## Genetic variability among soybean lineages based on seed morphological and biochemical markers

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#### ABSTRACT

Understanding genetic variability in soybean lineages allows breeding programs to be guided by the precise identification of desirable characteristics and the selection of superior genotypes adapted to Brazilian conditions. This work aimed to identify the genetic dissimilarity of soybean lineages based on morphological and biochemical markers of the seed. The study was conducted at the UNIJUÍ Farm School using 145 F7 generation lineages and 86 F10 generation lineages. Qualitative traits related to the seed were measured. Wide genetic variability was evaluated for color of halo and hilum color. The dataset was submitted to multivariate statistical analysis to verify the associations between the variables and the genetic dissimilarity of the evaluated lineages. Forming four large groups of genotypes was possible based on the genetic dissimilarity dendrogram obtained with the UPGMA method. Centroids 11, 16, and 17 differed from all the others, with 16 being the most distant. Genetic variability exists in F7 and F10 lineages for seed morphological and biochemical markers. Lineages 135F7IRC33, 62F10IRC41, and 25F10IRC35 showed the greatest dissimilarity related to the other lineages.

Keywords: Glycine max L. (Merr.), UPGMA method, Kohonen's Map, Genetic variability.

# Dissimilaridade genética de linhagens da soja baseada em marcadores genéticos morfológicos e bioquímicos

#### **RESUMO**

O entendimento da variabilidade genética nas linhagens de soja permite que os programas de melhoramento sejam orientados pela identificação precisa de características desejáveis e pela seleção de genótipos superiores adaptados às condições brasileiras. Este trabalho teve como objetivo identificar a dissimilaridade genética de linhagens de soja com base em marcadores morfológicos e bioquímicos da semente. O estudo foi realizado na Fazenda Escola da UNIJUÍ utilizando 145 linhas de geração F7 e 86 linhas de geração F10. Foram medidas características qualitativas relacionadas à semente. Ampla variabilidade genética foi avaliada para cor do halo e cor do hilo. O conjunto de dados foi submetido a análises de estatística multivariada para verificar as associações entre as variáveis e a dissimilaridade genética das linhagens avaliadas. A formação de quatro grandes grupos de genótipos foi possível com base no dendrograma de dissimilaridade genética obtido pelo método UPGMA. Os centroides 11, 16 e 17 diferiram de todos os demais, sendo 16 o mais distante. Existe variabilidade genética nas linhagens F7 e F10 para marcadores morfológicos e bioquímicos de sementes. As linhagens 135F7IRC33, 62F10IRC41 e 25F10IRC35 apresentaram a maior dissimilaridade em relação com as demais linhagens.

Palavras-chave: Glycine max L. (Merr.), Método UPGMA, Mapa de Kohonen, Variabilidade genética.



#### 1. Introduction

Soy is one of the most important crops worldwide (Lin et al., 2022). It contributes 69.4% of the world's protein meal production and 28.3% of vegetable oil. The development and selection of soybean genotypes adapted to Brazilian conditions with increased yield have been the key factors for the growth in the cultivation and production of soybeans in Brazil (Milioli et al., 2022).

One of the requirements to grant protection for a particular cultivar, is that it must be distinguishable from other cultivars (MAPA, 2009). The assessment of variability can be carried out using morphological and biochemical markers (Vieira et al., 2009; Kacharé et al., 2020) in addition to providing information on the genetic variability of genotypes in breeding programs (Guimarães et al., 2007). Various morphological traits are used to differentiate individual genotypes: seed coat color, color of halo, hilum color, shape of seed, and reaction to peroxidase. Due to their reliability in germplasm evaluation and trait selection, these morphological and biochemical markers maintain consistent expression across different environmental conditions (Kachare et al., 2020).

The existence and knowledge of genetic dissimilarity between different genotypes is essential for effectively selecting superior individuals with the desired traits (Oliveira et al., 2017). Sá et al. (2022) and Kachare et al. (2020) studied genetic variability between soybean lineages for morphological and biochemical markers. These authors revealed the presence of genetic variability for morphological and biochemical markers and the possibility of selecting lineages to launch cultivars.

