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Simultaneous multivariate selection with genotypic stability index of grain sorghum hybrids

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Short title
Selection and genotypic stability index of grain sorghum

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The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Chayane Bezerra Alves: Writing – original draft; Writing – review and editing; Data curation.
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Abstract
This study aimed to evaluate and select grain sorghum hybrids with adaptability, stability, and high grain productivity in diverse environments through multivariate analyses. A total of 51 experimental genotypes and 4 commercial controls were evaluated in 33 environments during the 2021-2024 growing seasons. In each growing season, 25 genotypes were evaluated, distributed across 7, 9, 10, and 7 environments in the 2021, 2022, 2023, and 2024 growing seasons, respectively. Furthermore, three scenarios were considered based on the combination of growing seasons and recurring genotypes: scenario 1 (2021-2022); scenario 2 (2023 and 2024); and scenario 3 (2021-2024), which were evaluated in 15, 17, and 33 environments, respectively. The traits of plant height (m), number of days to flowering, and grain yield (kg/ha) were evaluated in a randomized complete block design (RCBD) with three replications. Stability and adaptability analysis was performed using the AMMI-2, WAASB, and WAASBY methodologies, and the MTSI multivariate selection index was used to select superior genotypes. The WAASB method proved efficient in determining adaptability and stability in grain sorghum, allowing the construction of the additional WAASBY index, which facilitates the identification of more stable and productive genotypes, and the MTSI index, which, due to its multivariate nature, allows the definition of the desired plant ideotype, making the interpretation of results more accessible. Among the genotypes evaluated, CMSXS3019 and CMSXS3017 stood out for their high stability, high productive performance, and desirable agronomic characteristics, such as plant size and cycle, proving promising for the development of future cultivars.
Keywords: Genotype; Multivariate selection index; Interaction (G×E); WAASB.

Introduction

Sorghum (Sorghum bicolor L. Moench) is a plant of African origin, standing out as an alternative to corn due to its high adaptability, especially in regions subject to water scarcity (Magalhães et al., 2021). In Brazil, its production has been boosted by the possibility of cultivation in the second crop season, in succession to summer crops, especially soybeans, meeting the demand and standing out as a viable alternative for supplying grains to agro-industries producing animal feed, which are increasingly seeking low-cost and good quality food.(Menezes, et al., 2021).

Sorghum cultivation in the second crop season allows for greater use of later planting windows, in which rainfall is insufficient for corn cultivation. Under these conditions, sorghum emerges as a strategy to reduce agricultural losses, thus ensuring productivity stability in water-restricted environments (Menezes et al., 2018). Even so, its cultivated area remains relatively small compared to corn, especially in regions with water limitations, due to the low availability of adapted and stable cultivars.

Despite the growth of sorghum cultivation in recent years, according to IBGE data (2023), the production and planted area of sorghum in Brazil is still relatively small compared to corn, especially in water-restricted regions such as the Brazilian Northeast. This scenario may be associated with the low availability of adapted and stable cultivars on the market. To expand cultivation in unfavorable environments, it is essential to identify stable and adapted genotypes, thus reducing the effects of genotype x environment (G×E) interaction on productivity.(Ramalho et al., 1993; Guerra et al., 2005).

The introduction of new cultivars to the market requires a careful evaluation of genotype performance under different environmental conditions. Genotype x environment (G×E) interactions represent one of the main challenges for recommending cultivars for different regions, making adaptability and stability analysis fundamental to selection. In this sense, these analyses allow the identification of genotypes that are stable in specific environments, as well as the selection of those that exhibit high responsiveness to positive environmental variations.(Souza et al., 2013).

Evaluating genotypes in multi-environment trials is essential for understanding genotype x environment (G×E) interactions, as well as for identifying stable, adapted materials with high agronomic value for cultivation in heterogeneous environmental conditions (Martins et al., 2025). In this context, multi-environment trials are carried out as part of the final stages of the plant breeding program, and aim to evaluate pre-commercial genotypes and identify potential future cultivars to be released on the market (Olivoto et al., 2018).

Adaptability refers to the ability of genotypes to respond favorably.to the conditionsand environmental stimuli, while stability correspondsto the abilityto maintain predictable performance in response to environmental variations. These analyses consist of essential statistical tools for identifying stable cultivars with predictable responses to environmental variations, and are therefore indispensable instruments in the selection process.materialsgenetic (Cavalcante et al., 2014).

Currently, there are several methods for analyzing stability and adaptability, including AMMI (additive main effect and multiplicative interaction) and GGE (genotypes plus genotype x environment interaction) analysis. These methods are based on fixed-effects models, meaning that both the main (additive) effects of genotypes and environments and the multiplicative effects of the genotype × environment interaction (G×E) are considered fixed (Olivoto et al., 2019).

However, recently, Olivoto (2019) proposed a new method called WAASB (Weighted Average of Absolute Scores), which corresponds to the weighted average of the absolute scores obtained from the singular value decomposition of the BLUP matrix for the G×E interaction effects, generated by a mixed linear model. This model allows the use of random effects, providing greater flexibility and precision to the analysis.

In this sense, the objective of this work is to recommend genotypes of grain sorghum hybrids with high adaptability and stability, through the use of simultaneous multivariate selection, evaluated in different Brazilian states.

Materials and Methods

Study location

The evaluated genotypes are part of the national grain sorghum trial coordinated by Embrapa Maize and Sorghum. The experiments were conducted in 11 cities (Teresina-PI, Sobral-CE, Barra-BA, Sete Lagoas-MG, Nova Porteirinha-MG, Jataí-GO, Rio Verde-GO, Goiânia-GO, Vilhena-RO, Dourado-MS, Sinop-MT) distributed across different regions during the 2021, 2022, 2023, and 2024 growing seasons (Table 2). Planting in the experiments was carried out under rainfed conditions, according to the agricultural window of their respective regions. Soil management and fertilization were performed as recommended in the soil analysis of each experimental location.

Experimental genotypes

In total, 51 different experimental genotypes and 4 commercial controls from the Value for Cultivation and Use (VCU) trial coordinated by Embrapa Maize and Sorghum were evaluated in 33 environments throughout the 2021-2024 growing seasons. In each growing season, 25 genotypes were evaluated, 22 experimental and 3 commercial controls. Furthermore, three scenarios were considered based on the combination of growing seasons and genotypes recurring from 2021 to 2024. The combinations of growing seasons (2021-2022) were designated as scenario 01 (2021-2022), evaluated in 15 environments; scenario 02 (2023 and 2024), evaluated in 17 environments; and scenario 03 (2021-2022-2023-2024), evaluated in 33 environments (Table 1).

Experimental design

The experimental design adopted was a randomized complete block design (RCBD) with three replications at each location. Each useful plot consisted of two rows, 5 m long, 0.75 m between rows and 0.10 m between plants, with a density of 133,333 plants/ha. Harvesting of the experiments occurred when most panicles had dry grains.

Phenotypic variables evaluated

The following characteristics were evaluated: plant height (Alt) in meters, days of flowering (DF), and estimated grain yield (ProdGrãos) in kilograms per hectare, obtained from the harvest of the useful plot of the experiment, which was corrected for stand according to Cruz et al. (2014). Using a moisture meter, the moisture content of a grain sample from each plot was measured on the day of harvest. Subsequently, the values were corrected to 13% moisture.

Statistical analysis

Once the data were obtained, they were checked for compliance with the assumptions of analysis of variance, for normality of errors, homogeneity of variances, and when these were not met, data transformations were performed according to Box and Cox (1964). Subsequently, analysis of variance (ANOVA) was performed (Ramalho et al., 2012).

