rr
10	CG152174R667rr
11	HOCristalinoIPRO

Source: Authors, 2024.

The experiment was carried out in a randomized block design with 3 replications at each location, totaling 165 plots. The soybean seeds were sown in 4 crop lines with 5 m length plus 1 m corridor and 0.5 m spacing, the plant population varied according to the cycle (240 thousand – 300 thousand), totaling an average between 12 and 15 plants per meter. Harvesting was carried out manually 96 days after planting as soon as the plants reached

physiological maturity. The harvest date varied according to the planting plot due to the lineage and location. The average duration of the experiment with the cultivars was 130 days.

Table 2. Details of the municipalities where the genotypes were studied, including information on geographic location and soil fertility level.

Municipality	FU	Macro	Micro	Height	Latitude	Longitude	Fertility level
Lucas do Rio Verde	MT	4	402	395	12° 59'52''S	55° 57'44'' W	High
Montividiu	GO	4	401	929	17° 16'34'' S	51° 25'54'' W	High
Querência	MT	4	403	360	12° 39'12'' S	52° 15'26'' W	High
Rio Verde	GO	3	301	856	17° 44'46''S	50° 57'52''W	High
Santa Helena de Goiás	GO	3	302	632	17° 53'35'' S	50° 44'11'' W	High

Source: Authors, 2024.

2.2 Data analysis

All statistical analyzes were performed using the R 4.3.0 software (R Core Team, 2023).

2.2.1 Descriptive statistics

Descriptive statistics of the productivity of the eleven genotypes in each of the five tested environments were measured using the R-based "metan" package, which offers a simple and intuitive pipeline. The mean value of each variable was calculated for all combinations of genotypes and environments.

2.2.2 AMMI Model

For data analysis, we followed the AMMI method based on the four-step Gauch (2013) protocol. The AMMI model equation (1) is as follows:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \mathcal{K}_{r(e)} + \mathcal{E}_{ger} \quad (1)$$

Where: Y_{ger} is the yield of genotype g in the environment e to replicate r , μ is the overall mean, α_g is the genotype deviation from μ , β_e is the environmental deviation from μ , λ_n is the singular value for the principal of interaction component axis (ICA) n , γ_{gn} and δ_{en} are the values of the eigenvectors of the genotype g and the environment e for the n axis, respectively, ρ_{ge} is the residual, $\mathcal{K}_{r(e)}$ is the effect of the block r within the environment j , \mathcal{E}_{ger} is the error.

2.2.3 BLUP technique

The BLUP technique is used to consider the effects of genotype and genotype by GEI as random. Unlike the AMMI model, a linear mixed model is used, and the formula (2) is as follows:

$$Y = X\beta + Zu + \varepsilon \quad (2)$$

Where: β is the fixed unknown effect data vector (the block mean value in each environment), u is the GEI + genotype effect, X and Z represent the matrix involving β , u and Y , and ε are the random errors vector. In order to better predict the AMMI family model and the BLUP model, root mean square prediction difference (RMSPD) estimates were used to compare (Piepho, 1994). The variance components of agronomic traits were estimated by restricted maximum likelihood (RMLd) using the metan 1.14.0 package (Olivoto; Lúcio, 2020).

2.2.4 Cross-validation procedure

To evaluate the predictive accuracy of the AMMI and BLUP models, a cross-validation procedure was carried out according to Piepho (1994). The original data was randomly divided into a training set – two complete, randomly selected blocks per environment – and a validation set – the remaining block per environment.

Depending on the experiment, n AMMI models (AMMI0, AMMI1, ..., AMMI n) were fitted to the modeling data according to equation (1).

Validation using BLUP considered the same steps and was based on equation (2). Although these 10 cultivars constitute a relatively small set of cultivars, they represent the majority of the area cultivated with oats in southern Brazil. Therefore, it is reasonable to assume that they constitute a random sample from a population. For all models (AMMI n and BLUP) predictive success was compared in relative terms using the root mean squared difference (RMSD) between model estimates and validation data, equation (3) as follows:

$$\text{RMSPD} = \left[\left(\sum_{i=1}^n (\hat{y}_{ij} - y_{ij})^2 \right) / n \right]^{0.5} \quad (3)$$

Where: \hat{y}_{ij} is the model predicted value of the i -th genotype in the j -th environment and y_{ij} is the observed value of the i -th genotype in the j -th environment in the validation set. For all models (AMMI and BLUP model family), this procedure was repeated 200 times. A boxplot was used to show the distribution of the 200 RMSPD for each model.

