

GENETIC DISSIMILARITY BETWEEN SOYBEAN GENOTYPES SCREENING OF SOYBEAN GENOTYPES USING SEEDS PHYSIOLOGICAL ATTRIBUTES THROUGH MULTIVARIATE ANALYSIS

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ABSTRACT: Soybean genotypes capable of producing seeds with high physiological performance constitute a strategy to enhance the use of environmental resources towards increasing crop sustainability. The objective of this study was to estimate the genetic dissimilarity between soybean genotypes, aiming to identify superior genotypes to act as possible progenitors in breeding programs. Fourteen soybean genotypes were evaluated by germination and seed vigor tests. The data were submitted to univariate statistical analysis. Multivariate statistical analyses were performed, such as the UPGMA hierarchical grouping method and canonical variables. The length of the shoot allowed the greatest discrimination between genotypes; and the characteristics that contributed to the dissimilarities were: normal seedlings (51.72%), abnormal seedlings (24.24%), dead seeds (11.29%) and accelerated aging (6.61%). It was concluded that the three genotypes isolated in groups II, IV and V were also the most genetically divergent and presented superior performance. The SW ATRIA RR genotype was considered the most promising.

KEYWORDS: *Glycine max*, seedling performance, seed vigor, dissimilarities measures.

RESUMO: Genótipos de soja capazes de produzir sementes com alto desempenho fisiológico constituem uma estratégia para potencializar o aproveitamento dos recursos ambientais e aumento da sustentabilidade da cultura. O objetivo deste estudo foi estimar a dissimilaridade genética entre genótipos de soja, visando identificar genótipos superiores para atuarem como possíveis progenitores em programas de melhoramento genético. Quatorze genótipos de soja foram avaliados por testes de germinação e vigor de sementes. Os dados foram submetidos à análise estatística univariada. Foram realizadas análises estatísticas multivariadas, como método de agrupamento hierárquico UPGMA e variáveis canônicas. O comprimento da parte aérea permitiu a maior discriminação entre os genótipos; e as características que contribuíram para as dissimilaridades foram: plântulas normais (51,72%), plântulas anormais (24,24%), sementes mortas (11,29%) e envelhecimento acelerado (6,61%). Concluiu-se que os três genótipos isolados nos grupos II, IV e V também foram os mais divergentes geneticamente e apresentaram desempenho superior. O genótipo SW ATRIA RR foi considerado o mais promissor.

PALAVRAS-CHAVE: *Glycine max*, desempenho de plântulas, vigor de sementes, medidas de dissimilaridades.

INTRODUCTION

Plant-based proteins still hold the promising potential to substitute animal products to achieve a more sustainable food production system. Although it is not conclusive that plant-based protein diets can fully replace meat products, among vegetables, soybean (*Glycine max* [L.] Merril) is one of the species most traditionally used and have good health-promoting benefits over animal proteins (Rizzo, Baroni 2018; Qin *et al.*, 2022).

Soybean (*Glycine max* [L.] Merr.) is rich in proteins, isoflavones, peptides with low molecular weight, polyunsaturated fatty acids and oligosaccharides, which help to reduce chronic and degenerative diseases, in addition to being good sources of minerals and B complex vitamins (Houx *et al.*, 2014). More than 54% of the vegetable oils used in the world are extracted from soybeans, in addition to providing various products, such as animal feed and biofuel (USDA-FAS 2023).

Although the soybean demand is growing worldwide, the production of high-quality soybean seeds is still a challenge, especially in tropical and subtropical regions. It is widely known that seed quality is relevant to achieve high plant establishment and effective use of environmental resources. Nonetheless, it is worth noting that, particularly for soybeans, seed quality is an important aspect to be considered in production planning.

However, soybean seed quality can be negatively affected by several factors during crop production such as climatic conditions (Scatolin, Masetto 2018; Wijewardana *et al.*, 2019) and nutritional aspects (Taliman *et al.*, 2019). In order to improve seed germination speed and withstand abiotic factor after sowing, recently, many efforts have focused on breeding varieties with improved seed performance. During genotype selection some physiological attributes of seed quality are relevant and include high seed germination and

vigor through the ability to cope stress factors (i.e., high temperature and relative humidity) and high seedling performance.