Adaptability and stability analyses of multi-environment tests (METs) were conducted according to the methodology implemented in the metan package (Olivoto et al., 2020), using the R software. AMMI (Additive Main Effect and Multiplicative Interaction) analyses were performed based on BLUP (Best Linear Unbiased Prediction) values. In addition, the WAASB (Weighted Average of Absolute Scores from the singular value decomposition of the matrix of the best linear unbiased BLUP predictor) model proposed by Olivoto & Lucio (2020) was employed for the GEI effects generated by a Linear Mixed Model (LMM), estimated according to Olivoto et al., (2019) as follows:

$$WAASB_i = \sum_{k=1}^{p} |IPCA_{ik} \times EP_k| / \sum_{k=1}^{p} EP_k$$

In what way:

*WAASB:*weighted average of the absolute scores of the i-th genotype (or environment);

*IPCA:*the score of the i-th genotype (or environment) in the k-th intraclass correlation analysis (IPCA);

EP: amount of variance explained by the k-th IPCA.

In order to select genotypes that combine high performance and stability, the WAASBY index was used (weighted average of the weighted average of the absolute scores of the singular value decomposition of the matrix of best linear unbiased predictions for the genotype x environment interaction effects generated by a linear mixed-effects model and response variable). This index allows the selection of superior genotypes by weighting performance and stability (Olivoto et al., 2019). The WAASBY is estimated as follows:

$$WAASBY_i = \frac{(rG_i \times 0_Y) + (rW_i \times 0_S)}{0_Y + 0_S}$$

Where:

*WAASBY:*Superiority index for the i-th genotype that weighs performance against stability;

These are the weights for the response variable;θ_y

These are the weights for stability;θ_s

G_i: are the response variables (GY);

: WAASB values for the i-th genotype.W_i

The average performance (Y) for the genotype x environment effects (G x E) generated by a mixed linear model and to select superior genotypes, the MTSI (Multivariate Trait Stability Index) was used, assigning 10% to height (Alt), 20% to flowering days (DF), and 70% to grain yield (ProdGrãos).

Results

Of the 33 environments evaluated, grain productivity exceeded 6,000 kg/ha in Sinop-MT.(2023), Teresina-PI (2021), Vilhena-RO (2022), Vilhena-RO (2023), Sobral-CE (2021) and Teresina-PI (2022) (Table 2). Productivity showedvariation substantial, varying between 1,980 kg/ha and 7,865.83 kg/ha, with

the lowest value recorded in Sete Lagoas-MG in the 2023 harvest and the highest in Sinop-MT in the 2023 harvest.

Considering the environments that had harvests from 2021 to 2024, Teresina-PI presented the highest accumulated productivity, with 23,440 kg/ha of sorghum, while Sete Lagoas-MG presented the lowest value, with 10,441.85 kg/ha. In descending order, the most productive environments were Teresina-PI (23,440 kg/ha), Vilhena-RO (22,984 kg/ha) and Sobral-CE (20,423 kg/ha), which are located at different latitudes, longitudes and altitudes. Teresina is located at 5°02'12"S, 42°47'52"W and 77 m altitude; Vilhena at 12°47'17"S, 60°05'39"W and 607 m; and Sobral at 3°44'54"S, 40°21'37"W and 83 m.

On the other hand, the Nova Porteirinha-MG and Sete Lagoas-MG environments presented the lowest accumulated productivities in the four harvests (2021 to 2024), totaling 18,402.15 kg/ha and 10,441.85 kg/ha, respectively. Both are located in similar geographical regions, with Nova Porteirinha located at 15°48'06"S, 43°17'52"W and 535 m, while Sete Lagoas is located at 19°26'39"S, 44°09'58"W and 703 m altitude.

Regarding statistical performance, the joint analysis of variance (Table 3) performed on all evaluated harvests and scenarios showed significant differences for all sources of variation. For the genotype x environment (G×E) interaction source of variation, significant differences were observed for most of the evaluated traits and harvests at the 1% probability level using the F-test (Table 3). The estimates of the coefficient of variation and selective accuracy (Table 3) showed values between 3% and 22% and 0.92 and 0.99, respectively.

Regarding genetic control, estimates of broad-sense genetic heritability ranged from 0.18 to 0.96 across the evaluated crops and scenarios (Table 3). It was observed that the DF trait presented the lowest heritability value in all evaluated crops.

Regarding the productive performance of the genotypes based on grain yield performance using BLUP, it was observed that, in the 2021 harvest, the Sobral-CE and Teresina-PI environments were classified as favorable, since most genotypes showed performance above the overall average of 5,000 kg. The Nova Porteirinha-MG and Vilhena-RO environments were considered intermediate, while Sete Lagoas-MG, Jataí-GO, and Rio Verde-GO showed inferior performance, and were therefore classified as unfavorable (Figure 1 A).

In the 2022 growing season, the Sobral-CE, Teresina-PI, and Vilhena environments were classified as favorable. Barra-BA and NovaPorteirinha-MG1 showed intermediate performance, while Dourado-MS, NovaPorteirinha-MG, SeteLagoas-MG, and Sinop-MT were unfavorable (Figure 1 B).

Similarly, in the 2023 crop season, the environments Barra-BA, Rio Verde-GO, Sinop-MT and Vilhena-RO were considered favorable, while Nova Porteirinha-MG1, Nova Porteirinha-MG2, Santo Antônio de Goiás, Sobral-CE1, Sobral-CE2 and Teresina were classified as unfavorable (Figure 1 C).

Finally, in the 2024 harvest, only Sinop-MT and Teresina showed favorable performance. The environments of Nova Porteirinha-MG, Sete Lagoas-MG and Vilhena were considered intermediate, while Goiânia-GO and Sobral-CE showed unfavorable performance (Figure 1 D).

Continuing the analysis, Figures 2, 3, and 4 present the performance of the genotypes from the perspective of stability and adaptability using the AMMI, WAASB, and WAASBY methods proposed by Olivoto et al. (2019), evaluated in the three scenarios (scenario 1: 2021-2022; scenario 2: 2023-2024; scenario 3: 2021-2022-2023-2024).

The AMMI-2 analysis indicated that, in scenario 01, the first two principal components (PC1 and PC2) explained 61% of the variation in the GXE interaction (Figure 2A). While for scenarios 02 and 03 (Figures 2 B and 2 C), these percentages were 53.25% and 70.35%, respectively.

The three biplots presented (Figure 2 A, B, and C) exhibit similar interpretation patterns. In scenario 01, the genotypes BRS3318, 2116037, and CMSXS3011 showed larger vectors relative to the point of origin, indicating a significant contribution to the interaction between genotypes and environments. In contrast, the genotypes 1920012 and CMSXS3019 were located closer to the point of origin, demonstrating greater stability and less interaction between genotypes and environments.

In scenario 02, the CMSXS3025 and CMSXS3015 genotypes showed the largest vectors, while BRS3318, CMSXS3012, and 2217G23 remained close to the point of origin, meaning they contributed the least to the genotype-environment interactions (Figure 2B). On the other hand, in scenario 03, no genotype is positioned near the center, indicating that its performance varies between environments, and therefore, these are materials with low genotypic stability (Figure 2C). The BRS3318 genotype showed a marked variation in its stability in the biplots presented, which may be associated with the low seed germination reported in the experimental trials.

