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Development of TRAP primers for *Ricinus communis* L.

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ABSTRACT. The objective of this article was to develop TRAP (target region amplification polymorphism) primers for castor bean, with the goal of making functional markers available for genetic studies about the species. To do this, oligonucleotides were designed based on ESTs, obtained from the NCBI (National Center for Biotechnology Information) databank, which code enzymes involved in metabolic routes of fatty acid synthesis, ricin synthesis, and resistance to castor bean pathogens. The forward primers were designed with the help of the Primer3 software and, for the reverse, six arbitrary primers were used. To standardize the amplification reactions, the following criteria were used to select the primers: sizes between 18 and 20 bp, guanine/cytosine (GC) in the range of 40 to 60%, and average annealing temperature between 55° and 62°C. The design quality of the primers was verified using the Net Primer application. Fifty-six primers were designed, which had an average GC percentage of 53.2%. A total of 336 combinations were obtained using the 56 fixed and 6 arbitrary primers. Based on polymerase chain reaction, 330 combinations (89%) presented good amplification patterns for the genomic DNA of castor

bean. The size of the fragments amplified varied between 50 and 2072 bp. The TRAP primers designed and validated in this study are the first for castor bean and represent a significant increase in the molecular markers for this species.

Key words: GenBank/NCBI; Castor bean; Expressed sequence tag

INTRODUCTION

Castor bean (*Ricinus communis* L., 2n = 2X = 20, Euphorbiaceae) is an oleaginous plant with high socioeconomic value because of its industrial and agricultural products and byproducts (Costa et al., 2006), and has been considered as an alternative source for the production of biodiesel. For this reason, it is indispensable to implement strategies to develop genotypes that produce high amounts of oil, are resistant to pathogens, and have low (making it easier for farmers to manage) or high (for chemotherapeutic treatments) concentrations of ricin (Audi et al., 2005). TRAP (target region amplification polymorphism) functional molecular markers are an auxiliary tool important in the process of developing and selecting castor bean genotypes with these characteristics. In addition, these markers can be used in various genetic studies, for example, genetic variability, population genetics, mapping quantitative trait loci (QTLs), associated maps, and assisted selection.

Different types of molecular markers have been used to study the genetic variability of castor bean: amplified fragment length polymorphism (AFLP) (Pecina-Quintero et al., 2013), random amplified DNA polymorphism (RAPD) (Gajera et al., 2010; Silva et al., 2012; Machado et al., 2013; Vivodík et al., 2014, 2015; Lakhani et al., 2015), single nucleotide polymorphism (Foster et al., 2010), simple sequence repeats (SSR) (Bajay et al., 2009, 2011; Seo et al., 2011; Machado and Silva, 2013; Tan et al., 2013, 2014; Gálová et al., 2015; Machado et al., 2016), and inter-simple sequence repeats (ISSR) (Wang et al., 2013; Tomar et al., 2014; Goodarzi et al., 2015; Kallamadi et al., 2015).

The TRAP technique uses bioinformatic tools and EST (expressed sequence tags) data to generate markers from target sequences of candidate genes. The advantages of the technique are high reproducibility, simplicity, access to regions related to genes, and the ability to produce band patterns similar to those using the AFLP technique. The polymorphism is generated from the combination of a fixed primer, designed from an EST of interest, and an arbitrary primer (Hu and Vick, 2003).

TRAP markers were initially developed to sample DNA of the common sunflower (Hu and Vick, 2003). The markers have already been used to study various species, such as lettuce (*Lactuca sativa* L.; Hu et al., 2005), wheat (*Triticum aestivum* L.; Liu et al., 2005), the common bean (*Phaseolus vulgaris* L.; Miklas et al., 2006), fava bean (*Vicia faba* L.; Kwon et al., 2010), durum wheat (*Triticum durum*; Al-Doss et al., 2011), and cassava (*Manihot esculenta* Crantz; Carmo et al., 2015). For castor bean (*Ricinus communis* L.), there are no reports of the development or use of TRAP markers, although the genome of this species has been sequenced (Chan et al., 2010). Thus, it is necessary to develop and optimize TRAP primers to saturate the genome of castor bean, and therefore, more diverse genetic studies can be conducted, such as genetic variability, markers associated with phenotypic characteristics, and mapping QTLs of populations.

The objectives of the present study were to design TRAP primers for castor bean from ESTs obtained from the National Center for Biotechnology Information (NCBI), and to optimize them using PCR to create functional markers that can be used for genetic studies of this species.

MATERIAL AND METHODS

Design of oligonucleotides

The oligonucleotides were designed using castor bean ESTs involved in metabolic routes of fatty acid synthesis, ricin synthesis, and resistance to pathogens (Table 1).

Table 1. Expressed sequence tags used to develop target region amplification polymorphism (TRAP) primers for castor bean.

