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Grain morphological traits and genetic variability of upland rice genotypes

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Abstract

Grain shape is characterized by a combination of grain length, grain width, grain length-to- width ratio, and grain thickness diverge according to the rice cultivar. The aim of this work was to evaluate in grains of upland rice genotypes the morphological traits and identifying the genetic divergence between them, using hierarchical and non-hierarchical clustering techniques. The experiment was carried out in randomized blocks, with 42 upland rice genotypes. The following parameters were determined with digital caliper in a sample of 20 paddy rice grains per plot: grain width (greater side dimension), grain trickness (smallest side dimension), grain length (distance between tips) and GLGW= length-to-width ratio. The data were first submitted to the F-test ($p<0.05$) and to the Scott-Knott multiple comparison test at $p<0.01$ and $p<0.05$. Correlogram of Pearson's linear relationship was carried out and Cluster analysis was performed in two stages, using a hierarchical and non-hierarchical method in R Core Team software version 4.0.2. It is concluded that there is high genetic divergence among the upland rice genotypes. The genotypes OSVR15029 and OSVR15044 have similar traits to the cultivar IAC 202. The genotypes which have grains classified as long-thin, meeting national preferences and can be used for the breeding programs of upland rice crop.

Additional keywords: *Oryza sativa*, Cluster analysis, grain evaluation

Resumo

A forma do grão é caracterizada por uma combinação de comprimento, largura, relação comprimento/largura e espessura, que variam de acordo com a cultivar de arroz. O objetivo deste trabalho foi avaliar, em grãos de genótipos de arroz de terras altas, as características morfológicas e identificar a divergência genética entre elas, utilizando técnicas de agrupamento hierárquico e não hierárquico. O experimento foi conduzido em blocos casualizados, com 42 genótipos de arroz de terras altas. Os seguintes parâmetros foram determinados com paquímetro digital em uma amostra de 20 grãos de arroz por parcela: largura do grão (maior dimensão lateral), espessura do grão (menor dimensão lateral), comprimento do grão (distância entre as pontas) e relação comprimento/largura (GLGW). Os dados foram submetidos ao teste F ($p<0,05$) e ao teste de comparações múltiplas de Scott-Knott ($p<0,01$ e $p<0,05$). Foi realizado um correlograma da relação linear de Pearson e uma análise de agrupamento em duas etapas, utilizando um método hierárquico e um não hierárquico no software R Core Team versão 4.0.2. Concluiu-se que existe alta divergência genética entre os genótipos de arroz de terras altas. Os genótipos OSVR15029 e OSVR15044 apresentam características semelhantes à cultivar IAC 202. Os genótipos que possuem grãos classificados como longos e finos, atendendo às preferências nacionais, podem ser utilizados em programas de melhoramento genético da cultura do arroz de terras altas.

Palavras-chave: *Oryza sativa, Análise de agrupamentos, avaliação de grãos*

Introduction

Rice (*Oryza sativa* L.) is the second most produced cereal in the world, reaching 796 million tons in 2022 in an area of 166 million hectares, second only to corn (Fao, 2023). Rice is a highly rustic crop that provides an excellent nutritional balance, making it the species with the greatest potential for increasing production to fight hunger in the world (Fao, 2018; Li, 2025).

Due to the importance of rice in the diet of Brazilians, Brazil has been paying attention to new cultivars to improve its quality, in order to meet consumer demands regarding the preference and acceptability of the grain (Boeno et al., 2011). Due to the population increase, there is a demand for cultivars that contribute to improve the population's dietary pattern and that present high yield and quality (Gupta et al., 2020). An ideal rice cultivar must have high potential for grain yield, with grains in good shape, nutritional value, resistance to diseases and stress (Zhao et al., 2018; Ferrari et al., 2022; Zhang et al., 2024).

In Brazil, the identification of cultivars is carried out, in a majority, according to morphological descriptors (Bonow et al., 2007), these being prescribed by brazilian law. Grain shape is characterized by a combination of grain length, grain width, grain length-to-width ratio, and grain thickness diverge according to the cultivar (Yang et al., 2019). These traits are important in yield, harvest and quality of grains (Sun et al., 2016; Feng et al., 2016; Xu et al., 2020; Ferrari et al., 2021).