Scientific research related to plant breeding programs, combined with modern statistical techniques, allowed the detailed analysis of cultivars with both high productivity and vigorous seeds, including different types of environments and edaphoclimatic conditions suitable for soybean genotypes (Martins *et al.*, 2016; Silva *et al.*, 2017; Szareski *et al.*, 2018; Loro *et al.*, 2021). Multivariate analysis can contribute to understand the effects of variables jointly related to the variance of agronomic characteristics, thus helping breeding programs in the selection of interestingly soybean genotypes.

Among the multivariate analyses, the use of the unweighted pair-group method using arithmetic averages (UPGMA), Tocher and canonical variables grouping methods, with the use of the generalized Mahalanobis distance, as a measure of dissimilarity. Cluster analyzes are intended to gather the genotypes into groups, using a classification criterion based on the similarity of the genotypes in relation to the variables used in the experiment, so that there is homogeneity within the group and heterogeneity between groups (Hair *et al.*, 2009; Kloster *et al.*, 2011). These analyzes allow the identification of dissimilar and promising soybean genotypes and progenitors for possible crosses in view to select genotypes with technological and agronomic characteristics that allow higher yields (Leite *et al.*, 2018).

Therefore, multivariate analyses represented by clustering methods might help in the selection of genotypes, aiming at improving the physiological quality of seeds. In addition, this is a characteristic related to plant establishment, with both great importance to the genetic improvement programs and to the seed industry. It was our objective to analyze fourteen soybean seeds genotypes, in order to estimate the genetic dissimilarity between genotypes, in relation to germination and vigor tests, aiming at identifying superior genotypes capable of producing high quality soybean seeds.

MATERIAL AND METHODS

Experiment assembly

The experiment was conducted in a completely randomized design with soybean (*Glycine max* [L.] Merr.) seeds genotypes.

The evaluated genotypes consisted of 14 commercial cultivars widely grown by different breeding companies in the central South and Cerrado regions of Brazil. The soybean genotypes evaluated were 63164 RSF IPRO, BMX POTÊNCIA RR, BMX TURBO RR, BRS 1001 IPRO, BRS 7390 RR, FPS JUPTERA RR, GM AS3730, GM MG210, NA 7337 RR, SW ATRIA RR, SW BRIZA RR, SYL 9070 RR, SYN 1552 IPRO and TMG 1180 RR.

In this study, the soybean seeds from the different genotypes were produced in traditional seed industries at Mato Grosso do Sul state, Brazil, during the summer crop (September to February) of 2019 (Figure 1). After harvesting and processing the seeds, the seed lots were taken to the Seed Technology Laboratory of the Federal University of Grande Dourados (UFGD), in Dourados, Mato Grosso do Sul, Brazil. The time interval between seed processing and the installation of treatments in the laboratory was 5 days, so the soybean seed samples were considered to have the same physiological status. During the experimental period, the samples were kept in a cold chamber (15 °C, 55% RU) in order to the maintenance the seeds physiological attributes.

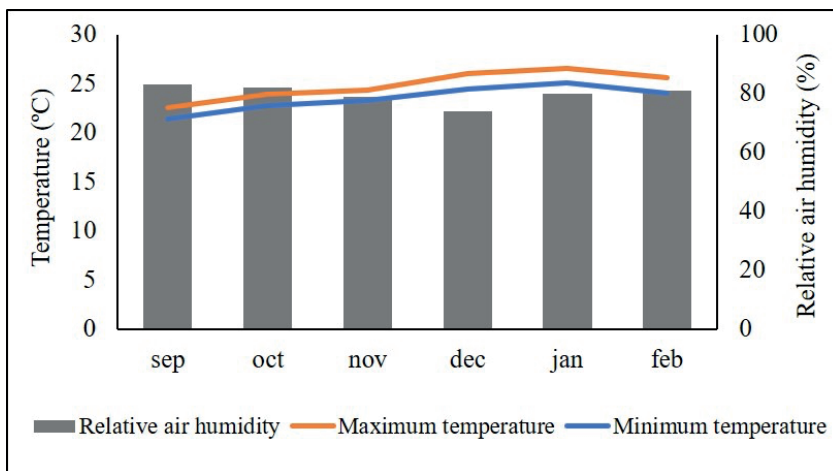


Figure 1: Maximum and minimum temperature and average relative air humidity observed during soybean genotypes summer crop (2018) in Dourados, MS, Brazil.

