

## Genetic diversity among 435 barley accessions based in morpho-agronomical characteristics under irrigation in the Brazilian savannah

Vitor Antunes Monteiro<sup>1</sup>, Renato Fernando Amabile<sup>2</sup>, Carlos Roberto Spehar<sup>3</sup>, Fábio Gelape Faleiro<sup>2</sup>, Eduardo Alano Vieira<sup>2</sup>, José Ricardo Peixoto<sup>3</sup>, Walter Quadros Ribeiro Junior<sup>2</sup> and Ana Paula Leite Montalvão<sup>4\*</sup>

<sup>1</sup>AmBev/Maltaria Passo Fundo - 99032-680, Passo Fundo, RS, Brazil

<sup>2</sup>Empresa Brasileira de Pesquisa Agropecuária/Embrapa Cerrados – BR 020, Km 18 - 73010-970, Planaltina, DF, Brazil

<sup>3</sup>Universidade de Brasília/UnB – Faculdade de Agronomia e Medicina Veterinária/FAV – 70910-900 – Brasília, DF Brazil

<sup>4</sup>University of Hamburg, Faculty of Mathematics, Informatics and Natural Sciences, Department of Biology, Hamburg, Germany

\*Corresponding author: ana.montalvao@thuenen.de

### Abstract

The success of barley (*Hordeum vulgare* L.) cultivation and its adaptation to cropping systems relies on the knowledge and utilization of existing variability in germplasm banks. The objective of this work was to analyse diversity among pre-selected barley accessions to organize a working collection and to identify genotypes for a breeding program for irrigated barley in the Brazilian Savannah. The field experiment was conducted under irrigation in Planaltina, DF, Brazil. The plant population consisted of 433 accessions plus BRS 180 and BRS 195 as checks. The accessions were evaluated using fifteen morpho agronomic descriptors, in which 11 quantitative and four discrete. The interpolated control design was used for the statistical analysis. A genetic distance matrix was calculated using Gower's coefficient. From the matrix, a grouping analysis was conducted, using the optimizing Tocher method and the graphic dispersion distance. The genetic distances varied between 0.025 and 0.572, with a mean of 0.256. The accessions were distributed in 18 groups by the Tocher method, which was directly related to the graphic dispersion. The existing genetic divergence in the collection under study helped the definition of accessions in crossing blocks from breeding programs directed to the savannah environment.

**Keywords:** *Hordeum vulgare* L.; germplasm bank; genetic resources; crop breeding; genetic distance.

**Abbreviations:** Al\_aluminum; Ca\_calcium; Mg\_magnesium; P\_phosphorus; K\_potassium; N\_nitrogen; M.O\_organic matter; P<sub>2</sub>O<sub>5</sub>, phosphorus pentoxide; K<sub>2</sub>O\_potassium oxide.

### Introduction

Barley was introduced as a winter crop to savannah. The main objective was to meet the demand for malt and offer farmers an alternative to diversify and integrate the irrigated production system guaranteeing a more stable total yield. Brazilian savannah has a great potential to supply the demand for barley grains, giving a chance and opening new frontiers for the agriculture business, together with modern commercial opportunities.

The adaptation and development of the crop in the Brazilian savannah is needed. However, a stronger investment in research, especially regarding the genetic improvement is necessary. The progress depends on the amplitude of diversity in germplasm and the heritability of the desirable characters for application in genetic improvement (Mohammadi and Prasanna, 2003). Thus, the characterization and evaluation of the genetic diversity are highly relevant to study the diversity (Wetzel and Ferreira, 2007). The knowledge of population variability, monitoring germplasm banks and generating useful information for

conservation of accessions (Cruz et al., 2004; Leistrumaite et al., 2010).

There are few studies regarding the genetic diversity in Brazilian savannah. Therefore, it is necessary to screen accessions based on their high variability, since they can present phenotypic and yield differences. The in-depth knowledge of the genetic variability of barley in the savannah can only be obtained through their characterization in field experiments, favouring its use in breeding programs in Brazil and allowing the possibility of the selection of superior genotypes for a specific environment (Verma and Sakar, 2010).

The knowledge of the variability allows the correct allocation of strategies for breeding. As highlighted by Moura (2003), one of the most used methods in breeding studies is the Principal Component Analysis (PCA). It is a statistical method to evaluate multivariate data. It transforms a series of raw data in a dataset of variables with a similar dimension, called principal components (Varella, 2008). It aims to analyse these groups of data inter-related, allowing the reduction of its size and facilitate the analysis (Regazzi, 2001; Vicini, 2005).

This study aimed to analyse the genetic diversity among 433 accessions plus checks, of barley to support the formation of a working collection and to select genotypes for its use in breeding programs of irrigated barley in the Brazilian savannah.

## Results and discussion

### *Morpho agronomic characterization*

The eleven quantitative traits collected from the 435 genetic materials demonstrated highly significant differences ( $p \leq 0.01$ ), except the HEIGHT, which was significant at a 5% level ( $p \leq 0.05$ ), based on the results verified with the F test (Table 1). This result revealed the existence of genetic variability among the accessions, with the possibility of genetic gains for the evaluated characteristics.

