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# Molecular characterization and identification of markers for toxic and non-toxic varieties of *Jatropha curcas* L. using RAPD, AFLP and SSR markers

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**Abstract** *Jatropha curcas* L., a multipurpose shrub has acquired significant economic importance for its seed oil which can be converted to biodiesel, is emerging as an alternative to petro-diesel. The deoiled seed cake remains after oil extraction is toxic and cannot be used as a feed despite having best nutritional contents. No quantitative and qualitative differences were observed between toxic and non-toxic varieties of *J. curcas* except for phorbol esters content. Development of molecular marker will enable to differentiate non-toxic from toxic variety in a mixed population and also help in improvement of the species through marker assisted breeding programs. The present investigation was undertaken to characterize the toxic and non-toxic varieties at molecular level and to develop PCR based molecular markers for distinguishing non-toxic from toxic or vice versa. The polymorphic markers were successfully identified specific to non-toxic and toxic variety using RAPD and AFLP techniques. Totally 371 RAPD, 1,442 AFLP markers were analyzed and 56 (15.09%) RAPD, 238 (16.49%) AFLP markers were found specific to either of the varieties. Genetic similarity between non-toxic and toxic verity was found to be 0.92 by RAPD and 0.90 by AFLP fingerprinting. In the present study out of 12 microsatellite markers analyzed, seven markers were found polymorphic. Among these seven, jcms21 showed homozygous allele in the toxic variety. The study demonstrated that both RAPD and AFLP techniques were equally competitive in identifying polymorphic markers and differentiating both the varieties of *J. curcas*. Polymorphism of SSR markers prevailed between the varieties of *J. curcas*. These RAPD and AFLP identified markers will help in selective cultivation of specific variety and along with SSRs these markers can be exploited for further improvement of the species through breeding and Marker Assisted Selection (MAS).

**Keywords** *Jatropha curcas* L. · Non-toxic · Toxic · RAPD (Random Amplified Polymorphic DNA) · AFLP (Amplified Fragment Length Polymorphism) and Microsatellites

## Introduction

*Jatropha curcas* L., belonging to the family Euphorbeaceae, is native to South America and widely distributed in South and Central Americas, Africa and Asia. *Jatropha curcas* is a multipurpose shrub with significant economic importance and having the capabilities to rehabilitate the degraded lands [1]. Since its seed oil can be converted to biodiesel, it is emerging as a renewable energy source, alternative to petro-diesel. Several reports have demonstrated better performance of the *Jatropha* biodiesel compared with the conventional petro-diesel [1–3]. The short gestation period, easy adaptation to different kinds of marginal and semi marginal lands, drought endurance and avoidance by animals, make this plant species more attractive for cultivation [4–7]. Inspite of best nutritional composition, seed cake obtained from the toxic *J. curcas* remains unutilized as an animal feed due to its toxic nature [8, 9] and no successful attempts are made till now for completely eliminating toxic principle [10]. Globally *J. curcas* is promoted for large acreage cultivation in a big way for biodiesel production

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[11, 12]. Selective cultivation of non-toxic variety reported from Mexico, whose innocuous nature was established [7, 8, 13], will add value to the crop through utilization of deoiled seed cake as a safe animal feed.

Cultivation of non-toxic variety of *J. curcas* could provide oil for biodiesel and deoiled seed cake as a live stock feed [9]. No significant morphological, qualitative and quantitative differences are known between toxic and non-toxic varieties except for the phorbol esters content in the toxic variety [1, 8]. Development of any simple marker will enable identification of non-toxic variety from toxic variety, which will not only add to the quality control for selective cultivation of non-toxic variety but also avoid any toxic adulteration in the animal feeds. In our previous study with multilocus marker systems RAPD and AFLP, we found both the marker systems equally competent [14]. The same marker systems have been selected for molecular characterization, estimation of genetic diversity between toxic and non-toxic varieties, possibility of distinguishing non-toxic from toxic variety or vice versa and identifying polymorphic microsatellite markers for both the varieties. The identified polymorphic markers can be exploit for genetic improvement of the species through breeding and Marker Assisted Selection (MAS).

