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Molecular and agronomic genetic diversity between barley genotypes under irrigation in the Brazilian Cerrado

Abstract – The objective of this work was to evaluate and compare the genetic diversity of 29 barley genotypes, on the basis of molecular markers and quantitative agronomic traits, under irrigation in the Brazilian Cerrado. The randomly amplified polymorphic DNA (RAPD), inter sequence simple repeats (ISSR), and simple sequence repeat (SSR) molecular markers were used. The quantitative agronomic traits were evaluated in two irrigated environments in the Brazilian Cerrado, for the following parameters: estimated grain yield, grain size, thousand seed weight, plant height, lodging degree, and days to heading. Marker polymorphisms were 91, 51.46, and 85% for RAPD, ISSR, and SSR, respectively. The RAPD, ISSR, and SSR markers are complementary for the identification of genetic variability among barley genotypes. The low correlations between the distances estimated on the basis of molecular markers and the distances estimated on the basis of agronomic traits emphasize the importance of using complementary analyses of molecular markers for more complete studies on genetic variability. The agronomic traits of the genotypes are different in the two environments. The selected genotypes can compose the working collection of irrigated barley in the Brazilian Cerrado because of their wide genetic variability.

Index terms: *Hordeum vulgare*, genetic variability, ISSR, molecular markers, RAPD, SSR.

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Diversidade genética molecular e agronômica entre genótipos de cevada sob irrigação no Cerrado brasileiro

Resumo – O objetivo deste trabalho foi avaliar e comparar a diversidade genética de 29 genótipos de cevada, com base em marcadores moleculares e características agronômicas quantitativas, sob irrigação no Cerrado. Foram utilizados os marcadores moleculares "randomly amplified polymorphic DNA" (RAPD), "inter simple sequence repeats" (ISSR) e "simple sequence repeat" (SSR). As características agronômicas quantitativas foram avaliadas em dois ambientes, sob irrigação no Cerrado, quanto aos seguintes parâmetros: rendimento estimado de grãos, tamanho do grão, peso de mil sementes, altura de plantas, grau de acamamento e dias para espigamento. Os polimorfismos dos marcadores foram 91, 51,46 e 85% para RAPD, ISSR e SSR, respectivamente. Os marcadores RAPD, ISSR e SSR são complementares quanto à identificação da variabilidade genética dos genótipos de cevada. As baixas correlações entre as distâncias calculadas com base nos marcadores moleculares e as distâncias calculadas com base nas características agronômicas enfatizam a importância da utilização de análises complementares de marcadores moleculares para estudos mais completos quanto à variabilidade genética. As características agronômicas dos genótipos são distintas nos dois ambientes. Os genótipos selecionados podem

compor a coleção de trabalho de cevada irrigada no Cerrado, em razão de sua ampla variabilidade genética.

Termos para indexação: *Hordeum vulgare*, variabilidade genética, ISSR, marcadores moleculares, RAPD, SSR.

Introduction

Barley (*Hordeum vulgare* L.) is one of the oldest domesticated plants in the human history and shows a high level of genetic variation (Al-Sayaydeh et al., 2019). However, in the last 100 years, the improved inbred lines have almost completely replaced the local varieties, resulting in less diversity. Numerous barley cultivars have been developed through extensive plant breeding efforts with strict selection parameters. Hybridizations aimed to obtain new cultivars are usually performed with adapted cultivars that are crossed with lineages obtained by crossbreeding between parental elite, which can lead to the reduction of genetic variability. Although the breeding of cultivars is mostly based on elite germplasm, some specific characteristics may be incorporated from crossbreeding of wild barley/local varieties through the backcrossing technique (Amabile et al., 2013).

In the search to incorporate high performance genotypes with high variability to breeding programs, genetic diversity analyses can be used as tools to attain the following goals: to identify the genetic variability existing among a group of genotypes; to facilitate the identification of inbred lines to be used to obtain hybrid cultivars or higher segregating populations; to avoid the use of genetically close germplasm, preventing the occurrence of genetic narrowing; and to assist with the introduction of desirable genes/alleles from diverse germplasm sources into elite germplasm (Sayd et al., 2017).

The understanding of the genetic resources is essential to ensure a continuous gain by the selection of genotypes that are superior to the existing ones, enabling more development of stable and productive cultivars with high industrial quality via genetic improvement programs. Genetic variability analysis is the basis for the development of breeding programs, and it can be performed using qualitative and quantitative data, as well as molecular data.

Embrapa – The Brazilian Agricultural Research Corporation – has a barley genetic improvement program, and its studies on genetic diversity of genetic resources and elite genotypes have contributed to the advancement of selection and recombination cycles. The molecular, agronomic, and malting genetic diversity was studied in 30 elite genotypes of barley, under irrigated conditions in the Brazilian Cerrado (savanna-like vegetation), which were classified according to their origin and separated into two and six-rowed genotypes (Amabile et al., 2013). From this work, we verified the need to expand the genetic basis of the barley genetic improvement program for Brazilian Cerrado at Embrapa.

The objective of this work was to evaluate and compare the genetic diversity of 29 barley genotypes, on the basis of molecular markers and quantitative agronomic traits, under irrigation in the Brazilian Cerrado.

Materials and Methods

The genetic variability studies based on molecular and agronomic data were carried out for 29 elite brewing barley genotypes, selected from 435 barley accessions and cultivars from the germplasm bank of Embrapa Recursos Genéticos e Biotecnologia analyzed by Monteiro (2020). Among the elite genotypes, 5 are cultivars adapted to the Brazilian Cerrado conditions which were used as controls, 20 genotypes were selected for their agronomic behavior, and 4 genotypes were selected to be used as genitors in future crosses (as they show some trait with optimal values) (Table 1). Selections based on local comparisons (Sayd et al., 2017) and temporal ones (over the years 2012, 2013, and 2014) were performed with the aid of 4 selection indexes, in 5 experiments, to obtain the elite genotypes.

