



Genetic divergence among castor bean lines and parental strains using ward's method based on morpho-agronomic descriptors

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ABSTRACT. This study sought to evaluate the genetic divergence among castor lines and parental strains in the germplasm bank at the Federal University of Recôncavo da Bahia (Universidade Federal do Recôncavo da Bahia) using morpho-agronomic descriptors. This experiment was conducted from 2014 to 2015 with 208 treatments represented by bank accessions that were analyzed using a randomized block design, which established homogeneity of the experimental conditions with all treatments repeated in four blocks. The genetic divergence analysis was performed using Ward's method and Tocher's method based on Gower's distance, which are two clustering methods used in studies with castor beans, with the help of the statistical programs R and GENES. The results revealed the formation of 21 groups for 13 morpho-agronomic descriptors, six qualitative and seven quantitative, indicating the presence of variability in the bank. The groups containing promising accessions for the characteristics of agronomic interest, including precociousness, fruit dehiscence, and plant height and yield were 2, 7, 8, 10, 14, 15, 16, and 17, which suggested potential hybrid crosses. The descriptors with the greatest contribution to variance were yield, plant height, the number of seeds per raceme, flowering and seed weight per raceme.

Keywords: *Ricinus communis* L., multivariate, variability.

Divergência genética entre linhagens e parentais de mamoneira pelo método Ward, baseada em descritores morfoagronômicos

RESUMO. Este trabalho objetivou avaliar a divergência genética entre linhagens e parentais de mamoneira do Banco de germoplasma da Universidade Federal do Recôncavo da Bahia, por meio de descritores morfoagronômicos. O experimento foi conduzido no período de 2014 a 2015, com 208 tratamentos representados pelos acessos do Banco, avaliados no delineamento em blocos casualizados, estabelecendo a homogeneidade das condições experimentais com todos os tratamentos igualmente repetidos em quatro blocos. A análise de divergência genética foi realizada pelo método Ward e pelo o método de Tocher por meio da distância de Gower, sendo esses métodos de agrupamento utilizados em estudos com mamoneira, com o auxílio dos programas estatísticos R e GENES. Os resultados revelam a formação de 21 grupos para 13 descritores morfoagronômicos, sendo seis qualitativos e sete quantitativos, indicando a presença de variabilidade no Banco. Os grupos que apresentam acessos promissores para as características de interesse agrônomo como precocidade, deiscência dos frutos, estatura e produtividade, são 2, 7, 8, 10, 14, 15, 16 e 17, sugerindo possíveis cruzamentos entre estes. Os descritores que mais contribuem para a divergência são produtividade, estatura da planta, número de sementes por racemo, florescimento e peso de sementes por racemo.

Palavras-chave: *Ricinus communis* L., multivariada, variabilidade.

Introduction

Castor oil is the major raw material, with economic value, for the species *Ricinus communis* L. and is used for the production of biodiesel and manufacturing of paint, varnishes, greases, lubricants, foams, plastics and cosmetics (Domingos et al., 2012).

According to 2013 data from the Food and Agriculture Organization (FAO) of the United

Nations, the five largest castor bean producers are India (1,644,000 ton), Mozambique (60,000 ton), China (50,000 ton), Ethiopia (13,000 ton) and Brazil (12,526 ton) (FAOSTAT, 2015). In Brazil, castor beans are mainly grown by farms in the semiarid region because this crop is well adapted to the soil and climate conditions in this region. The Northeast region is the largest producer of castor beans (94.3% of the national production value), particularly the

states of Bahia and Ceará, which each contribute 42.2% of the national production value (IBGE, 2015).

The Experimental Center of Genetic Improvement and Biotechnology (Núcleo de Melhoramento Genético e Biotecnologia-NBIO) of the Center for Agricultural, Environmental and Biological Sciences (Centro de Ciências Agrárias, Ambientais e Biológicas-CCAAB) at the Federal University of Recôncavo da Bahia (Universidade Federal do Recôncavo da Bahia-UFRB) has developed a castor bean genetic breeding program where lines are conserved *in vivo* in the germplasm bank through annual field plantings and seeds stored under refrigeration.