2.2.5 Combining features of AMMI and BLUP techniques

To combine the characteristics of the BLUP and MMII techniques, we followed the method presented by Olivoto et al. (2019). In this method, WAASB statistics were used, equation (4) as follows:

$$\text{WAASB}_g = \sum_{n=1}^p |\text{IPCA}_{gn} \times \text{EP}_n| / \sum_{n=1}^p \text{EP}_n \quad (4)$$

Where: WAASB_g is the weighted average of the absolute scores of genotype g , IPCA_{gn} is the score of genotype g on the n -th interaction principal component axis (IPCA) and EP_n is the value of the variance explained by the n -th IPCA. As achieving stable, high-yielding genotypes has become an important objective, we use the WAASBY index, equation (5) as follows:

$$\text{WAASBY}_g = \frac{(rG_g \times \theta_Y) + (rW_g \times \theta_S)}{\theta_Y + \theta_S} \quad (5)$$

Where: WAASBY_g is the superiority index with different weights between yield and stability for genotype g , θ_Y and θ_S are the weights for yield and stability, respectively. rG_g and rW_g are the rescaled values of the g th genotype for yield and WAASB, respectively, and are equations (6 and 7) as follows:

$$rG_g = \frac{100 - 0}{G_{max} - G_{min}} \times (G_g - G_{max}) + 100 \quad (6)$$

$$rW_g = \frac{0 - 100}{W_{max} - W_{min}} \times (W_g - W_{max}) + 0 \quad (7)$$

Where: G_g and W_g are the yield and WAASB values for genotype g , respectively. The mentioned method was carried out in the R software with the metan package (Olivoto; Lúcio, 2020).

2.2.6 Mixed linear model

The yield component data were analyzed using the linear mixed model, represented by the following equation (8):

$$Y_{ger} = \mu + \alpha_g + \beta_e + (\alpha\beta)_{ge} + w_{er} + \varepsilon_{ger} \quad (8)$$

Where: Y_{ger} are the observations of the yield components of genotype g in environment e and block r , μ is the average effect, α_g is the fixed effect of genotype g , β_e is the random effect of the environment and, $(\alpha\beta)_{ge}$ is the interaction of random effect of genotype g in environment e , w_{er} is the random effect of block r in environment e , ε_{ger} and the effect of the experimental error associated with the g -th genotype, the r -th block and the i -th environment, which is assumed as an independent normal distribution, with mean 0 and variance of σ^2 (Hilmarsson et al., 2021). The linear mixed model analysis was performed using the metan 1.14.0 packages (Olivoto; Lúcio, 2020).

2.2.7 GGE biplot

The difference in $G \times E$ effects facilitated the biplot methodology, as described by Yan (2001), for stability

analysis using the R software. The biplot was based on the following model (Yan, 2001) equation (9):

$$Y_{ij} - y_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij} \quad (9)$$

Where: Y_{ij} is the average yield of genotype i in environment j ; y_j is the average yield of all genotypes in environment j ; $\lambda_1 \xi_{i1} \eta_{j1}$ and $\lambda_2 \xi_{i2} \eta_{j2}$ are collectively the first principal component (PC1) and the second principal component (PC2), respectively; λ_1 and λ_2 are the singular values for PC1 and PC2, respectively; ξ_{i1} and ξ_{i2} are the eigenvectors PC1 and PC2, respectively, for genotype i ; η_{j1} and η_{j2} are the eigenvectors PC1 and PC2, respectively, for environment j ; and ε_{ij} is the model residue associated with genotype i and environment j . The interpretation of the GGE biplot was first described by Yan (1999).

Productivity data from five environments were used to construct the GGE biplot (Genotype Main Effect plus Genotype-Environment Interaction) using the main components PCA1 and PCA2 according to the model (Yan et al., 2000; Yan et al., 2006; Singamsetti et al., 2021). GGE biplots were established based on site regression analysis (Cossa et al., 2002; Singamsetti et al., 2021), and GGE biplot tools were used to identify highly adaptable soybean genotypes with maximum average productivity per 'Average vs. Stability' (Yan et al., 2001). Visualization of the mean environment coordinates (AEC) of the GGE biplot revealed the genotypes with the highest average and stable productivity.