For the RAPD markers, seven decamer primers (Operon Technologies Inc., Alameda, CA, USA) were used: OPD (04, 07, and 19); OPE (20); and OPF (1, 14, and 20). The amplifications were performed in a thermocycler programmed for 40 cycles, each consisting of the following sequence: 15 s at 94ºC; 30 s at 35ºC; and 90 s at 72ºC. After the 40 cycles, a final extension step of 6 min at 72ºC was performed, and finally, the temperature was reduced to 4ºC.

For the ISSR markers (Alpha DNA, Montreal, Canada), the DNA was amplified using 8 primers (ISSR-2, ISSR-3, ISSR-4, ISSR-5, ISSR-8, ISSR-13, ISSR-14, and ISSR-15). The amplifications were performed in a thermocycler programmed for 5 min at

94ºC, with 35 cycles each consisting of the following sequence: 40 s at 94ºC; 40 s at 48ºC; and 1 min at 72ºC. After the 35 cycles, a final extension step of 2 min at 72ºC was performed and, finally, the temperature was reduced to 4ºC.

To obtain the SSR markers (Alpha DNA), DNA was amplified using 7 primers (MAG 149, GB371, BMAC624, MAG210, MAG13, GB318, and V13GEIII), and the program of 30 cycles consisted of 30 s at 94°C, 30 s at 65-56°C, and 5 s at 72°C. The annealing temperature started at 65°C and decreased by 0.3°C with each cycle, followed by 3 cycles with annealing at 56°C.

Table 1. Barley (*Hordeum vulgare*) genotypes used to study its genetic diversity, by country of origin (Origin) and grain-row number (GRN). Distrito Federal, Brazil.

Id	Genotype	Origin	GRN
1	MCU 3870 PI 402348	Colombia	6
2	MCU 3502 PI 401980	Colombia	6
3	MCU 3449 PI 401927	Colombia	6
4	CI 09952	Russia	6
5	MCU 3884 PI 402362	Colombia	6
6	MCU 3852 PI 402330	Colombia	6
7	MCU 3865 PI 402343	Colombia	6
8	MCU 3634 PI 402112	Colombia	6
9	MCU 3750 PI 402228	Colombia	6
10	MCU 3484 PI 401962	Colombia	6
11	MCU 3461 PI 401939	Colombia	6
12	CI 15565 QB 136-20	Canada	6
13	CI 13715	Colombia	6
14	MCU 3851 PI 402329	Colombia	6
15	MCU 3469 PI 401947	Colombia	6
16	MCU 3489 PI 401967	Colombia	6
17	MCU 3571 PI 402049	Colombia	6
18	MCU 3452 PI 401930	Colombia	6
19	MCU 3832 PI 402310	Colombia	6
20	MCU 3592 PI 402070	Colombia	6
21*	BRS 180	Brazil	6
22*	BRS 195	Brazil	2
23*	BRS Deméter	Brazil	2
24*	BRS Sampa	Brazil	2
25*	BRS Savanna	Brazil	6
26	CI 09958	Morocco	6
27	MCU 3827 PI 402305	Colombia	6
28	CI 13683 NUMAR	EUA	6
29	H HOR 2325/58 PI 329126	Afghanistan	6

*Genotypes used as control.

After the amplifications to obtain the RAPD, ISSR, and SSR markers, 3 µL of a mixture of bromophenol blue (0.25%) (Vetec Química Fina Ltda., Duque de Caxias, RJ, Brazil) and glycerol (60%) (Sigma, Steinheim, Germany) in water were added to each sample. These samples were applied in agarose gel (1.2%) (Invitrogen Corporation, Auckland, New Zealand) for RAPD and ISSR, and agarose gel (3.0%) for SSR, stained with ethidium bromide (Invitrogen), submerged in 1 mmol L^{-1} EDTA TBE buffer (Biogen – Biotecnologia e Química, Fair Lawn, NJ, USA) and 90 mmol L^{-1} Tris-Borate to be subjected to electrophoresis. After the electrophoresis, gels were photographed under ultraviolet light.

Two experiments were carried out under center pivot irrigation from May to September 2015. The experiment 1 was conducted in the experimental field of Embrapa Cerrados, in Planaltina, DF, Brazil, at 1,007 m altitude, in a clayey Latossolo Vermelho distrófico típico, classified according to the Brazilian soil classification system (Santos et al., 2018), i.e., an Oxisol. The experiment 2 was conducted in the experimental field of Embrapa Cerrados - Centro de Inovação e Genética Vegetal), Riacho Fundo II, DF, Brazil, at 1,254 m altitude, in a clayey Latossolo Vermelho distrófico típico, classified according to the Brazilian soil classification system (Santos et al., 2018), i.e., an Oxisol.

The experimental design was carried out in a randomized complete block with four replicates. The plots consisted of were six rows of 5 m length, spaced at 20 cm apart, with a useful area of 4.8 m^2 for each plot (discarding the first and last rows), with 300 plants m^{-2} density.

Six agronomic characteristics were evaluated as follows: estimated grain yield (kg ha^{l}), at 13% of humidity, harvested by hand (Yield); grain size (% >2.5 mm) (GS, %), according to Brasil (1996); thousand seed weight (TSW, g), according to Brasil (2009); plant height (average of three plants per plot) (Height); degree of lodging (data transformed into arcsen $X^{0.5}$ 100^{-1}, where x is the value, in %, of lodging) (Lodge); days to heading (period from emergence until 50% of the spike in the plot were visible) (Cycle, days).

