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Genetic divergence in elite castor bean lineages based on TRAP markers

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ABSTRACT. Castor bean (*Ricinus communis* L.) is a tropical plant of great commercial interest and a potential source of biodiesel. The development of genetically improved cultivars with high amounts of oil in the seeds and low ricin toxicity is crucial to increase the productivity of this crop. The use of TRAP (target region amplification polymorphism) markers to develop elite lineages and study genetic divergence is fundamental to advance the genetic improvement of this species. The goal of this study was to evaluate the genetic divergence among 40 elite lineages of *R. communis*, which belong to the NBIO-UFRB Genetic Improvement Program, using TRAP markers involved in the biosynthesis of oil and ricin. Total DNA was extracted and quantified from the leaf tissue of the castor bean plants, and 70 TRAP combinations (fixed and arbitrary primers) were used to genotype the 40 lineages. Of the 580 fragments amplified, 335 were polymorphic (58%). The genetic dissimilarity among the lineages was calculated by the Jaccard dissimilarity index using the UPGMA grouping method. A dendrogram was generated, and four groups formed, showing divergence among the elite lineages that favors selection. The TRAP

molecular markers were efficient at characterizing the genetic variability among the lineages and, because TRAP markers are functional markers involved in the biosynthesis of oil and ricin, they are important when studying the association between a marker and a candidate gene.

Key words: Oil biosynthesis; Ricin; *Ricinus communis* L.

INTRODUCTION

Castor bean (*Ricinus communis* L., 2n = 2x = 20, Euphorbiaceae), an important non-edible oil seed, is predominantly cultivated in arid and semi-arid regions of the world and is used by industrial and agricultural industries.

The seeds of castor bean have around 35-55% oil, and the commercial standard is 44%. The oil percentage of the seeds varies depending on the cultivation environment and the cultivar (Costa and Ramos, 2004). The hydroxylated fatty acid ricinoleic is approximately 80-90% of the total fatty acids, which gives castor bean oil (ricin oil) unique chemical and physical properties. Ricin oil is a renewable resource and raw material with various industrial applications (e.g., to manufacture paints, lubricants, cosmetics, pharmaceutical drugs, dyes, anilines, disinfectants, germicides, low-temperature lubricating oils, glues and adhesives, fungicide and insecticide bases, printing inks and varnishes, nylon and plastic), and more recently its use as a biodiesel has been explored (Mutlu and Meier, 2010).

Castor bean seeds have a high concentration of ricin protein. This protein is extremely toxic and considered one of the most lethal poisons if used intravenously or ingested. Ricin inhibits protein synthesis by interacting with ribosomes (Chan et al., 2010), and castor bean cake contains this toxic protein (Audi et al., 2005), which limits its use as animal food.

With the goals of increasing productivity and the amount of oil in the seeds (main product), as well as reducing ricin toxicity, it is necessary to have programs that genetically improve castor bean and develop elite lineages that meet the needs of the market and farmers. Now that some of these lineages have been developed, there is a need to study the genetic divergence among them. The genetic divergence among genotypes of any species can be evaluated with molecular markers, for example, target region amplification polymorphism (TRAP) markers.

TRAP markers are functional markers that allow combining fixed and specific primers with arbitrary primers (Hu and Vick, 2003). These markers have high levels of polymorphism, which makes them a promising option for the genotypification of germplasm and identification of genes related to desirable agronomic characteristics. Besides, TRAP markers optimize the genetic gains in genetic improvement programs and are a valuable tool used by these programs to study genetic divergence (Agarwal et al., 2008).

Different molecular markers have been used to estimate the degree of genetic divergence among genotypes of castor bean, for example, AFLP and simple sequence repeats (SSR) (Pecina-Quintero et al., 2013; Machado and Silva, 2013; Tan et al., 2013; Gálová et al., 2015; Machado et al., 2016), and RAPD and inter-simple sequence repeats (ISSR) (Silva et al., 2012; Machado et al., 2013; Tomar et al., 2014; Lakhani et al., 2015; Vivodík et al., 2015; Goodarzi et al., 2015; Kallamadi et al., 2015). However, functional TRAP markers have never been used to evaluate the genetic divergence in castor bean.