## Materials and methods

Genomic DNA was extracted from six diverged toxic *J. curcas* accessions including one toxic variety collected from Mexico and a Mexican non-toxic variety. The extraction was carried from fresh leaves as described by Pamidiamarri et al. [14]. About 0.1 g of leaf tissue was ground in liquid nitrogen and put in a 2 ml eppendorf tube. To the ground sample 0.5 ml of extraction buffer (2% CTAB, 100 mM Tris–HCl, 3.5 M NaCl, 20 mM EDTA, 0.2 M $\beta$-Mercaptoethanol, 2% PVP, pH 8.0.) was added and incubated at 65°C for 90 min. The above sample was extracted with equal volume of Chloroform: Isoamyl alcohol (24:1) and supernatant was transferred to a new tube. The sample was treated with RNase and was extracted with Tris saturated phenol. The supernatant was further extracted with chloroform: Isoamyl alcohol (24:1) twice, and precipitated with 80% ethanol. The pellet was air dried and was dissolved in 100 µl of Milli Q water.

Amplification of RAPD fragments was performed according to Williams et al. [15] using decamer arbitrary primer (Operon Technologies Inc, USA; IDT, USA). The reaction was carried out in a volume of 25 µl containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 0.2 mM each dNTPs, 3.0 mM $MgCl_2$, 0.4 µM primer, 25 ng template, 1 unit Taq DNA polymerase (Biogene, USA). Amplification was performed in a thermal cycler (master cycle, eppendorf, Germany) with program of initial denaturation at 94°C for 3 min, 42 cycles of denaturation at 94°C for 30 s, primer annealing at 32°C for 1 min, extension at 72°C for 2.5 min, and final extension at 72°C for 4 min. Amplified products were separated in 1.5% TBE agarose gel. The gels were stained with ethidium bromide and documented using gel documentation system (Syngene, UK). Experiment with each primer was done three times and those primers gave reproducible fingerprints were considered for data analysis.

AFLP analysis system-II kit (Invitrogen Life Science Ltd, USA) was used for AFLP fingerprinting [16]. The genomic DNA (300 ng) was digested with *Eco*RI and *Mse*I at 37°C for 2 h and digested aliquot was ligated to *Eco*RI and *Mse*I specific adapters at 20°C for 3 h. The ligated DNA was preamplified using *Eco*RI and *Mse*I with one selective nucleotide at 3′ of primer each. The pre-amplified product was diluted 1:20 with sterile TE buffer. The diluted product was amplified using primers with three selective nucleotides for *Eco*RI primer and three selective nucleotides for *Mse*I primer at the 3′ end. PCR was performed using 65°C as the initial annealing temperature for the first cycle and for subsequent 11 cycles; the annealing temperature was successively reduced by 0.7°C. Twenty-three cycles were run at 56°C annealing temperature. To the PCR product appropriate formamide dye was added and subjected to electrophoretic separation on 6% denaturing polyacrylamide gel in 1× TBE buffer in a sequencing gel system (LKB, Sweden). The gels were stained with silver nitrate using silver staining kit (Sigma, USA). Experiment with each primer combination of *Eco*RI and *Mse*I was done three times and those primers which gave reproducible fingerprints were considered for data analysis.

Microsatellite markers were amplified from toxic and non-toxic *J. curcas* varieties in a volume of 25 µl containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 0.2 mM each dNTP, 3.0 mM $MgCl_2$, 0.4 µM primer, 25 ng template, 1 unit Taq DNA polymerase (Biogene,USA). Amplification was performed in a thermal cycler (master cycle, eppendorf, Germany) with program of initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 30 s, primer annealing (Table 2) for 30 s, extension at 72°C for 40 s, and final extension at 72°C for 4 min. To the PCR product appropriate formamide dye was added and subjected to electrophoretic separation on 10% denaturing polyacrylamide gel in 1× TBE buffer in a sequencing gel system (LKB, Sweden). The gels were stained with silver nitrate using silver staining kit (Sigma, USA).