The existence of genetic variability among individuals in a population is essential for crop breeding, where the selection of genetic material is effective when there is a significant amount of genetic variability between individuals (Sumanth et al., 2017). One of the ways of verifying genetic divergences between individuals is the use of multivariate analysis (Carvalho, 2020; Hair et al., 2009). This analysis allows to transform a group of heterogeneous individuals into homogeneous groups with similar characteristics (Oliveira, 2019).

In view of the above, the aim of this work was to evaluate in grains of upland rice genotypes the morphological traits, identifying the genetic divergence between them, using hierarchical and non-hierarchical clustering techniques and indicate possible genotypes for use in genetic improvement.

Material and methods

Obtaining seeds

Seeds of 41 upland rice genotypes were collected between January and June 2015 directly from rural properties located in the municipalities of Registro, Barra do Turvo, Eldorado, Iporanga, Iguape, and Cananéia, located in São Paulo State of. These seeds were then sent to the Campus of Registro of the São

Paulo State University, located in Registro, São Paulo State, at the geographic coordinates 24°31′ S and 47°51′ W, with an altitude of 25 m, a slope between 0 and 12%, mean temperature of 27 °C, and mean annual precipitation of 1500 mm.

Experiment set up

The experiment was carried out in randomized blocks, with 41 upland rice genotypes, as follows: OSVR15001, OSVR15003, OSVR15004, OSVR15005, OSVR15006, OSRV15008, OSRV15009, OSVR15010, OSVR15011, OSVR15016, OSVR15018, OSVR15019, OSVR15020, OSVR15021, OSVR15023, OSVR15024, OSVR15026, OSVR15027, OSVR15029, OSVR15030, OSVR15031, OSVR15032, OSVR15033, OSVR15034, OSVR15035, OSVR15036, OSVR15037, OSVR15038, OSVR15039, OSVR15040, OSVR15041, OSVR15042, OSVR15044, OSVR15045, OSVR15046, OSVR15047, OSVR15048, OSVR15049, OSVR15051, OSVR15052, and OSVR15053. The upland rice cultivar IAC 202 was used as a control group, as it is a material widely used in commercial areas in Brazil. The genotypes maturation cycle is shown in Table 1. Therefore, the experimental design consisted of 42 treatments and 4 replications, totaling 168 experimental plots. Each experimental plot consisted of six sowing rows, with 4 m long and inter- row spacing of 0.35 m, totaling 12.6 m^2 of total area. The useful area was composed of 3 m of the four central rows, totaling 4.2 m^2.

Table 1. Identity and characteristics of rice upland genotypes used in this study based on maturation cycle

Genotypes	ID	Maturation cycle	Genotypes	ID	Maturation cycle
OSVR15001	1	Early	OSVR15032	22	Semi late
OSVR15003	2	Early	OSVR15033	23	Semi late
OSVR15004	3	Early	OSVR15034	24	Super early
OSVR15005	4	Super early	OSVR15035	25	Super early
OSVR15006	5	Médium	OSVR15036	26	Médium
OSVR15008	6	Early	OSVR15037	27	Super early
OSVR15009	7	Early	OSVR15038	28	Médium
OSVR15010	8	Early	OSVR15039	29	Semi late
OSVR15011	9	Early	OSVR15040	30	Early
OSVR15016	10	Early	OSVR15041	31	Early
OSVR15018	11	Semi late	OSVR15042	32	Early
OSVR15019	12	Early	OSVR15044	33	Semi late
OSVR15020	13	Early	OSVR15045	34	Early
OSVR15021	14	Semi late	OSVR15046	35	Early
OSVR15023	15	Early	OSVR15047	36	Early
OSVR15024	16	Early	OSVR15048	37	Médium
OSVR15026	17	Early	OSVR15049	38	Early
OSVR15027	18	Early	OSVR15051	39	Early
OSVR15029	19	Early	OSVR15052	40	Semi late
OSVR15030	20	Early	OSVR15053	41	Early
OSVR15031	21	Semi late	IAC202	42	Early

