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CROP SCIENCE

Genetic variability in Brazilian castor (*Ricinus communis*) germplasm assessed by morphoagronomic traits and gray mold reaction

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Abstract: The characterization and conservation of castor accessions in germplasm bank are essential in order to breeding programs achieve its goals. Despite Brazil having the 4th largest castor germplasm bank in the world, castor diversity in Brazil remains little explored. Thus, this study aimed at characterize castor accessions collected in different Brazilian regions by means of 31 morphoagronomic traits and gray mold reaction. Forty accessions of the Universidade do Estado de São Paulo (UNESP), Botucatu, SP, Brazil, germplasm bank were evaluated. Genetic parameters were estimated for the quantitative traits, and the accessions were grouped by Ward method using the standardized Euclidean distance and the simple coincidence index for quantitative and qualitative data, respectively. Qualitative and quantitative traits were important to understand and differentiate castor accessions. The accessions showed a high variation regarding the castor gray mold reaction. The accessions assessed in this study have been preserved and can be used as a source for genetic variability in the development of new castor varieties in breeding programs.

Key words: Genetic improvement, genetic variability, qualitative descriptors, quantitative descriptors, REML/BLUP.

INTRODUCTION

Castor (*Ricinus communis* L.) is an important oleaginous from Eastern Africa and currently is cultivated in different tropical and subtropical regions around the world (Allan et al. 2008, Severino et al. 2012). Castor seeds have from 40 to 50% of oil content, which is the only commercial source of ricinoleic acid, which is used in high-quality lubricants, cosmetics, pharmaceutical products and polymers (Suhail et al. 2015, Venegas-Calerón et al. 2016). Although India has been responsible for more than 80% of the world production of castor seeds, the crop also plays an important role in Brazil, which

produces around 25,000 metric tons per year (FAO 2016).

In Brazil, castor crop is especially important for family smallholders, mainly in the northeast region (Florin et al. 2012). As the national industry of castor has been significantly suffering from the decrease of raw material in the past decades, the Brazilian government launched a program of incentive in order to promote the castor production in other regions (Ribeiro & Raiher 2013). Due to this fact, Brazilian breeding programs start to focus in the development of new dwarf, short cycle and highly productive castor varieties and hybrids with potential to be used in the Brazilian cerrado (Severino et al. 2012).

Studies of genetic diversity characterization are very important for the conservation and utilization of these genetic resources in breeding programs (Saadaoui et al. 2017). Different morphoagronomic traits and molecular markers have been widely used for characterization of the castor germplasm (Wang et al. 2016, Simões et al. 2017, Silva et al. 2017, Rukhsar et al. 2018). However, there are few data about the reactions of these accessions to *Botryotinia ricini* (Godfrey) Whetzel, which is the causal agent of the castor gray mold, the most import castor disease (Dange et al. 2005, Sussel et al. 2009, Soares 2012).

The castor gray mold can cause yield losses up to 100% when highly susceptible cultivars are growing under favorable conditions to the disease development, even though, there are only few studies about castor gray mold management and, so far, there is no effective ways to control this disease (Sussel et al. 2009, Soares 2012, Sá et al. 2015, Oliveira Datovo & Soares 2019). Attempts to identify sources of resistance to this pathogen have been made since the first reported castor gray mold outbreak, and some works published in the early 20th century, have claimed, based on field observation, that "spontaneous varieties" or wild genotypes are highly resistant to the pathogen, however, such claims were never corroborated by further studies (Soares 2012).

Although partial resistance to castor gray mold had been reported in some castor cultivars and hybrids (Anjani et al. 2018), only few studies have been conducted aiming to develop resistant genotypes, coupled with the agronomic traits required by the intensive agricultural systems, such as Brazilian cerrado (Severino et al. 2012, Soares 2012). The main reason for this is the lack of reliable genetic resistance source for castor gray mold (Soares 2012, Soares et al. 2010). Thus, the identification of genetic resistance sources

is crucial for castor crop expansion through Brazilian cerrado.

The present study had the objective to characterize castor accessions, collected in different regions of Brazil, by several morphoagronomic traits, as well as verify the reaction of these accessions to castor gray mold.

MATERIALS AND METHODS

Plant material

Forty wild accessions of castor which has been maintained in the germplasm bank of the Universidade do Estado de São Paulo (UNESP), Botucatu, Brazil, were evaluated. The accessions were collected on three different States (São Paulo, Minas Gerais and Rio Grande do Norte) of the Southeast and Northeast Brazil, comprising 23 different municipalities, during expeditions carried out in 2015 (Figure 1).