Acquired RAPD and AFLP finger prints were individually scored and statistically analyzed by assuming that fragment size as a locus was considered as biallelic (present = 1, absent = 0) and made the binary matrix.

Only those loci amplified strongly in each instance with reproducibility were scored and included in the analyses. Genetic similarity was calculated according to $F = 2Nxy/(Nx + Ny)$, where $Nxy$ was the number of bands shared by two species and $Nx$ and $Ny$ are the number for fragments in each sample. Genetic distance was calculated by formula $P = 1 - F$. The percentage of polymorphism was calculated by using formula PP = total number of polymorphic bands/total number of bands multiplied with 100. Percentage of Similarity was obtained by formula PS = 100 − PP. The allele size of the each SSR markers were determined by GeneTool analysis software (Syngene, UK).

## Results

Totally 180 RAPD primers were screened and 52 primers those resulted more than 6 scorable bands were selected for further study. In AFLP analysis 64 selective primer combinations were tried and out of them 56 combinations resulted with polymorphic bands between the toxic and non-toxic varieties. For identification of specific markers and to calculate the percentage of polymorphism and genetic similarity between toxic and non-toxic varieties the marker present only in non-toxic but not in toxic accessions and the marker present in all the *J. curcas* toxic accessions but not in non-toxic variety were taken. Out of total 371 RAPD and 1,442 AFLP markers analyzed 56 (15.09%) RAPD and 238 (16.49%) AFLP markers were found to be polymorphic. The percentage of similarity was 84.91 by RAPD and 83.51 by AFLP fingerprinting. Genetic similarity between toxic and non-toxic variety was found to be 0.92 by RAPD and 0.90 by AFLP fingerprinting techniques. The results obtained by both these techniques are comparable and showed the competitive validity of their application in molecular characterization of *J. curcas* varieties.

Out of 52 RAPD primers screened for identification of selective markers between toxic and non-toxic varieties, no polymorphic markers were observed between these two varieties with 13 primers and remaining 39 primers resulted in total 66 polymorphic markers. Primer OPO19 has given highest (5) number of polymorphic markers specific to toxic variety. The lowest number (1) of polymorphic markers was observed with 13 primers IDT E-12, 18, OPJ20, OPL1, OPN3, 8, 12, OPP1, 2, 15, OPQ7, 15 and 20. Use of primer OPQ15 has resulted in one specific marker each to toxic (approximately 810 bp) and non-toxic (approximately 425 bp) variety. Primers IDT E-18, OPL14 resulted in one marker each of approximately 900 and 2,100 bp, respectively; whereas, OPR8 resulted in two specific markers of approximately 1,450 and 700 bp (Figs. 1, 2). In case of AFLP the primers E-ACC/M-CAC combination resulted in maximum number of polymorphic markers (3) each for toxic and non-toxic and minimum marker (1) for non-toxic variety was recorded when primers combination E-AAG/M-CTG was used. Prominently amplified AFLP markers specific to toxic and non-toxic variety and their molecular weights were recorded (Table 1, Fig. 3). In this study 12 SSR markers (personal

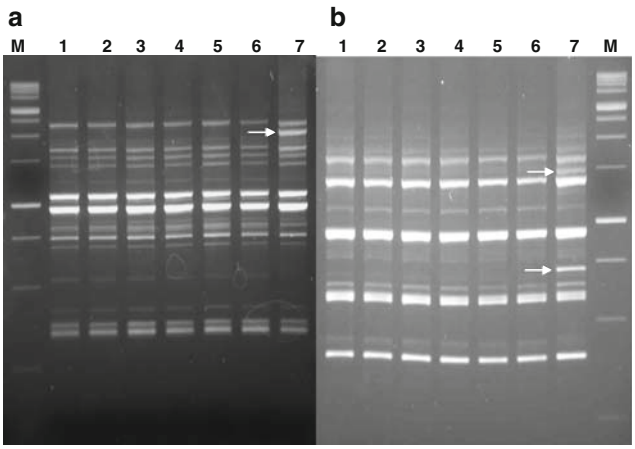


**Fig. 2** (**a**) RAPD profile with primer OPL14, (**b**) RAPD profile with primer OPR8; 1–6: Toxic and 7: Non-toxic variety of *J. curcas*; M: 1 kb Marker(Biogene, USA)