A composite soil sample (0-0.2m depth) was collected in July 2015 from the experimental area and the following soil chemical attributes were obtained: pH ($CaCl2$) = 4.5 ± 0.5; Presina= 18 ± 0.3 mg dm^{-3}; M.O.= 14 ± 0.6 g dm^{-3}; K= 1.3 ± 0.2 mmolc dm^{-3}; Ca= 23 ± 0.5 mmolc dm^{-3}; Mg= 11 ± 0.3 mmolc dm^{-3}; H+Al= 50 ± 0.7 mmolc dm^{-3}; Al = 2 ± 0.1 mmolc dm^{-3}; CTC= 85 ± 0.9 mmolc dm^{-3}; V= 41 ± 0.5 %; S= 3 ± 0.1 mg dm^{-3}; B=0,26 ± 0.04 mg dm^{-3}; Cu= 0,2 ± 0.03 mg dm^{-3}; Fe= 257 ± 2.4 mg dm^{-3}; Mn= 0,7 ± 0.04 mg dm^{-3} e Zn= 1,6 ± 0.1 mg dm^{-3}. Subsequently, 1.0 t ha^{-1} of dolomitic limestone (75% total neutralizing power) was applied to increase base saturation to 50%.

In the experimental area soil tillage was carried out in November 2015 using a chisel plow and harrow at a working depth of 0.30 and 0.20 m, respectively. Manual sowing of the rice genotypes was carried out at a density of approximately 1.72 million plants per hectare in December 11, 2015, according to Ferrari et al. (2018). Seeds were treated with carbofuran and carboxin + thiram at a proportion of 15 and 3.0 mL per kilogram of seeds, respectively, of the commercial product.

Base fertilization was carried out at the sowing furrows, with application of 500 kg ha^{-1} 04–14–08 (N–P–K) fertilizer. Plant emergence was observed 7 days after sowing. Top dressing fertilization was performed at 30 days after seedling emergence using 120 kg N ha^{-1}, with ammonium sulfate (21% N and 24% S) and urea (45% N) as the fertilizer sources at a proportion of 1:1 (v/v).

Conducting the experiment

Weeds were controlled with the pre-emergence herbicide (pendimethalin) 3.0 L ha^{-1} of the commercial product, in addition to manual weeding was performed. A sprinkler irrigation system with a water depth of 12 mm $hour^{-1}$ was used whenever necessary, meeting the crop demand. Fungicide was applied three times: two with azoxystrobin at 0.4 L commercial product (c.p.) ha^{-1} and one with tebuconazole at 0.15 L ha^{-1} for control of *Pyricularia grisea* and *Bipolaris oryzea.*

Assessments

After harvest time in may 2016, the following parameters were determined with digital caliper in sample of 20 paddy rice grains per plot: GW= grain width (greater side dimension), GT= grain trickness (smallest side dimension), GL= grain length (distance between tips) and GLGW= length-to-width ratio.

Statistical analysis

The data were first submitted to the F-test ($p<0.05$) and, subsequently, to the Scott-Knott multiple comparison test at p<0.01 and p<0.05.

To perform the cluster analysis, it consisted of the division into 2 stages. The first stage was characterized by determining the optimal number of clusters. For this, the hierarchical method of the middle link (UPGMA) from the Euclidean distance was used, where the optimal number of clusters was adopted based on the graphical index of Lebart et al. (2000), of the “NbClust” function of the NbClust package (Charrad et al., 2014) R Core Team software version 4.0.2. The adoption of this hierarchical grouping to verify the optimal number of clusters, was based on the value of the cofenetic correlation (Mantel, 1963) presented.

The second stage in the grouping process used a hierarchical method (stage 1) in combination with a non-hierarchical method (Hair et al., 2009) and correlogram of Pearson's linear relationship. Based on the hierarchical method for determining the optimal number of clusters, the non-hierarchical method of clustering (*Kmeans*) was used to develop an optimal solution for each number of aggregates.