The highest grain yield (YIELD) was obtained by CI 10022 (9108.3 kg ha<sup>-1</sup>), which performed well above the average of the genotypes, checks and the national average yield of 3145.0 kg ha<sup>-1</sup> (Companhia Nacional de Abastecimento, 2011; FAOSTAT, 2012). High values were acquired with the exotic genetic material of barley in other studies (Amabile et al., 2007), presenting grain yield up to 9700.0 kg ha<sup>-1</sup> for Mexican genotypes. In this study, 10 most productive accessions, including six are from Colombia, showed that similarities between environments are valid (Leistrumaite et al., 2010). Not more than 5% of the genotypes showed productivity lower than 1000.0 kg ha<sup>-1</sup>. The lowest grain yield was observed (233.3 kg ha<sup>-1</sup>) in an Epitope and six-row accession (CI 14146) (Table 1).

For the first commercial classification (CLASS1), 28 six-row Colombian accessions were placed among the best performing genotypes. However, the greater value (95%) was reached by the Marroquin CI 09959, which also exhibited the lowest second commercial classification (3%). In irrigated barley in savannah, the grain classification should be above 80% (Amabile et al., 2011). From the tested materials, 16% were within the ideal range (Table 1). On average, the first commercial classification was 56.7%, with about one-third of accessions below 50%. The access CI 09459 revealed only 2% falling into the first commercial grains category, a figure that never achieved in the Brazilian savannah (Amabile et al., 2011). With 20% of its grains in the second commercial class, its inability to produce malt was demonstrated. The highest second commercial class (CLASS2) was 60%, achieved by CI 11622 BALKAN 1942-922 (Table 1).

The thousand weight seed (TWS) varied between 21.0 g (CI 09459) and 65.0 g (CI 12577), with an average of 38.7 g, below the average of the checks (39.2 g). The studied accessions reported minimum, average and maximum values of 37.0 cm (PI 371464 2110B and CI 12997 SEKITORIKII), 6.74 cm (CI 06450 OZIERY) for spike length (SL) and 14.4 (CI 07100), 46.7 and 76.8 (CI 13655 PURPLE NUDUM B24) for number of grains by spike (NGS), respectively (Table 1). The amplitude among the maximum and minimum values of spike length (8.9 cm) was observed and the number of grains per spike (62.4) evidenced genetic variability, reflected by the phenotype, at the working collection (Setotaw et al., 2010). For NGS, this variation can be connected to the number of tillers (Windes et al., 2011), but not necessarily the increase in the number of grains by spike will affect the yield (Simmons et al., 1981). The height of plants (HEIGHT), ranged from 40 cm (CI 07100, CI 15536 IV 363 SIGNAL and CI

02082 WHITE SMYRNA) to 95 cm (MCU 3461 PI 401939, CI 12585, MCU 3469 PI 401947 and CI 07210 ABYSSINIAN 24), with an average of 72.2 cm (Table 1). The improvement of barley accessions for the savannah seeks low-sized cultivars, highly productive and quality of grains, prioritizing appropriate plant structure, thicker stems and near internodes (Sakamoto and Matsuoka, 2004; Amabile et al., 2011). Among the evaluated genotypes, few of them do not present those characteristics (14%), proving the enormous potential of the accessions to compose the cross block.

Lodging (LOD) is an essential trait to be evaluated in barley, since it is directly related to quality and yield and may cause losses to the farmer (Amabile et al., 2011; Manjunatha et al., 2007). In this context, approximately half of the genotypes demonstrated straight, although the trait was not included in pre-selection. This information, combined with other genetic parameters, such as heritability, can be translated into quantitative gains, when incorporated into the genetic improvement (Mohammadi and Prasanna, 2003).

The character days to heading (DH) pointed the accession CI 10389 originated from Ethiopia as the earlier one with 38 days, contrasting with 95 days for the late genotypes CI 14146 and CI 10670 with Ethiopian and Iranian origin, respectively (Table 1). Negassa (1985), Demissie and Bjornstad (1996), Allemayehu and Labuschagne (2004) and Setotaw et al. (2010) highlighted the great genetic variability present in Ethiopian lineages. In irrigated barley in savannah, the objective is to achieve shorter cycles of production ensuring the full physiological process of the plants (Amabile et al., 2011). More than half of the genotypes fitted within the ideal value pre-established for barley.

The flag leaf area (FLA) varied between 1.72 cm<sup>2</sup> (CI 10075 YORK) to 30.54 cm<sup>2</sup> (CI 07210 ABYSSINIAN 24) (Table 1). The average presented by the genotypes (9.29 cm<sup>2</sup>) was higher than the checks (7.90 cm<sup>2</sup>), showing the diversity of the accessions, a positive aspect in the working collection, once this feature is related to the yield (Thorne, 1965; Tungland et al., 1987) and the carbohydrate synthesis of cereals (Sicher, 1993).

The protein content (PROT) ranged from 10.68% (CI 13015) to 24.14% (CI 14146) (Table 1). Grains with low protein content are crucial to malt quality and consequently to beer production (BRASIL, 1996; Moral et al., 1998; Brewing and Malting Barley Research Institute, 2011). In this experiment, approximately 6% of the accessions reported values below the recommended rate, showing effects of climatic variables, such as temperature peaks and low air humidity, which is common during the winter in the savannah at Distrito Federal, as conditioning of more protein content (Chapman and Carter, 1976; Correll et al., 1994; Passarella et al., 2005). A high percentage of protein in barley grains is a characteristic present in varieties for feeding. Therefore, it can be inferred that the accession CI 14146 meets this purpose (Leistrumaite et al., 2010). Thus, barley with high protein can be used in malt blends, becoming appropriate for its use in breweries.