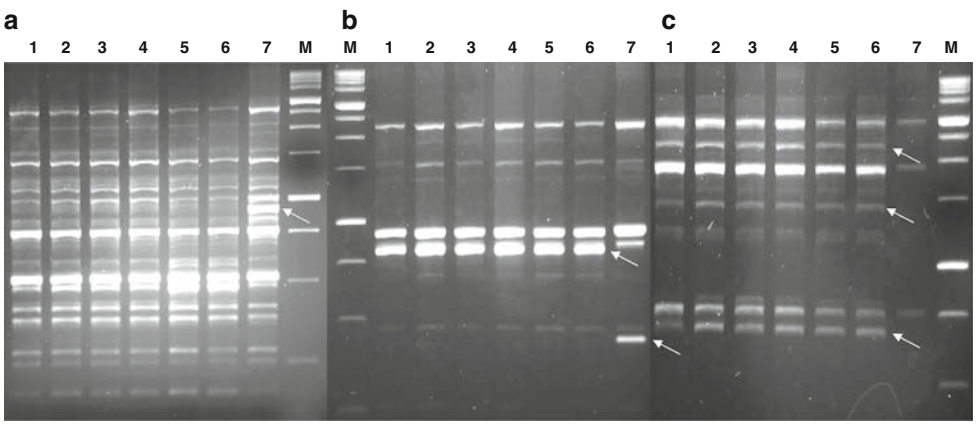


**Fig. 1** (**a**) RAPD profile with primer IDT E-18, (**b**) RAPD profile with primer OPQ15, (**c**) RAPD profile with primer OPO19;1–6: Toxic and 7: Non-toxic variety of *J. curcas;* M: 1 kb marker(Biogene, USA)

**Table 1** AFLP molecular markers specific to non-toxic and toxic variety of *J. curcas*

| AFLP specific primer set combination | Number of specific markers | Molecular weight of specific marker to non-toxic variety (bp) | Molecular weight of specific marker to toxic variety (bp) |
|---|---|---|---|
| E-AAC/M-CAA | 3 | 882, 446 | 957 |
| E-AAC/M-CAC | 1 | 1054 | Nil |
| E-AAC/M-CAG | 2 | Nil | 559, 85 |
| E-AAC/M-CAT | 1 | Nil | 575 |
| E-AAC/M-CTC | 2 | Nil | 584, 259 |
| E-AAC/M-CTG | 1 | 59 | Nil |
| E-AAC/M-CTT | 3 | 678, 98 | 484 |
| E-ACC/M-CAA | 5 | 529, 275 | 916, 373, 279 |
| E-ACC/M-CAC | 6 | 1533, 553, 521 | 1067, 593, 323 |
| E-ACC/M-CAG | 5 | 98 | 1435, 1247, 911, 567 |
| E-ACC/M-CAT | 1 | 1427 | Nil |
| E-ACC/M-CTA | 1 | 602 | Nil |
| EACC/M-CTC | 4 | 624, 529, 394 | 516 |
| E-ACC/M-CTG | 1 | 1612 | Nil |
| E-ACC/M-CTT | 5 | 1341, 1047, 623, 496, 172 | Nil |
| E-AGC/M-CAA | 2 | 926, 441, | Nil |
| E-AGC/M-CAC | 5 | 665, 624, 150, 251 | 251 |
| E-AGC/M-CTA | 2 | 64 | 133 |
| E-AGC/M-CTC | 3 | 293, 91 | 228 |
| E-AGC/M-CTG | 2 | 633 | 590 |
| E-AGC/M-CTT | 3 | 1110 | 502, 213 |

communication) were analyzed (Table 2) to find out their size polymorphism among non-toxic and toxic varieties. Miss-amplifications were minimized by gradient PCR and optimized the annealing temperature (Table 2). The polymorphic nature of SSR markers were characterized for toxic and non-toxic varieties. The results showed that out of 12 makers tested, 7 (jcms21, jcds24, jcms30, jcps20, jcps21, jcps6, jcps3) found to be size polymorphic. Among the seven markers, jcms21 showed homozygous allele in the toxic variety. The markers, jcms30, jcps21, jcps3 and jcps20 showed at least one allele of same size repeats common in both non-toxic and toxic varieties. The highest size allele polymorphism was found with jcds24, followed by jcms30 and jcps6 (Fig. 4, Table 2).