Several studies have demonstrated the variability of the castor bean germplasm, which has allowed it to be used in breeding programs and increased its genetic basis. The morphological characterization of castor bean accessions was reported by Rodrigues et al. (2014), who characterized the genetic variability of 15 castor bean accessions using binary and multicategorical traits. The authors identified three groups containing accessions with an average genetic distance of 0.35, which indicated that homogenous groups were formed. In a study characterizing cultivars specified for planting in Southern Brazil based on physiological, adaptive and flowering traits, Da Silva et al. (2011) reported that cultivar IAC 80 produced more seeds, had a larger harvest index and a shorter harvest duration than the cultivars AL Guarany 2002, CPACT 40, IAC 226, BRS Energia, IAC Guarani, Vinema T1, and IAC 2028.

Castor bean yield was analyzed by Bahia et al. (2008), who reported that of the five genotypes analyzed (BRS 149 Nordestina, BRS 188 Paraguaçu, EBDA MPA 17, Mirante 10, and Sipeal 28) in the Recôncavo Baiano region, Sipeal 28 had the highest performance for nearly all of the production components: raceme weight per plant, fruit weight per raceme, seed weight per raceme, fruit weight per plot, seed weight per plant, seed weight per plot and seed production potential. Sampaio Filho et al. (2011) analyzed four castor bean cultivars (EBDA MPA 17, Sipeal 28, BRS 188 Paraguaçu, and BRS 149 Nordestina) over two crop years in the Recôncavo Baiano region and showed that most of the variability for the descriptors was due to environmental factors stemming from different climatic conditions between the two crop years analyzed, which indicated that the genotypes had the flexibility necessary to adapt to climatic fluctuations.

Using agronomic descriptors, Zorzenoni et al. (2011) analyzed the performance of nine castor bean cultivars in Londrina, Paraná, including two hybrids (Savana and Íris), four varieties (IAC Guarany, IAC 80, IAC 226, and AL Guarany 2002) and three local varieties (Preta, Coti, and Sangue de Boi), and reported that the hybrids were more precocious than the varieties and that the cultivar IAC Guarany had the highest yield. The cultivars with the lowest yields were IAC 80, AL Guarany 2002, and Sangue de Boi.

Thus, the objective of this study was to analyze the genetic variability among the 203 castor bean lines and five parental strains from the UFRB/CCAAB/NBIO gene bank using morpho-agronomic descriptors recommended by the Brazilian Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento-MAPA) and NBIO using Ward's clustering method.

Material and methods

The experiment was conducted from May 2013 to January 2015 at the UFRB/CCAAB/NBIO experimental area, which is located in Cruz das Almas, Bahia State, Brazil. This municipality is located at 12° 40' 39" South and 39° 40' 23" West, at an altitude of 220 m. The mean temperature is 24.5°C, the relative humidity is 82%, and the mean annual rainfall is 1,197 mm. According to the Köppen classification, the climate is type C1 dry and subhumid, transitioning from zone Am to Aw. The soil is classified as Dystrophic Yellow Latosol A, which is moderate, with a sandy-clay-loam texture (EMBRAPA, 1993).

The experiment used a randomized block design, which consisted of 208 treatments of 203 lines and five parental strains, with four replications.

Thirteen morpho-agronomic descriptors were used for the analysis and characterization, including six qualitative descriptors proposed by MAPA (Brasil, 2008) and their corresponding phenotypic classes: PRI – primary raceme insertion height (Low < 50 cm; Medium 51 to 100 cm; High > 100 cm); SD – stem diameter (Thin < 3 cm; Medium 3 to 6 cm; Wide > 5 cm); MIL – mean internode length (Short < 2 cm; Medium 3 to 5 cm; Long > 5 cm); NI – number of internodes (Low < 15; Medium 16 to 18; High > 19); NRH – number of racemes harvested (Low < 3; Medium 4 to 7; High > 7); FD – fruit dehiscence (Dehiscent, Semidehiscent, Indehiscent), and seven quantitative descriptors recommended by NBIO: RL – raceme length (cm); ERL – effective raceme length (cm); SWR – seed weight per raceme

(g); NSR – number of seeds per raceme (units); YIELD - yield (kg ha^{-1}); FLO – flowering (days); and PH – plant height (cm).

The descriptors PRI, SD, MIL, NI, and FLO were analyzed during full flowering of the primary raceme, whereas the descriptors NRH, PH and FD were observed when the racemes were mature to the point of harvest. The other quantitative descriptors were analyzed at the NBIO processing area.