Six-rowed barley predominated in all locations, most in 68% of the evaluated origins. Two-row barley grains originated from Austria (100%), Belgium (100%), Brazil (88%), Czech Republic (100%), Denmark (75%), England (100%), France (100%), Germany (70%), Romania (100%), Slovenia (100%), Sweden (83%) and Switzerland (60%). The greater proportion of irregular spikes was occurred for the accessions originated from Denmark (25%), followed by Ethiopia (7%) and the USA (2%). Most of the accessions had

cream-colored grains. Genotypes with black grains are described in Argentina, China, Hungary, England, Iran, Iraq and Ethiopia, being this last one, the only to exhibit red grains. As the adherence of palea and lemma to the grain, we observed hulled grains from only 11 countries, while the largest proportion of them occurred among Brazilian genotypes (75%).

The Colombian genetic materials had the best yield, with 57% of the 76 accessions reporting higher results than 4500 kg ha<sup>-1</sup>, with exception of the low first commercial class. They presented excellent values for other agronomic traits, such height (78%), lodging (74%), days to heading (95%) and protein content (63%). The Ethiopian accessions described large variability, with genetic materials figuring in all phenotypic classes, prevailing the great variability of this place, considered as one of the centres of origin of barley (Poehlman, 1959; Setotaw et al., 2010).

### **Genetic dissimilarity**

The genetic distances among all pairs of accessions ranged from 0.025 to 0.572, with an average of 0.256. The smallest distance was obtained between the accessions CI 15356 JEBALI 1234-170 and CI 15335 JEBALI 4138-105 (six-row barley, with palea and lemma attached to the grain, cream-colored, originated from Tunisia) while the CI 10114 CARLSBERG II (two-row, cream colour, covered and from Denmark) and PI 356466 E 362 (hulled, six-row, black, Ethiopian) formed the most divergent pair.

Eighteen distinct groups were formed. The group I was the most numerous, constituted by 291 accessions, while the groups XIV, XV, XVI, XVII, and XVIII had only one accession each, including CI 07100, CI 07291 KRASNOYARSKI 74, CI 09459, CI 13704 and CI 13655 PURPLE NUDUM B24, respectively. The intergroup distances varied from 0.13 (group VI) to 0.22. The group VII stood out as the one with the largest divergences. Though the intergroup distance, more dissimilarities were found among the groups XVI and XVII (0.49) and VII and XVII (0.48), with lower values in the groups IX and XV (0.24) and I and IX (0.24). Amongst the most divergent ones, the most distances were observed among the accession CI 13704 and CI 09940 (0.56803), CI 09459 (0.49172), PI 370799 179A (0.51497), CI 07650 (0.53279), CI 12453 KAMAMUGI (0.53975) and CI 09976 (0.52515), evidencing the diversity.

The first group was formed only by accessions with cream colour, six-rowed, except the CI 15278 JEBALI 1206-7, CI 15281 3102-16 and CI 15280 2728-25, with two-row and six-row regardless of their origin, showing great divergence between accessions from the same origin. 291 accessions (66.9%), including the check BRS 180, were originated from all continents. The genetic materials that formed the group I presented low coefficient of variation (CV) for TSW, SL, HEIGHT, DH and PROT, varying from 25.5 to 54.0 g for the first, 4.0 to 9.6 cm for the second, 40 to 95 cm for HEIGHT, 44 to 78 days for the fourth and 10.69 to 20.68% for the last. The averages for HEIGHT and DH can be compared to the figures obtained from other studies (Amabile et al., 2011), being those 72.3 cm and 58.7 days, respectively. The grain yield of the first group (3785.4 kg ha<sup>-1</sup>) was higher than the national average (3100.0 kg ha<sup>-1</sup>) (Companhia Nacional de Abastecimento, 2011; FAOSTAT, 2012). In addition, the genotype of six-row most productive (CI 10022) and lower-sized (CI 15536 IV 363 UC SIGNAL) were found in this group, allowing its use in future hybridizations in the barley

breeding programs under irrigation in the Brazilian savannah. The group also had the presence of the check BRS 180, revealing common points among accessions to be used in breeding programs.

The group II is the second bigger group, with 46 genotypes from Iran, USA, Austria, Sweden, Australia, Canada, Yugoslavia, Germany, Belgium, Switzerland, Denmark, France, Czech Republic, Serbia, Hungary, Japan, Slovenia, and Brazil. The two-row genotypes and cream-colour stand out for irrigated conditions of savannah as well as satisfactory average height (Amabile et al., 2011). A low CV is observed for TWS, SL, NGS, HEIGHT, DH, and PROT. The best results in two-rowed barley for the evaluated parameters are included in this group, with grain yield of 6441.7 kg ha<sup>-1</sup> (PI 371091 1026B), 94% of the first-class grains (PI 370899 532C), height of 40 cm (CI 02082 WHITE SMYRNA), heading in 51 days (CI 11407) and protein content of 13.3% (CI 15039). The check BRS 195 also fall into this group.