The integrity and efficiency of TRAP markers linked to disease resistance have been

recorded in other crops, such as the common bean (Miklas et al., 2006). These markers have also been used to study the following: genetic maps of wheat (Liu et al., 2005), germplasm in spinach (Hu et al., 2007), and sugarcane (Suman et al., 2012); polymorphisms in cassava (Carmo et al., 2015); and the design and optimization of castor bean (Simões et al., 2017).

The elite lineages used in this study were obtained from crossing parents (BRS Nordestina, Sipeal, EBDA MPA-17, Mirante, and Paraguaçu) developed by the genetic improvement program at the Núcleo de Melhoramento Genético e Biotecnologia in the Universidade Federal do Recôncavo da Bahia (NBIO-UFRB), in the city of Cruz das Almas, Bahia, Brazil. The improvement method used was single seed descent (SSD) until the advanced F_6 generation, when the plants were highly homozygous. This method allowed for individual selection of plants from the advanced population, with the formation of elite lineages.

The objective of the present study was to evaluate the genetic divergence among 40 elite lineages of *R. communis* L., which belong to the NBIO-UFRB genetic improvement program, using TRAP markers involved in the biosynthesis of oil and ricin.

MATERIAL AND METHODS

Plant material

Forty elite lineages of castor bean developed by the NBIO-UFRB genetic improvement program in Cruz das Almas, Bahia, Brazil, were used. The lineages were obtained by crossing the parents BRS Nordestina, Sipeal, EBDA MPA-17, Mirante, and Paraguaçu.

The municipality of Cruz das Almas is located at 12°40'19''S, 39°06'23''W and has an average elevation of 220 m. The climate is sub-humid with an average annual rainfall of 1170 mm (varying between 900 and 1300 mm). It rains the most from March to August and is drier from September to February. The average annual temperature is 24.1°C (Almeida, 1999).

The improvement method used to create the populations was SSD until the F_6 generation when the degree of homozygosity is constant. SSD allowed for the individual selection of plants belonging to the advanced population (F_6). Of 240 lineages, 40 were selected, which suggests a selection pressure of approximately 17%. The selection considered morphological characters, based on individuals that presented the best averages for oil content and seed weight.

The lineages were called the following: UFRB5, UFRB6, UFRB28, UFRB29, UFRB36, UFRB43, UFRB45, UFRB55, UFRB65, UFRB67, UFRB117, UFRB118, UFRB119, UFRB121, UFRB128, UFRB129, UFRB144, UFRB176, UFRB181, UFRB183, UFRB186, UFRB195, UFRB198, UFRB205, UFRB209, UFRB214, UFRB223, UFRB229, UFRB230, UFRB231, UFRB232, UFRB235, UFRB237, UFRB240, UFRB249, UFRB250, UFRB252, UFRB256, UFRB259, and UFRB265.

DNA extraction

Before extraction, the castor bean leaves were disinfected in 20% sodium hypochlorite, washed with distilled water, and stored in an ultra-freezer at -80°C. DNA was extracted following the protocol described by Doyle and Doyle (1990).

Approximately 300 mg plant tissue was macerated with a mortar and pestle and liquid nitrogen. The macerated product was transferred to 2-mL microtubes, and 700 µL extraction

buffer was added to each tube (2.0% CTAB, 1.4 M NaCl, 0.1 M Tris-HCl, pH 8.0, 20 mM EDTA, 0.4% 2-mercaptoethanol, 1.0% PVP, and ultrapure water q.s.) at 65°C. The samples were incubated in a water bath at 65°C for 45 min, and homogenized every 15 min. After 45 min, 700 µL chloroform:isoamyl acid (24:1) was added to the samples. The material was gently homogenized and then centrifuged for 10 min at 10,000 rpm using a micro high-speed refrigerated centrifuge (VS-15000c, FNII). Subsequently, the supernatant was collected.