To analyze the genetic diversity among the lines and parental strains, multivariate clustering analysis using Tocher's optimization and Ward's hierarchical clustering was conducted using the Gower genetic dissimilarity distance for the qualitative and quantitative traits simultaneously. Next, a dendrogram was constructed, and the cophenetic correlation coefficient (CCC) was calculated to validate the clustering. These analyses were performed using the "Cluster" and "Rcmdr" packages for the R program (R Core Team, 2014). The dendrogram was cut using the pseudo t_2 index in the "NbClust" package for R (Charrad et al., 2014). In addition, the Singh's contribution of each quantitative descriptor to the genetic diversity was

calculated using the average Euclidean distance. These analyses were performed using the GENES program (Cruz, 2014).

Results and discussion

The Ward's method indicated that the breeding program, which seeks to develop novel cultivars for the species, is yielding satisfactory results. As shown in the dendrogram for the castor bean plants in the UFRB/CCAAB/NBIO germplasm bank (Figure 1), there were several distinct accessions, which indicated that the parental strains with the traits of interest can be selected from the analyzed population, increasing their ability to be used in hybrid crosses to exploit heterosis. In the Tocher's optimization method, the 203 lines and five parental strains of castor beans were divided into 21 groups (Table 1), which indicated that there is variability in the germplasm bank. This may be due to the crosses between the five parental strains used to generate the lines.

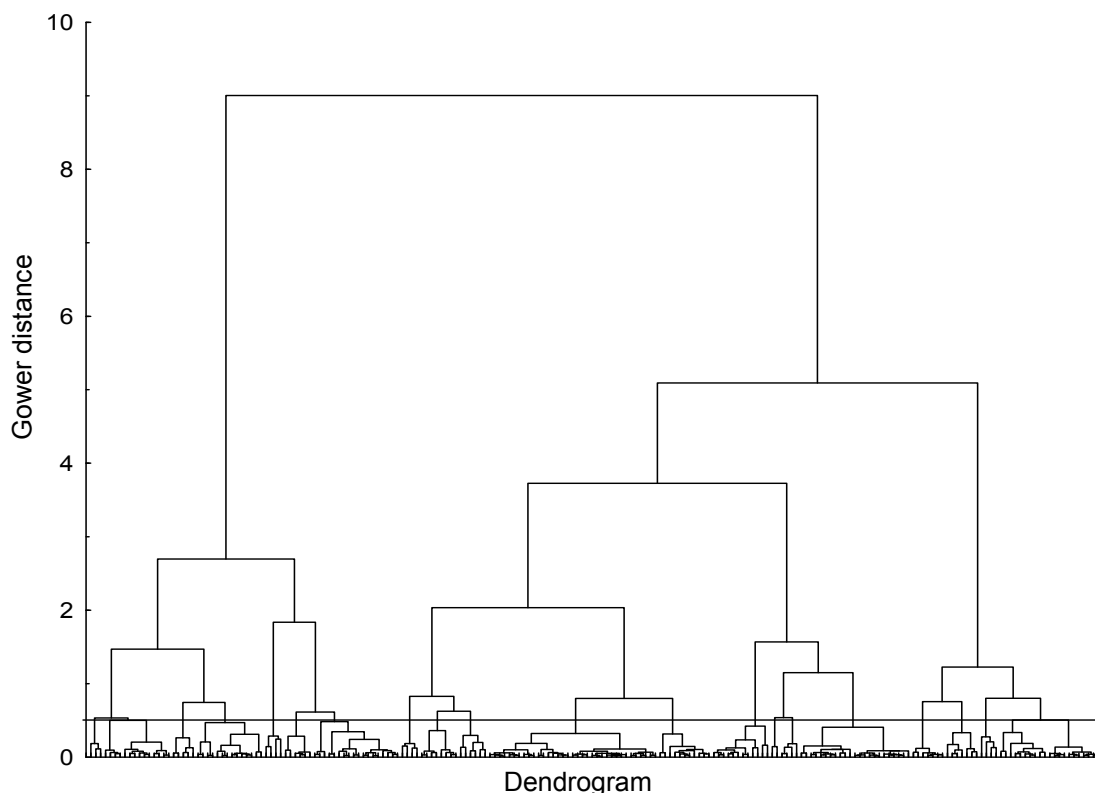


Figure 1. Graphical representation of the genetic divergence among the 203 lines and five parental strains of castor beans in the UFRB/CCAAB/NBIO germplasm bank based on the morpho-agronomic descriptors generated using Ward's clustering method and the Gower genetic dissimilarity distance. Cruz das Almas, Bahia, 2014-2015.