## Discussion

*Jatropha curcas*, a multipurpose shrub, has acquired high agro-industrial significance globally because of its seed oil which is a potential source of biodiesel and also for its beneficial by-products [3–6]. The seed cake remaining after oil extraction is toxic and owing to the presence of different toxic substances in seeds, oil and deoiled cake are not suitable for animal consumption [9] and cannot be used as fodder/feed despite having best protein composition and favorable amino acid profile [17]. Short gestation period, easy adaptation to different kinds of marginal and semi marginal lands, drought endurance and avoidance by animals, made the species more attractive for cultivation [4–7]. Though various processing techniques have been attempted to detoxify, no treatment has been successful in completely eliminating the toxic principles of defatted kernel meal [10]. In addition to common toxic cultivar, a non-toxic *J. curcas* has been reported from Mexico whose innocuous nature was established [7, 8, 13]. However, no qualitative and quantitative differences were reported between these two cultivars. Development of any simple marker will enable identification of non-toxic variety from toxic variety which will not only add the quality control for selective cultivation of non-toxic variety, but also avoid any toxic adulteration in the animal feeds.

In the present study a non-toxic Mexican variety was compared with five accessions of *J. curcas* collected from different geographical regions of India and one Mexican toxic variety using RAPD and AFLP technique and identified markers specific to non-toxic and toxic varieties. Sujatha et al. [12] reported 94.6% of similarity between toxic and non-toxic varieties using RAPD fingerprinting whereas, in the present study the percentage of similarity was 84.91 by RAPD and 83.59 by AFLP fingerprinting. Using the RAPD primers IDT E-18, OPL14 and AFLP

selective primer combination E-ACC/M-CAC resulted in polymorphic markers for both toxic and non-toxic varieties can be used for identification. Though the marker related studies for toxic *J. curcas* has been reported using ISSR [18]; in the present study markers were identified specific to both toxic and non-toxic varieties using RAPD and AFLP fingerprinting which have better application in molecular breeding studies.

From the last one decade microsatellites have become one of the most popular molecular markers utilized in different fields. High polymorphism and the relative ease of scoring represent the two major features that make microsatellites of large interest for many genetic studies. Soon after their first description [19–21] SSRs were being widely employed in many fields because of their high variability which made them very powerful genetic markers. Microsatellites have proven to be an extremely valuable tool for genome mapping in many organisms [22, 23]. In the present study, 12 markers isolated [personal communication] (Table 2) showed average length of the microsatellites between 75 and 250 bps which is similar to results reported in other species [24]. Among 12 markers analyzed, seven markers found polymorphic, which implies the high polymorphism of microsatellites between two varieties and their applicability in MAS and QTL analysis studies.

This is the first report on molecular characterization of non-toxic and toxic *J. curcas* using both multilocus and single locus maker systems. The specific markers generated using RAPD and AFLP fingerprinting will help to distinguish non-toxic from toxic varieties of *J. curcas* or vice versa and markers of RAPD and AFLP together with the