GenBank No.	Gene/process	Biological process	Primer
	Ricin		
RCOM_0792550	RIP superfamily	Inactivates ribosomes	TRAPRC-01
RCOM_1110780	RIP superfamily	Inactivates ribosomes	TRAPRC-02
RCOM_1110790	RIP superfamily	Inactivates ribosomes	TRAPRC-03
RCOM_1180980	Ricin superfamily	Ricin-type beta-trefoil	TRAPRC-04
RCOM_1960510	Ricin superfamily	Ricin-type beta-trefoil	TRAPRC-05
RCOM_2105270	Ricin superfamily	Ricin-type beta-trefoil	TRAPRC-06
RCOM_2159810	RIP superfamily	Inactivates ribosomes	TRAPRC-07
RCOM_2159910	RIP superfamily	Inactivates ribosomes	TRAPRC-08
RCOM_2160120	RIP superfamily	Inactivates ribosomes	TRAPRC-09
RCOM_2160860	RIP superfamily	Inactivates ribosomes	TRAPRC-10
RCOM_2160530	RIP superfamily	Inactivates ribosomes	TRAPRC-11
	Amount of oil		
RCOM_0040840	LPLAT superfamily	Glycerophospholipid biosynthesis	TRAPRC-12
RCOM_0138550	Ferritin superfamily	Fatty acid denaturation	TRAPRC-13
RCOM_0251360	Ferritin superfamily	Fatty acid denaturation	TRAPRC-14
RCOM_0612610	PLN superfamily	Omega-6 fatty acids denaturation	TRAPRC-15
RCOM_0724080	PLN2250 superfamily	Lipid phosphate phosphatase	TRAPRC-16
RCOM_0853360	LPLAT superfamily	Glycerophospholipid biosynthesis	TRAPRC-17
RCOM_0893800	PLA2 subfamily	Phospholipase biosynthesis	TRAPRC-18
RCOM_0900600	PAP2 superfamily	Encodes protein	TRAPRC-19
RCOM_0925410	PLN superfamily	Acyl-ACP thioesterase biosynthesis	TRAPRC-20
RCOM_1076810	Ferritin superfamily	Fatty acid denaturation	TRAPRC-21
RCOM_1081890	PLN superfamily	Xanthoxin dehydrogenase	TRAPRC-22
RCOM_1403260	RVT superfamily	Reverse transcriptase	TRAPRC-23
RCOM_1431520	PLN superfamily	Acetyl-CoA synthesis	TRAPRC-24
RCOM_1464650	PLN superfamily	Ethanolamine phosphotransferase synthesis	TRAPRC-25
RCOM_1502140	Oleosin superfamily	Oleosin synthesis	TRAPRC-26
RCOM_1593790	LPLAT superfamily	Glycerophospholipid synthesis	TRAPRC-27
RCOM_1712710	FABZ superfamily	ACP protein synthesis	TRAPRC-28
	Resistance to pathogen		
RCOM_0024370	Resistance protein RPM1	Encodes protein RPM1	TRAPRC-29
RCOM_0464860	Resistance protein RPP8	Encodes protein RPP8	TRAPRC-30
RCOM_0534550	Resistance protein RGA2	Encodes protein RGA2	TRAPRC-31
RCOM_0585390	Resistance protein RPH8A	Encodes protein RPH8A	TRAPRC-32
RCOM_0585520	Resistance protein RGA2	Encodes protein RGA2	TRAPRC-33
RCOM_0645130	Resistance protein	Encodes protein kinase	TRAPRC-34
RCOM_0687360	Resistance protein LRRK2	Encodes protein LRRK2	TRAPRC-35
RCOM_0739250	Resistance protein 4	Encodes protein 4	TRAPRC-36
RCOM_0739360	Resistance protein 23	Encodes protein 23	TRAPRC-37
RCOM_0742650	Resistance protein RPM1	Encodes protein RPM1	TRAPRC-38
RCOM_0820400	Resistance protein RPP8	Encodes protein RPP8	TRAPRC-39
RCOM_0898300	Resistance protein RPS5	Encodes protein RPS5	TRAPRC-40
RCOM_0900670	Resistance protein RGA2	Encodes protein RGA2	TRAPRC-41
RCOM_1045150	Resistance protein TMV	Encodes protein TMV	TRAPRC-42
RCOM_1122050	Resistance protein RGA3	Encodes protein RGA3	TRAPRC-43
RCOM_1184850	Resistance protein RPP13	Encodes protein RPP13	TRAPRC-44
RCOM_1195490	Resistance protein RPH8A	Encodes protein RPH8A	TRAPRC-45
RCOM_1292200	Resistance protein RGA2	Encodes protein RGA2	TRAPRC-46
RCOM_1329890	Resistance protein TMV	Encodes protein TMV	TRAPRC-47
RCOM_1330160	Resistance protein TMV	Encodes protein TMV	TRAPRC-48
RCOM_1397630	Resistance protein RGA3	Encodes protein RGA3	TRAPRC-49
RCOM_1452690	Resistance protein RGA3	Encodes protein RGA3	TRAPRC-50
RCOM_1481570	Resistance protein 206	Encodes protein 206	TRAPRC-51
RCOM_1487620	Resistance protein RGA3	Encodes protein RGA3	TRAPRC-52
RCOM_1508820	Resistance protein RGA3	Encodes protein RGA3	TRAPRC-53
RCOM_1579060	Resistance protein RGA3	Encodes protein RGA3	TRAPRC-54
RCOM_1680500	Resistance protein RGA3	Encodes protein RGA3	TRAPRC-55
RCOM_1689520	Resistance protein 206	Encodes protein 206	TRAPRC-56

The castor bean ESTs were obtained from the NCBI (National Center for Biotechnology Information, 2016) databank. The forward primers were designed with the help of the Primer3 software (Rozen and Skaletsky, 2000). The forward primers are specific and fixed. For the reverse, six arbitrary primers were used, according to Li and Quiros (2001) and adapted by Hu and Vick (2003) (Table 2). The arbitrary primers comprise three selective nucleotides at the 3'-end, four nucleotides rich in adenine/thimine (AT; corresponding to intron regions) or guanine/cytosine (GC; corresponding to exon regions) sequences in the central region, and 11 random nucleotides at the 5'-end.