The extraction steps with chloroform:isoamyl acid were repeated to purify the material. Cold isopropyl acid (400 µL) was added to the supernatant, which was equivalent to approximately 2/3 of the volume collected.

The samples were incubated at -20°C for 20 min and then centrifuged for 10 min at 12,000 rpm. The precipitate was resuspended in 600 µL TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), 200 µL 7.5 M ammonium acetate was added, and the solution was incubated on ice for 15 min.

The samples were then centrifuged for 15 min at 12,000 rpm. The supernatant was collected, and 800 µL absolute ethanol was added. Following this, the samples were incubated for 1 h at -20°C, and then centrifuged for 10 min at 12,000 rpm. The precipitate was washed with 500 µL 70% ethanol (v/v), centrifuged for 5 min at 12,000 rpm, and dried at room temperature. The precipitate was resuspended in TE buffer with 1 µL RNase (10 mg/mL), and the samples were incubated in a water bath at 37°C for 1 h. The samples were then stored in a freezer at -20°C.

Quantification of the genomic DNA

To evaluate the quality and amount of extracted DNA, 3 µL DNA was added to 5 µL dye (30% glycerol and 0.25% bromophenol blue). The samples were applied to 0.8% agarose gel stained with ethidium bromide (0.5 mg/mL) and submitted to electrophorese for approximately 1 hand 20 min at 80 V. The amount of DNA was evaluated by comparative analysis with a known concentration (lambda DNA; Invitrogen, Carlsbad, CA, USA). Before amplification, the samples were diluted in TE buffer to adjust the DNA concentration to 5 ng/µL.

Amplification of the genomic DNA with TRAP markers

The amplification process used 28 fixed primers (Table 1) combined with 6 arbitrary primers (Table 2), for a total of 168 combinations. Seventy combinations that presented a good amplification pattern and sharp bands were selected.

The fixed primers were developed for the metabolic routes of fatty acid and ricin synthesis of the castor bean crop.

ESTs (expressed sequence tags) were retrieved from the NCBI database (National Center for Biotechnology Information, 2016) to design the fixed and direct primers using the Primer3 software (Rozen and Skaletsky, 2000). For the reverse, six arbitrary primers were used according to Li and Quiros (2001) and adapted by Hu and Vick (2003). The arbitrary primers comprise three selective nucleotides at the 3'-end, four AT-rich nucleotides (intron regions) or GC-rich nucleotides (exon regions) in the center, and 11 random nucleotides at the 5'-end.

The amplification reactions were made in a final volume of 15 µL containing the following: 1X buffer (50 mM Tris-HCl, 20 mM KCl), 1.0 U Taq DNA Polymerase (Invitrogen, Brazil); 2.0 mM $MgCl_2$; 0.2 mM dNTP (Invitrogen, Brazil); 0.2 µM primers (IDT); and 10 ng DNA.

Table 1. Characterization of the fixed primers (target region amplification polymorphisms, TRAPs) used to genotype 40 lineages of castor bean.