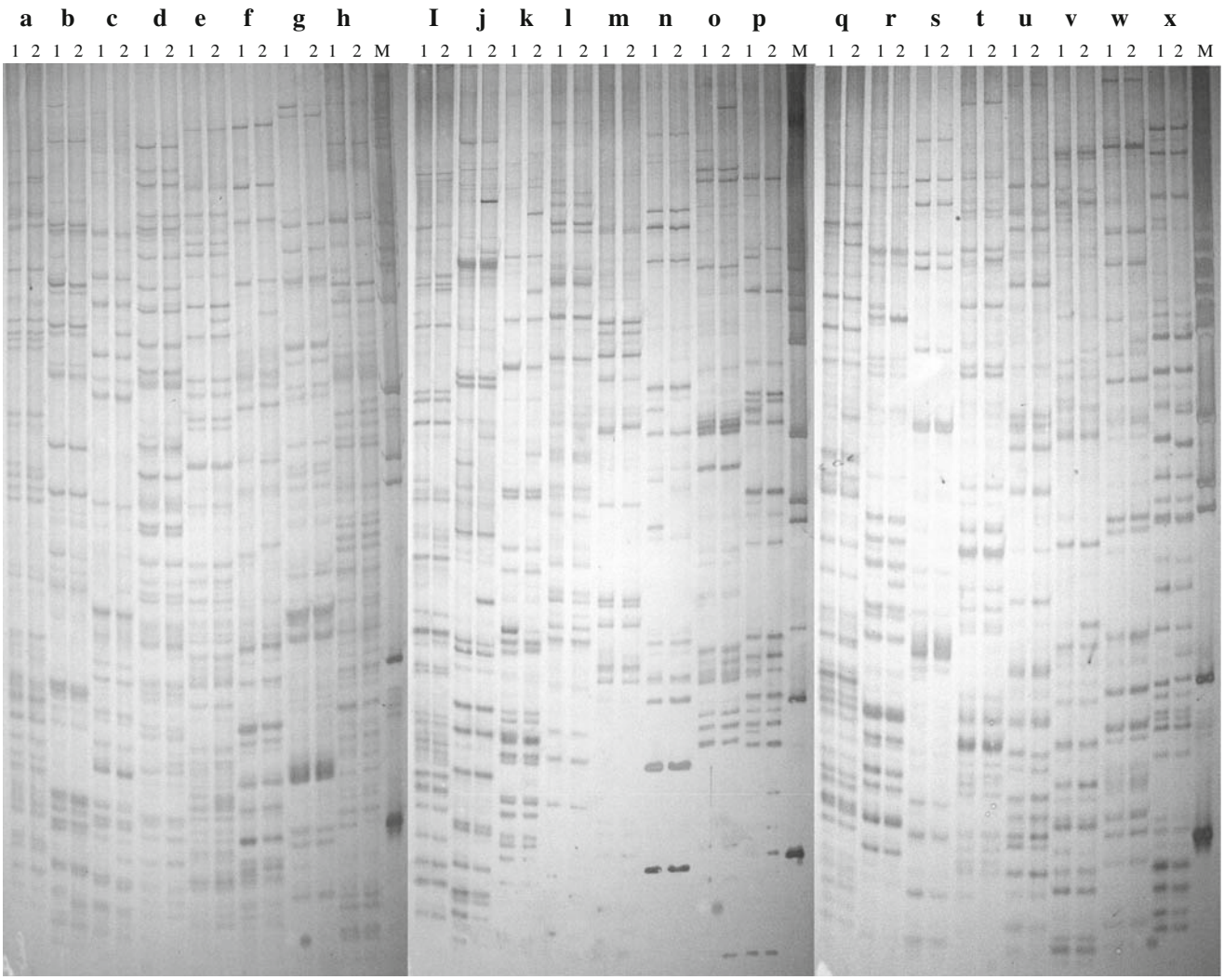


**Fig. 3** 1-Non-toxic variety of *J. curcas*, 2-Toxic variety of *J. curcas*. (**a**–**x**) Selective amplification with AFLP primers. (**a**) E-AAC/M-CAA; (**b**) E-AAC/M-CAC; (**c**) E-AAC/M-CAG; (**d**) E-AAC/M-CAT; (**e**) E-AAC/M-CTA; (**f**) E-AAC/M-CTC; (**g**) E-AAC/M-CTG; (**h**) E-AAC/M-CTT; (**i**) E-ACC/M-CAA; (**j**) E-ACC/M-CAC; (**k**) E-ACC/M-CAG; (**l**) E-ACC/M-CAT; (**m**) E-ACC/M-CTA; (**n**) E-ACC/M-CTC; (**o**) E-ACC/M-CTG; (**p**) E-ACC/M-CTT; (**q**) E-AGC/M-CAA; (**r**) E-AGC/M-CAC; (**s**) E-AGC/M-CAG; (**t**) E-AGC/M-CAT; (**u**) E-AGC/M-CTA; (**v**) E-AGC/M-CTC; (**w**) E-AGC/M-CTG; (**x**) E-AGC/M-CTT)