Binary data matrices were generated from the data obtained by RAPD and ISSR markers and,

with the aid of the Genes Program (Cruz, 2013), the genetic dissimilarities between the accessions were estimated, using the similarity coefficient of Nei & Li (1979). For SSR markers, the genetic dissimilarities were calculated with the help of the Genes Program (Cruz, 2013) on the basis of the following formula $GD_{ij} = 1 - (NCL/TNL)$, where: GD_{ij} is the genetic distance between accessions i and j; NCL is the number of coincident locus; and TNL is the total number of loci.

The NCL is the sum of allelic coincidences of each analyzed locus, and each coincidence can assume the value 1 (two coincident alleles), 0.5 (one coincident allele), or 0 (no coincident allele) for matches (0 1) and (1 0).

Data of the agronomic traits were subjected to analysis of variance, and the genetic dissimilarity was estimated among all pairs of genotypes, using the generalized Mahalanobis distance ($D_{2\ ij}$) in both environments (Planaltina and Riacho Fundo II).

For the grouping analyses, the genetic dissimilarity matrices based on each molecular marker and agronomic characteristics in each environment were standardized. The genetic distances were adjusted in values for which the largest distance was considered 100, and the other distances were considered proportional to it. From each standardized dissimilarity matrix, a cluster analysis was performed using the hierarchical agglomerative method of the unweighted pair group method with arithmetic mean (UPGMA) represented in a dendrogram; and the graphic dispersion was based on multidimensional scales using the method of principal coordinates, with the aid of the SAS Program (SAS Institute Inc., 2020) and Statistica (Statsoft Inc, 1999).

The values for the genetic dissimilarity estimated using molecular markers (RAPD, ISSR, and SSR) were correlated with the agronomic traits evaluated at the two sites (Planaltina and Riacho Fundo II) on the basis of Pearson's correlation coefficient at 1% probability, with the help of the Genes program.

Results and Discussion

Sixty-seven RAPD markers were obtained, out of which 91% were polymorphic. The polymorphism levels observed for RAPD markers were considered high and of the same magnitude as reported by Giancarla et al. (2012) of 91%, and Olgun et al. (2015), of 93%. The dendrogram based on RAPD markers (Figure 1 A) separated the genotypes into five distinct groups. The genotypes from the selection by high agronomic performance were mostly distributed in groups 1 and 2. Group 3, formed by nine genotypes, grouped the four genotypes chosen as parents, in addition to four Colombian genotypes and the 'BRS 195' track control. The controls were distributed in different similarity groups.

The most dissimilar genotypes were the Colombian ones MCU 3634 PI 402112 and MCU 3469 PI 401947 (100.0). The most similar genotypes were the parents CI 09958 with MCU 3827 PI 402305 (19.78), and among the Colombian MCU 3851 PI 402329 and MCU 3469 PI 401947 (22.41).

Through the graphical distribution of genotypes (Figure 1 B), it is possible to visualize that there was no tendency to group them by geographic origin. However, the controls and genotypes selected as parents are positioned in the third and fourth quadrant. The genotypes were distributed spatially throughout the graph. These results show the existence of high variability among the selected genotypes. From the point of view of genetic improvement, the genotypes considered both the most agronomically promising and genetically distant can be used in crossing blocks, in such a way, that a genetic complementarity can occur and these genotypes can be selected in segregating populations.

The ISSR molecular marker analyses generated 70 bands, out of which 36 (51.46%) were polymorphic; this value is considered low for barley, in comparison with the works published by Olgun et al. (2015), with 85% in 12 two- and six-rowed barley genotypes, and Giancarla et al. (2012), with 89% in 19 barley genotypes of different origins, but of greater magnitude than that obtained by El-Awady et al. (2012), in six local barley genotypes from Saudi Arabia (30%).

The 29 genotypes were distributed in three distinct groups in the dendrogram generated from the ISSR markers (Figure 2 A). The first group was composed of 14 genotypes, among which five are controls, seven are six-rowed Colombian genotypes, and two are genotypes selected as parents – one of Moroccan origin, and other of American origin. The smallest genetic distances occurred between the Colombian genotype MCU 3592 PI 402070 and the 'BRS Sampa' (12.74), the 'BRS 195' and 'BRS Deméter' (16.42), as well as between the MCU 3592 PI 402070 genotype

and the six-rowed barley cultivar BRS 180 (17.60). The greatest distances occurred between the genotype selected as parent of Afghan origin H HOR 2325/58 PI 329126 and the Colombian genotypes MCU 3870 PI 402348 (100.0) and MCU 3502 PI 401980 (97.7). The greatest genetic dissimilarity of the Afghan genotype is probably explained by the fact that it is the only genotype of Asian origin among the 29 ones. The scatter plot (Figure 2) shows a tendency of grouping of the controls (genotypes 21 to 25) in the first quadrant. The genotypes (1 to 20), selected by their high agronomic performance, are positioned spacedly in the second quadrant, which represents variability between them. The genotype of Afghan origin H HOR 2325/58 PI 329126 was isolated in the third quadrant.