Primer	Sequence (5'-3')	%GC	GenBank number	Gene/process
Metabolic pathway of ricin				
TRAP1	CCACATCCAGCACCTTTTG	52.63	RCOM_0792550	RIP superfamily
TRAP2	TGTGGAGCGTTGAGGATTC	52.63	RCOM_1110780	RIP superfamily
TRAP3	TGCTCGCAGGCAAAGATAC	52.63	RCOM_1110790	RIP superfamily
TRAP4	TGTCCCATATTTGCCAACG	47.37	RCOM_1180980	Ricin superfamily
TRAP5	TGACGACTGCTCCTTCCAC	57.89	RCOM_1960510	Ricin superfamily
TRAP6	GACGACTGCTCCTTCCACA	57.89	RCOM_2105270	Ricin superfamily
TRAP7	TACGCACTATGGGCTCAGG	57.89	RCOM_2159810	RIP superfamily
TRAP8	CCCTGATGTCGCTGCTAAA	52.63	RCOM_2159910	RIP superfamily
TRAP9	AACCGCAAGTGGTCAAACA	47.37	RCOM_2160120	RIP superfamily
TRAP10	CGGGTGGCATCAGTTACAG	57.89	RCOM_2160860	RIP superfamily
TRAP11	GGCGGATGCTATCTGTGAA	52.63	RCOM_2160530	RIP superfamily
Metabolic pathway of fatty acid synthesis				
TRAP12	GACACCTTTGTTGCCATCG	52.63	RCOM_0040840	LPLAT superfamily
TRAP13	ATCCCCAACAAGCACAACA	47.37	RCOM_0138550	Ferritin superfamily
TRAP14	TTTCCTTGCTGCCTCTGTG	52.63	RCOM_0251360	Ferritin superfamily
TRAP15	CCGTGATTCTGGTGGTGAG	57.89	RCOM_0612610	PLN superfamily
TRAP16	TTACAACTGCGGCATCTCC	52.63	RCOM_0724080	PLN2250 superfamily
TRAP17	TCCATCCCTTTCCATCCTC	52.63	RCOM_0853360	LPLAT superfamily
TRAP18	TGGCATTTGCTTCCTTTGA	42.11	RCOM_0893800	PLA2 subfamily
TRAP19	AATGCCAGCACCTACACCA	52.63	RCOM_0900600	PAP2 superfamily
TRAP20	TTATCTTGGGAGGGGCTTG	52.63	RCOM_0925410	PLN superfamily
TRAP21	ATCCTTCCAGGCAATCCAC	52.63	RCOM_1076810	Ferritin superfamily
TRAP22	CACTCGCCTGTTCAGCACT	57.89	RCOM_1081890	PLN superfamily
TRAP23	AGCAAGCCGCACCTAAGAT	52.63	RCOM_1403260	RVT superfamily
TRAP24	GTCCAAGCAAAAGCCACCT	52.63	RCOM_1431520	PLN superfamily
TRAP25	CCACCAATCCAACGCATAG	52.63	RCOM_1464650	PLN superfamily
TRAP26	TTCATCTCCCTTGCCTTCC	52.63	RCOM_1502140	Oleosin superfamily
TRAP27	CGAAATCCTCCTGCTCCTC	52.63	RCOM_1593790	LPLAT superfamily
TRAP28	GCCACCATCTTCACCACAG	57.89	RCOM_1712710	FABZ superfamily

The amplification was made with a Veriti® 96-well thermocycler (Applied Biosystems) and a touch-up program with the following cycle: 94 °C for 2 min; 5 cycles at 94°C for 45 s, 35°C for 45 s and 72°C for 1 min; followed by 30 cycles at 94°C for 45 s, 40°C for 45 s, 72°C for 1 min; and a final extension of 72°C for 7 min (Carmo et al., 2015).

Electrophoresis was done on 4% agarose gel stained with ethidium bromide (0.5 mg/mL). The amplified products were observed with a transilluminator and photographed with the Kodak Science digital system. The size of the fragments was estimated by visual comparison with a 100-bp Ladder (Invitrogen, Brazil).

Table 2. Arbitrary primers used to genotype the 40 lineages of castor bean.

Name	Nucleotide sequence (3'-5')
Arb1	GACTGCGTACGAATTGAC
Arb2	GACTGCGTACGAATTTGA
Arb3	GACTGCGTACGAATTGCA
Arb4	GACTGCGTACGAATTAATT
Arb5	GACTGCGTACGAATTTGCC
Arb6	GACTGCGTACGAATTGACC

Data analyses

Because TRAP is a dominant marker, data were computed as absence (0) and presence (1) of bands. The genetic diversity of the lineages was determined by the genetic dissimilarity

matrix using the Jaccard index generated by the Genes program (Cruz, 2013). The Jaccard index is formulated as:

$$IAB = A / (A + B + C)$$

where A = same band for both individuals; B = presence of a band in individual 1 and absence in individual 2; C = absence of a band in individual 1 and presence in individual 2.