Morphological characterization

The research was conducted in greenhouse conditions in the Department of Production and Genetic Improvement of Universidade do Estado de São Paulo (UNESP), School of Agriculture, Botucatu, SP, Brazil (22°50′59.0″S and 48°25′55.6″W and altitude of 786 m) in 2016. The experiments were performed using a completely randomized design with three repetitions. The plots were constituted by one plant per hole, with a spacing of 0.5 m between plants and 1.0 m between rows. Weed control, hydric and mineral supplementation, insecticide and fungicide applications were performed according to the plants need in order to keep satisfactory levels of plant health.

The accessions characterization was done using 32 descriptors (Savy Filho et al. 1999, MAPA 2008, Milani 2008): 23 qualitative and nine quantitative traits. The quantitative traits evaluated were: plant height (cm), primary

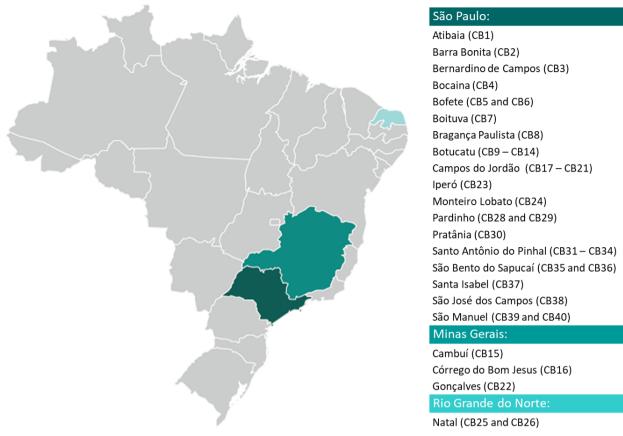


Figure 1. Identification and origin of the collection of the 40 accessions of castor (*Ricinus communis* L.) characterized by morphoagronomic descriptors and castor gray mold reaction.

raceme insertion height (cm), stem diameter (cm), internodes number, commercial raceme number, primary raceme length (cm), 100-seed weight (g), spores number of *Botryotinia ricini*, and seed oil content (%) using a bench-top NMR spectrometer model SLK-100 (SpinLock, Cordoba, Argentina), determined by nuclear magnetic resonance in time domain using ~15 g of seeds of each treatment were employed. The determination of the oil content was performed using the calibration curve for castor oil.

The qualitative traits were: anthocyanin pigmentation on the hypocotyl, stem waxy, stem color, face format of the limb, leaf vein pigmentation, face waxy limb, upper face color of the limb, stigma color, presence of male flowers on the raceme, predominance of male flowers on the raceme, raceme density, raceme format,

capsule waxy, capsule color, presence of spines on the capsule, spines density, spines color, capsule dehiscence, main seed color, secondary seed color, type of secondary color, seed format and caruncle protuberance.

Reaction to castor gray mold

The reaction of castor accessions to castor gray mold was verified using the Soares methodology (Soares et al. 2010), with modifications. Green capsules, between stages V and VII (Greenwood & Bewley 1982), were collected, conducted to the laboratory facilities, washed in running water, and then kept during 30 seconds in alcohol 70% followed by 30 seconds in sodium hypochlorite 0.5%. After that, the capsules were washed with sterilized distilled water and dried in room temperature (25±2°C) for 2 hours. After dried, the

capsules were sprayed with a manual atomizer driven by compressed air pump calibrated for a pressure of 1.5 bar with *B. ricini* spores suspension adjusted for 2×10⁵ spores.ml⁻¹. After inoculation, the capsules were placed in acrylic boxes, which were sealed with plastic film and maintained in growth chamber at 25±1 °C with a photoperiod of 12 h for 7 days. The boxes had previously been sterilized using sodium hypochlorite 0.5%, left to dry, and then lined with a double layer of sterilized wet filter paper and a polyethylene mesh used to avoid direct contact of the capsules with the wet paper. The experiment was performed using a complete randomized block design with four replicates, each replicate consisting of a box with four capsules. After the incubation period, the four capsules of each replicate were vigorously shaken in 100 mL of alcohol 50% to remove the spores. The obtained suspensions were then filtered in double-layered cheesecloth to remove the mycelial mat. The number of spores per mL, of each suspension, was determined using a Neubauer chamber by means of two independent readings. In order to standardize the spore readings due to the difference in capsule size, the number of spore were divide by the average capsule volume of each accession.