Table 1. Clustering of the 203 lines and five parental strains of castor beans from the UFRB/CCAAB/NBIO germplasm bank using the Tocher optimization method based on the morpho-agronomic descriptors. Cruz das Almas, Bahia, 2014-2015.

Groups	Lines and Parental Strains	Number of accessions	Mean intragroup dissimilarity
1	42 90 254 144 145 69 227 29 83	93	0.139
	170 183 165 121 179 181 19 57		
	43 176 95 51 192 34 47 6 35		
	109 61 160 232 159 139 173 13		
	213 45 4 71 60 147 70 73 175		
	17 NORDESTINA 180 197 119		
	15 40 138 177 223 174 1 178		
	14 80 195 66 77 PARAGUAÇU		
	184 151 193 128 224 129 63 149		
	9 264 114 3 236 23 53 28 113		
2	18 187 124 135 262 258 191 44	33	0.1463
	87 56 136 48 244 16		
	74 214 239 11 MPA/EBDA 228		
	252 206 241 208 86 255 117		
	259 84 182 101 32 256 96 33		
3	171 SIPEAL 220 245 97 78 237	22	0.1577
	263 72 62 94 240		
	126 238 108 231 230 148 211		
4	137 242 222 217 257 125 216	16	0.1488
	201 209 46 7 2 146 234 25		
5	39 249 133 38 112 55 140 67	10	0.0979
6	141 202 152 250 52 186 65 131	5	0.1622
7	188 204 132 116 205 93 91 190	4	0.1468
8	81 115	4	0.1638
9	189 229 153 203 199	2	0.1027
10	246 247 54 MIRANTE	2	0.1139
11	31 79 154 219	4	0.154
12	10 185	2	0.1431
13	82 89	2	0.1437
14	123 212 130 169	2	0.1582
15	198 253	1	0.0000
16	41 102	1	0.0000
17	5 265	1	0.0000
18	134	1	0.0000
19	248	1	0.0000
20	122	1	0.0000
21	36	1	0.0000
	235	1	0.0000
	24	1	0.0000
	251	1	0.0000

Castor bean lines in the UFRB/CCAAB/NBIO germplasm bank numbered 1 to 265 and the parental strains PAR (BRS 188 Paraguaçu), NOR (BRS 149 Nordestina), MIR (Mirante 10), MPA (EBDA MPA - 17) and SIP (Sipeal 28).

The intragroup distances were small (Table 1), which indicated that the lines and parental strains from the same group are genetically similar. However, the lines and the parental strains that were placed in different groups are divergent; therefore, these can be used in future breeding studies to develop novel cultivars.

Thus, the Ward (Figure 1) and Tocher optimization (Table 1) methods were similarly able to discriminate between the castor bean accessions in the germplasm bank. However, the Ward's method established larger subgroups.

In the Tocher method, group 1, with 93 lines, contained the largest number of germplasm accessions, indicating that 44.71% of the germplasm bank castor beans were placed in this group. However, groups 15, 16, 17, 18, 19, 20, and 21 contained only one line each, indicating that these lines are more divergent than the others (Table 1).

The groups exhibited a wide distribution for the descriptors primary raceme insertion height,

mean internode length, number of internodes, number of racemes harvested and fruit dehiscence (Table 2). Pivetta et al. (2015) explained that these descriptors are correlated with each other and with precociousness because insertion of the primary raceme begins at the reproductive stage, appearing after the primary stem grows and new leaves and internodes sprout, and is affected by the environment. Thus, one of the objectives of the breeding program is to generate lines with lower primary raceme insertion height, fewer internodes and reduced number of vegetative sinks.

To allow for mechanical harvesting, castor bean plants with thin stems are ideal because they facilitate processing (Soratto et al., 2011). This trait was widely distributed in all groups, except for group 12, which consisted of two lines that did not have any qualitative traits of interest. For the number of racemes harvested, the groups that contained a large number of lines with high numbers of racemes, with productive potential, were 1, 2, 3, 4, 6, 7, 8, 10, 14, 15, and 19 (Table 2).