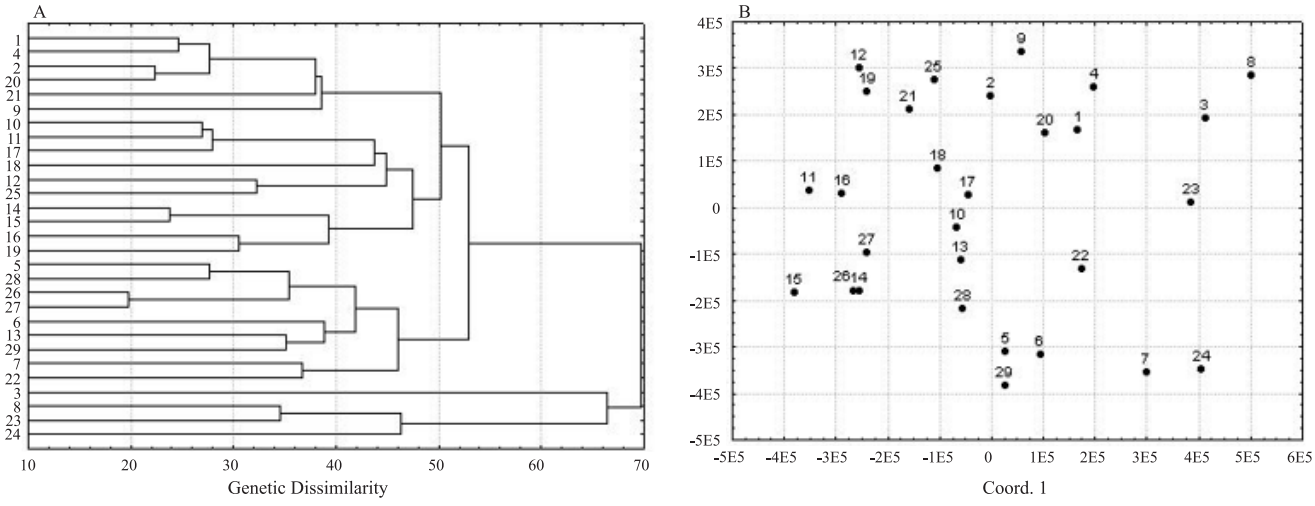

Figure 1. Dendrogram (A) and scatter plot (B) obtained from the matrix of genetic dissimilarities (arithmetic complement of the coefficient of Nei and Li) among 29 elite genotypes of barley (*Hordeum vulgare*) estimated by RAPD molecular markers.

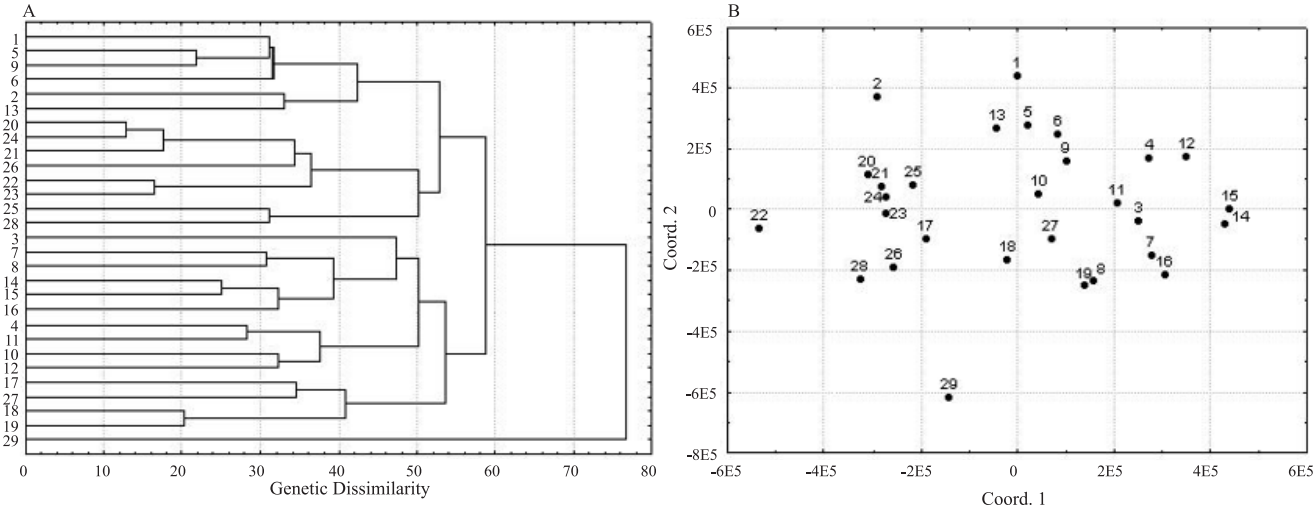

Figure 2. Dendrogram (A) and scatter plot (B), obtained from the matrix of genetic dissimilarities (arithmetic complement of the coefficient of Nei and Li) among 29 elite genotypes of barley (*Hordeum vulgare*) estimated by ISSR molecular markers.

The study of diversity of microsatellite markers (SSR) was performed using 07 primer pairs, out of which 06 were polymorphic (85%). The content of polymorphic information (PIC) ranged from 0.07 ('BMAC624') to 0.52 ('MAG149'), with 0.22 average. The observed PIC values are considered low in comparison with the mean value of 0.57 (0.07 to 0.86) of 34 SRH markers reported by Ferreira et al. (2016), for 64 barley cultivars in southern Brazil, and with values from 0.28 to 0.98 of 12 markers, in 25 Indian barley genotypes reported by Chourasia et al. (2016).

The dendrogram (Figure 3 A) that represents the genetic distances of the SSR markers separates the genotypes into seven similarity groups. The first group was composed of six Colombian genotypes, and the American genotype selected as genitor CI 13683 NUMAR. The second group was formed by 15 genotypes, out of which four are controls and two are parents. The closest genetic genotypes (MCU 3469 PI 401947 and 'BRS Savanna') are found in these first two groups, with 0 value. This fact can be explained by the small number of analyzed holes and, also, by the low resolution existing in the use of agarose gel in electrophoresis and photodocumentation, which hinders the differentiation of alleles with small size differences. Groups 3, 4, and 5 were formed by the six-rowed Colombian genotypes, while groups 6 and 7 were formed by only one genotype each. Group 6 was formed by the 'BRS Sampa', and group 7 by the Russian genotype CI 09952.

The dispersion plot obtained from the genetic dissimilarities of the estimated genotypes with SSR markers (Figure 3 B) shows a higher distribution of genotypes than that in the graph of the Figure 1 B. There was a tendency to group two genotypes in the central region of the graph, grouping together the controls and the Afghan genotype H HOR 2325/58 PI 329126, a behavior opposite to that observed in the ISSR markers.