The hierarchical cluster analysis was performed using unweighted pair-group method averages (UPGMA) (Sneath and Sokal, 1973). All results were obtained using the programs R® 2.6.2 (http://www.r-project.org), STATISTICA version 7.1, and Genes (Cruz, 2013).

The dendrogram was constructed using the MEGA 5 software (Tamura et al., 2011), and the number of groups was defined using the package NbClust of R (Charrad et al., 2014) using the pseudo-t^2 criterion; analyses were made with the statistical program R (R Core Team, 2017). The PIC (polymorphism information content) was calculated with the GENALEX 6.1 software (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

Of the 70 combinations of TRAP primers evaluated, 64 (91.0%) presented a good amplification pattern for the 40 lineages of castor bean genotyped (Table 3). A total of 580 fragments were obtained, including 335 (58.0%) that were polymorphic. The total number of fragments ranged from 3 to 15, with an average of 1.23 per primer combination (Table 3). The polymorphic fragments ranged from 1 to 10 (11.0 to 100.0% polymorphic bands), and the molecular size ranged from 50 to 2000 bp. Figure 1 shows the DNA amplification of the elite lineages of castor bean using TRAP markers.

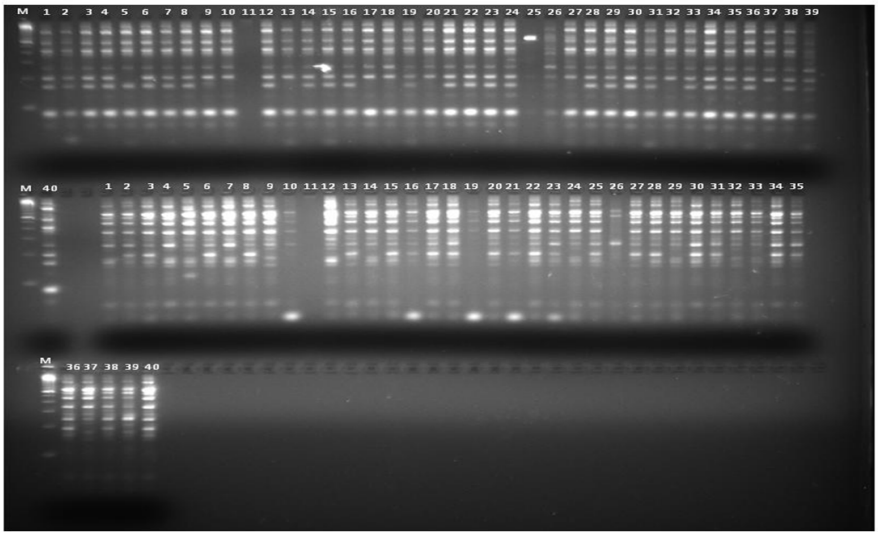

Figure 1. Electrophoretic profile, on 4% agarose gel, obtained from the amplification of the genomic DNA of 40 elite lineages of *Ricinus communis* L. (lanes 1-40) using TRAP primers. Lane M: 100-bp molecular weight marker and the combinations: 1 - TRAPC12+ARB5; 2 - TRAPC12+ARB6.

Table 3. Combinations of target region amplification polymorphism (TRAP) primers selected to analyze the polymorphism in 40 lineages of castor bean.