3. Results and Discussion

The productivity of the genotypes in the tested sites was highlighted by the following results (Figure 1): Lucas do Rio Verde (Genotype 3), Montividiu (Genotype 1), Querência (Genotype 8), Rio Verde (Genotype 8) and Santa Helena (Genotype 9). Numerous studies have previously shown the potential for simultaneously increasing soybean grain yield and grain filling rate under different conditions (Zanon et al., 2016; Vogel et al., 2021; Schoving et al., 2022). The variations observed in productivity between experimental genotypes imply the possibility of identifying preferred genotypes that present favorable characteristics in both environments (Oladosu et al., 2017; Ayed et al., 2021).

For productivity, the prediction accuracy of the BLUP and AMMI models was tested based on the RMSPD. The results shown (Figure 2) are based on the average of 200 RMSPD estimates for each model tested. The model with the lowest RMSPD value is considered the most accurate model and vice versa (Yue et al., 2022; Reddy et al., 2024). In the current study, compared with AMMI family models, the BLUP model was found to be the most accurate prediction model.

The GGE (Genotype plus Genotype vs Environment Interaction) model is widely used in evaluating genotypes and identifying megaenvironments in multi-environmental trials. The GGE biplot analysis revealed that, considering the average performance, the most suitable genotype for Rio Verde is genotype 8, while for Querência it is genotype 10, for Montividiu it is genotype 2, for Lucas do Rio Verde it is genotype 1 and, for Santa Helena, genotypes 1, 2 and 3.

The GGE model, considering average performance versus stability (Figure 4), demonstrated that, for Montividiu and Rio Verde, the most promising genotype was 8; for Querência, genotype 10 proved to be more suitable; for Lucas do Rio Verde, genotype 1 was identified as more favorable; and for Santa Helena, genotypes 1 and 3 stood out. Previous investigations have documented significant variations among tested soybean genotypes for traits examined in different environments (Li et al., 2020; Rani et al., 2023). The discernible interactions observed between the soybean genotype and the environment suggest a dynamic shift in the response and classification patterns of this soybean genotype in response to diverse environmental conditions (Happ et al., 2023; Hyten et al., 2024).

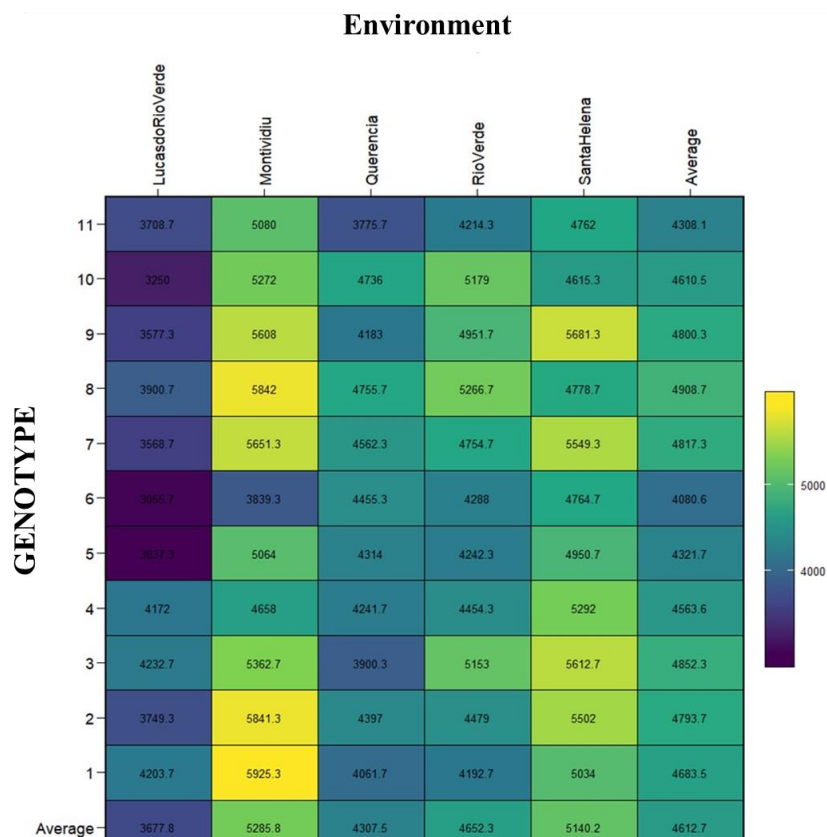


Figure 1. Heat map of productivity scores of different soybean genotypes (*Glycine max*) in different environments. Note: GEN = genotype; ENV = environment. Source: Authors, 2024.