The classification of soybean genotypes by dissimilarity and genetic distance can be done by measuring the divergence based on the phenotype of the individuals. Due to that, it is possible to quantify the similarity and dissimilarity between genotypes (Meira et al., 2016). Multivariate exploratory techniques can, therefore, be used to study genetic variability and select superior genotypes. The aim is to analyze all agronomic traits and their relationships simultaneously (Carvalho et al., 2016; Leite et al., 2018; Moura et al., 2021). Understanding the genetic variability in soybean lineages allows breeding programs to be guided by the precise identification of desirable traits and the selection of superior genotypes adapted to Brazilian conditions essential for the sustainability and competitiveness of agriculture in the country. Furthermore, the data obtained can be incorporated by breeding programs to direct future crossings and selections; the aim is to develop even more robust and efficient cultivars. This study aimed to identify the genetic variability of F7 and F10 soybean lineages based on morphological and biochemical seed markers.

#### 2. Material and Methods

The study was conducted at Escola Fazenda da Unijuí in Augusto Pestana, Rio Grande do Sul, Brazil. 145 F7 generation soybean lineages and 86 F10 generation lineages were evaluated from the UNIJUÍ Grain Genetic Improvement Program (Table 1). The lineages were sown by a seed drill on December 22, 2022. F7 lineages were sown in 10 m x 5 m plots, while F10 lineages were sown in 20 m x 5 m plots. 200 kg ha<sup>-1</sup> of potassium chloride was applied to the V3 stage of the plants. Phytosanitary management was carried out according to soybean crop requirements. Plants were harvested manually at full physiological maturity. The seeds were conditioned in a forced-air oven to correct grain moisture and maintain seed quality. Threshing was carried out in a thresher machine, where the seeds of each line were identified and stored separately.

The measurement classes of seed coat bright (BR), seed coat color (CC), shape of seed (SS), and color of hilum (CH) were determined according to the instructions for carrying out the distinctiveness, homogeneity, and stability trials of soybean cultivars (MAPA, 2009). The peroxidase (PX) reaction was carried out according to the methodology described by Campos & Silveira (2003). The variables color of halo (CHA), green seeds (GS), leaf rust (LR), damage caused by fungus (DF), seed coat damage (DB), bedbug damage (DP), and humidity damage (HD) were also evaluated. All of them were determined on a binary qualitative basis (presence or absence), except for the color of halo variable (CHA). The frequency of each variable was analyzed descriptively by using stratified frequency graphs for the F7 and F10 generations.

Table 1. Genealogy of the F7 and F10 soybean lineages used in the study.