Table 2. Arbitrary primers used to validate the TRAP markers for castor bean.

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

To standardize the PCR, the following criteria were used when selecting the primers: primer size between 18 and 20 bp; GC percentage ranging from 40 to 60%; and average annealing temperature between 55° and 62°C.

The quality of the design of the primers was verified using the Net Primer application (Premier Biosoft, 2015). The most suitable designs free of clamps, dimers, cross dimers, palindrome sequences, and nucleotide repeats were selected to synthesize the primers. To amplify the genomic DNA of castor bean, the synthesis was made at a scale of 25 nmol (Invitrogen, Carlsbad, CA, USA).

Plant material and DNA extraction

Genomic DNA was extracted from young, healthy leaves of three elite linages of castor bean developed by the genetic improvement program of Núcleo de Melhoramento Genético e Biotecnologia at Universidade Federal do Recôncavo da Bahia - NBIO/UFRB, in the city of Cruz das Almas, Bahia, Brazil.

Before the extraction, the leaves were disinfected in a solution of 20% sodium hypochlorite, rinsed with plenty of distilled water and stored in an ultra-freezer at -80°C. The DNA was extracted following the protocol described by Doyle and Doyle (1990). For this, approximately 300 mg plant tissue was macerated in a mortar with liquid nitrogen. The macerated material was then transferred to 2-mL microtubes and 700 µL extraction buffer was added (2.0% CTAB, 1.4 M NaCl, 0.1 M Tris-HCl, pH 8.0, 20 mM EDTA, 0.4% 2-mercaptoetanol, 1.0% PVP and ultrapure water q.s.p.) at 65°C. The samples were incubated in a water bath at 65°C for 45 min and homogenized every 15 min. After this, 700 µL chloroform: isoamyl alcohol (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 model VS-15000c). Soon after, the supernatant was collected.

To further purify the material, the extraction step using the chloroform and isoamyl alcohol was repeated. Then, 400 µL ice-cold isopropyl alcohol was added to the supernatant, which is 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 8.9, 1 mM EDTA), 200 µL 7.5 M ammonium acetate was added, and the solution was incubated on ice for 15 min.

After this, the samples were centrifuged for 15 min at 12,000 rpm. The supernatant was recovered by adding 800 µL absolute ethanol. The samples were then incubated for 1 h at -20°C and 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. It was then resuspended in TE buffer containing 1 µL RNAse (10 mg/mL) and the samples were incubated in a water bath at 37°C for 1 h. Subsequently, the samples were stored in a freezer at -20°C until they were used.

Quantification of the genomic DNA

To evaluate the quality and quantity of the extracted DNA, a total of 3 µL DNA was added to 5 µL dye solution (30% glycerol and 0.25% bromophenol blue). The samples were then applied to 0.8% agarose gel, stained with ethidium bromide (0.5 mg/mL), and electrophoresed for approximately 1 h and 20 min at 80 V. The quantity of DNA was evaluated using a comparative analysis with a known concentration of DNA (lambda DNA, Invitrogen, USA). To carry out the amplification reactions, the samples were diluted in TE buffer to adjust the concentration to 5 ng/µL.

Optimization of the PCRs for the TRAP primers

The amplification reactions were optimized to a final volume of 15 µL containing a 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.

The amplifications were made using the following program: 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 at 72°C for 7 min (Carmo et al., 2015). This was done in a thermocycler, model Veriti® 96-well (Applied Biosystems). Electrophoresis was performed on 4% agarose gel stained with ethidium bromide (0.5 mg/mL). The amplified products were viewed using a transilluminator and photographed using the Kodak Science digital software. The size of the fragments was estimated by visual comparison with a 100-bp ladder (Invitrogen, Brazil).

RESULTS AND DISCUSSION

Selecting the TRAP combinations

A total of 56 TRAP primers were developed for castor bean, which are denominated by the abbreviations TRAPRC followed by numbers (Table 3). The primers have an average GC percentage of 53.20% (Table 3). This result is close to what was found by Bajay et al. (2009), who reported 47.56% for 41 pairs of SSR primers for caster bean, and Machado and Silva (2013), who found an average GC percentage of 47.29% for 30 pairs of SSR primers for the same species.

Table 3. Fixed target region amplification polymorphism (TRAP) primers developed for castor bean.