The group III reunited 22 genotypes, with 20 of them from Ethiopia, approximately 30% of all Ethiopian accessions. A variability regarding grain colour can be observed since was possible to find cream, black and red grains in this group. The lowest variation coefficients were described in CLASS2, TSW, SL, HEIGHT, DH, and PROT. The earliest six-rowed accession harvest in the experiment (CI 10389) was allocated to this group.

The fourth group presented the latest accession (CI 10670), with average values of grain yield (2116.3 kg ha<sup>-1</sup>) lower than the national average (Companhia Nacional de Abastecimento, 2011; FAOSTAT, 2012). Besides, the average values for CLASS1 (18.1%), DH (67.7 days) and PROT (17.1%) are undesired for barley (Amabile et al., 2011; BRASIL, 1996; Brewing and Malting Barley Research Institute, 2011). Altogether, the group contains the less favourable accessions for barley genetic improvement for the savannah. They are originated from Iran, USA, India, Turkey, Russia, China, Canada, Greece, Yugoslavia, Germany, Sweden, South Korea, Hungary and Mongolia, 21 of them had six-row grains and one two-rowed (CI 06779), all cream-coloured.

The accessions of group V, on average presented, better grain yield (4647.6 kg ha<sup>-1</sup>), commercial classification (88.1%) and heading (55.9 days), besides the favourable height to growing (66.4 cm) and protein content suitable for its use in breweries (13.2%) (Amabile et al., 2011; BRASIL, 1996; Brewing and Malting Barley Research Institute, 2011). The group was composed of seven six-rowed accessions with cream colour grains, originated from Iran, India, Colombia, Morocco, Italy, and Afghanistan, whereas one of them stand out as the one with the highest grain classification among all others in the experiment (CI 09959).

The groups VI and VII included the hull-less barley, being two-rowed and cream coloured in group VI and six-row and cream and black coloured in group VII (CI 09977, PI 356466 E 362/2 and PI 356474 E 363/3), both presenting height within the desired range, and protein content above 13% (Amabile et al., 2011; BRASIL, 1996; Brewing and Malting Barley Research Institute, 2011). However, their grain yield was below the national average. In the seventh group, the accessions presented comparatively higher average yield. However, the heading average (59.7 days) was satisfactory and did not present lodging.

The eighth group was composed of six Ethiopian accessions, cream-coloured, with four of them six-rows and two irregular ones. The group present grain yields higher than the national average (3406.9 kg ha<sup>-1</sup>) (Companhia Nacional

de Abastecimento, 2011; FAOSTAT, 2012) and the accession with the lowest protein content among all others - accession 73, with 10.68%. However, this group does not exhibit any advantages regarding the genetic improvement for the irrigated system, once most of them showed a late cycle, lodging sharp and high protein content.

The genotypes from group IX are originated from Turkey, South Africa, and Poland. Even with satisfactory protein content and plant height in the studied environment (Amabile et al., 2011; BRASIL, 1996; Brewing and Malting Barley Research Institute, 2011), only one out of the five accessions demonstrated low lodging (CI 06450 OZIERY). On the other hand, even with high lodging, the accessions, except the CI 11264 (1050.0 kg ha<sup>-1</sup>) recorded a grain yield superior to the national and world averages (3471.1 kg ha<sup>-1</sup>). The groups X and XI are composed of three genetic materials each, with irregular rows in the first one and six-row in the second. Besides the difference between the numbers of rows, the grain colour was also distinct, being cream in the tenth and black in the eleventh one. In addition, the group X demonstrated higher yield than the others on average, with 4500.0 kg ha<sup>-1</sup>. The barley from this group comes from Ethiopia, the USA and Denmark, and the group XI, Iran, Hungary and Iraq. On both, the height, the heading, and protein content are within the desirable range for cultivation in the savannah (Amabile et al., 2011; BRASIL, 1996; Brewing and Malting Barley Research Institute, 2011).

The groups XII and XIII have two accessions each. In the first one, there are hull-less grains, two-row, cream grains and originated from Ethiopia and Poland, and lodged, even with the height near the desirable (73.0 cm) (Amabile et al., 2011). The second one presents six-row, cream grains, and Ethiopian and Canadian origins. The protein content is high, the low productivity and late cycle make this group the less recommendable to use in cross blocks aiming superior lineages in the savannah.

The projection of distances in the plot allowed observing the dispersion of accessions, regarding their qualitative characteristics, from clustering using the Tocher method and the agronomic traits for the savannah environment.

From the visual inspection of Figure 1, we concluded that the optimization method from Tocher was coherent to set apart the groups. Since there is graphic proximity among the accessing in each group, the reliability of the obtained data ensured and corroborates the genetic divergence that exists in the working collection enhancing the potential for its use in breeding programs.

The graphic containing the geographic origins dispersion (Figure 2), points proximity between some genotypes with the same origin, standing out the Ethiopian, Tunisian, Colombian and Brazilian accessions. The distribution of some accessions with the same origin occurred in a dispersive way, evidencing the divergence for centres of origin (Cross, 1994). Most of the accessions did not group based on their geographic origin and that can be explained by the agronomic traits used to estimate the genetic dissimilarities, complex variables and genic expression, with environmental effect (Ceccarelli et al., 2007). These results demonstrated that crosses among most divergent accessions can provide an increase in the variability, and possibly obtaining superior individuals.