Results and Discussion

The genotypes OSVR1515005, OSVR15034, OSVR15035, and OSVR15037 had an early cycle of 90 days between emergence to harvest under the Vale do Ribeira cultivation conditions. The genotypes OSVR15023, OSVR15029, OSVR15040, IAC202 had a cicle of 102 days. The other genotypes had a cicle of 110 days (OSVR15001, OSVR15004, OSVR15008, OSVR15010, OSVR15011, OSVR15016, OSVR15019, OSVR15027, OSVR15045, OSVR15053), 115 days (OSVR15003, OSVR15009, OSVR15020, OSVR15024, OSVR15026, OSVR15027, OSVR15030, OSVR15041, OSVR15042, OSVR15047, OSVR15049, OSVR15051), 118 days (OSVR15036, OSVR15038, OSVR15048) and 133 days between emergence to harvest (OSVR15006, OSVR15018, OSVR15021, OSVR15031, OSVR15032, OSVR15033, OSVR15039, OSVR15044, OSVR15052).

The lowest averages for grain length were related to the genotypes OSVR15003, OSVR15006, OSVR15018, OSVR15027, OSVR15029, OSVR15030, OSVR15033, OSVR15035, OSVR15039, OSVR15044, OSVR15051 and IAC 202 (Figure 1). The variation coefficient for this trait (3.59%) was less than 8%, demonstrating that the grains of these genotypes do not have a defined pattern in their dimensions (Boeno et al., 2011).

Regarding the grain width evaluations, the lowest averages found were for the genotypes: OSVR15011, OSVR15029, OSVR15044, IAC 202 (Figure 1). According to Facchinello (2017), the length and width of grains are characters of high genotypic variance.

For the trait grain thickness (Figure 1), the genotypes that obtained the lowest averages were OSVR1506, OSVR15024, OSVR15029, OSVR15031, OSVR15032, OSVR15044, OSVR15049 and IAC 202.