The original matrix distances based on markers show that the SSR and RAPD markers detected a greater variability that the ISSR marker. The mean genetic dissimilarities obtained with basis on SRH and RAPD were 0.46 and 0.40, respectively, while that obtained with basis on SRHS was only 0.14. Hamidi (2018) used RAPD markers in 10 Iranian barley genotypes, and reported 0.71 average dissimilarity. In another study, eight genotypes from various regions of the world showed 0.61 mean genetic dissimilarity (Drine et al., 2016). An evaluation using RAPD markers, in the Brazilian Cerrado, showed 0.28 mean genetic dissimilarity for 21 naked barley genotypes from different continents (Sayd et al., 2015). In this scenario, the genetic dissimilarity values obtained in the present study show that the genotypes have a considerable degree of variability, although most of them are of Colombian origin and have been selected for the same agronomic characteristics.

Genetic distances based on the agronomic characteristics evaluated in two Planaltina (Figure 4)

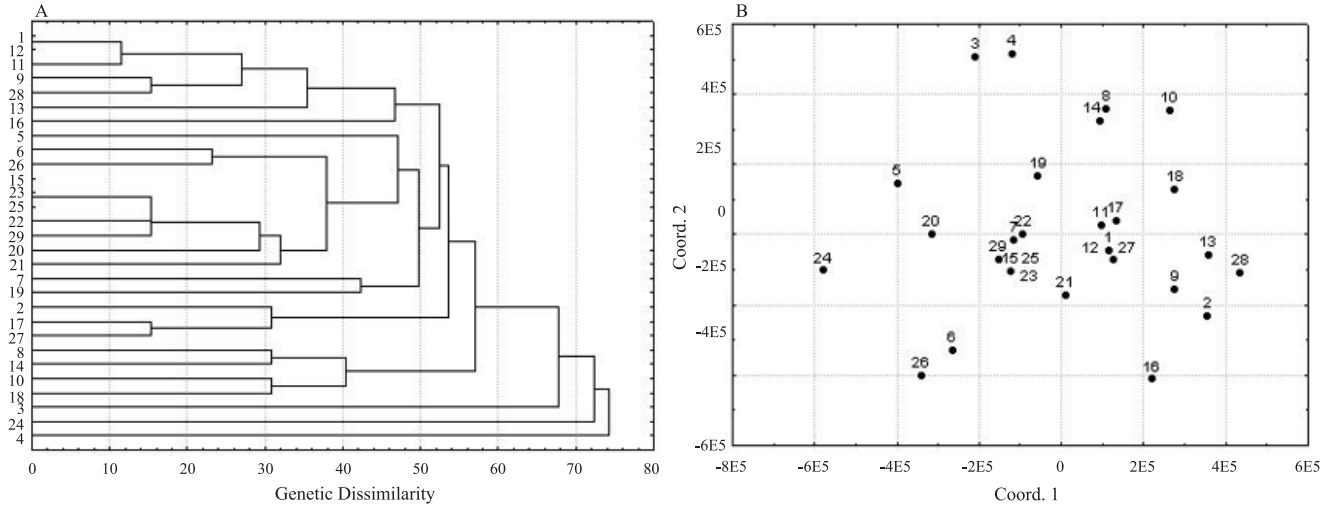

Figure 3. Dendrogram (A) and scatter plot (B) obtained from the matrix of genetic dissimilarities (arithmetic complement of the coefficient of Nei and Li) among 29 elite genotypes of barley (*Hordeum vulgare*) estimated by SSR molecular markers.

and Riacho Fundo II (Figure 5) environments were also used for cluster and graphical dispersion analyses.

Despite the correlation between agronomic genetic distances in the two environments (Table 2), the elite genotypes were distributed differently in the dendrograms and dispersion graphs. In the Planaltina environment, there was a concentration of genotypes to the right of the graph (Figure 4). In the Riacho Fundo II, the genotypes were uniformly distributed to the center of the graph (Figure 5). In both cases, the Afghan genotype H HOR 2325/58 PI 329126 was kept away from the others, as it was in the ISSR marker. In both graphs, the controls were very close, mainly due to the high agronomic performance showed for grain size and grain yield.

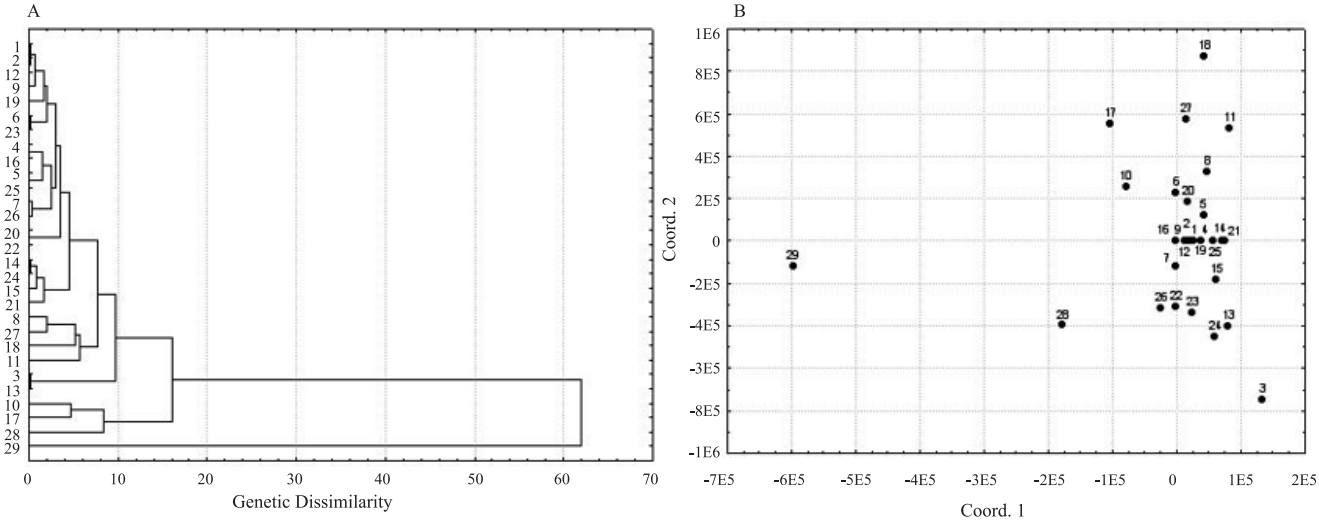

Figure 4. Dendrogram (A) and scatter plot (B) obtained from the genetic dissimilarity matrix (generalized Mahalanobis distance) among 29 elite genotypes of barley (*Hordeum vulgare*), estimated in agronomic characteristics evaluated in the Planaltina environment.