Maternal parent	Paternal parent	F2	Li
BMX POWER RR	NA 5909 RG	IRC 050	59F10, 20F7, 93F7, 103F7, 44F7, 121F7, 2F7, 74F7, 119F7, 53F7
TMG 7166 RR	DM 5.9	IRC 049	121F7, 2F7, 74F7, 119F7, 33F7 127F7, 48F7, 29F10
ROOS AVANCE RR	M 5892	IRC 048	85F7, 125F7
TMG 7166 RR	DM 5.9	IRC 047	69F7, 98F7
BROWN		IRC 045	25F7
BMX TURBO RR	9471 IPRO	IRC 044	122F7, 7F7, 141F7, 62F7, 32F7, 77F7, 31F7, 117F7, 108F7, 126F7
DM 5.8	DM 5.9	IRC 043	132F7
6968 RSF 6968 RSF	ROOS AVANCE DM 5.8	IRC 042	81F7, 122F7, 3F7, 137F7, 118F7, 142F7
	COLETA DP	IRC 041	62F10
BMX TURBO RR	TMG 7166 RR	IRC 040	78F10, 38F10, 81F10
TMG 7166 RR	NA 5909 RG	IRC 039	37F7, 95F7, 28F7, 55F7, 139F7, 35F10 47F10 49F10 66F7
NS 6700 IPRO	MAR.M3	IRC 038	67F10, 28F10, 10F10
NA 5909 RG	MAR.M4	IRC 037	26F10
DM 5.8 BMX APOLO RR	TMG 7166 RR	IRC 036	44F10, 63F10
BMX POWER RR	MAR.M4	IRC 035	38F7, 105F7, 131F7, 92F7, 11F7,
			58F10, 40F10, 13F10, 25F10
NS 5958 RR	NS 6700 IPRO	IRC 034	39F7, 38F7, 101F7, 03F7, 22F7, 128F7 83F7 56F10 60F7 80F10
NS 5750 KK		ite 054	30F10, 39F10
			18F7, 35F7, 64F7, 136F7, 86F7,
MAR.M4	NA 5909 RG	IRC 033	27F7, 90F7, 13F7, 84F7, 26F7,
	N.4. 5000 D.C.	The close	116F7, 135F7, 88F7
DM 7.0 BMX MAGNA RR	NA 5909 RG	IRC 032	43F10, 14F10, 12F10 129F7, 1F7, 61F7, 75F7, 52F7
NS 5958 RR	MAR.M2	IRC 031	16F7, 24F7, 23F10, 65F10, 7F10,
		<b>D</b> C 020	71F10, 32F10
BMX POWER RR	MAR.M4 MAR M6 C	IRC 030	20F10, 8F7, 66F10, 110F7 34E10
MAR.MOC	MAKIMOC	IRC 029	63F7 11F10 77F10 37F10
	MASSAL	IRC 026	61F10
			49F7, 42F7, 47F7, 130F7, 96F7,
	MASSAL	IRC 025	133F7, 6F7, 114F7, 15F10,52F10,
NS 6700 IPRO	5106 IPRO	IRC 023	/3F7, 35F7 22F10 36F10
NS 6700 IPRO	BMX ATIVA RR	IRC 022	45F7, 115F7, 67F7
TMG 7062 RR	NS 6700 IPRO	IRC 021	24F10, 16F10, 27F10, 8F10,
11010 7002 KK		INC 021	21F10, 74F10
,		IRC 020	33F10 124F7 120F7 12F7 17F7
FPS JUPITER RR	MONASCA RR	IRC 017	134F7, 4F10, 55F10,
		IRC 016	45F10
DM 7.0 BMX MAGNA RR	DM 5.8 BMX APOLO RR	IRC 013	3F10, 51F10, 17F10, 29F7,
			/0F10,9F10 10F7 21F7 138F7 145F7
FPS NETUNO RR	DM 5.8 BMX APOLO RR	IRC 012	123F7, 2F10, 42F10, 91F7, 6F10,
			68F10, 50F10, 70F7
BMX POWER	MAR.M4	IRC 011	9F7, 79F7, 99F7, 1F10, 78F7
DM 5.8 BMX APOLO RR	MAR.M4	IRC 007	102F7, 144F7, 76F10, 23F7, 50F7, 19F10
DOOS CAMINO DD	ΕΦΣ ΦΑΦΑΝΑΦΑΝΕΜΑ ΦΦ	IPC 005	84F10, 89F7, 82F7, 51F7, 5F7,
		IRC 005	19F7
DM 7.0 BMA MAGNA KK	MONASCA KK	IRC 005	57F7, 57F10 87F7, 80F7, 107F7, 71F7, 54F7,
DM 5.8 BMX APOLO RR	FUNDACEP 66 RR	IRC 002	15F7, 100F7, 30F7, 104F7, 11F7,
			79F10, 109F7, 43F7, 41F10
			4F/, 40F7, 34F7, 48F10, 5F10, 30F7, 94F7, 106F7, 83F10, 64F10
DM 7.0 BMX MAGNA RR	FUNDACEP 66 RR	IRC 001	85F10, 75F10, 69F10, 31F10, 73F10
			86F10, 46F10, 53F10, 76F7

F2: population F2; Li: lineages.

Pearson's correlation coefficients were calculated between the pairs of traits, and significance was assessed using Student's t-test at 5%. The principal components analysis was stratified by segregation levels (F7 and F10), and the categorical variables were numbered according to class. For example, for BR it was assigned a value of 1 to the classes with the lowest brightness; the ones with the highest brightness were assigned an ascending order. Binary variables received 0 (zero) for absence and 1 (one) for presence.

All lineages from both generations were considered for the dissimilarity analyses, using the UPGMA clustering method (Unweighted Pair Group Method using Arithmetic Averages) and the Self Organizing Maps (Kohonen map) methodology to classify the lineages into large groups and determine the proximity between genotypes. The analyses were carried through the packages from MultivariateAnalysis, dendextend, ape, metan, kohonen, ggplot2, and EnvRtype, using the RStudio software (R Core Team, 2022).

#### 3. Results and Discussion

The results showed genetic variability between the F7 lineages, especially concerning descriptors color of hilum and color of halo (Figure 1). All lineages showed yellow seed coat color with more than 30% demonstrating black CH. The shape of the seeds was predominantly sphericalflattened (98% of observations). More than 50% of the lineages showed green seeds, which is common in times of water deficit. The presence of leaf rust was predominant (in more than 75% of the lineages), while damage caused by fungus was observed in around 35% of the progenies. Variables seed coat damage and humidity damage occurred in more than 95% of the lineages. Seeds with low seed coat bright were predominant. More than half of the samples for peroxidase were classified as reagents, an important enzyme for plant defense against pathogens and cell elongation (Campos e Silveira, 2003). There was a predominance of lineages with a brown halo in more than 75% of observations.