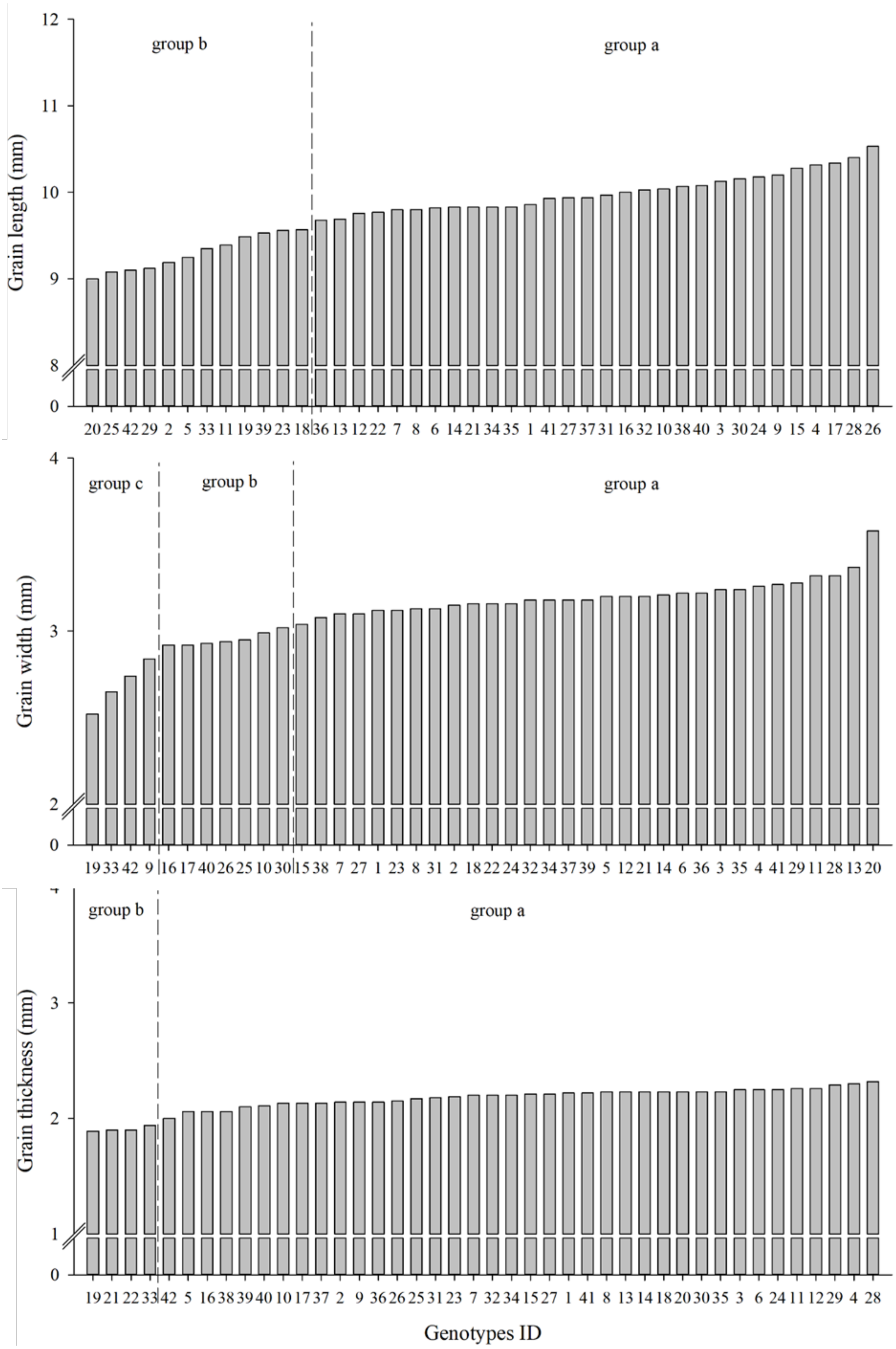

Figure 1. Averages of grain lenght (mm), grain width (mm) and grain thickness (mm). Averages followed by the same letter do not differ by Scott Knott test ($p < 0.05$).

For the length / width ratio of the grains (Figure 2) the highest averages were found for the genotypes: OSVR15011, OSVR15016, OSVR15023, OSVR15052, OSVR15024, OSVR15026, OSVR15029, OSVR15036, OSVR15040, OSVR15044, OSVR15049, IAC 202.