Combinations	No. of fragments		Polymorphism (%)	PIC
	Total	Polymorphism		
Trap1 x Arb1	9	3	33	0.03
Trap1 x Arb5	12	3	25	0.30
Trap1 x Arb6	13	3	23	0.08
Trap2 x Arb1	9	1	11	0.21
Trap2 x Arb3	10	4	40	0.30
Trap2 x Arb4	9	4	44	0.37
Trap2 x Arb6	8	4	50	0.37
Trap3 x Arb4	7	5	71	0.17
Trap4 x Arb6	9	5	55	0.10
Trap5 x Arb6	7	1	14	0.38
Trap6 x Arb2	7	5	71	0.20
Trap6 x Arb6	6	6	100	0.30
Trap7 x Arb1	5	3	60	0.15
Trap7 x Arb2	5	3	60	0.26
Trap7 x Arb3	6	3	50	0.27
Trap8 x Arb2	6	4	66	0.16
Trap8 x Arb5	7	5	71	0.20
Trap9 x Arb4	4	4	100	0.24
Trap9 x Arb6	9	7	77	0.16
Trap10 x Arb3	10	4	40	0.16
Trap10 x Arb4	5	5	100	0.26
Trap10 x Arb5	8	8	100	0.23
Trap10 x Arb6	6	5	83	0.29
Trap11 x Arb2	9	2	22	0.09
Trap11 x Arb3	8	5	62	0.11
Trap11 x Arb6	7	3	43	0.28
Trap12 x Arb2	7	4	57	0.09
Trap12 x Arb3	8	3	38	0.30
Trap12 x Arb5	9	6	67	0.22
Trap12 x Arb6	11	10	91	0.24
Trap13 x Arb1	8	5	63	0.33
Trap13 x Arb2	9	7	78	0.31
Trap13 x Arb3	12	5	42	0.31
Trap13 x Arb4	10	5	50	0.24
Trap13 x Arb5	12	6	50	0.23
Trap13 x Arb6	8	3	38	0.23
Trap14 x Arb1	4	3	75	0.33
Trap14 x Arb4	3	3	100	0.21
Trap14 x Arb6	8	5	63	0.27
Trap15 x Arb2	7	6	86	0.27
Trap15 x Arb5	13	4	31	0.18
Trap16 x Arb2	9	4	44	0.14
Trap16 x Arb3	8	3	38	0.28
Trap16 x Arb4	8	4	50	0.22
Trap17 x Arb2	7	2	29	0.24
Trap17 x Arb6	6	5	83	0.32
Trap18 x Arb2	7	3	43	0.25
Trap18 x Arb3	6	4	67	0.27
Trap19 x Arb3	9	8	89	0.21
Trap19 x Arb5	9	3	33	0.29
Trap20 x Arb1	7	4	57	0.25
Trap20 x Arb3	6	6	100	0.25
Trap20 x Arb4	4	2	50	0.26
Trap21 x Arb2	7	6	86	0.22
Trap21 x Arb5	9	7	78	0.29
Trap22 x Arb1	10	7	70	0.33
Trap22 x Arb6	15	5	33	0.31
Trap23 x Arb2	6	4	67	0.24
Trap23 x Arb3	10	5	50	0.20
Trap24 x Arb1	12	4	33	0.33
Trap24 x Arb5	10	5	50	0.27
Trap25 x Arb1	11	5	45	0.18
Trap25 x Arb4	8	8	100	0.29
Trap26 x Arb2	7	6	86	0.27
Trap26 x Arb4	7	3	43	0.32
Trap27 x Arb1	12	6	50	0.32
Trap27 x Arb2	10	9	90	0.20
Trap27 x Arb3	10	8	80	0.33
Trap28 x Arb2	11	9	82	0.31
Trap28 x Arb4	10	10	100	0.28
Averages	8.3	4.8		0.24

PIC = polymorphism information content.

Studies using TRAP markers have been conducted with other crops: guaraná (total number of polymorphic bands ranged from 12 to 33, with 79% polymorphism, and fragment size between 100 and 800 bp) (Da Silva et al., 2016); *Dendrobium* (genotyped with 13 TRAP markers, obtaining 510 fragments, including 500 polymorphic fragments, and 97.8% polymorphism) (Feng et al., 2015); *Diospyros kaki* (genotyped with 36 combinations of TRAP primers with an amplification of 2184 fragments, of which 2072 were polymorphic fragments and 94.87% polymorphism) (Luo et al., 2013); and sugarcane (21 TRAP combinations, for sucrose synthesis, with an amplification of 340 polymorphic fragments, with an average of 16.2 fragments, and 9 TRAP combinations for drought tolerance, resulting in 155 polymorphic fragments, with an average of 17.2 fragments per primer combination) (Creste et al., 2010). These studies corroborate the results obtained for castor bean, because they show that TRAP markers are highly polymorphic and appropriate for studying genetic divergence and other applications, for example, mapping associations and QTLs. It is worth noting that TRAP markers for castor bean, developed in this study, have never been published.