Initially, the water content of the seeds was determined by the gravimetric method after drying in an oven at 105 ± 3 °C, 24 h according to the International Seed Testing Association (ISTA, 2016). The seeds presented moisture content of 13.4%, in average. In order to evaluate the physiological attributes of the soybean seeds, each genotype was evaluated for seed germination and vigor (accelerated aging and seedlings biometric), according to the procedures detailed below.

Standard germination test

Seeds were distributed in rolls of germination paper (substrate) moistened with distilled water equivalent to 2.5 times the dry substrate mass, and kept in a germination chamber at 25 °C in the constant light. Seedling evaluations were performed at 8 days after sowing; results were expressed as percentage of normal seedlings, abnormal seedlings and dead seeds (ISTA, 2016).

Accelerated aging

Samples of 250 seeds per lot were spread in a single layer on stainless-steel screens and placed in plastic germination boxes (11 × 11 × 3.5 cm) containing 40 ml of deionized water at the bottom. The boxes were maintained in a germination chamber at 41°C in the dark for 48 h (ISTA, 2016). After the ageing period, two replications of 25 seeds were used to determine seed water content; four replications of 50 seeds were submitted to the standard germination test. The normal seedlings were evaluated at five days after sowing and the results were expressed as a percentage.

Seedling performance

The seeds were germinated in paper towel rolls at 25 °C for five days. After this period, measurements (with a millimetric ruler) of the lengths of the aerial part of the plant (stem) and root were made (Nakagawa, 1999). Data regarding aerial seedling length (cm) and root length were expressed as mean values for each genotype.

After the measurement of seedlings length, the normal seedlings were divided into different parts and weighed to determine their fresh mass through of the precision balance (0.0001 g). The results were expressed in grams (g).

In order to assess seedling mass dry matter, the seedlings parts were placed in paper bags. Then, they were subsequently kept in a forced-air oven at 40 °C for 48 hours. The samples were then removed from the oven and weighed to determine their dry mass; the results were expressed in grams (g).

Statistical Assessment

Data were subjected to univariate statistical analyses; such as analysis of variance and Scott-Knott mean grouping test performed using the GENES program (Cruz, 2013). Analysis of variance was performed based on a completely randomized design, with four replications for each genotype. Multivariate statistical analyses, such as the UPGMA hierarchical grouping method and canonical variables were performed using the v. 2015.5.0 (Cruz, 2013). The distance matrix used in both analyzes was the generalized Mahalanobis distance.

RESULTS

The analysis of variance revealed significant differences between the soybean genotypes for all evaluated traits, indicating the presence of variability at 1% probability in the “test f” (Table 1). The results of seed germination of the 14 genotypes were 91%, in average, considered above the minimum standard of 80% germination required for the commercialization of soybean seeds in Brazil (BRASIL, 2013).

	Mean Square									
	NS	AS	DS	AA	SL	RL	SFM	RFM	SDM	RDM
Genotype 13	400 ^{**}	197 ^{**}	76 ^{**}	968.1 ^{**}	21.5 ^{**}	25.7 ^{**}	55.3 ^{**}	2.3 ^{**}	0.6 ^{**}	0.02 ^{**}
Residual 42	24.8	17.3	14.9	189.6	0.9	1.9	4.4	0.6	0.1	0.011
Mean	91.2	6.14	3.85	59,71	8.60	9.62	8.77	1.27	1.09	8.67
VC (%)	5.61	16.2	18.6	17.0	11.5	14.6	14	12.4	14.1	12.3

¹Normal seedlings (NS), Abnormal seedlings (AS), Dead seeds (DS), Accelerated aging (AA), Shoot length (SL), Root length (RL), Root fresh mass (RFM), Shoot fresh mass (SFM), Shoot dry mass (SDM) and Root dry mass (RDM). ** and * significant at $P < 0.01$ e $P < 0.05$, respectively, by F test.

Table 1: Analysis of variance for seed quality evaluation tests¹ of soybean genotypes.