The indehiscent lines were placed in groups 1, 2, 3, 5, 7, 8, 9, 10, 13, 15 and 17 (Table 2). According to Savy Filho et al. (2007), castor bean cultivars with uniformly ripening and indehiscent fruits reduce seed loss because the fruit does not open easily.

Table 2. Descriptors and number of lines per qualitative trait class in each of the 21 groups formed using the Ward method from the 203 lines and five parental strains of castor beans in the UFRB/CCAAB/NBIO germplasm bank. Cruz das Almas, Bahia, 2014-2015.

Descriptors	Groups																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
PRI																						
Low	3	33					4		2	2			2			1			1	1	1	
Medium	90	22	16	10	5	4	2		2			2	1			1	1					
High											2											
SD																						
Thin	92	33	22	16	9	4	4	2	2	4		2	2	1	1	1	1	1	1	1	1	
Medium	1			1	5						2											
Thick																						
MIL																						
Short	1								2		2									1	1	
Medium	91	32	21	16	10	5	4	4	2	4	1					1	1	1	1			
Long	2	1									1	2	1									
NI																						
Low	2	33	15				4		2	2					1	1		1	1	1	1	
Medium	68	4	8	1	4				2	2	2							1				
High	23	3	8	10	4			2			2			1							1	
NRH																						
Low	1		1	10								2							1		1	
Medium	78	20	5	2	1	2	2	4	2						1	1				1		
High	14	13	22	10	3	3	2	2				2	1							1		
FD																						
Dehiscent	3	7	2	16			11			4									1			
Semidehiscent	88	25	18	9	4	1	1		2	1	2		1	1					1	1	1	
Indehiscent	2	1	2	1	3	3	2	1				1	1	1								

PRI: primary raceme insertion height (Low: < 50 cm; Medium: 51 to 100 cm; High: > 100 cm); SD: stem diameter (Thin: < 3 cm; Medium: 3 to 6 cm; Thick: > 6 cm); MIL: mean internode length (Short: < 2 cm; Medium: 3 to 5 cm; Long: > 5 cm); NI: number of internodes (Low: < 15; Medium: 16 to 18; High: > 7); NRH: number of racemes harvested (Low: < 3; Medium: 4 to 7; High: > 7) and FD: fruit dehiscence (Dehiscent; Semidehiscent; Indehiscent).

Groups 11, 13, 19, 20, and 21 had lower mean values for total length, effective raceme length, seed weight per raceme, number of seeds per raceme and plant height and showed low performance (Table 3).

In contrast, the group with the largest mean total length and effective raceme length was group 12. The accessions with the largest values for seed weight per raceme, number of seeds per raceme and yield were placed in groups 8, 14, 15, 16, and 17, which therefore made them promising germplasms for the breeding program. In addition to these groups, other groups showed strong productive potential because they produced more than the national average for castor beans in August 2015, which was 737 kg ha⁻¹ (IBGE, 2015). These groups were 3, 4, 6, 7, 9, 10, and 12. Oliveira et al. (2013) measured yields of 596.81 to 823.26 kg ha⁻¹ in castor bean genotypes analyzed in Cruz das Almas, Bahia State. Bezerra Neto et al.

(2010), who analyzed two hybrids and nine cultivars of castor beans in Goytacazes, Rio de Janeiro State, found yields of 450 to 1617.5 kg ha⁻¹.

Table 3. Mean for the quantitative descriptors for each of the 21 groups formed by analyzing the 203 lines and five parental strains of castor beans in the UFRB/CCAAB/NBIO germplasm bank. Cruz das Almas, Bahia, 2014-2015.

Groups	Descriptors						
	RL	ERL	SWR	NSR	YIELD	FLO	PH
1	17.52	10.59	36.30	66	663.82	88	123.44
2	16.81	10.84	39.63	66	720.67	69	113.69
3	20.29	12.80	48.17	82	937.01	76	138.57
4	20.03	11.13	38.36	75	742.61	89	142.51
5	20.74	13.16	37.39	74	516.60	109	131.95
6	21.21	11.07	40.79	81	830.97	88	156.15
7	21.78	13.94	50.50	89	991.41	69	133.52
8	22.96	16.85	61.92	111	1100.73	83	153.55
9	17.00	9.60	36.90	64	770.08	97	138.63
10	18.69	9.75	41.23	61	782.99	50	116.75
11	12.34	6.74	18.26	34	308.89	89	101.33
12	27.22	17.60	46.44	90	986.29	99	189.08
13	11.76	6.07	15.91	32	254.48	101	65.00
14	22.95	15.57	64.98	97	1404.83	68	179.63
15	20.05	16.00	46.32	91	1013.33	94	159.50
16	24.30	17.83	66.18	104	1270.67	68	111.50
17	25.88	15.68	44.19	127	889.67	74	130.00
18	12.00	7.83	11.12	44	214.67	116	66.00
19	16.35	6.91	23.74	42	395.37	62	124.00
20	9.40	4.73	9.40	17	163.00	98	82.00
21	6.25	3.60	3.88	12	34.50	52	45.00