Variations in DNA sequences lead to greater genetic diversity. The PIC varied from 0.03 (Trap1 x Arb1 combination) to 0.33 (Trap5 x Arb6 combination), with an average of 0.24 (Table 3).

Considering that TRAP markers are dominant, the maximum PIC value observed was 0.50; therefore, the closer to this value, the greater the discriminatory power of the primer. Thus, 35 TRAP combinations had a PIC above 0.25 (Table 3), with higher discrimination power among the genotypes evaluated.

The PIC values found for castor bean are comparable to those for the TRAP technique used for cassava, where PIC values ranged from 0.03 to 0.38, with an average of 0.23, for 31 TRAP combinations (Carmo et al., 2015). For other crops, higher PIC values were found: guaraná, PIC ranged from 0.29 to 0.36, average of 0.33, for 5 TRAP combinations (Da Silva et al., 2016), and sunflower, PIC ranged from 0.02 to 0.50, average of 0.39, for 12 TRAP combinations (Yue et al., 2009).

Besides, it was observed that the PIC values for TRAP markers vary based on the arbitrary primer. In general, the Arb2 and Arb6 primers had the highest number of fragments per marker, with averages of 14.25 (Arb2) and 16.14 (Arb6), and polymorphic fragment averages of 4.93 (Arb2) and 8.86 (Arb6) (Table 4). However, Arb4 had a higher percentage of polymorphism (70.67%).

Table 4. Polymorphism and the total number of fragments concerning the arbitrary primers with 70 TRAP combinations.

Arbitrary primer	No. of fragments		Polymorphic fragments		%
	Total	Average	Total	Average	
Arb 1	87	15.90	41	4.10	47.12
Arb 2	117	14.25	74	4.93	63.24
Arb 3	99	15.23	58	4.83	58.60
Arb 4	75	12.50	53	8.83	70.67
Arb 5	89	17.80	47	9.40	52.80
Arb 6	113	16.14	62	8.86	54.87

The genetic distance, calculated by the amount of discordance between lineages (for the genetic dissimilarity of the 40 lineages), varied between 0.20 and 0.98, with an average of 0.66. The high average genetic distance (0.66) reveals significant genetic dissimilarity among the elite lineages evaluated, which is important for decision making within the genetic improvement program for the species.

The most genetically similar lineages were UFRB45 and UFRB55 (genetic distance of 0.20) and the most dissimilar were the lineages UFRB214 and UFRB252 (genetic distance of 0.98). Furthermore, the lineages UFRB5 and UFRB214 (0.97), UFRB121 and UFRB 214 (0.96), and UFRB214 and UFRB240 (0.96) stood out for having high genetic distance values.

Based on the dendrogram obtained, it is possible to separate the 40 lineages of castor bean into four groups (Figure 2): Group 1 (UFRB45, UFRB55, UFRB43, UFRB65, UFRB29, UFRB231, UFRB240, UFRB198, UFRB186, UFRB195, UFRB144, UFRB36, UFRB250, UFRB121, UFRB118, UFRB67, UFRB128, UFRB129, UFRB265, UFRB28, UFRB223, UFRB229, UFRB230, UFRB205, UFRB209, UFRB117, UFRB235, UFRB119, UFRB181, UFRB176, UFRB183, UFRB252, UFRB232, UFRB237, UFRB249); Group 2 (UFRB5, UFRB6); Group 3 (UFRB256, UFRB259); and Group 4 (UFRB214).