The results of normal seedlings and abnormal seedlings are presented in the Table 2. The BRS 7390 RR genotype presented the lowest performance according to the normal seedlings, followed by the GM AS3730 and SW BRIZA RR genotypes, which were lower than the total average of 91% of normal seedlings (Table 2). In addition, the average of those three genotypes for the abnormal seedlings was greater than 6.14%. These results indicate a value above average for an unwanted characteristic that is detrimental to seed quality. The results of dead seeds reinforce the low germination of the BRS 7390 RR and SW BRIZA RR seeds genotypes presented the highest results in relation to the other genotypes. The results of dead seeds constitute an undesirable characteristic for the physiological performance of seed lots.

Genotypes	N	A	D	A	SL	RL	RFM	SFM	SDM	RDM
(1) SYN	90	6 ^c	4 ^b	54	7.14 ^d	10.31 ^b	0.352 ^b	3.285 ^c	0.566 ^b	0.031 ^b
(2) SYL	95	1 ^c	4 ^b	54	5.70 ^e	9.28 ^b	0.742 ^b	5.657 ^c	0.745 ^b	0.056 ^b
(3) BRS	93	4 ^c	3 ^b	77	9.98 ^c	13.47 ^a	2.467 ^a	14.85 ^a	2.027 ^a	0.189 ^a
(4) BMX	93	5 ^c	2 ^b	60	10.42 ^c	11.96 ^a	1.285 ^b	8.612 ^b	0.791 ^b	0.08 ^b
(5) 63164	96	3 ^c	1 ^b	54	6.04 ^e	9.04 ^b	1.25 ^b	8.257 ^b	1.582 ^a	0.089 ^b
(6) NA	95	3 ^c	1 ^b	51	7.09 ^d	6.15 ^c	0.905 ^b	9.24 ^b	1.477 ^a	0.061 ^b
(7) GM	85	12 ^b	3 ^b	57	5.63 ^e	5.29 ^c	0.38 ^b	6.175 ^c	1.138 ^b	0.025 ^b
(8) TMG	90	4 ^c	5 ^b	59	9.89 ^c	9.05 ^b	2.05 ^a	5.407 ^c	0.730 ^b	0.038 ^b
(9) BMX	89	5 ^c	5 ^b	68	9.08 ^c	9.42 ^b	0.97 ^b	7.867 ^b	1.048 ^b	0.0611 ^b
(10) FPS	98	1 ^c	0 ^b	69	9.26 ^c	9.37 ^b	1.477 ^b	10.347 ^b	1.127 ^b	0.262 ^a
(11) GM	93	3 ^c	3 ^b	50	8.51 ^c	10.15 ^b	1.085 ^b	7.872 ^b	0.962 ^b	0.049 ^b
(12) SW	85	9 ^c	14 ^a	51	11.33 ^b	10.45 ^b	1.66 ^a	13.792 ^a	1.4458 ^a	0.103 ^b
(13) SW	96	4 ^c	0 ^b	80	13.47 ^a	14.20 ^a	2.822 ^a	15.595 ^a	0.983 ^b	0.156 ^a
(14) BRS	80	16 ^a	9 ^a	52	6.86 ^d	6.54 ^c	0.452 ^b	5.84 ^c	0.714 ^b	0.017 ^b

Normal seedlings (NS, %), Abnormal seedlings (AS, %), Dead seeds (DS, %), Accelerated aging (AA, %), Shoot length (SL, cm), Root length (RL, cm), Root fresh mass (RFM, mg plântula⁻¹), Shoot fresh mass (SFM, mg plântula⁻¹), Shoot dry mass (SDM, mg plântula⁻¹) and Root dry mass (RDM, mg plântula⁻¹). Equal letters in the column indicate statistically homogeneous groups at 5% probability by the Scott-Knott test.

Table 2: Results of germination and seed vigor tests of 14 soybean genotypes.

The accelerated aging (AA) was applied to determine seed performance after exposure to high temperature and relative humidity. Through this parameter the genotype SW ATRIA RR presented the highest result in relation to the other evaluated genotypes. The BMX TURBO, FPS JUPITER RR and SW BRIZA RR genotypes showed intermediate performance and the other genotypes showed low vigor without differences between them (Table 2).