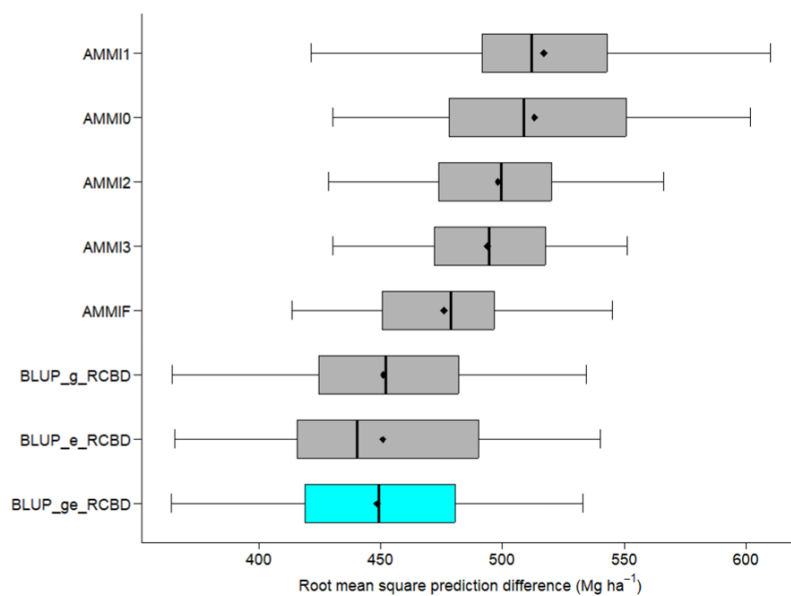


Figure 2. Boxplot showing RMSPD estimates from different prediction models tested for productivity. Source: Authors, 2024.

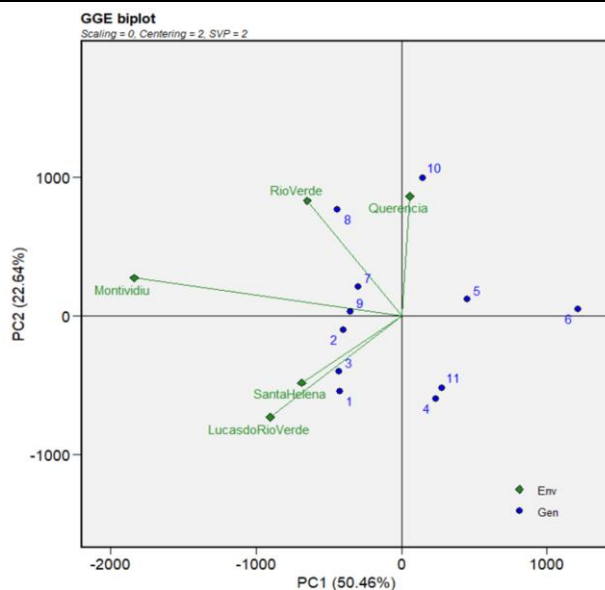


Figure 3. Biplot of productivity (kg/ha^{-1}) versus weighted average of absolute scores. Source: Authors, 2024.