Regarding the environments, it was observed that Teresina-PI2022 was positioned near the center of the biplot in scenarios 01 and 02, indicating greater representativeness in relation to the overall average (Figure 2 A and B). In scenario 01, the environments Dourados-MS2023, Jataí-GO2022, and Teresina-PI2022 are located near the center of the biplot. In scenario 02, the environments Sobral-CE12023, Sobral-

CE2024, and Sete Lagoas-MG2024 showed similar behavior. In scenario 03, Teresina-PI2022, Rio Verde-GO2023, Barra-BA2023, and Vilhena-RO2023 remained close to the central region of the biplot.

The combined analysis of the biplot of scenario 1 (Figure 3A) allows visualization of the relationship between productivity and stability (WAASB x Grain Productivity). In this scenario, the genotypes located in quadrant I are those that presented low stability and adaptability. In scenario 1, BRS3318 and 2116037 are located in quadrant I, characterized by low productivity and low stability. In scenario 2, CMSXS3025, CMSXS3021, CMSXS3020, and 1822053 are located in quadrant I (Figure 3B). While in scenario 3, only the CMSXS3011 genotype presented low productivity and stability (Figure 3C).

In contrast, the genotypes located in quadrant IV combined high grain productivity and low instability scores, thus being the most stable and productive genotypes (Figure 3). In scenario 01, the genotypes CMSXS3016, 2120043, CMSXS3017, CMSXS3021, CMSXS3019, CMSXS3004, BRS373, and 1920012 stood out (Figure 3A). In scenario 02, the genotypes CMSXS3019, 1621057, CMSXS3017, BRS3318, CMSXS3013, CMSXS3011, and CMSXS3012 (Figure 3B). In scenario 03, only the genotypes CMSXS3017 and CMSXS3021 stood out (Figure 3C).

Considering the behavior of the environments, in scenario 01, it is noted that Teresina-PI 2021 and 2022, Sobral-CE 2021 and 2022, Vilhena-RO 2022, and Barra-BA 2022 presented higher productivities. In the Sobral-CE 2021 environment, the evaluated genotypes showed greater variation in productivity when compared to the Nova Porteirinha-MG 2022 environment (Figure 3 A). In scenario 02 (Figure 3 B), it was observed that the Teresina-PI 2024, Vilhena-RO 2023, Rio Verde-GO 2023, and Sete Lagoas-MG 2024 environments presented stability and satisfactory productivity. In scenario 03 (Figure 3 C), the environments Teresina-PI2021, Teresina-PI2022, Vilhena-RO2022, Sinop-MT2022, RioVerde-GO2023, Vilhena-RO2023, Barra-BA2023, SeteLagoas-MG2024, and Teresina-PI2024 showed favorable performance with greater stability and good productivity.

Complementary evaluation based on absolute WAASB scores showed that values above 60 indicated greater stability and high productive performance. In this criterion, the genotypes CMSXS3019, CMSXS3017, and 1920012 stood out in scenario 01 (Figure 6A); CMSXS3012, CMSXS3011, and BRS3318 in scenario 02 (Figure 4B); and CMSXS3019, CMSXS3017, and CMSXS3011 in scenario 03 (Figure 4C). Overall, all genotypes classified as having high performance and stability in the WAASB x Productivity biplot presented WAASBY scores greater than 60.

Based on AMMI, WAASB, and WAASBY analyses, the desired ideotype was defined as one that exhibits low height, early flowering, high productivity, and stability. Selection via MTSI considered an intensity of 25% and weights of 10%, 15%, and 75% for Height, Flowering Difference, and Grain Production, respectively. In scenario 01 (2021–2022), the genotypes CMSXS3019, CMSXS3017, CMSXS3020, and CMSXS3004 were selected (Figure 5A). In scenario 02 (2023–2024), the selected genotypes were CMSXS3029, CMSXS3023, CMSXS3019, CMSXS3021, CMSXS3017, and CMSXS3013 (Figure 5B). In scenario 3 (2021-2022-2023-2024The CMSXS3017 and CMSXS3019 genotypes were selected (Figure 5 C).

Discussion

It was found that latitude and altitude did not show a direct relationship with the most productive crop (Table 2). This suggests that, although environments with more favorable photoperiods and higher altitudes (due to cooler nights) were expected to favor the productive potential of sorghum, other environmental factors may have been decisive in determining the most productive crops.

The significant effect for all sources of variation and all evaluated harvests demonstrates the presence of genetic variability, which allows the selection of superior genotypes (Birhanu et al., 2024)The significance observed for the "environments" factor may be associated with the heterogeneity of environmental conditions at the experimental sites.

This environmental variation can be explained by both climatic differences (temperature and precipitation) and by soil fertility and photoperiod of the evaluation sites. According to Allard et al. (1964), such variations can be grouped into predictable and unpredictable. Predictable variations characterize the environment itself, being permanent factors such as soil fertility and photoperiod, as well as aspects controllable by humans, such as planting date, seeding density, harvesting methods, and other cultural practices. Unpredictable variations, on the other hand, occur randomly, such as final stand, rainfall distribution, temperatures, and the occurrence of pests and diseases. Similar results were seen by Gomes et al. (2019), where there was a significant effect for all hybrids and environments evaluated.

In general, the "environmental" sources of variation showed higher F values in all evaluated harvests (Table 3), thus reflecting the influence of environmental conditions on the evaluated traits, especially for flowering days (FD). This analysis demonstrates the importance of the evaluated environments in identifying the best genotypes for the different regions of Brazil.

The significant interaction (G×E) observed in Table 3 occurs when the behavior of the cultivars is not consistent across different environments, that is, it implies differential performance of the genotypes in

each environment. Therefore, it is necessary to identify the most stable genotypes, i.e., those that exhibit the most stable behavior in the face of environmental changes, and also the most adapted genotypes, i.e., those that are able to advantageously assimilate the environmental stimulus (Ramalho et al., 2012; Aruna et al., 2024).Bocianowski, 2024).

Additionally, the estimates of the coefficient of variation and selective accuracy (Table 3) showed a high magnitude of experimental precision for all characters in all crops and scenarios evaluated, with values between 3% and 22% and 0.92 and 0.99, respectively. According to Resende and Duarte (2007), accuracies greater than 0.9 are considered ideal for reliable statistical inference.

A variation in broad-sense heritability was observed between 0.18 and 0.95. The lowest heritability values are associated with the flowering days (FD) trait.(Table 3)In general, traits with higher heritability are strongly influenced by genetic factors, which gives them a greater potential for direct genetic selection. On the other hand, traits with low heritability estimates reflect greater environmental influence and genetic complexity.(Aruna et al., 2024)

The broad-sense heritability for the DF trait showed inconsistency in its estimates, with values of 0.19, 0.18, 0.51, and 0.47 for the 2021, 2022, 2024, and scenario 03 crops, respectively, although it presented a low coefficient of variation and a high selective accuracy value (Table 3). This behavior may be associated with the differential response of each genotype to the photoperiod effect in different environments. According to Magalhães et al. (2021), floral differentiation in sorghum is influenced by photoperiod and temperature. In sorghum, six maturity genes are known to affect flowering time and the photoperiodic response to flowering: Ma1, Ma2, Ma3, Ma4, Ma5, and Ma6. The first four genes inhibit flowering under long-day conditions but allow early flowering under short-day conditions. Among them, the Ma1 gene stands out for causing greater sensitivity to long-day conditions compared to the other three.(Calviño & Messing, 2012).

In genetic improvement programs, value for cultivation and use (VCU) trials aim to select the best lines or cultivars based on ranking, rather than simply comparing means. For this ranking to reflect true genotypic effects as accurately as possible, it is necessary to use methods that consider genotypic effects as random. The BLUP (Best Linear Unbiased Prediction) method is widely recommended in this context, as it allows for accurate prediction of genetic values, closely approximating the actual classifications of cultivars.(Olivoto et al., 2019).