The genetic variability for morphological and biochemical markers among F10 lineages was lower than the F7 generation. The predominance of yellow color remained for the seed coat color descriptor; only two lineages had brown seed coats. The lineages of the color of hilum descriptor were characterized as yellow, medium brown, black, and imperfect black, in descending order in terms of observed frequency. All the lineages with the SS descriptor were classified as sphericalflattened. Seed coat damage and color of halo obtained a similar result, with more than 95% of the lineages showing no seed coat damage and brown color of halo. The green seeds variable was present in more than 70% of the lineages. A similar frequency was found for damage caused by fungus, humidity damage, and peroxidase. The peroxidase reaction was related to genotypes with higher levels of linoleic acid and protein in soybean seeds (Sangiovo et al., 2023). Therefore, peroxidase can be used as a biochemical marker to improve the nutritional quality of soybean seeds indirectly.

Contrary to what was observed for the F7 generation, leaf rust was present in less than 40% of the samples. The lower occurrence of leaf rust in later generations suggests the need for continuous monitoring and adjustments to selection strategies to ensure the long-lasting resistance of the varieties. The seed coat bright descriptor showed greater variability, with nearly 1:3 of the samples with medium brightness and 2:3 with low brightness.

Multi-categorical traits are commonly evaluated in plant breeding, mainly related to plant morphological and structural traits (Cruz et al., 2014). This involves selecting variables with high heritability and easy measurement, which are highly correlated with the desired trait, to increase the gains from selecting superior genotypes. There was a significant positive correlation between heterozygosity with seed coat damage, leaf rust, and bedbug damage (Figure 3). This indicates that the higher the level of heterozygosity in a soybean population, the higher its seed coat damage, leaf rust, and bedbug damage tend to be. There was a positive relationship between seed coat damage with bedbug damage and leaf rust, indicating that the presence of seed coat damage is associated with the occurrence of bedbug damage and leaf rust. The incidence of damage caused by fungus was associated with the presence of humidity damage in the seeds. Seeds with higher seed coat bright were negatively associated with the presence of leaf rust. Thus, it can be inferred that the seed coat bright level of the seeds can define the presence of leaf rust. Therefore, it is possible to select superior genotypes through correlations, especially for traits negatively impacting soybean yield and quality (fungus damage, green seeds, seed coat damage, bedbug damage, and leaf rust).

The humidity damage and seed coat damage variables were important for grouping F7 generation lineages (Figure 4). The lineages grouped close to the vector of variables humidity damage and seed coat damage had true logical values, i.e., equal to 1 (presence). Thus, it can be inferred that seed coat damage and humidity damage were present in most lineages, while it was possible to identify the absence of seed coat damage and humidity damage in line 66F7IRC39. This indicates the greater potential of this line to reduce of these variables.



Figure 1. (A) Qualitative morphological and biochemical markers for F7 generation soybean lineages. LR: leaf rust; GS: green seeds; CH: hilum color; SS: shape of seed; CC: seed coat color; DF: damage caused by fungus. (B) Qualitative morphological and biochemical markers for F7 generation soybean lineages. CD: seed coat damage; BR: seed brightness; PX: peroxidase reaction; HD: humidity damage; CHA: color of halo.

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Figure 2. (A) Qualitative morphological and biochemical markers for F10 generation soybean lineages. LR: leaf rust; GS: green seeds; CH: hilum color; SS: shape of seed; CC: seed coat color; DF: damage caused by fungus. (B) Qualitative morphological and biochemical markers for F10 generation soybean lineages. CD: seed coat damage; BR: seed brightness; PX: peroxidase reaction; HD: humidity damage; CHA: color of halo.



**Figure 3.** Pearson's linear correlation coefficient matrix for the traits evaluated in soybean lineages from the F7 and F10 generations. LR: leaf rust; DP: bedbug damage; HET: heterozygosity; CD: seed coat damage; GS: green seeds; BR: seed brightness; CH: hilum color; PX: peroxidase reaction; SS: shape of seed; CC: seed coat color; DF: damage caused by fungus; HD: humidity damage; CHA: color of halo.