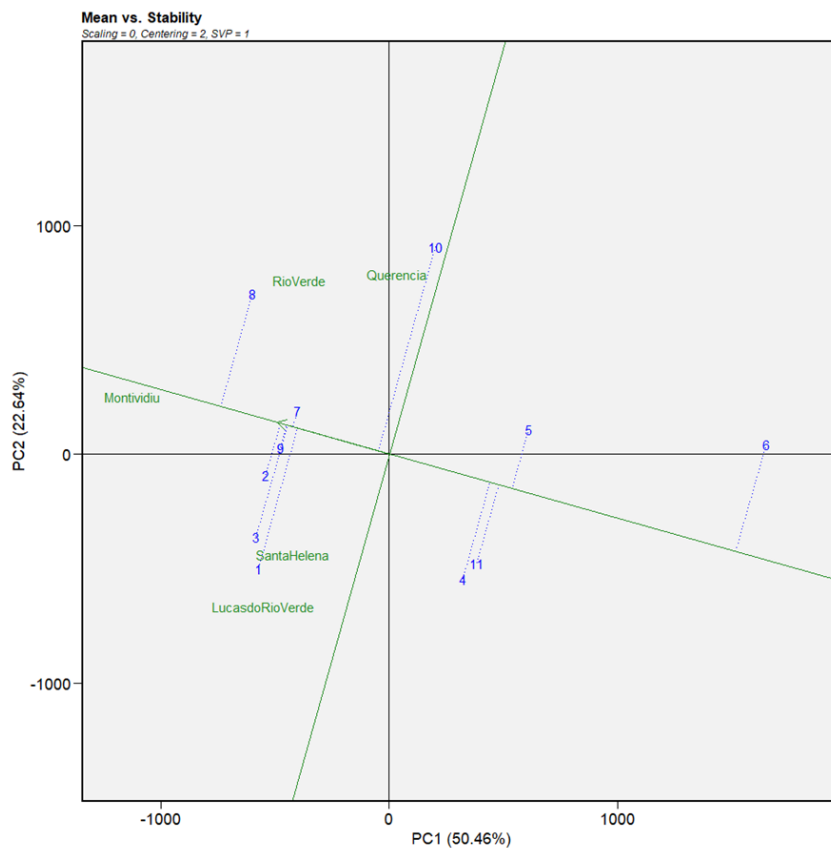


Figure 4. Biplot of average performance vs stability for productivity. Source: Authors, 2024.

When applying mixed models and BLUP, known variance components and random genetic effects are considered. This methodology increases the prediction of genetic value with the true value, reducing the prediction error (Alves et al., 2018). When this methodology was applied to the productivity character (Figure 3), cultivars 8, 3, 7, 9, 2 and 1 stood out with the highest grain yield regardless of the environment evaluated, showing greater adaptability to different environments.

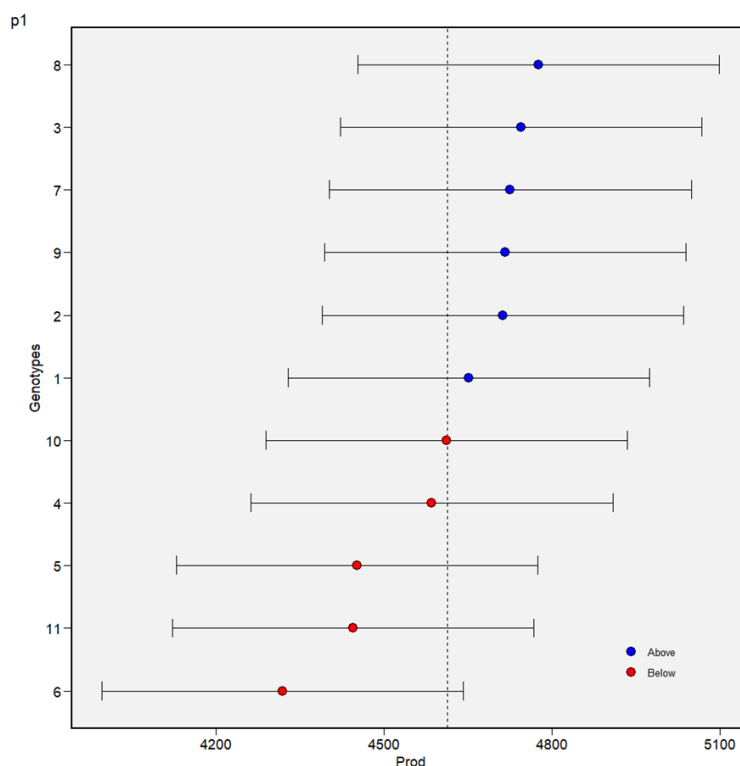


Figure 5. BLUP means of the unbiased linear prediction for the grain yield character of soybean genotypes (*Glycine max*). Source: Authors, 2024.

The simultaneous interpretation of productivity, stability and environments is done using the WAASB biplot with quadrants containing four different classes of genotypes and environments (Figure 6). In the case of the first quadrant, highly unstable genotypes and highly discriminatory environments are included (Olivoto et al., 2019).