In Figure 1, there is a formation of two distinct groups regarding the number of rows in the spike. Most of the accessions (six-row) are in the middle of the graphic, while in the superior left side groups the two-row genotypes. In

addition, the irregular grains are included among the six and two-rowed grains. Therefore, it was considered because they demonstrated spike of two and six rows in the same genotypes (Gymer, 1978). Only three irregular genetic materials, from Tunisia (CI 15278 JEBALI 1206-7, CI 15281 3102-6 and CI 15280 2728-25), were not that way and can be found at the inferior part of the geographic regions.

Concerning to the colour of the grains, there is a distribution of black and cream all over the graphical dispersion. However, the red ones present themselves near to each other (Figure 2). The hull-less barley can be found in the superior part of the graphical region, showing there is a substantial difference between them and the ones with the palea and lemma attached.

Considering that the multivariate analysis allows predicting heterosis, several crosses can be suggested based on the principle of crossing the most distant accessions and with better agronomic traits (Sudré et al., 2005). In this context, the selection index based on the distance from the ideotype helps to recommend genotypes that are more promising, and dissimilar and better for hybridizations with greater possibilities to obtain superior genotypes with heterotic potential. Five genetic materials were more similar to the ideotype 1<sup>o</sup> MCU 3502 PI 401980, 2<sup>o</sup> CI 09962, 3<sup>o</sup> CI 15560 QB 136-4-1, 4<sup>o</sup> CI 10256 and 5<sup>o</sup> MCU 3870 PI 402348, where the first one can be found in group V and the others at the first group. The presence of the check BRS 180 as the 14<sup>o</sup> cultivar nearest to the ideotype indicated efficiency in the selection (Amabile et al., 2011). Therefore, the use of genetic diversity studied in this experiment is essential to maintain and increase the competitiveness of the agribusiness of barley in Brazil.

The analysis of the matrix of genetic distances of the 30 accessions closest to the ideotype allows, predicting which cross can provide a greater chance of success to obtain superior varieties, based on the heterotic potential and achieved complementarity from the hybridization of the most divergent genotypes. Thus, the most divergent pair is composed by the accessions MCU 3502 PI 401980 and CI 02082 WHITE SMYRNA, belonging to the groups V and II respectively, followed by GALOVER (C A N 1126) PI 361636 x MCU 3700 PI 402178 and MCU 3502 PI 401980 (groups X, I and V, respectively), CI 02082 WHITE SMYRNA x CI 1591 QB 139-1 and MCU 3469 PI 401947 (groups II, I and I, respectively). The most similar pairs were CI 15560 QB 136-4-1 and CI 15580 QB 136-41, both Canadian and in the first group, MCU 3852 PI 402330 and MCU 3478 PI 401956, MCU 3484 PI 401962 and MCU 3452 PI 401930, all Colombian from the group I.

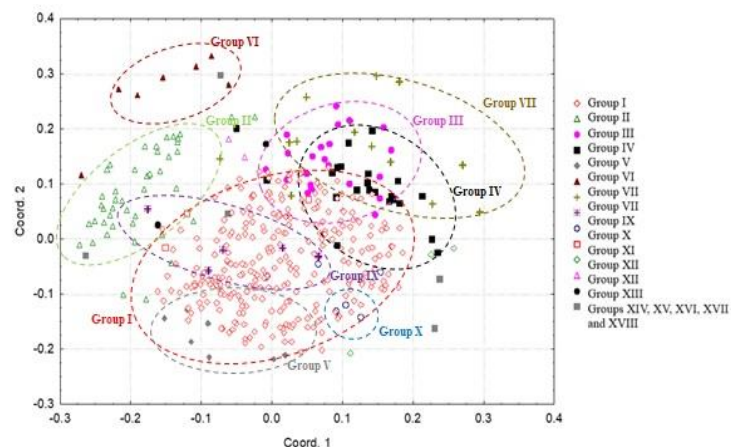
### **Principal Component Analysis (PCA)**

The PCA results for the variables evaluated in this study are shown in Figure 3. It demonstrates that the first two principal components are responsible for 35.7% of the total variability. By carefully observing the eigenvalues (Table 2), from the first component, (associated to the x axis) (21%), the variables CLASS2 (0.783), DH (0.635) and PROT (0.561) presented the highest contributions and are positively associated while CLASS1 (-0.831), YIELD (-0.616), TSW (-0.520) and NAKED (-0.328) are negatively associated, being inversely proportional to the positive ones. For the second component (in the y axis) with a smaller contribution (14.7%), the variables TSW (0.454), PROT (0.490), CLASS1

**Table 1.** Mean square (QM) of genotypes (g), check (t) and error (e), F value for genotypes and checks, minimum and maximum values, genotypes and checks average for each evaluated parameter for the 435 barley accessions. Embrapa Cerrados, Planaltina, DF.