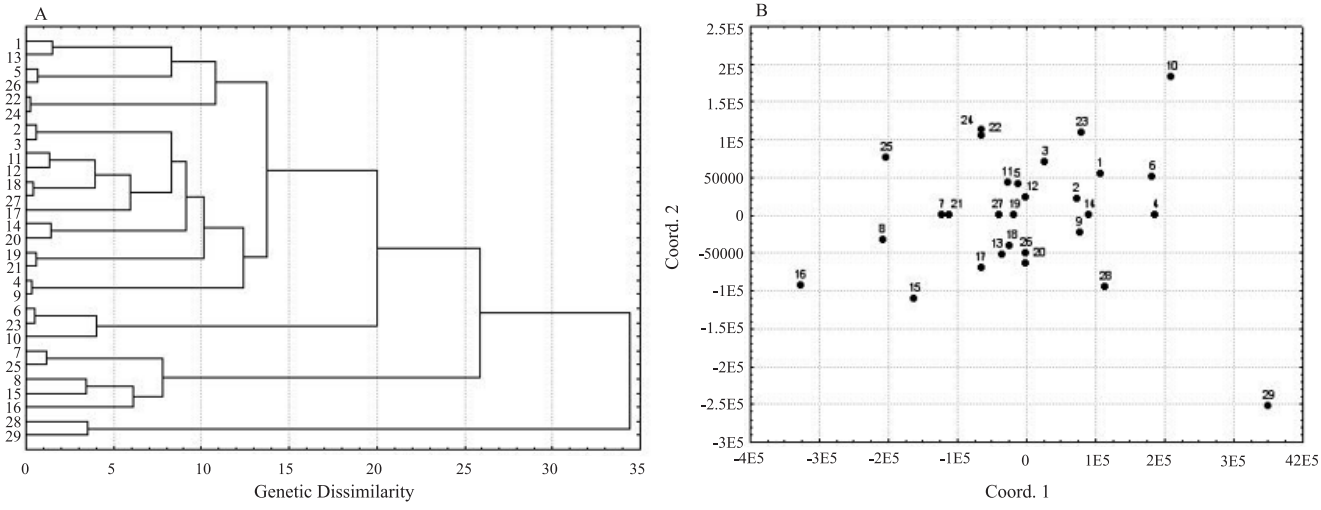

Figure 5. Dendrogram (A) and scatter plot (B) obtained from the genetic dissimilarity matrix (generalized Mahalanobis distance) among 29 elite genotypes of barley (*Hordeum vulgare*), estimated in agronomic characteristics evaluated in the Riacho Fundo II environment.

The cluster analysis obtained with basis on the agronomic characteristics evaluated in the Planaltina (Figure 4) shows the formation of three similarity groups at 11.28 cut-off point. Out of the 29 evaluated genotypes, 25 were located in group I with 8.69 average distance. In general, the genotypes of this group showed grain yield above 3,500 kg ha^{-1} and above 70% grain size.

Group II established the genotypes MCU 3484 PI 401962, MCU 3571 PI 402049 and CI 13683 NUMAR, with mean distance of 15.96. They differed from the others mainly by the low grain size values (55%) and grain yield.

In group III, the Afghan genotype had the lowest values for grain yield and grain size, and therefore grouped separately from the other genotypes.

In both the Planaltina and Riacho Fundo II, there was no Yield towards grouping of two or six-rowed genotypes, opposing to what was observed by Amabile et al. (2017) in 39 elite genotypes of brewing barley in the Brazilian Cerrado. In Riacho Fundo II, the dendrogram was structured into four groups, considering the average distance (19.27) as a cutoff point. 'BRS 195' and 'BRS Sampa' were the closest genotypes and grouped within the first group. This fact can be explained by the fact that 'BRS Sampa' is a daughter of 'BRS 195' and has very close agronomic performance with this last cultivar (Figure 5). Group I had 15.61 mean distance and included 19 of the 29 genotypes. The group was characterized by high values of grain yield, grain size, and height.

Group II had 24.10 mean distance and contains three genotypes (MCU 3571 PI 402049, MCU 3852 PI 402330, and the control 'BRS Deméter'). The main characteristic of this group is the small degree of lodging, associated with low grain size values and grain yield, in addition to an early cycle of approximately 51 days.

Group III with 24.67 average distance ('BRS Savanna', MCU 3489 PI 401967, MCU 3469 PI 401947, MCU 3634 PI 402112 and MCU 3865 PI 402343) showed highly productive genotypes, with grain yield above 8,000 kg ha^{-1}, grain size ranging from 70% to 78%, and thousand seed weight close to 42 g.

In group IV, the genotype H HOR 2325/58 PI 329126 was again the most distant, but grouped with the American genotype CI 13683 NUMAR. They obtained grain yield around 5,000 kg ha^{-1}, grain size below 70%, and a high degree of mating and early spike cycle.

Because genotypes passed through a high degree of selection for agronomic characteristics, a higher agronomic grouping was expected in comparison with molecular data. This fact may be beneficial for improvement, since we found genotypes with high productive potential that still have genetic variability and can be exploited through crossbreeding, providing considerable selection gains.