Data analysis

The quantitative traits were analyzed by restricted maximum likelihood (REML) and best linear unbiased prediction (BLUP) methods using the software Selegen-REML/BLUP (Resende 2016). The predicted genotypic values were calculated after verification of normality and homogeneity of data by Shapiro & Wilk (1965) and Hartley (1950) tests, respectively. The data that did not present the assumptions of normality and homogeneity where changed by Box-Cox transformation (Box & Cox 1964). The

deviance analysis was performed considering the following statistic model:

$$y = Xb + Za + e$$

where: *y* is the data vector, *a* is the vector of block effects (assumed as fixed) added with the total average, *b* is the vector of genotypic effects (assumed as aleatory), *e* is the vector of error (aleatory), *X* and *Z* represents the matrices of incidence for *b* and *a*, respectively.

The estimators REML for attainment of phenotypic (σ_p^2) , genotypic (σ_g^2) and environmental (σ_e^2) variance using the algorithm EM (Expectation-Maximization) were:

$$\begin{split} \sigma_e^2 &= \left[y'y - b'X'y - g'Z'y \right] / \left[N - r(X) \right]_; \\ \sigma_g^2 &= \left[g'g + \sigma_e^2 \ tr \ C^{22} \right] / N_{g;} \\ \sigma_v^2 &= \sigma_g^2 + \sigma_e^2 \end{split}$$

where: N s is the number of aleatory elements (individuals), tr is the dot matrix operator (sum of elements of the diagonal matrix), N is the total number of data, $^{r}(^{X})$ is the number of independent linear columns X and, $^{C^{22}}$ is the formula:

$$\begin{bmatrix} C^{11} & C^{12} \\ C^{21} & C^{22} \end{bmatrix} = \begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + A^{-1} (\sigma_e^2/\sigma_a^2) \end{bmatrix}^{-1}$$

Broad-sense heritability (h²) and the selective accuracy of genotypes (AS_g) were calculated in the following way:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

$$AS_g = \left(h^2\right)^{1/2}$$

The coefficient of genotypic (CV_g) and environmental (CV_e) variation was determined, respectively, by the following formulas:

$$CV_g(\%) = \frac{100 \, \sqrt{\sigma_g^2}}{\overline{m}}$$

$$CV_e(\%) = \frac{100 \sqrt{\sigma_e^2}}{\overline{m}}$$

Pearson linear correlation, Principal Components Analysis (PCA) and Singh's relative importance (Singh 1981), analysis were performed using the predicted genotypic values. The estimation of the genetic distance matrix among the accessions for the quantitative traits was performed using the standardized Euclidean distance, while for the qualitative traits it was used the simple coincidence index. The Ward method (Ward 1963), was used in the hierarchical groupings of the accessions for both quantitative and qualitative data. The correlation between the matrices of the quantitative and qualitative data was verified by Mantel test (Mantel 1967), using 2,000 permutations. The optimal number of groups, formed in the dendrograms, was choose using the Milligan and Cooper methods (Milligan & Cooper 1885). Statistical analyses were performed using Genes (Cruz 2013) and R software (R Core Team 2019) through packages 'NbClust' (Charrad et al. 2014), 'dendextend' (Galili 2015), 'corrplot' (Wei et al. 2017) and 'FactoMineR' (Lê et al. 2008).

RESULTS

Qualitative descriptors

Polymorphism was observed for all qualitative traits evaluated, except for anthocyanin pigmentation on the hypocotyl and male flowers on the raceme (Table I). Stem wax was present on 67.5% of the accessions, while 32.5% did not show wax. Stem color varied from light green (10.0%), green (32.5%), pinky green (42.5%), pinky (10.0%), and red (5.0%). Funneled limb was observed on 72.5% of the accessions, while the

other 27.5% were not funneled. The presence of wax on the limb was observed in 97.5% and only 2.5% of the accessions did not showed this trait. Limb color varied from light green (17.5%), green (65.0%) and dark green (17.5%), while the vein colors were greenish (52.5%) and reddish (47.5%). Concerning the inflorescence traits, 10.0% of the accessions showed stigmas with greenish color, 27.5% orange, 37.5% reddish and 25.0% pinky. The male flowers were mainly observed on the lower part of the raceme (95.0%) and only 5.0% of the accessions showed male flowers interspersed with female flowers. The predominant racemes form was conical (60.0%), followed by globose (32.5%) and cylindrical (7.5%), while their densities were predominantly intermediate (42.5%), followed by sparse (37.5%) and compact (20.0%).