Genotypes 6 and 10, being the most unstable and with an average below the general average, are included in this quadrant. Ambientes, Lucas do Rio Verde and Querência were considered effective in discriminating between the genotypes under study. Unstable genotypes, namely Genotype 1, which otherwise have a mean greater than the overall mean, are included in the second quadrant. Although the Rio Verde, Santa Helena and Montividiu environments were discriminative, their environmental averages were higher than the general average.

The third quadrant includes stable, widely adapted and low-yielding genotypes, with an average lower than the general average. A lower WAASB value indicates a greater magnitude of stability. Stable genotypes included in this quadrant are 4, 5 and 1. No environment fell into the third quadrant, which means that no environment can be considered less productive, with less discriminatory capacity.

Likewise, in the case of the fourth quadrant, widely adapted and high-yielding genotypes (genotypic averages greater than the overall average) with low WAASB scores are included. They are 2, 3, 7, 8 and 9. No environments were included in this quadrant, so none were considered less discriminating but more productive.

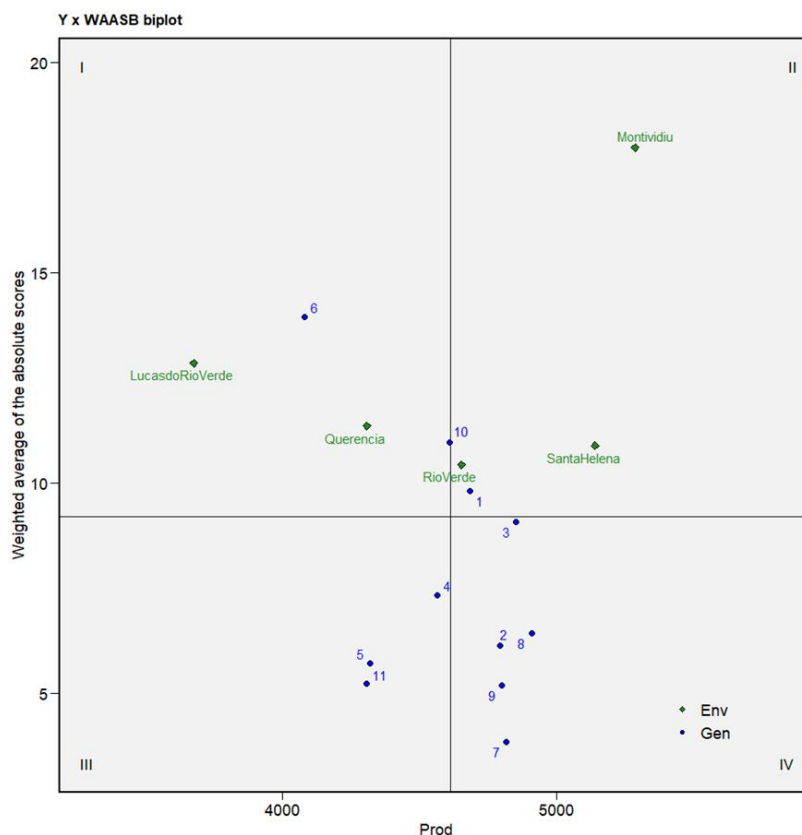


Figure 6. BLUP-based WAASB stability index for genotype productivity for different locations. Source: Authors, 2024.

In Figure 7, the pre-tuned BLUP_model was used to design several WAASBY estimation situations, modifying the coefficients assigned to stability and average performance. In this scenario, the WAASBY superiority index is calculated considering the following coefficients: stability = 100; average performance = 0. In other words, only stability is taken into account for the classification of genotypes.

In the next iteration, the coefficients become 95/5 (since the increment is 5). In the third scenario, the coefficients become 90/10, and so on, until these coefficients are 0/100. In the last iteration, the genotype classification for WAASBY perfectly matches the classifications of the response variable. As observed, the genotypes that stood out when taking only stability into account were genotypes 1, 2 and 8.

While the genotypes that stood out when taking only average performance into account were genotypes 4, 5, 6 and 11. In overall, genotypes 1, 2 and 8 demonstrated good performance in terms of stability and productivity. The good classification in WAASBY observed in genotype 8, demonstrating high productivity in this study, suggests its greater adaptability to high-yield environments.