Primer	Sequence (5'- 3')	GC%
TRAPRC-01	CCACATCCAGCACCTTTTG	52.63
TRAPRC-02	TGTGGAGCGTTGAGGATTC	52.63
TRAPRC-03	TGCTCGCAGGCAAAGATAC	52.63
TRAPRC-04	TGTCCCATATTTGCCAACG	47.37
TRAPRC-05	TGACGACTGCTCCTTCCAC	57.89
TRAPRC-06	GACGACTGCTCCTTCCACA	57.89
TRAPRC-07	TACGCACTATGGGCTCAGG	57.89
TRAPRC-08	CCCTGATGTCGCTGCTAAA	52.63
TRAPRC-09	AACCGCAAGTGGTCAAACA	47.37
TRAPRC-10	CGGGTGGCATCAGTTACAG	57.89
TRAPRC-11	GGCGGATGCTATCTGTGAA	52.63
TRAPRC-12	GACACCTTTGTTGCCATCG	52.63
TRAPRC-13	ATCCCCAACAAGCACAACA	47.37
TRAPRC-14	TTTCCTTGCTGCCTCTGTG	52.63
TRAPRC-15	CCGTGATTCTGGTGGTGAG	57.89
TRAPRC-16	TTACAACTGCGGCATCTCC	52.63
TRAPRC-17	TCCATCCCTTTCCATCCTC	52.63
TRAPRC-18	TGGCATTTGCTTCCTTTGA	42.11
TRAPRC-19	AATGCCAGCACCTACACCA	52.63
TRAPRC-20	TTATCTTGGGAGGGGCTTG	52.63
TRAPRC-21	ATCCTTCCAGGCAATCCAC	52.63
TRAPRC-22	CACTCGCCTGTTCAGCACT	57.89
TRAPRC-23	AGCAAGCCGCACCTAAGAT	52.63
TRAPRC-24	GTCCAAGCAAAAGCCACCT	52.63
TRAPRC-25	CCACCAATCCAACGCATAG	52.63
TRAPRC-26	TTCATCTCCCTTGCCTTCC	52.63
TRAPRC-27	CGAAATCCTCCTGCTCCTC	52.63
TRAPRC-28	GCCACCATCTTCACCACAG	57.89
TRAPRC-29	ACAGCGTTCAGCAGTCACC	57.89
TRAPRC-30	CTTCTCAGTTGCCCGTTCA	52.63
TRAPRC-31	CCACCAATGAACCAACTGC	52.63
TRAPRC-32	TGCCGACTTCTCCTTTCCT	52.63
TRAPRC-33	CAGTCACAATCGCCAAAGG	52.63
TRAPRC-34	TGGGCATTTTCTTGGTTGA	42.11
TRAPRC-35	CCTCATCATCGTTGCTGCT	52.63
TRAPRC-36	TCCCTCCAACAACAGCAAG	52.63
TRAPRC-37	GCTCACGCACTGGACTCAT	57.89
TRAPRC-38	CAAACTCCTCTGCCATCCA	52.63
TRAPRC-39	GCACCCGAAATCTTCCACT	52.63
TRAPRC-40	CCACTCAACACCGTTCCAC	57.89
TRAPRC-41	AGGATGTTGTCGCTCACCA	52.63
TRAPRC-42	TTTGGGCAACAAGCAAGAG	47.37
TRAPRC-43	ATGCCAAGAACCCCAAGAC	52.63
TRAPRC-44	CGTCCACCCACACTTTCAC	57.89
TRAPRC-45	TTCGCTGGATGGATAGTCG	52.63
TRAPRC-46	CCAGTCACCGTTTGTTGCT	52.63
TRAPRC-47	CGCCTCTGACAAATCTGGA	52.63
TRAPRC-48	GGCTAAGGCTTCCAACTGC	57.89
TRAPRC-49	TCCTGTCCAATGCTGAACC	52.63
TRAPRC-50	ATTCCACCCTCCAGTTTGC	52.63
TRAPRC-51	CCACCGAGAGAGCATACCA	57.89
TRAPRC-52	GTGGCAAATGCTCACAGGT	52.63
TRAPRC-53	TACAACTTCGGGTGGTGGA	52.63
TRAPRC-54	ATCCTTTTCCGTCGCAGTC	52.63
TRAPRC-55	TGATGGAAACCCTTGTGGA	47.37
TRAPRC-56	CTTGTGCCCTACCAACTGC	57.89

With the 56 fixed primers developed, it was possible to make 336 combinations using 6 arbitrary primers, and 330 combinations (89%) presented an adequate amplification pattern for the three linages analyzed (Figure 1). Low percentage of combinations of fixed and arbitrary primers was found by Carmo et al. (2015) when developing TRAP markers for cassava. These authors made 396 combinations, of which 253 (64%) had a good amplification pattern.