In this context, the performance of the genotypes was visualized based on the BLUP-predicted estimates for grain yield, broken down by crop season and environment, as illustrated in Figure 1. This type of graphical representation allows for a detailed identification of the genotypes' behavior in each environment. Therefore, it is possible to classify each environment as favorable, intermediate, or unfavorable, based on the relative performance of the genotypes compared to the average of the respective crop season.

Based on the analysis, it was found that there was no stability in environmental behavior over the years; in other words, an environment considered favorable in a given crop season was not necessarily favorable in the following crop season, and vice versa (Figure 1). Furthermore, genetic materials respond differently to biotic and abiotic factors; the same genotype may exhibit excellent performance in a given environment and inferior performance in another, highlighting the need to perform adaptability and stability analysis (Olivoto et al., 2019; Aruna et al., 2024).Dosho et al., 2022).

Traditionally, breeders consider the effects of years and their interactions with genotypes as random. However, there is still discussion about how the effects of locations should be treated. Given that the goal of most breeding programs is to infer the future performance of genotypes in locations not yet tested, it is recommended that the effects of locations also be considered random. Thus, when conducting a trial with a series of genotypes evaluated in different environments, the ideal model would be one that assumes all effects as random, as proposed in several studies.(Ortiz et al., 2023;Lado et al., 2016).

Additionally, the recommendation to evaluate cultivars in at least three environments over two years is supported by extensive research on genotype-by-environment interactions in various crops.(González-Barrios et al., 2017; Ortiz et al., 2023; Aruna et al., 2024). This approach increases the reliability of cultivar performance evaluations and optimizes breeding strategies, even serving as a basis for the national cultivar registry – RNC established by MAPA Ordinance No. 294/1998 (Brazil, 1998). Thus, three crop groupings were defined (2021–2022, 2023–2024 and 2021 to 2024), prioritizing those that presented the greatest number of genotypes in common in consecutive crops (Tables 1).

These findings reinforce the relevance of a more detailed environmental characterization, encompassing a more diverse set of variables, such as temperature, solar radiation, wind speed, rainfall regime, and soil fertility attributes, among other factors. The incorporation of this information broadens the understanding of the environmental influence on the performance of genotypes and contributes to improving the accuracy of predictive estimates, resulting in more consistent and efficient cultivar selection and recommendation strategies (Rebollo et al., 2023;Dosho et al., 2022).

To perform the adaptability and stability analysis, the metan package, proposed by Olivoto (2019), was used. This package allows for AMMI (Additive Main Effects and Multiplicative Interaction) analyses based on the predictive values of REML/BLUP (Restricted Maximum Likelihood/Best Linear Unbiased Predictor). Furthermore, for multi-environment analyses using a linear mixed-effects model, the author proposed new quantitative measures of genotypic stability, called WAASB (Weighted Average of Absolute Scores from the BLUP), as well as the WAASBY index (Weighted Average of Absolute Scores and Yield).

These analyses were performed to verify the stability and productivity of grain sorghum. It is important to note that grain productivity is a complex quantitative trait, influenced by genetic and environmental factors and their interactions (G×E).This complexity increases due to the differentiated response of genotypes to different environmental conditions throughout the crop cycle (Bocianowski et al., 2024).

Additive main effects and multiplicative interaction analysis (AMMI) is a methodological approach that decomposes genotype, environmental, and G×E interaction effects, and offers a robust framework for characterizing the stability and adaptability of genetic materials in different environments.Yue et al., 2025)The AMMI2 model allows for inferring complex genotype x environment interactions, involving multiple environments, as well as identifying genotypes with broad or specific adaptability (Oroain et al., 2023). AMMI analysis has proven efficient in several studies of genotype x environment interaction assays and in identifying stable and high-performing genotypes of grain sorghum and other crops (Enyew et al., 2021;Shahriari et al., 2018)

In this study, conducted in three distinct scenarios, a total of 14 grain sorghum genotypes were evaluated in scenario 1 in 15 environments, employing Additive Main Effects and Multiplicative Interaction (AMMI-2) analysis (Figure 2A). In scenario 2 (Figure 2B), 23 genotypes were evaluated in 17 environments. The materials that showed the greatest stability were 920012 and CMSXS3019 in scenario 1, and BRS3318, CMSXS3012, and 2217G23 in scenario 2. In scenario 3, evaluating 9 genotypes in 33 environments, it was observed that all materials showed a significant genotype x environment interaction (Figure 2C). Although some genotypes show greater stability, it is essential to consider, in addition to this aspect, productivity and grain yield in the selection process for superior materials (Danakuma et al., 2023; Artiaga et al., 2025).

In the AMMI-2 biplot, environments with low IPCA1 and IPCA2 scores are strong contributors to genotype stability and, consequently, show a low contribution to the genotype x environment interaction.Enyew et al., 2021).Therefore, the 33 environments evaluated in scenario 03 were highly responsive, contributing the most to the genotype x environment interaction effect. This behavior is evidenced by the fact that the first two principal components (PC1 and PC2) explained 70.35% of the interaction variation, indicating a high level of environmental heterogeneity and, consequently, greater complexity in the genotype response (Figure 2C). In this context, to facilitate interpretation and provide greater statistical rigor to the results, the use of the WAASB index is highlighted (Figure 3).

The WAASB (Weighted Average of Absolute Scores from the BLUP) index represents a recent methodological approach developed to assess genotypic stability. This methodology combines the principles of the AMMI (Additive Main Effects and Multiplicative Interaction) model with the REML/BLUP (Restricted Maximum Likelihood/Best Linear Unbiased Predictor) procedures, allowing, in addition to stability analysis, the identification of genotypes that are simultaneously productive and stable. According to Olivoto et al. (2019), the WAASB index presents several advantages, including: being a quantitative measure of stability, allowing the classification of genotypes according to the consistency of their production; enabling the joint interpretation of productivity and stability by considering all multiplicative terms of the model; and allowing analysis even in unbalanced databases. A study conducted by Olivoto et al. (2019) demonstrated that the WAASB index is a robust statistical tool for identifying productive genotypes with broad environmental stability.

The combined interpretation of stability and productivity is made from the biplot represented in Figure 3, in which all PCs (principal components) were considered, thus explaining the entire pattern of genotype x environment interaction. Based on productivity and stability, the genotypes were distributed into four quadrants. Those positioned in quadrant I are considered to have low productivity and low stability, presenting yields below the overall average and WAASB values above the average, indicating greater variation in relation to the environments; genotypes included in quadrant II are considered productive but not very stable; genotypes included in quadrant III are considered stable but not very productive; and finally, the ideal genotypes are those included in quadrant IV.

To select genotypes that combine high performance and stability, the authors also proposed the WAASBY index (Weighted Average of Absolute Scores and Yield), a tool that combines, in a weighted manner, the effects of stability and productivity. This index allows adjusting the weight assigned to each of these characteristics, according to the selection objectives. This flexibility in assigning weights to stability

and average performance in the classification of genotypes can facilitate selection, especially in situations where it is desired to prioritize one of these characteristics (Olivoto et al., 2019).

The index ranges from 0 to 100, with 100 corresponding to the desired ideotype, that is, the genotype considered simultaneously the most productive and the most stable within the set of environments evaluated. Finally, both WAASB and WAASBY can be estimated independently of the assumption of fixed or random effects in the model. This flexibility allows stability analysis and simultaneous selection for stability and average performance to be conducted in a way that is compatible with different experimental designs and statistical models (Olivoto et al., 2019).