Parameters	Characters <sup>(1)</sup>											
	YIELD	CLASS1	CLASS2	TSW	SL	NGS	HEIGHT	LOD <sup>(2)</sup>	DH	FLA	PROT	
QM	g	2,275,806.93	490.05	173.82	35.47	2.18	168.53	91.08	0.00	53.31	26.37	4.51
	t	34,848,076.30	653.33	197.63	95.05	2.41	11,952.05	1,032.53	0.00	1,009.20	248.03	161.33
	e	442,276.18	132.10	40.92	6.36	0.68	25.96	50.62	0.00	1.15	1.25	1.04
F	g	5.14**	3.71**	4.25**	5.58**	3.20**	6.49**	1.80*	29.92**	46.26**	21.09**	4.32**
	t	78.79**	4.95*	4.83*	14.95**	3.53 <sup>NS</sup>	460.46**	20.40**	2.15 <sup>NS</sup>	875.75**	198.44**	154.68**
Min		233.3	2.00	3.00	21.00	3.70	14.40	40.00	0.00	38.00	1.72	10.68
Max		9108.3	95.00	60.00	65.00	12.6	76.80	95.00	0.10	95.00	30.54	24.14
Average	g	3,527.42	56.68	32.04	38.70	6.74	46.68	72.24	0.04	60.46	9.29	15.84
	t	3,986.12	75.00	19.57	39.21	7.90	43.84	69.00	0.00	63.47	7.90	14.45

<sup>1</sup> Estimated grain yield (YIELD). First commercial classification (CLASS1) and second (CLASS2). Thousand seed weight (TSW). Spike length at harvesting stage (SL). Number of grains per spike (NGS). Plant height (HEIGHT). Lodging degree (LOD). Days to heading (DH). Flag leaf area (FLA). Protein content (PROT). <sup>2</sup> Transformed data in  $\arcsin x^{0.5} \cdot 100^{-1}$ , where x = the value, in %, of lodging. \*\*, Significant at 1%, \* significant at 5% of probability by F test and NS not significant.

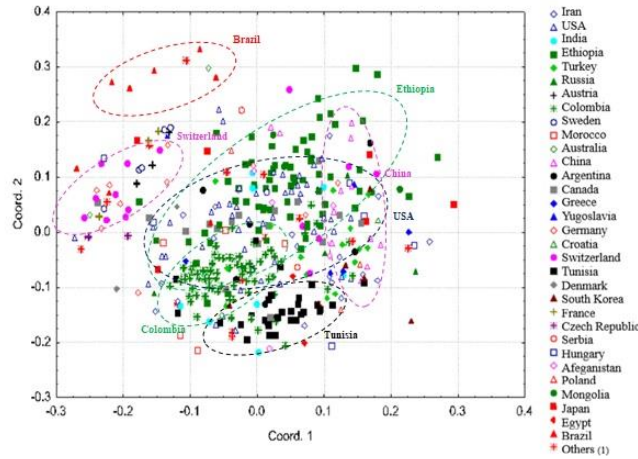


**Fig 1.** Graphical dispersion of the 435 barley accessions based on the genetic dissimilarities using the Gower index for 11 qualitative and four categorical morpho-agronomic descriptors, from multidimensional scales using the principal coordinates method. The legends were obtained based on the groups by the Tocher method.

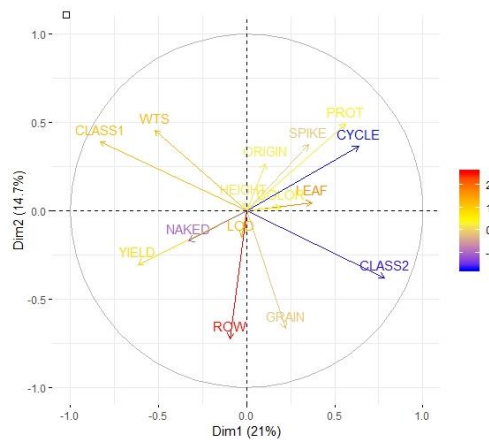
**Table 2.** Eigenvalues corresponding to the percentage of variance ( $\lambda$ ) and the cumulative variance ( $\lambda_j$ ) from the principal components and respective correlations and eigenvalues of the 15 evaluated parameters for the 435 barley accessions.

	$\lambda$ (%)	$\lambda_j$ (%)	YIELD	CLASS1	CLASS2	TSW	SL	NGS	HEIGHT	LOD	DH	LEAF	PROT	ROW	COLOR	ORIGIN	NAKED
Dim 1	21.0	21.0	-0.616	-0.831	0.783	-0.520	0.352	0.220	-0.013	-0.028	0.635	0.370	0.561	-0.094	0.195	0.106	-0.328
Dim 2	14.0	35.7	-0.303	0.389	-0.379	0.454	0.374	-0.665	0.052	-0.150	0.364	0.046	0.490	-0.725	0.022	0.266	-0.170
Dim 3	12.4	48.1	0.091	0.199	-0.117	0.360	0.295	0.296	0.619	-0.032	0.234	0.603	0.174	0.281	0.376	-0.564	0.358
Dim 4	8.9	57.1	-0.471	0.085	-0.048	0.369	-0.479	-0.092	-0.471	0.452	0.220	0.015	0.221	0.413	0.136	-0.086	0.129
Dim 5	7.4	64.6	0.281	-0.122	0.100	0.154	-0.161	-0.245	0.090	0.194	-0.338	0.260	0.007	0.031	0.617	0.199	-0.507

<sup>1</sup> Estimated grain yield (YIELD). First commercial classification (CLASS1) and second (CLASS2). Thousand seed weight (TSW). Spike length at harvesting stage (SL). Number of grains per spike (GRAIN). Plant height (HEIGHT). Lodging degree (LOD). Days to heading (CYCLE). Flag leaf area (LEAF). Protein content (PROT).