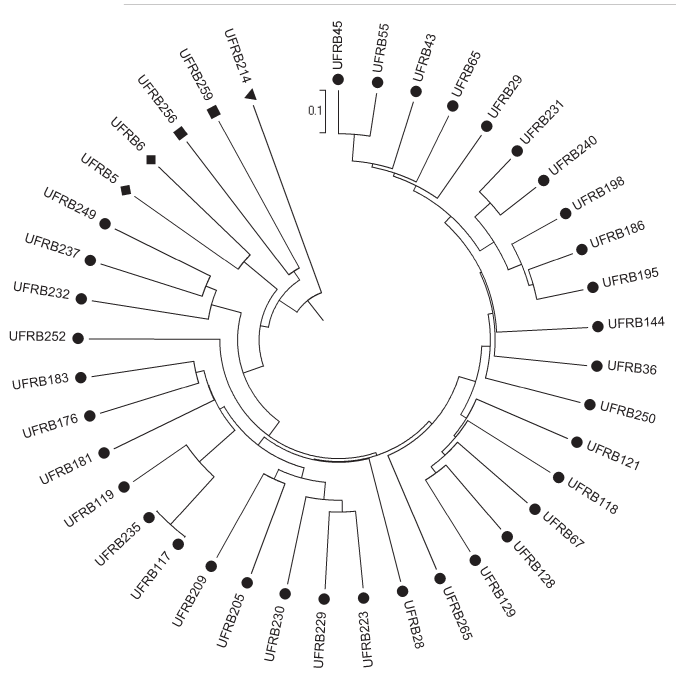

Figure 2. Genetic dissimilarity among 40 elite lineages of the active germplasm bank (AGB) of castor bean. Circles, group 1; lozenges, group 2; squares, group 3; and triangles, group 4.

Group 1 represents 87.5% of the 40 lineages evaluated and groups 2 and 3 represent 10%. Group 4 is notable for having only one lineage, UFRB 214, which corresponds to 2.5% of the lineages evaluated. These results show the genetic dissimilarity among the elite lineages of castor bean, especially UFRB 214 that had the greatest dissimilarity with the others. This is an important fact for the genetic improvement program of the species because all the genotypes constitute advanced generations from self-fertilization with high degrees of homozygosity.

Studies of genetic divergence of accessions and cultivars of castor bean using molecular markers have been conducted; however, articles with improved, advanced (6 generations of self-fertilization), highly homozygous (elite lineages) populations that use TRAP markers have not been published.

Genetic divergence studies of castor bean cultivars have been made using RAPD markers by the following: Machado et al. (2013) genotyped 15 cultivars and obtained 5 distinct groups; Silva et al. (2012) studied 5 cultivars and observed 2 distinct groups; Lakhani et al. (2015) evaluated 13 accessions and, like the previous authors, identified 2 groups; and Vivodík et al. (2015) evaluated 32 genotypes and also found 2 groups.

Another dominant marker, ISSR, was used to study genetic divergence of castor bean by Goodarzi et al. (2015), who found 4 groups among 12 accessions, and Wang et al. (2013), who evaluated 39 genotypes that separated into 4 groups.

In addition, SSR were used to evaluate genetic divergence of castor bean: Machado et al. (2016) studied 32 improved genotypes (F4), which formed 5 distinct groups; Gálová et al. (2015), for 60 accessions, found 6 groups; and Tan et al. (2013), with 53 clones, also found 6 groups.

The number of groups formed in this study, compared to the studies cited above, shows the efficiency of TRAP markers at detecting genetic variability of castor bean. Also, it is worth noting that the methodology of improvement and self-fertilization of the population resulted in divergent lineages that formed 4 groups.

TRAP markers are efficient when studying genetic variability of castor bean and are an important tool for genetic studies of the species.

Further, because TRAPs are functional markers involved in the biosynthesis of oil and ricin, they are promising for studies of associations between a marker and candidate gene.

The elite lineages in this study, developed by the UFRB Genetic Improvement Program, showed genetic divergence among them (especially lineage 214), a favorable result for the success of the program.

Conflicts of interest

The authors declare no conflict of interest.

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