Regarding the capsule traits, the presence of wax was observed in 67.5% of the accessions. Color varied from light green (35.0%), to green (35.0%), to dark green (25.0%), and red (5.0%). Capsule dehiscence was observed in 87.5% of the evaluated accessions, while semi-dehiscence and 7.5% indehiscence were observed on 5.0 and 7.5% of the accessions, respectively. The presence of spines on the capsules was observed in 97.5% of the accessions. Spine density varied from low (5.0%) to high (27.5%), being the medium the most observed density (67.5%). Color of spines was light green (40.0%), green (40.0%), dark green (12.5%), pinky (5.0%), and red (2.5%). Concerning the seeds, the accessions showed seeds with rounded (47.5%) or ellipsoid (52.5%) shape. The caruncle protuberance was conspicuous for 87.5% and inconspicuous on 12.5% of the accessions. Seed primary color was white (10.0%), yellow (10.0%), light brown (5.0%), brown (2.5%), dark brown (2.5%), and grayish (70.0%). Striped seeds (97.5%) were most frequently observed, and only 2.5% of the seeds were painted. The secondary colors observed were white (2.5%), yellow (2.5%), light brown (7.5%), brown (47.5%), dark brown (30.0%), reddish brown (7.5%) and black (2.5%).

Table I. Accessions number per group for qualitative morphoagronomic traits in each of the three groups (G1, G2 and G3) formed by the Milligan & Cooper (1985) method from 40 castor (*Ricinus communis* L.) accessions.

	Groups				Groups		
Trait	G1 G2 G3 (12) (18) (10)			Trait	G1 (12)	G2 (18)	G3 (10)
Anthocyanin on hypocotyl				Capsule color			
Presence	12	18	10	Light green	1	12	1
Absence	-	-	-	Green	3	3	8
Male flowers on the raceme				Dark green	7	2	1
Presence	12	18	10	Red	1	1	-
Absence	-	-	-	Spines on capsules			
Stem waxy				Presence	12	17	10
Presence	1	16	10	Absence	-	1	-
Absence	11	2	-	Spines density			
Stem color				Low	-	1	1
Light green	_	4	-	Medium	8	10	8
Green	3	5	5	High	4	6	1
Pinky green	5	7	5	Spines color	<u> </u>		<u> </u>
Pinky	2	2	_	Light green	1	14	1
Reddish	2	_	_	Green	5	2	9
Limb face				Dark green	3	2	
Plain or not funnel-shaped	4	6	1	Pink	2	_	_
Funneled or funnel-shaped	8	12	9	Red	1	_	_
	0	12	9		1	_	_
Leaf vein pigmentation		12	2	Capsule dehiscence	10	15	10
Greenish	6	12	3	Dehiscent	10	15	10
Reddish	6	6	7	Semi-dehiscent	1	1	-
Limb waxy	44	40	40	Indehiscent	1	2	-
Presence	11	18	10	Seed main color			
Absence	1	-	-	White	2	2	-
Foliar lamina color				Yellow	1	2	1
Light green	2	5	-	Light brown	-	2	-
Green	8	12	6	Brown	1	-	-
Dark green	2	1	4	Dark brown	-	1	-
Stigma color				Grayish	8	11	9
Light green	-	1	3	Black	-	-	-
Orange	1	9	1	Seed secondary color			
Reddish	4	7	4	White	-	1	-
Pinky	7	1	2	Yellow	-	-	1
Male flower predominance				Light brown	1	-	2
Lower part predominant	12	16	10	Brown	6	7	6
nterspersed with female flowers	_	2	-	Dark brown	1	10	1
Raceme shape				Reddish brown	3	-	-
Globose	7	4	2	Black	1	-	-
Cylindrical	-	2	1	Secondary color type			
Conical	5	12	7	Painted	-	1	_
Raceme density				Striped	12	17	10
Sparse	6	4	5	Seed shape			
Intermediate	3	13	1	Rounded	7	8	4
Compact	3	1	4	Ellipsoid	5	10	6
Capsule waxy			İ	Caruncle protuberance			
Absence	10	3	-	Inconspicuous	2	1	2
Presence	2	15	10	Conspicuous	10	17	8

Quantitative traits

The predicted genotypic values regarding the nine quantitative traits are presented in Figure 2. The plant height varied from 64.2 (CB12) to 248.7 cm (CB14), while the insertion height from the primary racemes and the length of the raceme varied from 52.2 (CB12) to 185.6 cm (CB13) and 19.1 (CB23) to 56.7 cm (CB14), respectively. Stem diameter varied from 1.63 (CB17) and 3.73 cm (CB14). Regarding the number of internodes and racemes, the genotypic means varied from 13.0 (CB37) a 24.0 (CB40) and 1.63 (CB20) to 10.33 (CB3), respectively. The weight of 100 seeds varied from 10.96 to 41.29 g, considering that accessions CB4, CB30 and CB40 showed the heaviest seeds. The seed oil content varied between 39.18 and

50.83%, considering that accessions CB10, CB30 and CB40 showed the higher percentage. The spores number of *B. ricini* varied from 2.19 to 6.14 spores.mL⁻¹ among the accessions evaluated, considering that accession CB40 was considered the most tolerant to the castor gray mold and the CB10 the most sensible.