**Fig 2.** Graphical dispersion of the 435 barley accessions based on the genetic dissimilarities using the Gower index for 11 qualitative and four categorical morpho agronomic descriptors, from multidimensional scales using the principal coordinates method. Legends were obtained considering the barley origin. Bosnia and Herzegovina, South Africa, Belgium, Macedonia, Italy, Romania, England, Cyprus, Slovenia, Iraq, Argelia, Mexico, Portugal, Bulgaria and Peru.



**Fig 3.** Principal Component Analysis (PCA) of the 11 quantitative and four categorical descriptors of 435 barley accessions. Estimated grain yield (YIELD), First commercial classification (CLASS1) and second (CLASS2), Thousand seed weight (TSW), Spike length at harvesting stage (SL), Number of grains per spike (GRAIN), Plant height (HEIGHT), Lodging degree (LOD), Days to heading (CYCLE), Flag leaf area (LEAF), Protein content (PROT).

(0.398), DH (0.364), SL (0.374) were the ones with higher positive association. YIELD (-0.303), CLASS 2 (-0.379), ROW (-0.725) and NGS (-0.665) are shown with negative results and with greater influence on the second component.

Observing the results for the first component, the negative association between CLASS2 against YIELD and TSW is natural, because the smaller grains in accession presents, the lower yield and thousand seed weight. The same is expected taking into consideration DH and CLASS1. Barley accessions with smaller cycle have less time to fill grains, implying that smaller grains will be obtained from those plants. For the second component, the same discussion must be taken in place. Six rows barley allows less space for kernel development; thus, TSW will be lower depending on the number of rows. Also, CLASS1 are bigger in two rows, when compared to six rows. Nevertheless, six row barley carries more NGS per head.

## Materials and methods

### Plant material and experiment conduction

The present study evaluated a total of 435 barley accessions, two-row and six-row, aiming the malting industry and animal

feed, hull-less barley, cream, red and black coloured, originated from 47 distinct countries, from a world working collection maintained at Embrapa Recursos Genéticos e Biotecnologia, including the cultivars BRS 180 and BRS 195 used as checks. The experiment was carried out at Embrapa Cerrados Planaltina, DF, situated at 15°35'30" South and 47°42'30" West, at an altitude of 1.007 meters. The soil was classified as Rhodic Hapludox, and the results of chemical analysis indicated 0.7 mmol<sub>c</sub> dm<sup>-3</sup> of Al; 32.2 mmol<sub>c</sub> dm<sup>-3</sup> of Ca; 6.8 mmol<sub>c</sub> dm<sup>-3</sup> of Mg; 41.9 mg kg<sup>-1</sup> of P; 6.2 mmol<sub>c</sub> dm<sup>-3</sup> of K; 24.7 g kg of M.O and pH<sub>(water)</sub> of 5.55; coarse sand = 60 g kg<sup>-1</sup>; fine sand = 380 g kg<sup>-1</sup>; silt = 130 g kg<sup>-1</sup> and clay = 430 g kg<sup>-1</sup>. According to the classification of Köppen, the region of the Savannah presents a tropical seasonal climate (Aw) (Nimer, 1989). The weather data, during the field trials were: minimum, average and maximum air temperature of 14.9 °C, 21.7 °C and 28.9 °C, respectively; minimum, average and maximum air humidity corresponding to 28.4%, 52.3% and 74.8%; airspeed of 2.2 m s<sup>-1</sup>, 462.2 cal cm<sup>-2</sup>/day of solar radiation and 43.2 mm of rain. The harvesting was conducted between 28 September and 17 October of 2016, according to the physiologic maturation of the accessions, under irrigation through sprinklers.

### Experimental design and morpho-agronomic characteristics

The chosen experimental design was to intercalate the checks, where the genotypes were evaluated in single parcels, using the checks as replicates, to support the estimates of environmental variation. The randomized block was considered to make the analysis, with each check repeated 15 times during the experiment, in which evaluations were made on an average basis or of the total of parcels (Cruz, 2007).

The plot had 4 lines spaced 20 cm apart and 1.5 meters long, with a useful area of 1.2 m<sup>2</sup> for each spot and density of 300 plants by m<sup>-2</sup>. The residue from the previous crop (soybean) was incorporated in the soil, using 32" disk plough, followed by a 20" leveling disk. The weed control was made by the application of the herbicide Pendimethalin [N-(1-ethyl propyl)-2, 6-dinitro-3, 4-xylydine] in a pre-emergency situation at a dose of 3.0 L ha<sup>-1</sup>. Also, based on the results from the soil analysis, we applied 14.0 kg ha<sup>-1</sup> of N; 105.0 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>; 56 kg ha<sup>-1</sup> of K<sub>2</sub>O; and 50.0 kg ha<sup>-1</sup> of N in the planting furrow, when the fifth leaf was expanded.