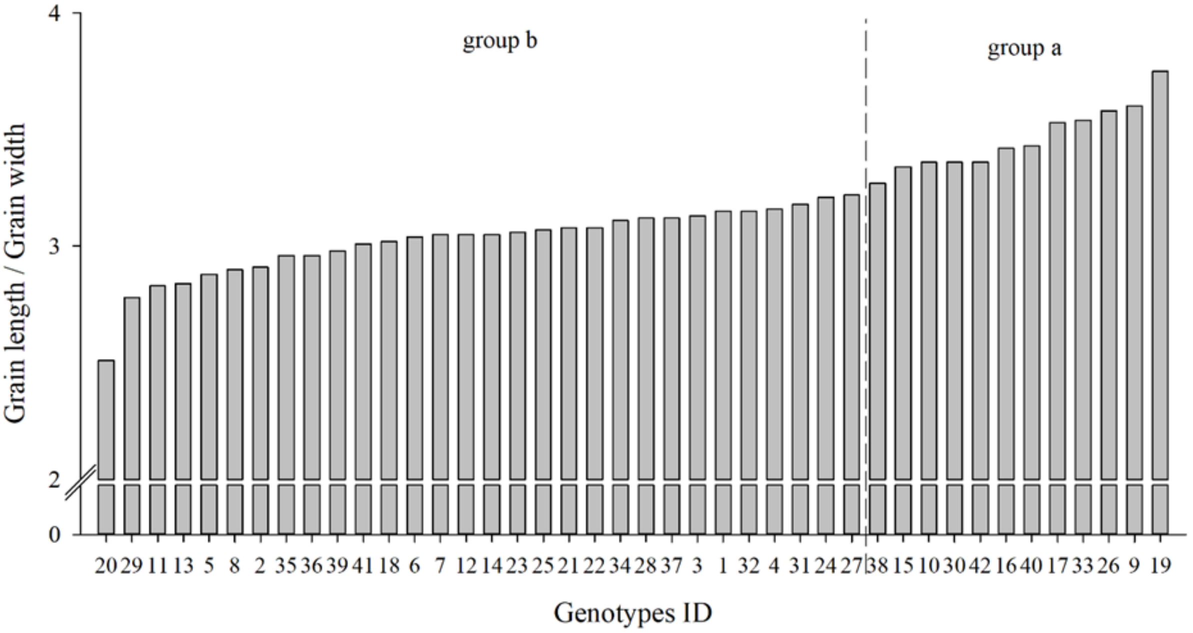

Figure 2. Averages of the grain lenght / grain width ratio. Averages followed by the same letter do not differ by Scott Knott test ($p < 0.05$).

Araujo et al. (2003) demonstrated that the grains that have the lowest grain length / width ratios, have the highest accumulations of crude protein. On the other hand, Soares and Camargos (2009) found that there was no correlation between the protein content and the length / width ratio of the grain.

The genotypes OSVR15029, OSVR15031 and OSVR15032 presented values of length greater than 6.0 mm, thickness less than or equal to 1.90 and length-to-width ratio greater than 2.75, being classified as long-fine grains, meeting national preferences. The consumers preference in Japan, Korea and northern China is for round and short rice grains, while consumers in Brazil, South China, USA and Southeast Southeast Asian countries have been for long and thin rice grains (needle), which clumps in the pan and remains loose and soft after cooking (Pereira and Rangel, 2001; Suwannaporn and Linnemann, 2008). This is due to the amylose content, so the bigger it is, the drier and more separated the beans will be after cooking (Yadav and Jindal, 2007; Batista, 2019).

The shape and size of rice grains is an important component of rice production, which determines the level of yield and quality, affecting the market value and acceptance by consumers (Yang et al., 2019; Wang et al., 2019; Jeong et al., 2020). Zhou et al. (2000) reported that the length, thickness and width of the grains, length / width ratio are controlled by QTLs (quantitative trait loci). In addition, these traits are positively correlated with grain weight, which is an important determinant of rice silage capacity and yield and can vary dramatically depending on the variety of rice. (Yang et al., 2019; Tan et al., 2000; Jeong et al., 2020).

The coenetic correlation coefficient of the hierarchical method (stage 1) was 0.82 and significant ($p < 0.01$) by the Mantel test (1963). Bussab et al. (2015) reported that cluster analyzes are acceptable when they produce a co-phenetic correlation coefficient above 0.80.

In the D index graph (Figure 3), was looked for a significant curve that corresponds to a significant increase in the measurement value. Thus, it was noted that the ideal number of clusters was 5 (Figure 4).

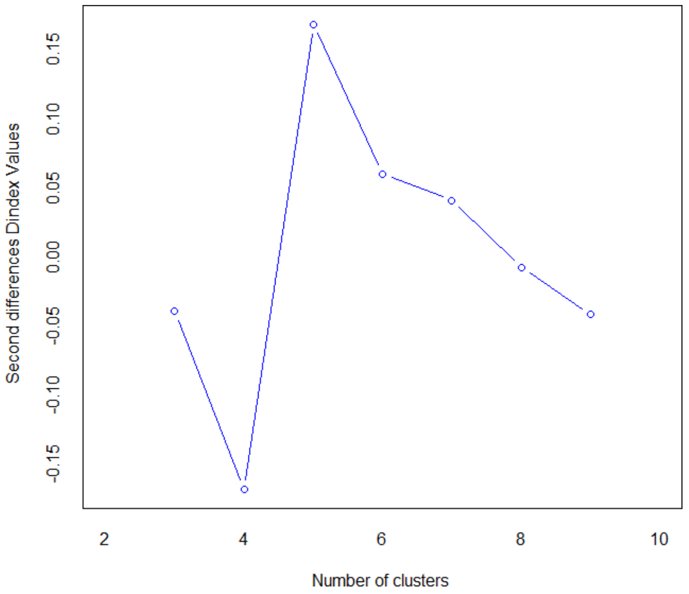

Figure 3. Lebart et al. (2000) to determine the optimal number of clusters.