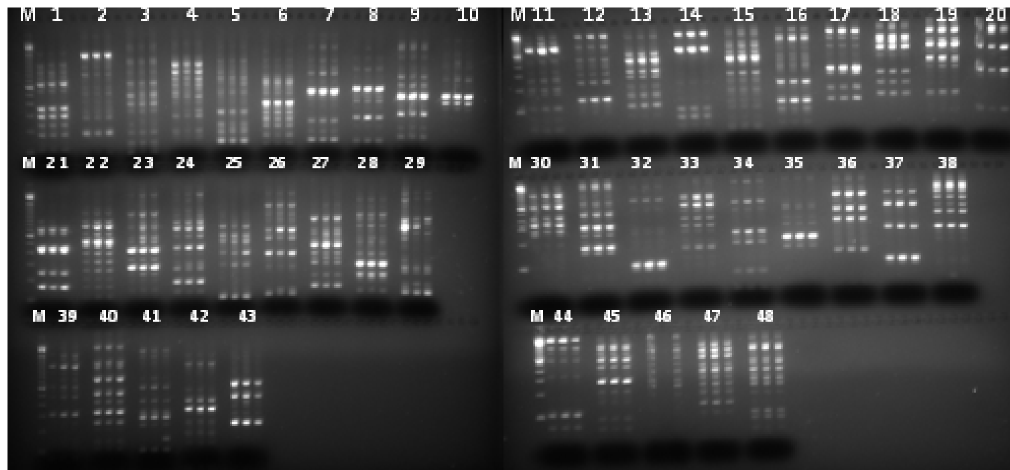

Figure 1. Electrophoretic profile, on 4% agarose gel, obtained after amplifying the genomic DNA of three lineages of *Ricinus communis* L. using target region amplification polymorphism (TRAP) primers. *Lane M*: 100-bp molecular weight marker. And the combinations: *Lane 1*- TRAPC22+ARB1, *lane 2*- TRAPC22+ARB2, *lane 3*- TRAPC22+ARB3, *lane 4*- TRAPC22+ARB4, *lane 5*- TRAPC22+ARB5, *lane 6*- TRAPC22+ARB6, *lane 7*- TRAPC23+ARB1, *lane 8*- TRAPC23+ARB2, *lane 9*- TRAPC23+ARB3, *lane 10*- TRAPC23+ARB4, *lane 11*- TRAPC23+ARB5, *lane 12*- TRAPC23+ARB6, *lane 13*- TRAPC24+ARB1, *lane 14*- TRAPC24+ARB2, *lane 15*- TRAPC24+ARB3, *lane 16*- TRAPC24+ARB4, *lane 17*- TRAPC24+ARB5, *lane 18*- TRAPC24+ARB6, *lane 19*- TRAPC25+ARB1, *lane 20*- TRAPC25+ARB2, *lane 21*- TRAPC25+ARB3, *lane 22*- TRAPC25+ARB4, *lane 23*- TRAPC25+ARB5, *lane 24*- TRAPC225+ARB6. *lane 25*- TRAPC46+ARB1, *lane 26*- TRAPC46+ARB2, *lane 27*- TRAPC46+ARB3, *lane 28*- TRAPC46+ARB4, *lane 29*- TRAPC46+ARB5, *lane 30*- TRAPC46+ARB6, *lane 31*- TRAPC47+ARB1, *lane 32*- TRAPC47+ARB2, *lane 33*- TRAPC47+ARB3, *lane 34*- TRAPC47+ARB4, *lane 35*- TRAPC47+ARB5, *lane 36*- TRAPC47+ARB6, *lane 37*- TRAPC48+ARB1, *lane 38*- TRAPC48+ARB2, *lane 39*- TRAPC48+ARB3, *lane 40*- TRAPC48+ARB4, *lane 41*- TRAPC48+ARB5, *lane 42*- TRAPC48+ARB6, *lane 43*- TRAPC49+ARB1, *lane 44*- TRAPC49+ARB2, *lane 45*- TRAPC49+ARB3, *lane 46*- TRAPC49+ARB4, *lane 47*- TRAPC49+ARB5, and *lane 48*- TRAPC49+ARB6.

Different percentages of combinations with fixed and arbitrary primers have been obtained for different species of plants. Luo et al. (2015) developed 91 combinations of TRAP primers (13 fixed and 7 arbitrary) for mango, of which 72.5% were consistent, and Kumar et al. (2014) reported 262 TRAP combinations for chickpea, of which 42% of the markers were valid. Similar combination percentage of fixed and arbitrary TRAP primers (49%) was found by Zhang et al. (2012) for the ornamental species *Paeonia suffruticosa*.

In the present study, the number of amplified loci, for the 330 combinations of the primers, varied from 2 (TRAP5 +ARB1; TRAP5 +ARB3; TRAP6+ARB3; TRAP14+ARB3; TRAP17+ARB4; TRAP20+ARB2; TRAP30+ARB1; TRAP30+ARB2; TRAP34+ARB3) to 15 (TRAP22+ARB6), with an average of 1.22 loci per combination (Table 4).

The size of the products amplified varied from 50 to 2072 bp. The combinations TRAP46+ARB4, TRAP46+ARB5, TRAP46+ARB6, TRAP49+ARB1, TRAP49+ARB2, TRAP51+ARB6, and TRAP52+ARB1 amplified fragments with 50 bp; the remaining amplified fragments with sizes above this value (Table 4).

Table 4. Number of amplified loci for the target region amplification polymorphism (TRAP) combinations (fixed and arbitrary primers).