Shoot length (SL) was the vigor parameter that enabled the greatest discrimination between genotypes, that is, the one in which the largest number of significantly different groups. The GM AS3730 genotype presented the lowest result, but not different statistically to the genotypes 63164 RSF IPRO and SYL 9070. In contrast, the SW ATRIA RR genotype presented the highest result (shoot length of 13.47 cm) as observed through the AA. These results indicate high seed physiological performance in relation to the analyzed genotypes (Table 2).

A highly similar pattern was observed to root length (RL) (Table 2). The SW ATRIA RR genotype presented the highest result and was statistically similar to the BRS 1001 IPRO and BMX POTENCIA RR genotypes. However, the GM AS3730, BRS 7390 RR and NA 7337 RR genotypes presented the lowest results than the others genotypes, without significant differences among them (Table 2).

For fresh root mass (FRM), the SW ATRIA RR genotype also presented the highest result, since these characteristics are correlated. However, SW ATRIA RR genotype was statistically similar to the BRS 1001 IPRO, TMG 1180 RR and SW BRIZA RR genotypes (Table 2). However, the lowest result was observed for the SYN 1552 IPRO genotype, but without statistical difference between the other genotypes analyzed (Table 2).

Similar results were observed for shoot fresh mass (SFM). The SW ATRIA RR genotype also presented the highest result, and it was statistically similar to the SW BRIZA RR and BRS 1001 IPRO genotypes. However, the SYN 1552 IPRO, SYL 9070 RR, GM AS3730, TMG 1180 RR and BRS 7390 RR genotypes presented the lowest results of SFM (Table 2).

For shoot dry mass (MSPA), the BRS 1001 IPRO genotype presented the highest result and was statistically similar to genotypes 63164 RSF IPRO, NA 7337 RR and SW BRIZA RR (Table 2). BRS 1001 IPRO and SW ATRIA RR genotypes also presented the highest results for root dry mass (RDM), but without statistical differences of the FPS JUPTERA RR (Table 2). These results indicate greater efficiency of mass translocation to the shoot seedlings. The other genotypes presented lower results without statistical differences between them (Table 2).

The relative contributions of each seed quality attribute can be observed in the analysis of the canonical variables (Table 3). The characteristics that most contributed to the dissimilarities between soybean genotypes were: normal seedlings (51.72%), abnormal seedlings (24.24%), dead seeds (11.29%) and accelerated aging (6.61%). These parameters comprised 93.87% of the variance observed between the evaluated genotypes. These results indicate that these characteristics are more efficient in explaining the existing dissimilarity between the studied genotypes.

Canonical Variables	Individual Variance (%)	Accumulated Variance (%)
Normal seedlings (%) (NS)	51.72	51.72
Abnormal seedlings (%) (AS)	24.24	75.97
Dead seeds (%) (DS)	11.29	87.26
Accelerated aging (%) (AG)	6.61	93.87
Shoot length (cm) (SL)	3.12	97
Root length (cm) (RL)	1.41	98.42
Root fresh mass (g) (RFM)	0.61	99.04
Shoot fresh mass (g) (SFM)	0.59	99.63
Shoot dry mass (g) (SDM)	0.36	100
Root dry mass (g) (RDM)	0	100

Table 3: Estimates of individual and cumulative variances of canonical variables, aiming to estimate the genetic dissimilarity between soybean genotypes.

The shoot length (SL) contributed with only 3.12% of the dissimilarity and the root length presented only 1.41% of dissimilarity (Table 3). The variables normal seedlings and abnormal seedlings were responsible for 75% of the variance of the genotypes. These results indicate such characteristics are the ones that most influence the existing difference between the seeds used in this study (Table 3) and the variance can be given from variables obtained through the standard germination test (BRASIL, 2009).

The hierarchical grouping of the average link between groups (or UPGMA) method was applied and enabled stratification into five groups; the cut in the dendrogram was performed considering 40% of the genetic variability (Fig. 2). Based on the first two canonical variables five groups was also observed in the genotype dispersion graph (Fig. 3).