To calculate the WAASBY index, equal weights were assigned to the stability (WAASB) and grain yield effects. The genotypes that presented the highest WAASBY indices were CMSXS3019, CMSXS3017, and 1920012, indicating that these genotypes possess both high stability and high grain yield (Figure 4). These WAASBY results confirm the interpretations previously obtained by the AMMI-2 and WAASB methods, but with a simple and direct interpretation.

Breeding programs seek genotype ideotypes that combine various characteristics, not limited to grain yield, although this is still one of the main selection indices. (Singamsetti et al., 2021;Zhang et al., 2026; Olivoto et al., 2019However, the occurrence of biotic and abiotic stresses, in addition to adaptation to crop management, requires the definition of specific ideotypes for grain sorghum, which integrate essential characteristics to overcome these factors.

In this context, it becomes fundamental to use a multivariate selection index capable of identifying superior genotypes. The MTSI (Multivariate Trait Stability Index) (Olivoto et al., 2019) presents properties that distinguish it from other indices in the literature, such as: (i) it allows weighting between stability and average performance; (ii) it classifies the correlation structure between variables; (iii) it is unambiguous, resulting in a single genotypic classification; (iv) it is easy to interpret, allowing the inclusion of hundreds of genotypes in the graph known as a radar plot. The MTSI can also be estimated considering both selection for multivariate stability and simultaneous multivariate selection, assigning specific weights to stability and average performance.

Based on the results of the three scenarios evaluated, two aspects stand out. The first is the consistent recommendation of the CMSXS3019 genotype in all scenarios analyzed (Figures 5 A, B, and C). This genotype is one of the few that contains tannin, which, although it offers some agronomic advantages such as resistance to birds and grain diseases, can also cause digestive problems in animals. This occurs because tannin forms complexes with proteins, reducing the digestibility of the feed. Furthermore, when present, tannin can inhibit the production of gases, especially methane, by ruminants (Rodrigues et al., 2021).

The CMSXS3019 genotype stood out in all three scenarios evaluated, demonstrating its great potential to be released as a commercial cultivar in the future. However, it is important to emphasize that, due to the presence of tannin, this cultivar is limited to a specific market niche. In this sense, the CMSXS3017 genotype emerges as a promising alternative, aimed at the agro-industry and animal feed production niche.

In this context, the MTSI (Figure 5) offered an innovative and easy-to-understand selection process, allowing the assignment of weights according to the economic importance of each trait. Furthermore, the MTSI demonstrated several practical applications for breeders and agronomists seeking to perform simultaneous selection for average performance when data on multiple traits are available (Yue et al., 2025).

The WAASB method was efficient in determining adaptability and stability in grain sorghum. The method enabled the construction ofindexesThe additional WAABY index enabled the identification of more stable and productive genotypes, as well as obtaining the MTSI index, which, due to its multivariate nature, allows the determination of the desired plant ideotype, making interpretation even more accessible.

The CMSXS3019 and CMSXS3017 genotypes stand out for their superior stability and productive performance, in addition to presenting desirable characteristics, such as plant size and cycle, which make them promising for future cultivars.

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Figure 1. Relative performance of genotypes based on predicted BLUP values

Note: Relative performance of genotypes based on predicted BLUP values for: A - Grain yield (Prodgrãos) for 25 genotypes in the 2021 crop season evaluated in 7 environments, B - Grain yield (Prodgrãos) for 25 genotypes in the 2022 crop season evaluated in 9 environments, C - Grain yield (Prodgrãos) for 25 genotypes in 2023 evaluated in 10 environments, and D - Grain yield (Prodgrãos) for 25 genotypes in the 2024 crop season evaluated in 7 environments. The blue and red circles represent the genotypes that presented BLUP above and below the BLUP averages, respectively.
Source: Metan Package (OLIVOTO, 2020)

Figure 2. Adaptability and stability analysis of grain productivity using the AMMI-2 biplot.

Note: Adaptability and stability analysis of grain productivity using the AMMI-2 biplot (PCA1 × PCA2), obtained from the decomposition of the BLUP matrix. A- Scenario 01, composed of 14 grain sorghum genotypes evaluated in 15 environments (2021–2022 growing seasons). B- Scenario 02, composed of 23 genotypes evaluated in 17 environments (2023–2024 growing seasons). C- Scenario 03, composed of 9 genotypes evaluated in 33 environments (2021–2022–2023–2024 growing seasons).
Source: Metan Package (OLIVOTO, 2020).

Figure 3. Biplot of WAASB analysis x Grain Productivity

Note: Biplot of the WAASB x Grain Productivity analysis. A- Scenario 01, composed of 14 grain sorghum genotypes evaluated in 15 environments (2021–2022 growing seasons). B- Scenario 02, composed of 23 genotypes evaluated in 17 environments (2023–2024 growing seasons). C- Scenario 03, composed of 9 genotypes evaluated in 33 environments (2021–2022–2023–2024 growing seasons).

Figure 4. Biplot of estimated WAASBY values

Note: Biplot of estimated WAASBY values considering a 50% stability and 50% performance percentage. A- Scenario 01, composed of 14 grain sorghum genotypes evaluated in 15 environments (2021–2022 growing seasons). B- Scenario 02, composed of 23 genotypes evaluated in 17 environments (2023–2024 growing seasons). C- Scenario 03, composed of 9 genotypes evaluated in 33 environments (2021–2022–2023–2024 growing seasons).

Figure 5. Multivariate Stability Index (MTSI)

Note: Ranking and selection of grain sorghum genotypes by the multivariate stability index (MTSI) (weights: Alt - 10%, DF - 15% and Grain Production - 75%) considering a selection intensity of 25% in different harvests and scenarios: A - scenario 01 (2021-2022), B - scenario 02 (2023-2024), C - scenario 03 (2021-2022-2023-2024).
Source: Metan Package (OLIVOTO, 2020).

Table 1. Genotypes grouped by crop and scenarios.

Harvests and scenarios						
2021	**2022**	**2023**	**2024**	**Scenario 01**	**Scenario 02**	**Scenario 03**
1822038	1921007	2217G23	2321007	2120043	1621057	CMSXS3020
1822039	1921008	1822048	1928011	1822044	1822053	CMSXS3021
1823039	2116017	CMSXS3020	CMSXS3020	CMSXS 3016	2217G33	CMSXS3017
1823044	2116019	CMSXS3021	CMSXS3021	1920012	2217G29	CMSXS3011
1921011	1822043	CMSXS3017	CMSXS3017	2116037	2217G25	CMSXS3019
1316013	2116029	CMSXS3011	CMSXS3011	CMSXS3020	2220034G	CMSXS3012
1719035	1822048	CMSXS3019	CMSXS3019	CMSXS3021	CMSXS3002	CMSXS3004
1921010	1920007	CMSXS3012	CMSXS3012	CMSXS3017	CMSXS3015	BRS3318
1236020	CMSXS3014	CMSXS3004	CMSXS3004	CMSXS3011	CMSXS3013	BRS373
CMSXS3013	1527057	BRS3318	BRS3318	CMSXS3019	CMSXS3018	
DKB550	AG1085	BRS373	BRS373	CMSXS3012	CMSXS3023	
CMSXS3020	CMSXS3020	1621057	1621057	CMSXS3004	CMSXS3025	
CMSXS3021	CMSXS3021	1822053	1822053	BRS3318	CMSXS3029	
CMSXS3017	CMSXS3017	2217G33	2217G33	BRS373	AG1085	
CMSXS3011	CMSXS3011	2217G29	2217G29		CMSXS3020	
CMSXS3019	CMSXS3019	2217G25	2217G25		CMSXS3021	
CMSXS3012	CMSXS3012	2220034G	2220034G		CMSXS3017	
CMSXS3004	CMSXS3004	CMSXS3002	CMSXS3002		CMSXS3011	
BRS3318	BRS3318	CMSXS3015	CMSXS3015		CMSXS3019	
BRS373	BRS373	CMSXS3013	CMSXS3013		CMSXS3012	
2120043	2120043	CMSXS3018	CMSXS3018		CMSXS3004	
1822044	1822044	CMSXS3023	CMSXS3023		BRS3318	
CMSXS 3016	CMSXS 3016	CMSXS3025	CMSXS3025		BRS373	
1920012	1920012	CMSXS3029	CMSXS3029			
2116037	2116037	AG1085	AG1085			

Table 2. Experimental locations, with geographic coordinates and altitude, evaluation crop season, and average productivity.