Primers	Number of loci	Size of loci (bp)	Primers	Number of loci	Size of loci (bp)
TRAP1+ARB1	9	97-1100	TRAP8+ARB1	3	80-1300
TRAP1+ARB2	12	100-720	TRAP8+ARB2	6	80-1470
TRAP1+ARB3	6	96-800	TRAP8+ARB3	8	85-1340
TRAP1+ARB4	6	91-500	TRAP8+ARB4	4	200-1540
TRAP1+ARB5	12	95-1500	TRAP8+ARB5	7	120-1760
TRAP1+ARB6	13	90-1100	TRAP8+ARB6	7	100-1290
TRAP2+ARB1	9	113-2072	TRAP9+ARB1	3	100-700
TRAP2+ARB2	6	180-955	TRAP9+ARB2	5	160-2000
TRAP2+ARB3	10	90-1180	TRAP9+ARB3	5	205-1300
TRAP2+ARB4	9	190-1675	TRAP9+ARB4	4	590-2000
TRAP2+ARB5	9	177-1500	TRAP9+ARB5	4	205-1400
TRAP2+ARB6	8	113-1200	TRAP9+ARB6	5	220-1400
TRAP3+ARB1	6	115-2000	TRAP10+ARB1	8	113-1900
TRAP3+ARB2	12	105-1660	TRAP10+ARB2	6	86-1780
TRAP3+ARB3	12	90-1700	TRAP10+ARB3	10	86-1530
TRAP3+ARB4	7	120-1100	TRAP10+ARB4	5	87-2072
TRAP3+ARB5	9	100-1600	TRAP10+ARB5	8	87-2000
TRAP3+ARB6	9	90-2072	TRAP10+ARB6	6	88-1100
TRAP4+ARB1	7	120-1800	TRAP11+ARB1	6	87-1800
TRAP4+ARB2	5	90-1400	TRAP11+ARB2	9	87-2025
TRAP4+ARB3	8	102-1400	TRAP11+ARB3	8	87-1060
TRAP4+ARB4	8	120-1500	TRAP11+ARB4	7	92-1400
TRAP4+ARB5	4	300-1100	TRAP11+ARB5	9	110-950
TRAP4+ARB6	9	200-1500	TRAP11+ARB6	7	110-930
TRAP5+ARB1	2	280-2000	TRAP12+ARB1	6	212-2045
TRAP5+ARB2	3	130-1200	TRAP12+ARB2	7	97-1700
TRAP5+ARB3	2	150-600	TRAP12+ARB3	4	86-2000
TRAP5+ARB4	6	130-1200	TRAP12+ARB4	4	107-1500
TRAP5+ARB5	6	250-1700	TRAP12+ARB5	9	87-1420
TRAP5+ARB6	7	120-2000	TRAP12+ARB6	9	87-1200
TRAP6+ARB1	4	100-1100	TRAP13+ARB1	0	0
TRAP6+ARB2	4	190-1460	TRAP13+ARB2	0	0
TRAP6+ARB3	2	86-1300	TRAP13+ARB3	0	0
TRAP6+ARB4	4	240-800	TRAP13+ARB4	0	0
TRAP6+ARB5	4	200-1200	TRAP13+ARB5	0	0
TRAP6+ARB6	6	240-1050	TRAP13+ARB6	0	0
TRAP7+ARB1	5	210-1150	TRAP14+ARB1	4	95-1000
TRAP7+ARB2	5	280-2072	TRAP14+ARB2	7	164-770
TRAP7+ARB3	6	94-1900	TRAP14+ARB3	2	100-700
TRAP7+ARB4	4	450-1900	TRAP14+ARB4	3	90-1330
TRAP7+ARB5	5	115-1500	TRAP14+ARB5	4	97-780
TRAP7+ARB6	7	90-1060	TRAP14+ARB6	8	110-830
TRAP15+ARB1	10	90-1360	TRAP22+ARB1	10	95-1540
TRAP15+ARB2	7	105-1660	TRAP22+ARB2	14	95-1430
TRAP15+ARB3	10	90-2045	TRAP22+ARB3	14	103-1630
TRAP15+ARB4	9	107-2000	TRAP22+ARB4	13	120-1900
TRAP15+ARB5	13	90-1600	TRAP22+ARB5	12	108-2072
TRAP15+ARB6	6	90-900	TRAP22+ARB6	15	107-1600
TRAP16+ARB1	8	100-865	TRAP23+ARB1	11	110-1550
TRAP16+ARB2	9	95-954	TRAP23+ARB2	9	110-1560
TRAP16+ARB3	8	90-1640	TRAP23+ARB3	10	114-1700
TRAP16+ARB4	8	110-1585	TRAP23+ARB4	6	300-1290
TRAP16+ARB5	6	95-1430	TRAP23+ARB5	7	113-785
TRAP16+ARB6	6	95-2072	TRAP23+ARB6	10	105-1270
TRAP17+ARB1	8	105-1650	TRAP24+ARB1	12	108-1560
TRAP17+ARB2	7	120-1370	TRAP24+ARB2	13	104-1830
TRAP17+ARB3	5	190-1590	TRAP24+ARB3	7	102-760
TRAP17+ARB4	2	195-1690	TRAP24+ARB4	10	104-1420
TRAP17+ARB5	7	95-1730	TRAP24+ARB5	10	110-1360
TRAP17+ARB6	6	92-1480	TRAP24+ARB6	10	107-1740
TRAP18+ARB1	5	230-1350	TRAP25+ARB1	11	103-2000
TRAP18+ARB2	7	107-1300	TRAP25+ARB2	8	100-1400
TRAP18+ARB3	6	92-750	TRAP25+ARB3	10	104-1530
TRAP18+ARB4	4	400-2000	TRAP25+ARB4	8	105-1600
TRAP18+ARB5	7	113-900	TRAP25+ARB5	8	108-1300

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Table 4. Continued.