The following quantitative traits of the main stem were evaluated: 1. YIELD – estimated grain yield (kg ha<sup>-1</sup>); 2. CLASS1 – first commercial classification (%); 3. CLASS2 – second commercial classification (%) (BRASIL, 1996); 4. TSW – thousand seed weight (g) (BRASIL, 2009); 5. SL – spike length at harvesting stage (cm); 6. NGS – number of grains by spike; 7. HEIGHT – plant height (cm); 8. LOD – lodging degree (%); 9. DH – days to heading (50% of the spikes, visible in 50% of the plot area); 10. FLA – flag leaf area (cm<sup>2</sup>), taken from the plant during the linear phase of grain filling, where the area was determined by the ImageJ software (Rasband, 2006); and 11. PROT – protein content (%), through the colorimetric method (Oliveira, 1981). The following features were also evaluated to estimate the diversity: 12. NRS – number of rows per spike; 13. HULL – palea, and lemma attached or not in the grain; 14. ORIG – origin from the genetic material, based on passport data (National Genetic Resources Program, 2012); and 15. COLOUR – grain colour (visual classification of the palea and lemma). The accessions were separated into three different colour categories: cream, black and red.

### Statistical analysis

For each character, an analysis of variance was performed using the following statistic model (Cruz, 2007): For checks:  $Y_{ij} = \mu + T_i + \varepsilon_{ij}$ , where  $Y_{ij}$  = valued obtained related to the  $i$ th check in the  $j$ th block;  $\mu$  = general average of the check;  $T_i$  = effect of  $i$ th check ( $i = 1, 2, \dots, t$ );  $\varepsilon_{ij}$  = random error (uncontrolled factors) which focuses on checks,  $\varepsilon_{ij} \sim \text{NID}(0, \sigma^2)$ . For the genotypes:  $y_i = \mu_g + G_i + \varepsilon_i$ , where:  $y_i$  = value obtained related to the  $i$ th genotype characteristic in the  $j$ th block;  $\mu_g$  = general average of genotypes;  $G_i$  = effect of  $i$ th genotype;  $\varepsilon_i$  = random error (uncontrolled factors) which focuses on genotypes,  $\varepsilon_i \sim \text{NID}(0, \sigma^2)$ .

To estimate the genetic dissimilarity among all pairs of accessions a similarity matrix was calculated using the Gower coefficient (1971), due to the presence of different variables (quantitative and qualitative), using the MULTIV software (Pillar, 2004). The dissimilarity matrix ( $d$ ) was estimated based on the similarity matrix ( $s$ ) using  $d_{ij} = 1 - s_{ij}$ . According to the dissimilarity matrix, a cluster analysis was performed using the Tocher criteria, obtained with the

GENES program (Cruz, 2007). This method applies to a great number of lineages, making easier the interpretation of the differences (Miranda et al., 1999). A scatter plot was made based on the dissimilarity matrix, from multidimensional scales using the principal coordinate analysis, with the support of the SAS program (SAS Institute Inc., 1989) and STATISTICA (Statsoft Inc., 1999).

Regarding the recommendation of hybrids based on genetic distance, an index was used in relation to the ideotype (ideal genotype proposed by breeders) from the best behaviours obtained for each parameter studied. The ideotype was defined based on the higher results for YIELD, CLASS1, CLASS2, TSW, SL, NGS, LOD and FLA, while smaller to CLASS2, HEIGHT, and DH; and proximity of the ideotype for PROT. In addition, the similarity distances were estimated using the Gower coefficient (1971), based on the data of the 435 genotypes and the ideotype using the software MULTIV (Pillar, 2004). The best accessions were those, which revealed lower distances in relation to the ideotype. A principal component analysis was performed to evaluate which traits may present greater contribution for the diversity among the evaluated genotypes. The coefficients were obtained through the eigenvalues from the matrix of correlation between the 15 the variables analysed in this study, standardized in a way which the variables were dimensionless. The analysis was performed using the statistical program R (v. 3.5.1)

### Conclusion

There is great genetic diversity among the accessions, enhanced in the 18 diverse groups, regardless of their origins. The genotypes CI 09952, CI 09960, CI 09961, CI 09962, CI 10256, CI 11493 FRUGHERSTE STANKAS, CI 13626 ARIMAR, MCU 3478 PI 401956, MCU 3484 PI 401962, MCU 3556 PI 402034, MCU 3592 PI 402070, MCU 3654 PI 402132, MCU 3719 PI 402197, MCU 3816 PI 402294, MCU 3827 PI 402305, MCU 3852 PI 402330 and MCU 3865 PI 402343 showed agronomic characteristics of interest, such productivity above 4000.0 kg ha<sup>-1</sup>, first commercial classification higher or equal to 80%, height lower or equal to 80 cm, lodging resistance, early heading up to 60 days and protein content below 15%. Selected genotypes by dissimilarity can be inter-crossed to obtain segregated populations, generating superior lineages for the savannah. The crossing between MCU 3502 PI 401980 and CI 02082 WHITE SMYRNA may possibility have bigger heterotic effect and complementarity amongst accessions equivalent to the ideotype. The application of the method of Tocher is efficient to group in blocks with a big number of accessions, supporting the multivariate analysis. The barley accessions were selected from a world working collection based on the amount of seed available. Thus, there was great diversity among accessions. To explain the variation, based on the principal component analysis, at least five dimensions would be necessary, implicating that none of the characters can be discarded for breeding purposes. Nevertheless, the rich diversity observed represented the beginning of a research to narrow the number of accessions and start a core collection for barley under irrigated conditions in Brazilian savanna, which was the aim of this study.

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