Deviance analysis and genetic parameters

The deviance analysis and the genetic parameters obtained are presented in Table II. The deviances were highly significant (P<0.01) for all the traits assessed. The CV_g varied between 6.91 and 58.31% to the seed oil content and number of commercial raceme, respectively. The highest CV_g were observed in the number

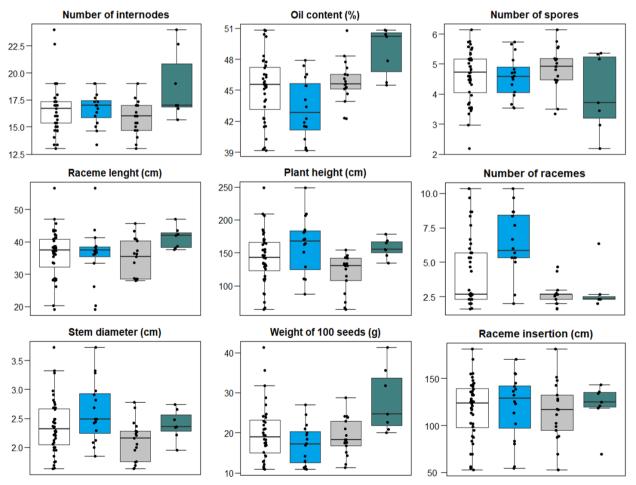


Figure 2. Boxplots of the predicted genotypic values from the nine traits evaluated in 40 accessions from the castor (*Ricinus communis*) grouped in all accessions (white), group 1 (blue), group 2 (gray), and group 3 (green).

of commercial racemes (14.38%) and in spores number of *B. ricini* (12.90%), while the lowest values were verified to the 100-seeds weight (4.99%) and in the seed oil content (4.68%). The h² estimates oscillated between 49.12% and 94.59%. The plant height (94.22%) and the commercial racemes numbers (94.59%) showed the highest h² estimates, while the lowest estimated were observed in the number of spores of *B. ricini* (49.12%) and stem diameter (71.44%). The estimates of AS_g were elevated to all assessed traits varying between 0.70 and 0.97 to the number of spores of *B. ricini* and plant height, respectively.

Correlations and relative importance of traits

The correlation coefficients among the nine quantitative traits are presented in Figure 3. The

plant height presented a positive and significant correlation with the variables: insertion of primary raceme (0.82**) and stem diameter (0.77**). A positive and significant correlation was also observed among traits such as stem diameter with insertion in the primary raceme (0.61*), the number of commercial raceme with stem diameter (0.60*) and seed oil content with the 100-seeds weight (0.64*). By means of the analysis of relative importance of traits of Singh (1981) it can be observed that the variables: number of racemes (16.96%) and seed oil content (15.52%) were the traits the most contributed to the differentiation of the accessions evaluated while the number of internodes (3.53%) and 100-seeds weight (7.42%) showed less importance (Figure 4).

Table II. Deviation analysis, estimate of variance components and genetic parameters of nine quantitative traits in 40 accessions of castor (*Ricinus communis* L.).

Parameters ^{2/}	Morphoagronomic traits ^{1/}											
	NS	PH	IR	SD	NI	NR	LR	WS	ос			
Deviance	145.95*	795.56*	754.55*	238.42*	331.71*	168.97*	656.76*	143.05*	466.71*			
$\sigma_{\rm g}^2$	0.60	1590.95	1020.01	0.21	4.46	6.00	52.14	46.99	9.91			
σ ² _e	0.43	98.76	137.84	0.09	1.54	0.37	10.51	0.15	0.05			
σ_{f}^{2}	1.03	1689.71	1157.85	0.30	6.00	6.37	63.65	47.14	9.96			
h² (%)	49.12	94.22	88.45	71.44	74.12	94.59	83.01	91.82	89.44			
AS_g	0.70	0.97	0.89	0.84	0.86	0.97	0.91	0.95	0.94			
CV _g (%)	16.90	28.19	27.36	19.71	12.74	58.31	19.96	34.92	6.91			
CV _e (%)	12.90	7.02	10.05	12.62	7.48	14.38	8.96	4.99	4.68			
Average	4.58	141.45	116.73	2.34	16.58	4.21	36.16	16.62	45.34			

^{1/}NS: number of spores of *Botryotinia ricini*, PH: plant height, IR: primary raceme insertion, SD: stem diameter, NI: number of internodes, NR: number of commercial racemes, LR: length of primary raceme, WS: 100-seeds weight and OC: seed oil content. $^{2/}\sigma_{g}^{2}$: genotypic variance, σ_{e}^{2} : environmental variance, σ_{f}^{2} : phenotypic variance, h^{2} : broad-sense heritability, AS_g: selective accuracy of genotypes, CV_g: coefficient of genotypic variation and CV_e: coefficient of residual variation.