Primers	Number of loci	Size of loci (bp)	Primers	Number of loci	Size of loci (bp)
TRAP18+ARB6	7	117-864	TRAP25+ARB6	8	220-590
TRAP19+ARB1	11	117-1800	TRAP26+ARB1	4	106-830
TRAP19+ARB2	8	110-1630	TRAP26+ARB2	7	100-1600
TRAP19+ARB3	8	86-1500	TRAP26+ARB3	9	108-1550
TRAP19+ARB4	8	86-1330	TRAP26+ARB4	7	207-1500
TRAP19+ARB5	9	91-1530	TRAP26+ARB5	8	123-1700
TRAP19+ARB6	12	91-1775	TRAP26+ARB6	6	106-952
TRAP20+ARB1	7	95-1400	TRAP27+ARB1	12	112-1300
TRAP20+ARB2	2	325-620	TRAP27+ARB2	10	122-1250
TRAP20+ARB3	6	93-600	TRAP27+ARB3	10	107-1430
TRAP20+ARB4	4	206-1800	TRAP27+ARB4	6	120-1400
TRAP20+ARB5	7	103-1100	TRAP27+ARB5	6	106-820
TRAP20+ARB6	3	170-545	TRAP27+ARB6	6	112-900
TRAP21+ARB1	8	174-1150	TRAP28+ARB1	8	108-1340
TRAP21+ARB2	7	110-990	TRAP28+ARB2	8	123-1360
TRAP21+ARB3	6	120-1360	TRAP28+ARB3	9	118-1600
TRAP21+ARB4	6	200-1445	TRAP28+ARB4	10	117-1600
TRAP21+ARB5	9	120-1200	TRAP28+ARB5	10	95-2072
TRAP21+ARB6	8	114-815	TRAP28+ARB6	8	114-1500
TRAP29+ARB1	7	100-1500	TRAP36+ARB1	7	170-1500
TRAP29+ARB2	6	110-1260	TRAP36+ARB2	8	120-2072
TRAP29+ARB3	4	107-1300	TRAP36+ARB3	7	120-1200
TRAP29+ARB4	7	116-1200	TRAP36+ARB4	6	480-1800
TRAP29+ARB5	7	107-1370	TRAP36+ARB5	8	130-800
TRAP29+ARB6	8	109-1100	TRAP36+ARB6	9	170-1600
TRAP30+ARB1	2	320-500	TRAP37+ARB1	8	140-1400
TRAP30+ARB2	2	216-1400	TRAP37+ARB2	9	110-1600
TRAP30+ARB3	10	110-1400	TRAP37+ARB3	6	100-600
TRAP30+ARB4	10	120-1530	TRAP37+ARB4	6	200-1500
TRAP30+ARB5	9	120-1700	TRAP37+ARB5	7	140-1500
TRAP30+ARB6	10	114-1800	TRAP37+ARB6	11	140-2000
TRAP31+ARB1	9	106-1300	TRAP38+ARB1	5	200-850
TRAP31+ARB2	11	110-1300	TRAP38+ARB2	5	200-1000
TRAP31+ARB3	10	107-1020	TRAP38+ARB3	8	180-1500
TRAP31+ARB4	8	112-1470	TRAP38+ARB4	8	130-800
TRAP31+ARB5	8	108-1440	TRAP38+ARB5	7	120-1200
TRAP31+ARB6	8	108-980	TRAP38+ARB6	7	130-1400
TRAP32+ARB1	8	105-1500	TRAP39+ARB1	7	180-1400
TRAP32+ARB2	11	109-1600	TRAP39+ARB2	9	160-2072
TRAP32+ARB3	7	112-1200	TRAP39+ARB3	9	170-1600
TRAP32+ARB4	8	109-1700	TRAP39+ARB4	5	180-1100
TRAP32+ARB5	9	113-1400	TRAP39+ARB5	10	150-1200
TRAP32+ARB6	9	103-1000	TRAP39+ARB6	3	160-1300
TRAP33+ARB1	8	230-1500	TRAP40+ARB1	8	130-1000
TRAP33+ARB2	8	110-1850	TRAP40+ARB2	5	90-1600
TRAP33+ARB3	7	105-1560	TRAP40+ARB3	5	300-1400
TRAP33+ARB4	5	110-1560	TRAP40+ARB4	7	210-700
TRAP33+ARB5	9	108-1400	TRAP40+ARB5	8	280-1700
TRAP33+ARB6	5	112-1570	TRAP40+ARB6	10	90-2000
TRAP34+ARB1	7	200-1400	TRAP41+ARB1	9	130-1700
TRAP34+ARB2	7	100-1100	TRAP41+ARB2	5	300-1400
TRAP34+ARB3	2	150-500	TRAP41+ARB3	4	180-800
TRAP34+ARB4	4	200-400	TRAP41+ARB4	4	120-1500
TRAP34+ARB5	6	110-1400	TRAP41+ARB5	8	170-1400
TRAP34+ARB6	8	150-1100	TRAP41+ARB6	9	110-1800
TRAP35+ARB1	8	90-1800	TRAP42+ARB1	5	200-1500
TRAP35+ARB2	10	130-1600	TRAP42+ARB2	8	180-1400
TRAP35+ARB3	9	120-800	TRAP42+ARB3	9	110-1400
TRAP35+ARB4	7	180-1500	TRAP42+ARB4	8	280-1400
TRAP35+ARB5	6	100-780	TRAP42+ARB5	7	70-1500
TRAP35+ARB6	6	60-450	TRAP42+ARB6	6	120-700
TRAP43+ARB1	7	230-1300	TRAP50+ARB1	8	100-2072
TRAP43+ARB2	7	120-1300	TRAP50+ARB2	7	80-1400
TRAP43+ARB3	3	120-1300	TRAP50+ARB3	7	180-1400
TRAP43+ARB4	4	180-1400	TRAP50+ARB4	8	60-1500

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Table 4. Continued.