RL: raceme length (cm); ERL: effective raceme length (cm); SWR: seed weight per raceme (g); NSR: number of seeds per raceme (units); YIELD: yield (kg ha⁻¹); FLO: flowering (days); and PH: plant height (cm).

Early flowering is desired to reduce production losses. The lowest means were found in groups 10 and 21 at 50 and 52 days, respectively. Measurements obtained by Oliveira et al. (2013) were between 89 and 92 days for an F3 population consisting of 259 castor bean genotypes in Recôncavo da Bahia. Lara, Zanotto, and Okita (2012) reported means ranging from 66 to 105 days for eight castor bean lines in the state of São Paulo. However, lower means were observed by Bahia et al. (2008), who observed flowering at 42 to 53 days for five castor bean cultivars analyzed in Recôncavo da Bahia.

Castor bean plants with short plant heights is a desired trait because it allows the plants to be grown in a crop rotation system with narrow plant spacing, which increases the plant population size (Soratto et al., 2011). We found that 57% of the clusters (1, 2, 3, 4, 5, 7, 9, 10, 11, 16, 17, and 19) had mean plant heights between 100 and 150 cm, making them short plants according to the MAPA classification criteria (Brasil, 2008). Sampaio Filho et al. (2011) analyzed four castor bean cultivars over two crop years in Recôncavo da Bahia and observed plant heights between 92 and 233 cm.

Groups 2, 7, and 10 contained accessions with more than four desired classes for the qualitative descriptors, and groups 8, 14, 15, 16, and 17 had

the best values for the quantitative descriptors. This result suggests that crosses between the accessions in these groups may be beneficial for hybrid generation because these accessions may represent divergent parental strains with complimentary characteristics.

The CCC from the Ward method was 0.60, which indicated a moderate fit of the clustering analysis to the Gower distance dissimilarity matrix.

Based on the Singh's contribution, the quantitative descriptors that has the highest contribution to genetic divergence between the lines and the parental strains were yield (97.96%), plant height (0.90%), number of seeds per raceme (0.56%), flowering (0.32%) and seed weight per raceme (0.22%). Of note was the descriptor yield, which had the largest effect on the discrimination. This may be because this descriptor is affected by several other descriptors. Furtado et al. (2014), in their study on yield and correlations in intercropped castor bean, found a significant positive correlation between bean yield and raceme length, number of fruits per raceme, and number of racemes per plant. Oliveira et al. (2013) analyzed the genetic diversity of a segregating population of castor beans in Recôncavo da Bahia and reported that the most important descriptors were fruit weight per plant, bunch weight per plant, yield, bunch weight and number of seeds per plant.

Conclusion

The lines and parental strains from the UFRB/CCAAB/NBIO castor bean germplasm bank possessed genetic variability for the morpho-agronomic descriptors analyzed.

There are promising genetic combinations in the germplasm bank, which have the genetic potential to be used in a breeding program that seeks to develop novel castor bean cultivars.

The descriptors with the highest contribution to the genetic diversity were yield, plant height, number of seeds per raceme, flowering and seed weight per raceme.

The Ward clustering method efficiently detected genetic diversity and effectively grouped the lines using qualitative and quantitative morpho-agronomic descriptors simultaneously.

Acknowledgements

We thank Petrobrás Biocombustível and the National Petroleum, Natural Gas and Biofuels Agency (*Agência Nacional do Petróleo, Gás Natural e Biocombustíveis-ANP*), the Federal University of Recôncavo da Bahia (*Universidade Federal do*

Recôncavo da Bahia-UFRB) and the Coordination for the Improvement of Higher Education Personnel (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES*) for their support.

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Received on July 1, 2016.

Accepted on November 3, 2016.

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