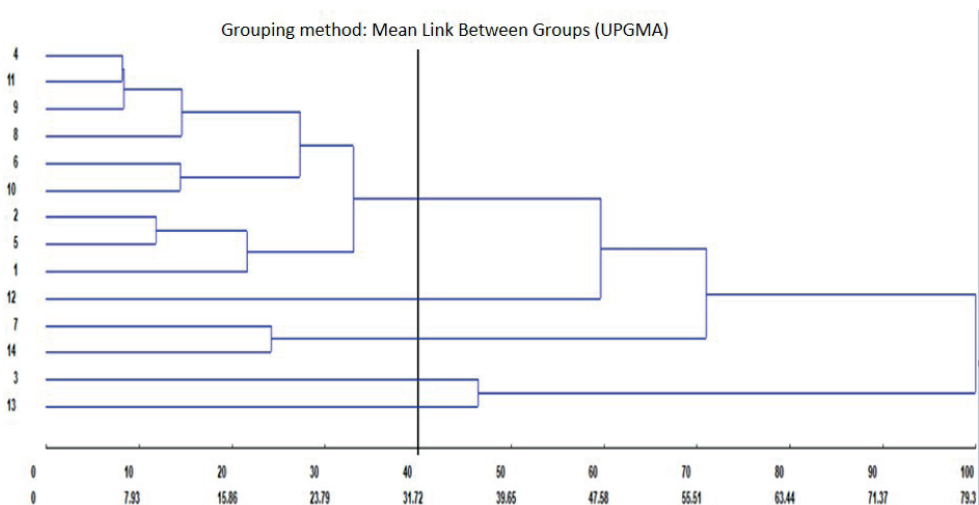


Figure 2. Representative dendrogram of genetic dissimilarity between 14 soybean genotypes and obtained by the UPGMA clustering method, using the Mahalanobis distance as a measure of dissimilarity.

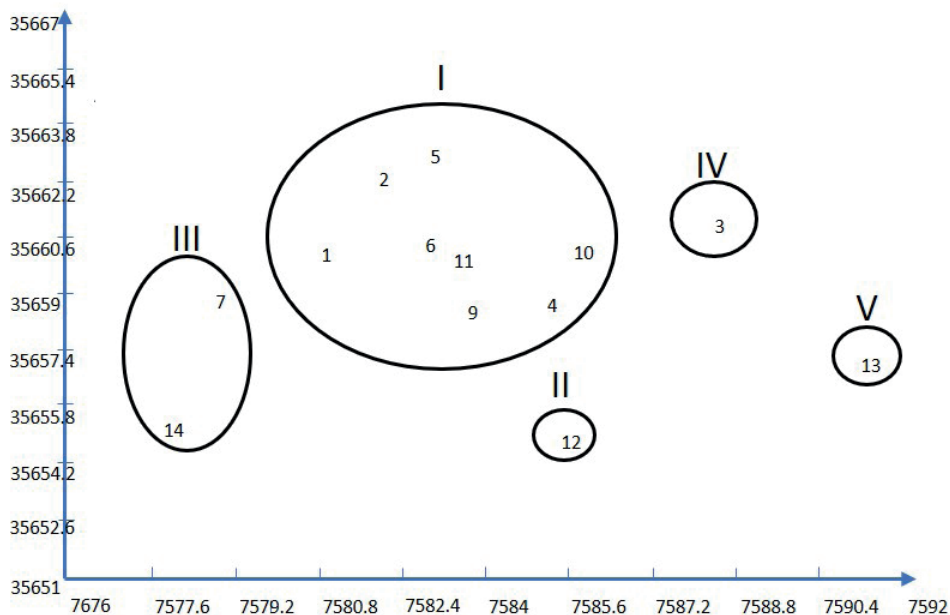


Figure 3. 2d graphic dispersion of the 14 soybean genotypes established by the linear combination of the variables used in the experiment

Thus, the same similarities relations between the genotypes were observed, since both methods were concordant in the distribution and obtained the same results regarding the groupings, increasing the reliability of the results. Group I was composed of 9 genotypes (totaling 64.28% of the individuals) (Fig. 3). This indicates the occurrence of homogeneity within the group related to the characteristics presented by the genotypes, and heterogeneity between the genotypes contained in the others groups. The genotypes SW BRIZA RR, SW ATRIA RR and BRS 1001 IPRO were allocated in isolated groups (groups II, V and IV, respectively), indicating these genotypes were the most divergent. Group III was composed of two genotypes, BRS 7390 RR and GM AS3730 (Fig. 3).