The analyses of the correlations between the standardized distances were significant ($p \leq 0.01$) between the molecular distance ISSR and the agronomic distances for Riacho Fundo II and Planaltina, between the agronomic distances for Riacho Fundo II and Planaltina, and between the molecular distances SSR and RAPD (Table 2). Among the significant correlations, the highest magnitude was observed between the agronomic distances for Riacho Fundo II and Planaltina (0.3764). The lowest correlation occurred between the molecular distances SSR and RAPD (0.1536). Significant correlations between RAPD and SSR have already been reported by Dar et al. (2017), and in a comparative assessment of genetic diversity in *Sesamum indicum* L., Zhang et al. (2019) in 10 fig varieties. Garcia et al. (2004) reported that the correlation between RAPD and SSR (r=0.33) was the lowest among the four types of markers tested in corn lines. Low intensity correlation (0.113) was also observed by Hou et al. (2005) between the ISSR and RAPD markers in barley from Western China, which is corroborated by the low magnitude and nonsignificant correlation obtained in the present study (Table 2). Amabile et al. (2013) found no significant correlations between the distances estimated on the basis of molecular markers (RAPD), morpho-agronomic

Table 2. Correlations between the genetic distances among 29 elite genotypes of barley (*Hordeum vulgare*) obtained from three molecular markers (ISSR, SSR, and RAPD) and agronomic characteristics evaluated in two environments (Planaltina and Riacho Fundo II, DF, Brazil).

Marker	ISSR	SSR	RAPD	Riacho Fundo II
SSR	-0.0427			
RAPD	-0.0158	0.1536**		
Riacho Fundo II	0.2093**	0.0213	0.0561	
Planaltina	0.3535**	-0.0459	0.0459	0.3764**

** Significant at 1% probability by the t-test.

and quality characters. This lack of correlation can also occur between different environments, which hinders the work of the breeder and shows the need for the complementary use of different groups of characteristics, in the process of selection of parents (Piepho & Williams, 2006).

After the individual analysis of each genetic marker (RAPD, SSR, and ISSR), the complementarity between them was verified, since they access different regions of the genome. Each marker has its advantages and disadvantages and, for this reason, an individual analysis of the molecular and agronomic genetic diversity was taken into account, since the correlation between them was considered low. Furthermore, the evaluation of both molecular and agronomic genetic diversity (in two environments) was a strategy used to increase the accuracy in the selection of genotypes and minimize eventual experimental errors in the evaluation of genetic markers. Thus, the strategy was a complementary analysis between the dendrograms generated by different types of markers (agronomic and molecular), for a more assertive analysis of diversity, in order to indicate the best crossings to be performed, therefore seeking the best gene complementarity in future segregating generations.

Conclusions

1. There is high genetic diversity among the 29 barley (*Hordeum vulgare*) genotypes, based on the three types of molecular markers and quantitative agronomic characteristics evaluated in two experimental irrigated environments in the Brazilian Cerrado.

2. The low correlations between distances based on molecular markers and distances based on agronomic traits emphasize the importance of the use of a complementary analysis of the molecular and agronomic markers in more complete studies of genetic variability.

3. The molecular markers ISSR, SSR, and RAPD access different regions of the genome of each genotype, and they should be analyzed in a complementary way; the agronomic characteristics of the genotypes are different in the two environments; in Riacho Fundo II, there is greater variation of characteristics among the analyzed genotypes.

4. Because of their molecular and agronomic variability, the selected genotypes can compose the working collection of irrigated barley in the Brazilian Cerrado.

References

AL-SAYAYDEH, R.; AL-BAWALIZE, A.; AL-AJLOUNI, Z.; AKASH, M.W.; ABU-ELENEIN, J.; AL-ABDALLAT, A.M. Agronomic evaluation and yield performance of selected barley (*Hordeum vulgare* L.) landraces from Jordan. **International Journal of Agronomy**, v.2019, art.9575081, 2019. DOI: https://doi.org/10.1155/2019/9575081.

AMABILE, R.F.; FALEIRO, F.G.; CAPETTINI, F.; PEIXOTO, J.R.; SAYD, R.M. Genetic variability in elite barley genotypes based on the agro-morphological characteristics evaluated under irrigated system. **Ciência e Agrotecnologia**, v.41, p.147-158, 2017. DOI: https://doi.org/10.1590/1413-70542017412010116.

AMABILE, R.F.; FALEIRO, F.G.; VIEIRA, E.A.; PEIXOTO, J.R.; CAPETTINI, F.; RIBEIRO JÚNIOR, W.Q. Genetic diversity of irrigated barley based on molecular and quantitative data and on malting quality. **Pesquisa Agropecuária Brasileira**, v.48, p.748-756, 2013.

BRASIL. Ministério da Agricultura e do Abastecimento. Portaria nº 691, de 22 de novembro de 1996. [Aprova a Norma de Identidade e Qualidade da Cevada, para comercialização interna]. **Diário Oficial [da] República Federativa do Brasil**, 25 nov. 1996. Seção 1, p.24751-24752.

BRASIL. Secretaria de Defesa Agropecuária. **Regras para análise de sementes**. Brasília: Mapa/ACS, 2009. 395p.

CHOURASIA, K.N.; PRASAD, L.C.; PRAKASH, O. Morphological and molecular characterization in diverse genotypes of barley (*Hordeum vulgare* L.). **The Bioscan**, v.11, p.1913-1916, 2016.

CRUZ, C.D. Genes: a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum. Agronomy**, v.35, p.271-276, 2013. DOI: https://doi.org/10.4025/actasciagron.v35i3.21251.

DAR, A.A.; MUDIGUNDA, S.; MITTAL, P.K.; ARUMUGAN, N. Comparative assessment of genetic diversity in *Sesamum indicum* L. using RAPD and SSR markers. **3 Biotech**, v.7, art.10, 2017. Doi: https://doi.org/10.1007/s13205-016-0578-4.