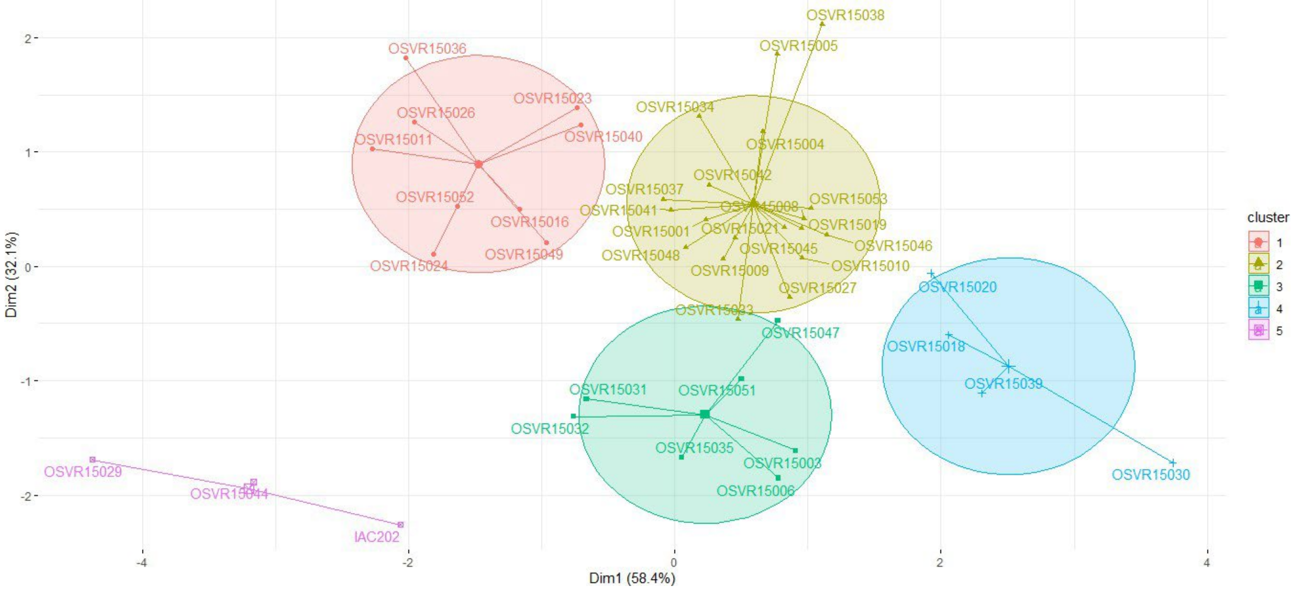

Figure 4. *Kmeans* non-hierarchical cluster for the 42 upland rice genotypes.

According to the non-hierarchical cluster of *Kmeans*, it was observed that cluster 1 was composed of 9 genotypes, OSVR15036, OSVR15026, OSVR15011, OSVR15052, OSVR15024, OSVR15049, OSVR15016, OSVR14040 and OSVR15023.

Cluster 2 was composed of 19 genotypes, OSVR15038, OSVR15005, OSVR15034, OSVR15037, OSVR15041, OSVR15001, OSVR15048, OSVR15033, OSVR15027, OSVR150310, OSVR15046, OSVR15053, OSVR15019, OSVR15045, OSVR15009, OSVR15021, OSVR15008, OSVR15042 and OSVR15004. Cluster 3 was composed of 7 genotypes, OSVR15047, OSVR15051, OSVR15031, OSVR15032, OSVR15035, OSVR15006 and OSVR15003. Cluster 4 was composed of 4 genotypes, OSVR15020, OSVR15018, OSVR15039 and OSVR15030. Finally, cluster 5 was composed of 3 genotypes OSVR15029, OSVR15044 and IAC 202. The averages of the morphological traits of the genotypes of each cluster are shown in Table 2.