^{*:} significant by the Chi-square test with a degree of freedom at the 1% probability level.

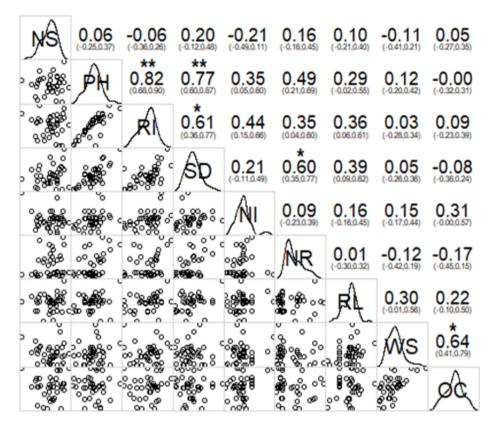


Figure 3. Estimation of the genotypic correlation coefficients with their respective 95% confidence intervals in nine traits evaluated in 40 accessions of castor (Ricinus communis). **NS:** number of spores of Botryotinia ricini, PH: plant height, RI: insertion of primary raceme in the stem, SD: stem diameter, NI: number of internodes, NR: number of commercial racemes, LR: length of primary racemes. WS: 100seed weight and OC: seed oil content. ** and *: significant at 1 and 5% probability levels by the t-test, respectively.

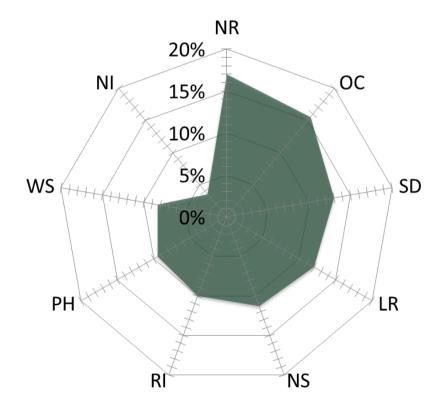


Figure 4. Relative importance by the Singh (1981) method of nine traits evaluated in 40 accessions of castor (Ricinus communis). NS: spores number of Botryotinia ricini, PH: plant height, RI: insertion of the primary racemes into the stem, SD: stem diameter, NI: internodes number, NR: number of commercial racemes, LR: length of primary racemes, WS: 100seed weight and OC: seed oil content.

Principal component analysis (PCA)

The first two principal components (PC) explained 77.03% of the total variation among the nine quantitative traits assessed (Figure 5). The first component (PC1) responded by 55.44% from the variation attributed to the traits such as plant height, number of commercial racemes and internodes number. On the other hand, the second component (PC2) absorbed 21.59% of the total variation, and are associated with seed oil content and the 100-seeds weight. In the two-dimensional graphic of PCA, it can be observed the distinction of 40 accessions of castor in three different groups. Generally, the groups 1 (blue) and 2 (gray) presented the highest and lowest averages, respectively, to the traits of primary raceme insertion, plant height, stem diameter and the number of commercial racemes. The groups 3 (green) presented associations with vectors of the variables seed oil content, 100-seeds weight, internode number and length of primary raceme, presenting the highest averages to those traits.

Grouping analysis

The genetic dissimilarity among the accessions obtained by the simple coincidence index had the average value of 0.38 (±0.08). The lower distance (0.12) was observed among accessions CB19 and CB24, being those accessions collected in the towns of Campos do Jordão and Natal (São Paulo and Rio Grande do Norte States. respectively). The CB13 (Botucatu, São Paulo State) and CB37 (Santa Isabel, São Paulo State) accessions were the more distanced (0.67). Using a dendrogram obtained by the Ward method, it can be observed the formation of three distinct groups (Figure 6a). The group I (green) was constituted by 12 accessions, presenting prevalence of accessions with an absence of waxy coating in the stem and capsules. Eighteen accessions constituted the group II

(gray), presenting in common accessions with a prevalence of light green capsules, plants with an intermediary density of racemes and stigmas with orange and red colors. The group III (blue) was formed by ten accessions that showed waxy coating in the stems and capsules, spines with green coloration and dehiscent capsules.