Primers	Number of loci	Size of loci (bp)	Primers	Number of loci	Size of loci (bp)
TRAP43+ARB5	5	180-2072	TRAP50+ARB5	7	120-1400
TRAP43+ARB6	4	170-1000	TRAP50+ARB6	7	130-2072
TRAP44+ARB1	7	180-1500	TRAP51+ARB1	10	180-1800
TRAP44+ARB2	6	70-1000	TRAP51+ARB2	8	130-1700
TRAP44+ARB3	9	110-1400	TRAP51+ARB3	6	90-1900
TRAP44+ARB4	4	270-1800	TRAP51+ARB4	8	110-2000
TRAP44+ARB5	10	90-1500	TRAP51+ARB5	9	90-2000
TRAP44+ARB6	9	130-1500	TRAP51+ARB6	9	50-2000
TRAP45+ARB1	6	300-2072	TRAP52+ARB1	7	50-2000
TRAP45+ARB2	7	180-1800	TRAP52+ARB2	8	70-2072
TRAP45+ARB3	8	180-2072	TRAP52+ARB3	7	70-1400
TRAP45+ARB4	5	280-900	TRAP52+ARB4	6	60-2072
TRAP45+ARB5	7	150-1400	TRAP52+ARB5	5	60-1400
TRAP45+ARB6	5	300-1000	TRAP52+ARB6	5	70-1900
TRAP46+ARB1	10	60-2000	TRAP53+ARB1	9	180-1900
TRAP46+ARB2	8	100-1900	TRAP53+ARB2	6	380-2000
TRAP46+ARB3	9	80-1800	TRAP53+ARB3	8	160-1100
TRAP46+ARB4	4	50-1400	TRAP53+ARB4	7	200-2000
TRAP46+ARB5	13	50-2000	TRAP53+ARB5	8	120-1900
TRAP46+ARB6	10	50-2000	TRAP53+ARB6	10	90-1800
TRAP47+ARB1	7	110-1400	TRAP54+ARB1	3	180-1400
TRAP47+ARB2	8	120-1600	TRAP54+ARB2	6	180-1300
TRAP47+ARB3	4	130-1500	TRAP54+ARB3	6	120-1000
TRAP47+ARB4	7	80-1500	TRAP54+ARB4	8	140-1400
TRAP47+ARB5	9	200-900	TRAP54+ARB5	7	200-1000
TRAP47+ARB6	7	170-2000	TRAP54+ARB6	7	110-1600
TRAP48+ARB1	6	100-1100	TRAP55+ARB1	6	140-600
TRAP48+ARB2	8	180-800	TRAP55+ARB2	8	120-800
TRAP48+ARB3	6	80-2000	TRAP55+ARB3	6	100-1800
TRAP48+ARB4	7	150-1500	TRAP55+ARB4	7	100-2000
TRAP48+ARB5	6	180-1600	TRAP55+ARB5	10	90-1400
TRAP48+ARB6	5	110-1000	TRAP55+ARB6	9	130-2000
TRAP49+ARB1	6	50-2072	TRAP56+ARB1	8	90-1000
TRAP49+ARB2	7	50-1500	TRAP56+ARB2	8	130-1200
TRAP49+ARB3	7	200-2072	TRAP56+ARB3	11	90-1600
TRAP49+ARB4	8	110-1200	TRAP56+ARB4	8	90-1500
TRAP49+ARB5	9	100-1000	TRAP56+ARB5	11	120-2000
TRAP49+ARB6	10	80-1900	TRAP56+ARB6	9	160-1300

Different results were obtained for sugarcane: an average of 33 fragments amplified based on 18 TRAP combinations, which varied between 100 and 700 bp (Alwala et al., 2006); and an average of 58 fragments with 16 TRAP combinations, which varied between 60 and 550 bp (Suman et al., 2012). Also, for fava bean, Kwon et al. (2010) used 12 combinations of TRAP primers and found a variation of 7 to 37 fragments that had between 100 and 850 bp.

In general, the primers Arb5 and Arb6, in combination with the fixed primers, enabled the amplification of the greatest number of loci, which had an average of 8.0 and 7.9 loci per combination, respectively (Table 5).

Table 5. Arbitrary primers that enabled the amplification of the greatest number of loci in combination with fixed primers.

Arbitrary primer	No. of bands	Average
	Total	
Arb 1	398	7.1
Arb 2	423	7.6
Arb 3	407	7.2
Arb 4	364	6.5
Arb 5	446	8.0
Arb 6	441	7.9

Liu et al. (2005) reported the efficiency of TRAP markers. According to these authors, TRAP markers are very efficient at generating rapid markers throughout the genome of an organism and allow linkage groups to be determined and many gaps to be filled. TRAP markers have shown the same capacity as microsatellites to assign linkage groups to chromosomes.

For the primer Arb1, 5 of the analyzed combinations did not result in an amplification product, and for the primers Arb2, Arb3, Arb4, Arb5, and Arb6 the number combinations that did not amplify were 3, 1, 3, 1, and 2, respectively. The lack of amplification can be explained by the existence of genetic differences in the amplification target site of the original source used for sequencing in relation to the genotypes evaluated.

The TRAP markers developed and optimized in this study are the first functional markers developed for castor bean (based on a literature review). These markers constitute another important tool for genetic improvement programs and studies about genetic variability and population genetics of castor bean. In addition to the advantages mentioned, TRAP markers allow polymorphisms to be detected in regions near genes of interest in castor bean, which makes them useful in QTL identification and associative mapping studies because they accelerate the process of marker-assisted selection.

Conflicts of interest

The authors declare no conflict of interest.

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