DISCUSSION

The interpretation of the genotypic values is of great importance. It is not enough for parents to be divergent; they also need to present high mean estimates for traits of interest (Casagrande *et al.*, 2020). The identification and differentiation of genotypes through grouping techniques is important to direct the choice of parents, due to genetic variability. In this sense, crosses between genotypes from divergent groups are recommended, but which also present superior performance in relation to the main agronomic characteristics (Abreu *et al.*, 2004).

In view of the characteristics that most confer variance to the genotypes, the increase of normal seedlings is a positive attribute in the physiological potential of soybean seeds, while increase of abnormal seedlings is a detrimental aspect to seed quality. In this study, although the variables were detected as those that most interfere with the quality attribute of the studied genotypes, not all comprise desirable characteristics, as the abnormal seedlings.

The abnormal seedlings are characterized by a range of phenotypes, such as impaired cotyledon expansion, altered root growth and overall stunted growth. Therefore, it could be extrapolated that abnormal seedlings were unlikely to complete seedling establishment, as previously demonstrated for soybean seeds (Basso *et al.*, 2018; Pereira *et al.*, 2021).

Shoot length was the variable with the highest number of cluster formations (5 groups), indicating this trait was the one with the greatest variation between genotypes, which shows the existing divergence between them. Besides, aerial part length can be considered as a factor that influences the seed quality.

In the present study, although shoot and root length showed poor contribution to genotypes dissimilarities, this attribute has fundamental importance to the crop establishment, since seedlings greater lengths in the initial stages of plant development possibly result in greater chances of stand establishment, crucial for high yields and productivity (Rehmani *et al.*, 2023).

When analyzing both methods of multivariate grouping and grouping of means (Table 3), it was concluded that the three genotypes that were isolated in groups II, IV and V were also the most genetically divergent and presented superior performance related to seed quality attributes. The SW ATRIA RR genotype was considered the most promising among the evaluated genotypes, according to the results in table 3 and the grouping in figure 3.

In this context, the analysis of the comparison of means, together with the groupings established by the UPGMA method and the canonical variables, can help to identify the most promising soybean genotypes for obtaining high quality seeds. Our results are in line with the premise that soybean seed vigor is associated with the genetic characteristics of seeds lot, as observed by Martins *et al.* (2016) and Feliceti *et al.* (2020).

The identification of divergent genotypes based on UPGMA and owing to high estimates of genotypic values for the traits of interest was also highlighted for wheat, whose seed is the propellant input for obtaining successful plant stand and productivity (Casagrande *et al.*, 2020). Similarly, the statistical significance observed for the canonical variables indicated a relation between agronomic and seed physiological traits in soybean; it was associated highly productive and tall plants with seeds exhibiting a greater capacity of emergence and tolerance to stress caused by accelerated aging, i.e., with high vigor (Pereira *et al.*, 2017). Besides, the genetic characteristics of the soybean genotypes influenced their seeds lots germination and vigor, which was more relevant in terms of physiological seed quality than the maturity group (Batista *et al.*, 2022).

Soybean breeding programs predominantly aim to select genotypes with technological and agronomic characteristics that allow higher yields. In spite of there is genetic variability for the soybean traits, allowing selection of genotypes with superior performance, and that the genotypes can exhibit differential performance according to the crop environment as previously assessed through multivariate analyzes (Gonçalves *et al.*, 2020) it can be argued that seeds with high ability to convert embryonic axis reserves for seedling growth also contribute to seedling vigor under stress conditions.

Therefore, it is concluded that soybean genotypes can still have similar germination percentages, but significant differences in seedling establishment. Besides, resistance to deterioration is not the only factor that needs to be considered for future breeding programs. From the viewpoint of maintaining germinability and acceptable use in the food industry, thereby increasing food security, increased resistance to deterioration is a desirable trait.

CONCLUSIONS

The most promising genotypes for obtaining high quality seeds, according to the UPGMA clustering method, canonical variables and mean clustering, were SW ATRIA RR and BRS 1001 IPRO. In addition, both presented the best potential for possible crosses.

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AUTHOR CONTRIBUTIONS

LSD and TEM planned the experiments, LSD and TEM interpreted the results, LSD, TEM, LGR and BAR made the write up, LSD and TEM statistically analyzed the data and made illustrations.

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