City	State	Year/crop	Latitude	Longitude	Altitude	Productivity (kg/ha)
Jataí	GO	2021	17°34'09"S	51°44'07"W	895 m	2525.40
Dourados	MS	2022	22°16'31"S	54°48'58"W	412 m	2842.41
Green River	GO	2021	17°46'56"S	50°57'35"W	759 m	2350.32
		2023				5166.22
Goiânia	GO	2023	16°30'21"S	49°16'49"W	830 m	3705.19
		2024				3431.44
Bar	BA	2021	11°04'11"S	43°09'24"W	411 m	5336.30
		2022				5336.30
		2023				5823.63
Sinop	MT	2022	11°52'27"S	55°35'44"W	376 m	4079.13
		2023				7865.83
		2024				6579.91
New Porteirinha	MG	2021	15°48'06"S	43°17'52"W	535 m	3733.33
		2022				4988.44
		2023				4243.70
		2024				5436.67
Sobral	CE	2021	3°44'54"S	40°21'37"W	83 m	6826.38
		2022				5289.67
		2023				4450.85
		2024				3856.32
Teresina	PI	2021	5°02'12"S	42°47'52"W	77 m	7619.85
		2022				6586.74
		2023				3122.42
		2024				6111.33
Vilhena	RO	2021	12°47'17"S	60°05'39"W	607 m	4028.28
		2022				7437.63
		2023				6886.33
		2024				4632.65
Seven Lakes	MG	2021	19°26'39"S	44°09'58"W	703 m	1092.22
		2022				2544.07
		2023				1980.00
		2024				4825.56

Source: geographic location data retrieved from Google Earth (2025).

Table 3. Individual analysis of variance for the years 2021, 2022, 2023, and 2024, and joint analyses for three different scenarios: 2021-2022, 2023-2024, and 2021-2024, with F values, genotype x environment interaction (GxA), coefficient of variation (CV), experimental accuracy, broad-sense heritability (h^2), and genetic correlation (r_g) using the multivariate stability index.

2021				
Source of Variation	**GL**	**Alt**	**DF**	**Grain Producers**
Genotypes	24	22,020**	27,426**	14,864**
Environments	6	178,458**	19589,365**	770,730**
G x A	144	5,546**	22,030**	3,800**
CV		6,821	4,317	18,617
Accuracy		0.977	0.981	0.965
h^2		0.74	0.19	0.74
r_g (Grain Producers)		0.52	0.65	1.00
2022				
Genotypes	24	3170,265**	9,359**	11,201**
Environments	8	3,177**	5225,107**	325,831**
G x E	192	3,428**	7,634**	3,035**
CV		8,970	6,822	16.55
Accuracy		0.98	0.945	0.954
h^2		0.86	0.18	0.72
r_g (Grain Producers)		0.44	0.74	1
2023				
Genotypes	24	49,378**	67,356**	16,182**
Environments	9	2529,388**	15146,925**	322,832**
G x E	216	2,240**	5,708**	2,264**
CV		6,796	3,721	19.41
Accuracy		0.989	0.992	0.968
h^2		0.95	0.91	0.86
r_g (Grain Producers)		0.31	0.23	1
2024				
Genotypes	24	79,377**	15,962**	17,642**
Environments	6	6670,8148**	40875,417**	453,877**
G x E	144	8,062**	7,683**	4,202**
CV		4,983	4,014	15,153
Accuracy		0.993	0.968	0.971
h^2		0.85	0.51	0.74
r_g (Grain Producers)		0.007	0.07	1
Scenario 01 - 2021 and 2022				
Genotypes	13	35,155**	51,563**	32,923**
Environments	14	665,430**	7431,088**	302,712**
G x E	182	4,628**	33,572**	3,203**
CV		7,635	3,964	17,128
Accuracy		0.985	0.99	0.984

h^2		0.019	0.32	0.90
r_g (Grain Producers)		-0.16	0.77	1.00
Scenario 02 - 2023 and 2024				
Genotypes	22	7,076*	19,973**	18,313**
Environments	16	534,978**	5090.98**	189,703**
G x E	352	0.339^{NS}	1,766*	1,787**
CV		14,512	8	22
Accuracy		0.92	0.974	0.972
h^2		0.96	0.91	0.84
r_g (Grain Producers)		0.86	0.16	1.00
Scenario 03 - 2021, 2022, 2023 and 2024				
Genotypes	8	84,121**	53,487**	28,587**
Environments	32	829,155**	6751,673**	153,507**
G x E	256	4,907**	28,112**	3,803**
CV		7,342	4,276	16,482
Accuracy		0.994	0.99	0.982
h^2		0.94	0.47	0.86
r_g (Grain Producers)		0.58	0.59	1.00

Note: NS, * and ** are not significant and significant at 5% and 1% probability by the F-test, respectively. Plant height in meters (Alt), number of days of flowering (DF) and grain yield in kg/ha (ProdGrãos). Source: Prepared by the author.