The genetic dissimilarity obtained by the standard Euclidean distance presented the average value of 0.31 (±0.08). The smaller distance was observed among the accessions CB5 and CB6 (0.04), being both accessions from Bofete city (São Paulo State). On the other hand, the accessions CB14 (Botucatu, São Paulo State) and CB37 (Santa Isabel, São Paulo State) were the most distanced (0.69). By the dendrogram obtained by the Ward method, it can be observed the formation of three distinct groups (Figure 6b). The group I (blue) was constituted by 16 accessions being those characterized for presenting low seed oil content and elevated averages of plant height and number of commercial racemes. Seventeen accessions constituted the groups II (gray), presenting in common with low plant height and number of commercial racemes, and intermediated values to the other traits. The group III (green) was formed by seven that presented a low number of commercial racemes and higher seed oil content, 100-seeds weight, and internodes number.

Comparing the dendrograms obtained by the quantitative and qualitative descriptors (Figure 6), it is verified that there was not concordance among the groups formed in both dendrograms. The discordance of the formed groups can be confirmed by the absence of the correlation in both distances matrices verified by the Mantel test ($r_m = 0.12$; P>0.05).

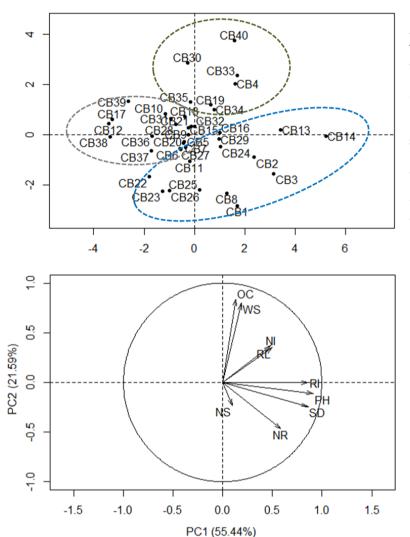


Figure 5. Principal component analysis (PCA) of nine traits assessed in 40 castor accessions (*Ricinus communis*). NS: spores number of *Botryotinia ricini*, PH: plant height, RI: insertion of the primary racemes into the stem, SD: stem diameter, NI: internodes number, NR: number of commercial racemes, LR: length of primary racemes, WS: 100-seed weight and OC: seed oil content.

DISCUSSION

The elevated polymorphism among the qualitative descriptors as well as the significance of deviance analysis indicated a wide genetic variability among the 40 accessions of castor collected in the 23 Brazilian municipalities. The variability observed regarding the different morphoagronomic traits evaluated was already expected and corroborate with previously studies (Silva et al. 2017, Goodarzi et al. 2012, Oliveira et al. 2013, Bezerra Neto et al. 2010, Rukhsar et al. 2017, 2018, Rodrigues et al. 2015). For instance, authors observed values of plant

height between 108.0 and 256.0 cm and racemes length between 13.71 and 44.38 cm (Bezerra Neto et al. 2010). Others reported values from 14.77 to 37.16 g to 100-seeds weight, 32.19 to 50.81% to the seed oil content, 4.17 to 7.33 number of racemes and 12.31 to 20.53 number of internodes in the castor accessions (Rukhsar et al. 2017). Generally, castor breeding programs seek to identify genotypes with high yield, high seed oil content, dwarf plants to facilitate mechanized harvest, beyond resistant to the main diseases, specially *Fusarium* wilt, charcoal rot and castor gray mold (Severino et al. 2012, Rodrigues et al. 2015, Saadaoui et al. 2017, Anjani et al. 2018).

In general, all others traits presented elevated estimates of SA_g and h², except the spores number of *B. ricini* for h². The SA_g it is a parameter of big relevance in the experimental quality assessment, taking into consideration not only the number of repetitions and the environmental quality but also the relation between the genetic and residual variations, being considered the most important parameter in the context of selective assessment (Ribeiro et al. 2017). The h² is a parameter of high importance in breeding programs since it is used to measure the phenotypic variation occurred by a genetic

factor which means that reflects the proportion of phenotypic variance inherited (Falconer & Mackay 1996). Authors studying genetics parameter in castor, reported h² values varying from 8.0 to 97.2%, being these values concordant to the values obtained in the current study for plant height, length of primary racemes, 100-seed weight and oil content (Rukhsar et al. 2018).

Although the castor gray mold is considered the main castor disease in Brazil, information regarding the genetics parameters and the heritage of castor resistance *B. ricini* is very scarce. The presence and the distribution of