The bedbug damage variable had the greatest influence on the line grouping of the F10 generation (Figure 5). The following lineages 11F10IRC28, 13F10IRC35, 58F10IRC35, 23F10IRC31, 3F10IRC13, 28F10IRC38, 4F10IRC17, 18F10IRC4, 42F10IRC12, 40F10IRC35, 45F10IRC16, 35F10IRC39, 83F10IRC1, and 26F10IRC37 showed humidity damage and damage caused by fungus 27F10IRC21. (presence). For individuals 37F10IRC28, and 29F10IRC49, with the highest seed coat bright, there was an absence of humidity damage and damage caused by fungus (values equal to 0).

The dendrogram obtained from the dissimilarity matrix of the soybean lineages (Figure 6) shows the formation of four large groups based on origin. Dellagostin et al. (2011) evaluated genetic dissimilarity in a segregating soybean population and found six groups formed from morphological and biochemical markers. However, 30 subgroups of soybean lineages were observed, differing between groups by morphological and biochemical markers. This indicates the presence of genetic variability in both F7 and F10 populations. Vieira et al. (2009) and Kacharé et al. (2020) also observed the presence of genetic variability for morphological and biochemical markers in soybeans, highlighting the efficiency of using these traits due to the smaller variation. This genetic variability offers opportunities to select promising lineages for preliminary trials to identify desirable traits and obtain superior genotypes. Therefore, these results provide a solid basis for guiding selection and improvement strategies, contributing to developing soybean cultivars.



**Figure 4**. Principal Component Analysis (PCA) for morphological and biochemical markers of F7 generation soybean lineages. CH: hilum color; CHA: color of halo; SS: shape of seed; GS: green seeds; LR: leaf rust; DF: damage caused by fungus; CD: seed coat damage; DP: bedbug damage; BR: seed brightness; HD: humidity damage; PX: peroxidase reaction.



**Figure 5.** Principal Component Analysis (PCA) for morphological and biochemical markers of F10 generation soybean lineages. CH: hilum color; CHA: color of halo; SS: shape of seed; GS: green seeds; LR: leaf rust; DF: damage caused by fungus; CD: seed coat damage; DP: bedbug damage; BR: seed brightness; HD: humidity damage; PX: peroxidase reaction.

The lineages were grouped into 20 neurons according to morphological and biochemical markers, demonstrating variability (Figure 7). The variability was higher than in studies with soybeans (Sá et al., 2022) and rice (Santos et al., 2019), which used this neural model and reached five and six clusters using a total of 36 and 13 genotypes, respectively.

While neurons 11, 16, and 17 were distant from the others, neuron 16 was the furthest away from the other clusters. This is because line 135F7IRC33 was the only

one that presented a different shape of seed. With their brown seed coat color, the 62F10IRC41 and 25F10IRC35 lineages were included in neuron 10. However, they did not present enough weight for the neuron to distance itself from the others. The divergence of neurons 11 and 17 from the others was mainly due to the absence of damage caused by fungus. Therefore, these results indicate the presence of genetic variability and the possibility of selecting genotypes for descriptor traits.



Figure 6. Dendrogram obtained from the dissimilarity matrix of soybean lineages from F7 and F10 generations, based on morphological and biochemical markers.



**Figure 7.** Kohonen classification (self-organizing map) for soybean lineages from F7 and F10 generations based on morphological and biochemical markers. CH: hilum color; CHA: color of halo; SS: shape of seed; GS: green seeds; LR: leaf rust; DF: damage caused by fungus; CD: seed coat damage; DP: bedbug damage; BR: seed brightness; HD: humidity damage; PX: peroxidase reaction.

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#### 4. Conclusions

There is genetic variability in both F7 and F10 lineages for seed morphological and biochemical markers.

The greatest genetic variability among the F7 generation individuals is caused by variables seed coat damage and humidity damage.

The bedbug damage variable determined the grouping of the lineages for the F10 generation.

Lineages 135F7IRC33, 62F10IRC41, and 25F10IRC35 showed the greatest dissimilarity to the other lineages.

#### **Authors' Contribution**

All authors contributed equally to the manuscript. Willyan Júnior Adorian Bandeira, Ivan Ricardo Carvalho and Jaqueline Piesanti Sangiovo for conducting the experiment, evaluating agronomic traits, data tabulation, statistical analyzes and writing the manuscript. Murilo Vieira Loro, João Pedro Dalla Roza and Leonardo Cesar Pradebon for reviewing the manuscript.

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