Table 2. Averages of the genotype variables of each cluster. GW= grain width, GT= grain trickness, GL= grain lenght and GLGW= length-to-width ratio.

Cluster	GL (mm)	GW (mm)	GT (mm)	GLGW
1	9.47	3.15	2.05	2.99
2	10.18	2.96	2.13	3.43
3	9.31	2.63	1.94	3.55
4	9.92	3.18	2.22	3.08
5	9.30	3.38	2.25	2.74

The *Kmeans* cluster showed good representativeness, characterized by the homogeneity of the genotypes which within the groups. It was observed that there was a great genetic divergence between the genotypes, due to the distances between the clusters. These divergences can be explored in breeding programs based on quantitative descriptors to obtain new cultivars. This cluster indicates that, if you do not want to use the cultivar IAC202 but also want to maintain its traits, the best choices are the genotypes OSVR15029 and OSVR15044.

The results of this work have important implications for the breeding programs of rice (Fan et al., 2006). The choice of genotypes belonging to the same similarity group should be avoided in order not to restrict the genetic variability and, therefore, the negative effects on gains to be obtained by selection (Cruz and Regazzi, 2004).

According to the correlogram (Figure 5), was observed that the width of the grains had a positive relationship with the thickness of the grains. The negative correlation of the length-to-width ratio with grain width and positive with grain length is explained by the fact that this relationship is a trait composed of the length and width of the grains.

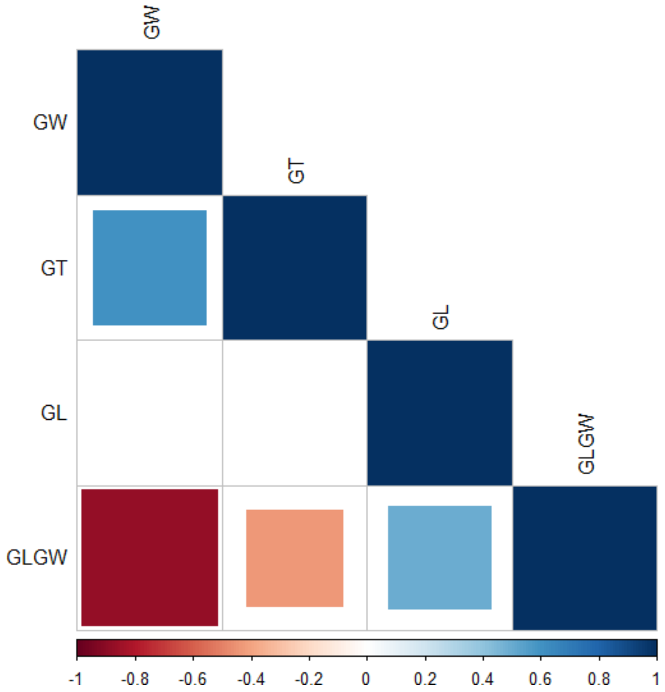

Figure 5. Correlogram of Pearson's linear relationship for the variables of the 42 upland rice genotypes. GW= grain width, GT= grain trickness, GL= grain lenght and GLGW= length-to- width ratio.

This dendrogram suggests that, in order to obtain needle-type grains, it is necessary that the grain has a low width and thickness and a high length, corroborating the national classification required to obtain this type of grain. Rocha et al. (2020) found that the thickness and length of rice grains had a negative correlation. According to the authors, the grain thickness presented itself as a trait antagonistic to the length, in the grains where greater thickness was observed, the shorter was its length, disagreeing with the results obtained in this work.

Conclusion

There is high genetic divergence among the upland rice genotypes.

The genotypes OSVR15029 and OSVR15044 have similar traits to the cultivar IAC 202.

The genotypes which have grains classified as long-thin, meeting national preferences and can be used for the breeding programs of upland rice crop.

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