Figure 1

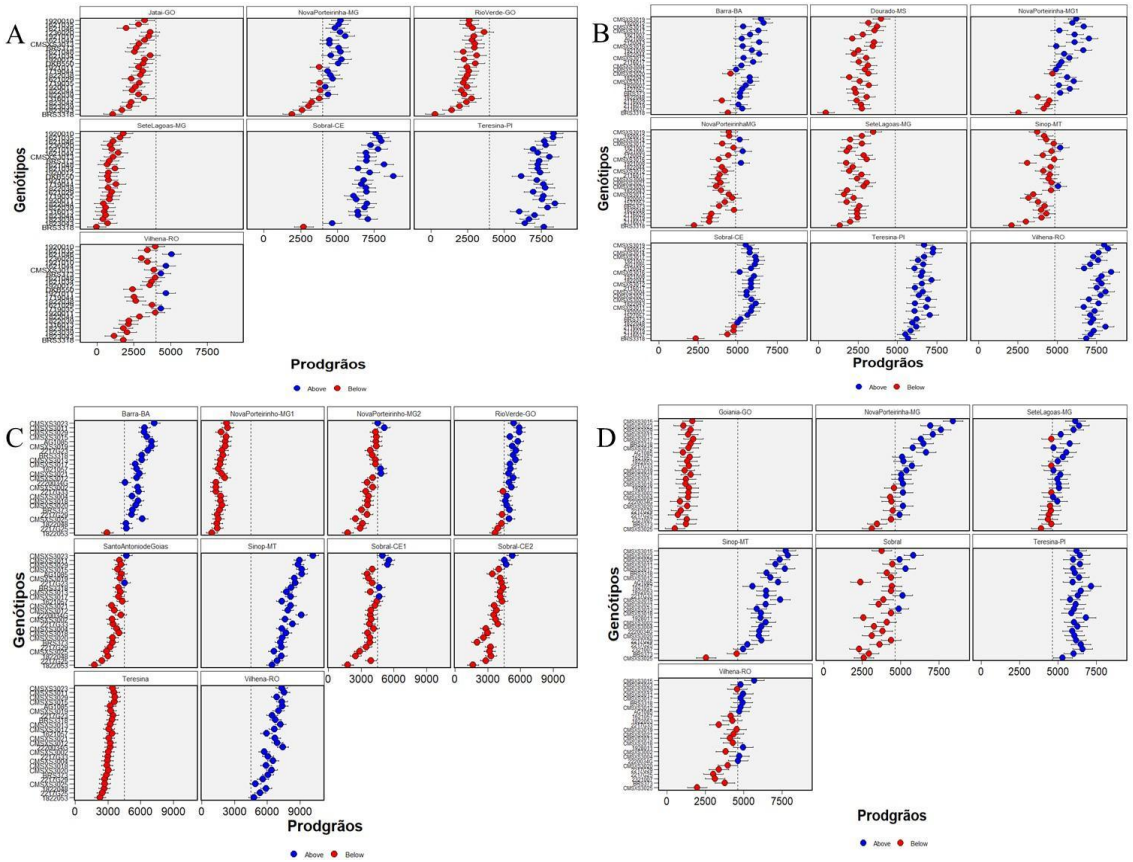


Figure 2

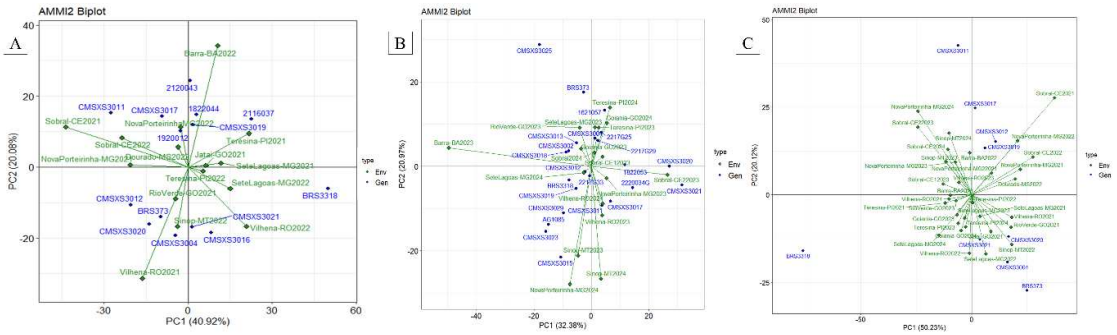


Figure 3

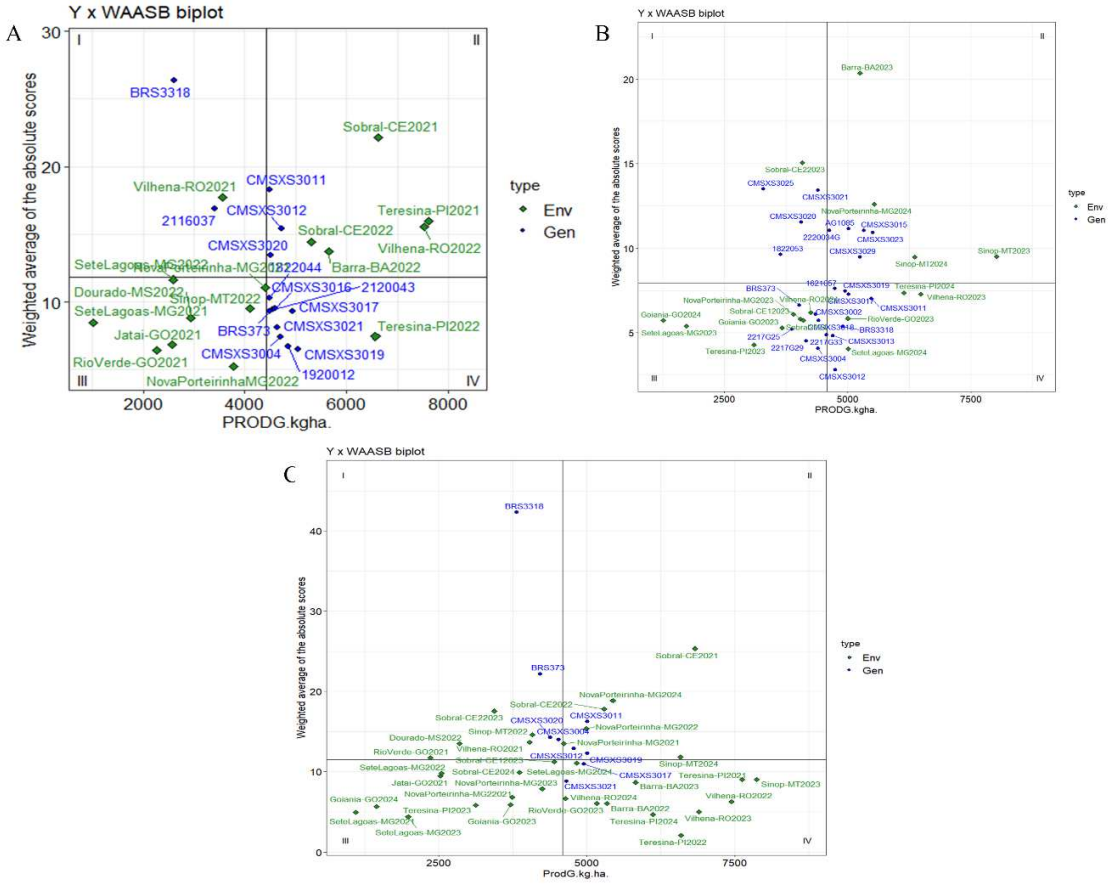
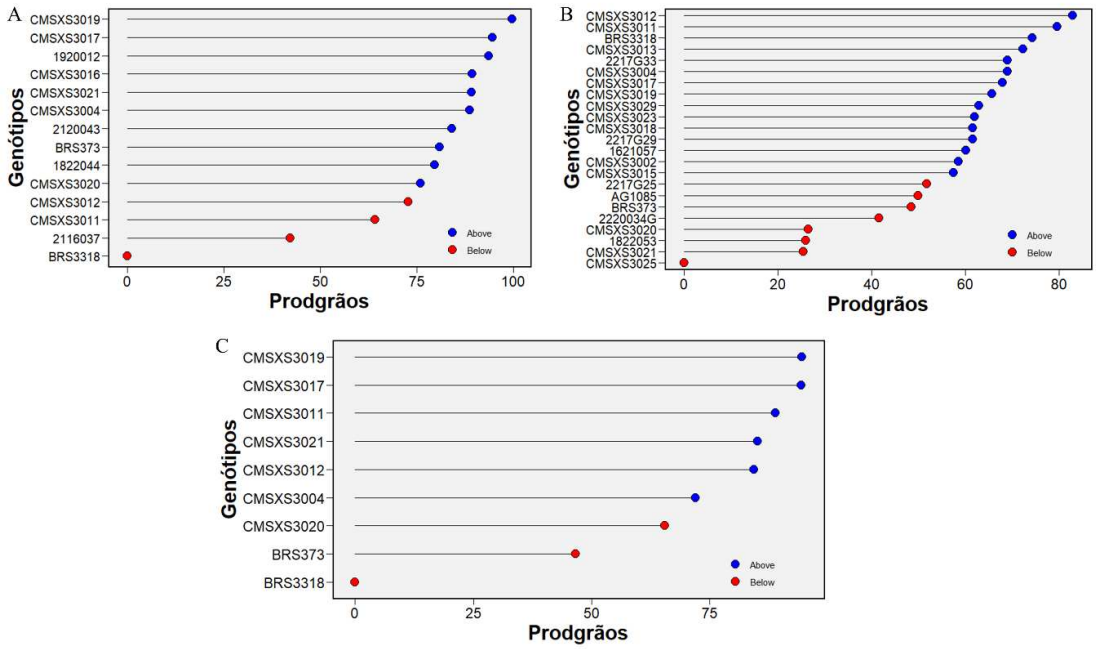
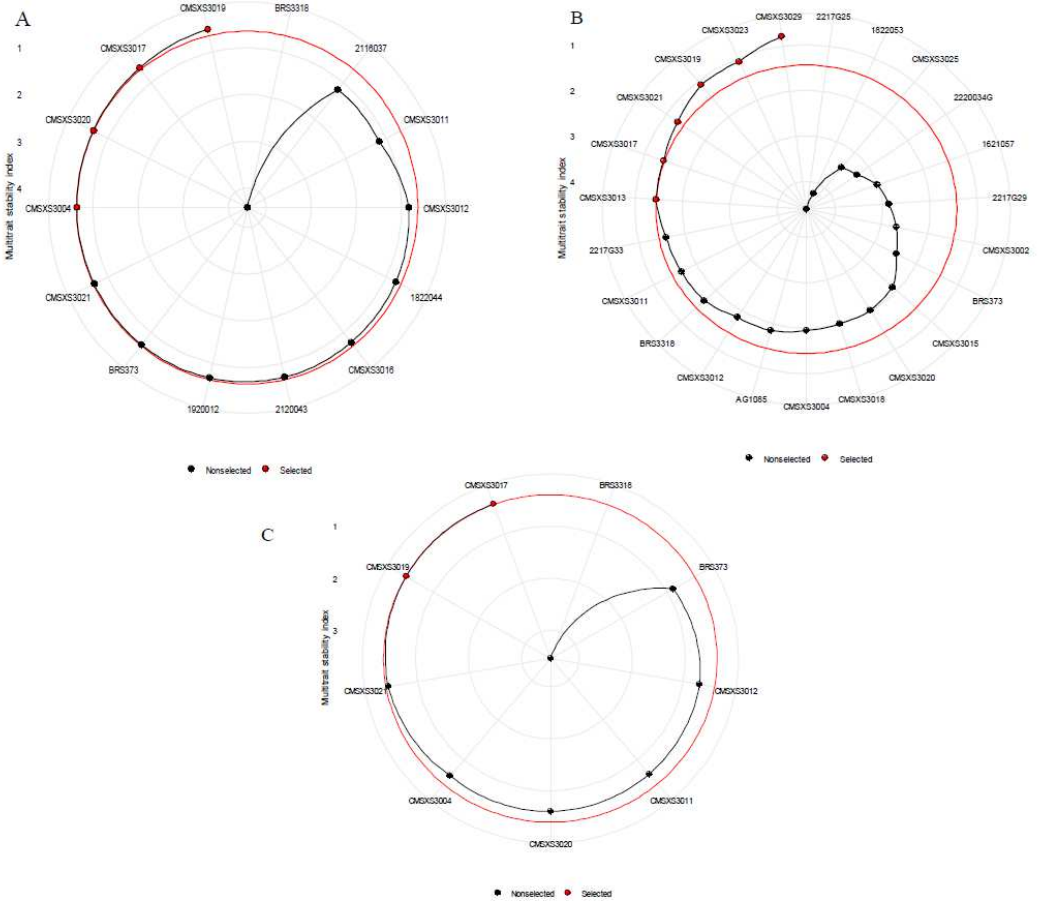