DRINE, S.; GUASMI, F.; BEN ALI, S.; TRIKI, T.; BOUSSORRA, F.; FERCHICHI, A. Genetic diversity analysis of different barley (*Hordeum vulgare* L.) genotypes from arid and humid regions using ISSR and RAPD markers. **Journal of New Sciences**, v.34, p.1930-1939, 2016.

EL-AWADY, M.A.H.M.; EL-TARRAS, A.A.E.-S; EL-ASSAL, S.E.-D. Genetic diversity of some Saudi barley (*Hordeum vulgare* L.) landraces based on two types of molecular markers. **American Journal of Applied Sciences**, v.9, p.752-758, 2012. DOI: https://doi.org/10.3844/ajassp.2012.752.758.

FERREIRA, J.R.; PEREIRA, J.F.; TURCHETTO, C.; MINELLA, E.; CONSOLI, L.; DELATORRE, C.A. Assessment of genetic diversity in Brazilian barley using SSR markers. **Genetics**

and Molecular Biology, v.39, p.86-96, 2016. DOI: https://doi.org/10.1590/1678-4685-GMB-2015-0148.

GARCIA, A.A.F.; BENCHIMOL, L.L.; BARBOSA, A.M.M.; GERALDI, I.O.; SOUZA JR., C.L.; SOUZA, A.P. de. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. **Genetics and Molecular Biology**, v.27, p.579-588, 2004. DOI: https://doi.org/10.1590/S1415-47572004000400019.

GIANCARLA, V.; EMILIAN, M.; RADU, S.; SORIN, C.; SORINA, P.; CERASELA, P. The use of RAPD and ISSR markers for genetic diversity among some barley cultivars. **Romanian Biotechnological Letters**, v.17, p.7493-7503, 2012.

HAMIDI, H. Genetic diversity study in some barley diverse landraces based on RAPD markers in Kurdistan region of Iraq. **International Journal of Farming and Allied Sciences**, v.7, p.100-106, 2018.

HOU, Y.C.; YAN, Z.H.; WEI, Y.M.; ZHENG, Y.L. Genetic diversity in barley from West China based on RAPD and ISSR analysis. **Barley Genetics Newsletter**, v.35, p.9-22, 2005.

MONTEIRO, V.A.; AMABILE, R.F.; SPEHAR, C.R.; FALEIRO, F.G.; VIEIRA, E.A.; PEIXOTO, J.R.; RIBEIRO JUNIOR, W.Q.; MONTALVÃO, A.P.L. Genetic diversity among 435 barley accessions based in morpho-agronomical characteristics under irrigation in the Brazilian savannah. **Australian Journal of Crop Science**, v.14, p.1385-1393, 2020. DOI: https://doi.org/10.21475/ajcs.20.14.09.p2281.

NEI, M.; LI, W.-H. Mathematical model for studying genetic variation in terms of restriction endonucleases. **Proceedings of the National Academy of Sciences of the United States of America**, v.76, p.5269-5273, 1979. DOI: https://doi.org/10.1073/pnas.76.10.5269.

OLGUN, M.; BUDAK BAŞÇİFTÇİ, Z.; AYTER, N.G.; TURAN, M.; KOYUNCU, O.; ARDIÇ, M.; AĞAR, G.; TAKIL, E. Genetic divergence in some barley (*Hordeum vulgare* L.) genotypes by RAPD and ISSR analyses. **Journal of the Faculty of Agriculture**, v.10, p.102-109, 2015.

PIEPHO, H.P.; WILLIAMS, E.R. A comparison of experimental designs for selection in breeding trials with nested treatment structure. **Theoretical and Applied Genetics**, v.113, p.1505-1513, 2006. DOI: https://doi.org/10.1007/s00122-006-0398-8.

SANTOS, H.G. dos; JACOMINE, P.K.T.; ANJOS, L.H.C. dos; OLIVEIRA, V.Á. de; LUMBRERAS, J.F.; COELHO, M.R.; ALMEIDA, J.A. de; ARAÚJO FILHO, J.C. de; OLIVEIRA, J.B. de; CUNHA, T.J.F. **Sistema brasileiro de classificação de solos**. 5.ed. rev. e ampl. Brasília: Embrapa, 2018. 356p.

SAS INSTITUTE INC. **SAS/STAT 9.2 user's guide**. p.7857. Available at: <http://support.sas.com/documentation/cdl/en/statug/59654/PDF/default/statug.pdf>. Accessed on: 20 mar. 2020.

SAYD, R.M.; AMABILE, R.F.; FALEIRO, F.G.; BELLON, G. Genetic variability of hull-less barley accessions based on molecular and quantitative data. **Pesquisa Agropecuária Brasileira**, v.50, p.160-167, 2015. DOI: https://doi.org/10.1590/S0100-204X2015000200008.

SAYD, R.M.; AMABILE, R.F.; FALEIRO, F.G.; MONTALVÃO, A.P.L.; COELHO, M.C. Agronomic characterization of high-yielding irrigated barley accessions in the Cerrado. **Pesquisa Agropecuária Brasileira**, v.52, p.84-94, 2017. DOI: https://doi.org/10.1590/s0100-204x2017000200002.

STATSOFT INC. **Statistica for Windows [Computer program manual].** Tulsa, 1999.

ZHANG, Z.; HU, Y.; LIU, L.; WANG, X.; WANG, W.; LIU, Y.; DENG, Q.; ZHANG, H.; WANG, X.; XIA, H.; LIANG, D. Classification and phylogenetic relationship of 10 fig varieties by RAPD and SSR molecular markers. **IOP Conference Series: Earth and Environmental Science**, v.332, art.032026, 2019. DOI: https://doi.org/10.1088/1755-1315/332/3/032026.