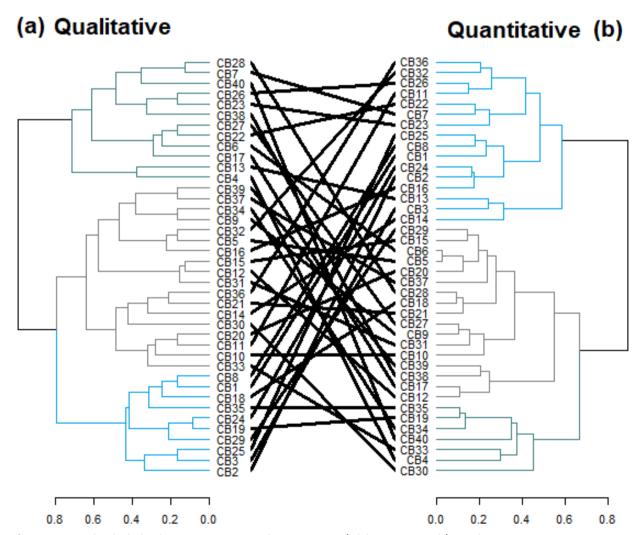


Figure 6. Genetic dissimilarity among 40 accessions of castor (*Ricinus communis*) obtained by the Ward method based on standard Euclidian distance (quantitative) and simple coincidence index (qualitative).

spines influence the disease development and the genotypes with a low number of spines were the most resistant (Lima & Soares 1990). Controversial results were observed in the current study, since accession CB40, herein the most tolerant, showed an average density of spines, on the other hand accession CB10, herein the most susceptible to castor gray mold pathogen, had no spines. The spine density is considered a recessive monogenic characteristic conditioning by the gene s ("spineless"), where a SS plant has high spine density, and Ss has intermediated density (Gurgel 1945). In the presence of S, a not determined number of modifiers genes affect the final number and distribution of spines on capsules. Others morphoagronomic traits can influence the disease development under field conditions, such as plant architecture, raceme compaction, and internodes length (Milani et al. 2005). However, usually in an opposite way to that seeking by breeding programs, i.e., dwarf plants, with compact racemes usually are more susceptible to the pathogen.

The knowledge of the relationship among different traits is of great importance in breeding programs, mainly if the selection for one of those traits is difficult due its low h², measurement takes and/or identification, especially for perennial crops such castor. In the current study, the seed oil content and 100-seed weight traits were positively correlated. In this way, the 100-seed weight can be considered an important trait to indirect selection of genotypes with higher seed oil content, since it is considered a highly inherited trait and of easily measured. This correlation had already been reported by previous studies (Rukhsar et al. 2017, Adeyanju et al. 2010).

Using Singh's relative importance analysis (Singh 1981) it was possible to classify the variables studied according to their respective contribution to the genetic divergences among

the accessions and to identify the traits that contributed the most and the less to differentiate the accessions. In the present study, the number of commercial racemes and seed oil content were the variables that most contributed to the differentiation of the accessions, while the 100-seed weight and the number of internodes were less important. Discordant results were presented by other authors, where 100-seed weight was the variable that most contributed for the differentiation of castor accessions evaluated (Cavalcante et al. 2008).

The first two components of PCA explained more than 75% of the total variation observed, and the main groups formed were in agreement with those obtained for the quantitative traits using the Ward method. Based on the dendrograms obtained by the qualitative and quantitative descriptors, the 40 accessions of castor were allocated in three different groups in each dendrogram. However, it was not possible to observe a relation among the formed groups with the geographic origin of the accessions. The discordance among the formed groups and the accession geographic origin was also reported by others authors, using morphoagronomic traits and single nucleotide polymorphism (SNP), respectively (Arif et al. 2015, Foster et al. 2010).

The absence of correlation among the distance matrices from the qualitative and quantitative traits indicates that both kind of traits should be used for the differentiation among castor accessions. In general, studies showing the genetic diversity of castor germoplasm banks by means of qualitative descriptors are very scarce. On the other hand, was reported the complementarity of molecular and phenotypic markers in the study of castor diversity in India, emphasizing that the use of both kind of markers allowed a more precise

distinction of genetic diversity present among the accessions evaluated (Rukhsar et al. 2017).

CONCLUSION

The present study revealed the presence of high genetic variability among the 32 traits assessed for the 40 castor accessions collected in Brazil. Those accession are been preserved and can be used as genetic variability source for castor breeding programs. The genetic parameters obtained in the present study, confirm that the variability observed was due to genetic factors rather than just environmental influence. The seed oil content and the 100-seed weight showed positive correlation inferring the possibility of indirect selection between these traits. The grouping analysis allocated the accessions in three different groups for the quantitative and qualitative traits. However, there was no concordance among the formed groups, indicating that both kind of traits are important in the accessions differentiation. Additionally, it was also not possible to verify concordance among the formed groups and origin of the accessions.

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SSON was responsible for collecting seeds, setting up experiments, obtaining data and contributed to the writing of the article. DMZ did the statistical analysis and was responsible for writing the article. MMPS and MDZ encouraged the work, contributed to the idea and the project and writing of the article. DJS was responsible for analysis to gray mold reaction and contributed to the writing of the work.