A
Y x WAASB biplot
Weighted average of the absolute scores
PRODG.kgha.
type
Env
Gen
BRS3318
Sobral-CE2021
Vilhena-RO2021
CMSXS3011
2116037
CMSXS3012
Teresina-PI2021
Sobral-CE2022
CMSXS3020
Vilhena-RO2022
SeteLagoas-MG2022
NovaPorteirinha-MG2022
1822044
Barra-BA2022
Dourado-MS2022
Sinop-MT2022
CMSXS3016
2120043
SeteLagoas-MG2021
CMSXS3017
Jatai-GO2021
BRS373
CMSXS3021
Teresina-PI2022
RioVerde-GO2021
CMSXS3004
CMSXS3019
1920012
NovaPorteirinhaMG2022
B
Y x WAASB biplot
Barra-BA2023
Sobral-CE22023
CMSXS3025
CMSXS3021
NovaPorteirinha-MG2024
CMSXS3020
AG1065
CMSXS3015
2220054G
CMSXS3023
1822053
CMSXS3029
Sinop-MT2023
Sinop-MT2024
BRS373
1821052
CMSXS3019
Teresina-PI2024
NovaPorteirinha-MG2023
Vilhena-RO2023
CMSXS3011
Goiania-GO2024
Sobral-CE12023
CMSXS3002
RioVerde-GO2023
Goiania-GO2023
SeteLagoas-MG2023
BRS3318
2217G25
2217G33
CMSXS3013
Teresina-PI2023
2217G29
SeteLagoas-MG2024
CMSXS3004
CMSXS3012
PRODG.kgha.
C
Y x WAASB biplot
Weighted average of the absolute scores
ProdG.kg.ha.
type
Env
Gen
BRS3318
Sobral-CE2021
BRS373
NovaPorteirinha-MG2024
Sobral-CE2022
Sobral-CE22023
CMSXS3020
CMSXS3011
Sinop-MT2022
CMSXS3002
NovaPorteirinha-MG2022
Dourado-MS2022
NovaPorteirinha-MG2021
RioVerde-GO2021
Vilhena-RO2021
Sobral-CE12023
CMSXS3012
CMSXS3019
Sinop-MT2024
SeteLagoas-MG2022
Sobral-CE2024
SeteLagoas-MG2024
CMSXS3017
Teresina-PI2021
Jatai-GO2021
NovaPorteirinha-MG2023
CMSXS3021
Barra-BA2023
Sinop-MT2023
NovaPorteirinha-MG2024
Vilhena-RO2024
Vilhena-RO2022
Goiania-GO2024
Teresina-PI2023
RioVerde-GO2023
Barra-BA2022
SeteLagoas-MG2021
Goiania-GO2023
Teresina-PI2024
Vilhena-RO2023
SeteLagoas-MG2023
Teresina-PI2022

Figure 4


A
CMSXS3019
CMSXS3017
1920012
CMSXS3016
CMSXS3021
CMSXS3004
2120043
BRS373
1822044
CMSXS3020
CMSXS3012
CMSXS3011
2116037
BRS3318
Genótipos
0
25
50
75
100
Prodgrãos
Above
Below
B
CMSXS3012
CMSXS3011
BRS3318
CMSXS3013
2217G33
CMSXS3004
CMSXS3017
CMSXS3019
CMSXS3029
CMSXS3023
CMSXS3018
2217G29
1621057
CMSXS3002
CMSXS3015
2217G25
AG1085
BRS373
2220034G
CMSXS3020
1822053
CMSXS3021
CMSXS3025
Genótipos
0
20
40
60
80
Prodgrãos
Above
Below
C
CMSXS3019
CMSXS3017
CMSXS3011
CMSXS3021
CMSXS3012
CMSXS3004
CMSXS3020
BRS373
BRS3318
Genótipos
0
25
50
75
Prodgrãos
Above
Below

839 **Figure 5**



840

841