According to Betrán et al. 2003, a positive correlation was observed between high average performance and regression coefficient in various environmental conditions. According to the univariate stability analysis, 1, 2 and 8 exhibited the highest stability for productivity, respectively, in the tested environments. For productivity, genotypes 5 and 6 showed superior average performance, when stability in all environments was not considered.

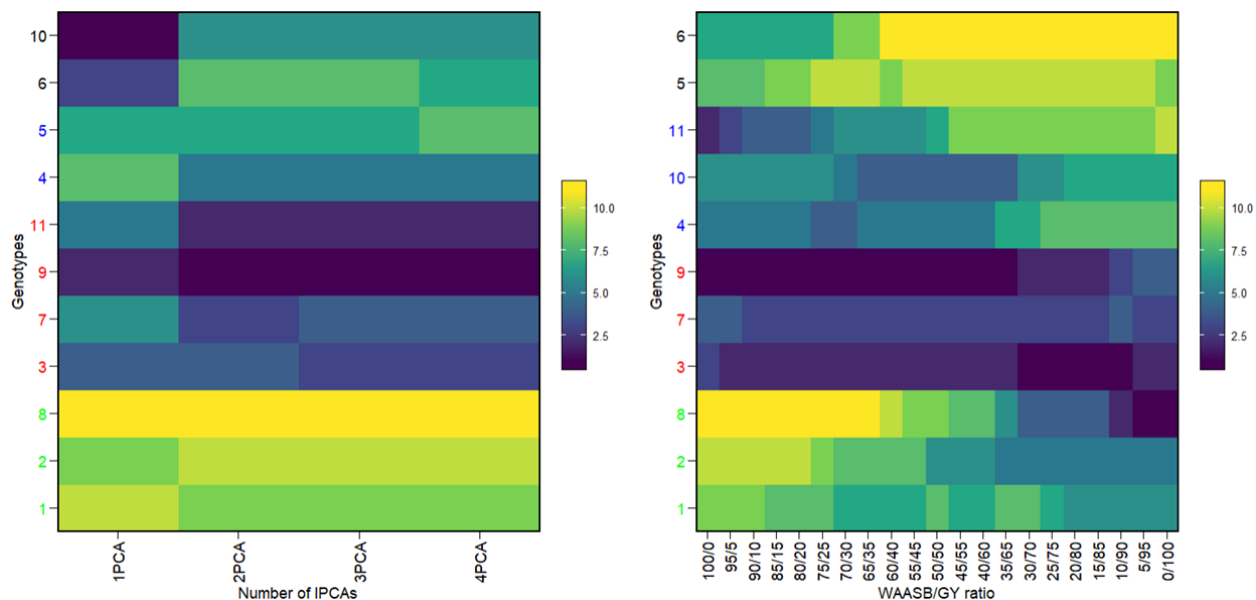


Figure 7. Different scenarios for WAASBY estimation of genotype productivity. Source: Authors, 2024.

4. Conclusions

It is concluded that the significant variation in productivity of soybean genotypes tested in different environments demonstrates that Genotypes 1, 2 and 8 proved to be highly productive and adaptable in high-yield environments, while genotypes 5 and 6 presented superior average performance. when stability was not considered in all environments. On the other hand, genotypes 4, 10 and 11 exhibited below average performance, while genotypes 3, 7 and 9 showed generally inferior performance in these environments for both stability and productivity.

5. Acknowledgments

We thank Sementes Caraíba, Goiás State, Brazil.

6. Authors' Contributions

Wanderson Barbosa da Silva: project design, writing, publication standards, final corrections. *Antonio Carlos Pereira de Menezes Filho*: translation, final corrections, post-evaluation corrections, submission and publication. *Mateus Neri Oliveira Reis*: data analysis, manuscript writing. *Samuel Leandro Soare*: data analysis, manuscript writing, post-evaluation corrections. *Ivandro Bertan*: data analysis, manuscript writing, post-evaluation corrections. *Cláudio Roberto Cardoso de Godoi*: data analysis, manuscript writing, post-evaluation corrections. *Mônica Christina Ferreira*: data analysis, manuscript writing, post-evaluation corrections. *Anaisa Kato Cavalcante*: data analysis, manuscript writing, post-evaluation corrections. *João Carlos Silva Ferreira*: data analysis, manuscript writing, post-evaluation corrections. *Matheus Vinícius Abadia Ventura*: advisor, writing of results, final analysis of the article, publication.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

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Funding

Not applicable.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

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