**Table 2** Characteristics of 12 microsatellite loci in non-toxic and toxic variety of *J. curcas*

| Marker GenBank No. | Primer sequence(5′–3′) | $T_a$ (°C) | Repeat motif | Allele size in range non-toxic (bp) | Allele size in Toxic (bp) |
|---|---|---|---|---|---|
| jcds10 (EU586340) | F:CATCAAATGCTAATGAAAGTACA<br>R:CACACCTAGCAAACTACTTGCA | 46.5 | $(TG)_6CACGCA(TG)_4$ | 108/122 | 108/122 |
| jcds24 (EU586341) | F:GGATATGAAGTTTCATGGGACAAG<br>R:TTCATTGAATGGATGGTTGTAAGG | 51.0 | $(CA)_5(TA)_8(CA)_4…(TA)_3GA(TA)_4$ | 204/210 | 204/216 |
| jcds41 (EU586342) | F: AACACACCATGGGCCACAGGT<br>R:TGCATGTGTGCGGGTTTGATTAC | 56.5 | $(CA)_6(TA)_2$ | 102/114 | 102/114 |
| jcds58 (EU586343) | F:TCCATGAAGTTTGCTGGCAAT<br>R:AGGTCATCTGGTAAAGCCATACC | 54.0 | $(GT)_4(GA)_5$ | 104/112 | 104/112 |
| jcds66 (EU586344) | F:CCTACGAGTGATTGGATAGTTTCTCA<br>R:TCTTCCATCAAGAGTCGTTGGGCA | 54.0 | $(CT)_2(GT)_3ATTGCA(AT)_4$ | 216/228 | 216/228 |
| jcps1 (EU586345) | F:GAGGATATTACAGCATGAATGTG<br>R:5′AATCAATCAATCTTTGGCAAA | 47.5 | $(TG)_4…(GT)_3…(GT)_4$ | 132/162 | 132/162 |
| jcps6 (EU586346) | F:CCAGAAGTAGAATTATAAATTAAA<br>R:AGCGGCTCTGACATTATGTAC | 44.0 | $(AT)_3G(TA)_3…(CT)_3…(GT)_5CT(GT)_3$ | 288/305 | 288/380 |
| jcps9 (EU586347) | F:GTACTTAGATCTCTTGTAACTAACAG<br>R:TATCTCTTGTTCAGAAATGGAT | 48.0 | $(GT)_3GC(TG)_2A(GT)_3$ | 140/132 | 140/132 |
| jcps20 (EU586348) | F:ACAGCAAGTGCACAACAATCTCA<br>R:TACTGCAGATGGATGGCATGA | 55.0 | $(TG)_{12}(GA)_{22}$ | 271/260 | 260/278 |
| jcps21 (EU586349) | F:CCTGCTGACAGGCCATGATT<br>R:TTTCACTGCAGAGGTAGCTTGTATA | 54.8 | $(CA)_2…(CA)_4$ | 189/200 | 189/208 |
| jcms21 (EU586350) | F:TAACCTCTTCCTGACA<br>R:ATAGGAAATAAGAGTTCAAA | 43.0 | $(CA)_7$ | 81/89 | 75 |
| jcms30 (EU586351) | F:GGGAAAGAGGCTCTTTGC<br>R:ATGAGTTCACATAAAATCATGCA | 48.5 | $(GT)_5T(TG)_2$ | 135/144 | 144/148 |

$T_a$, annealing temperature; bp, basepairs

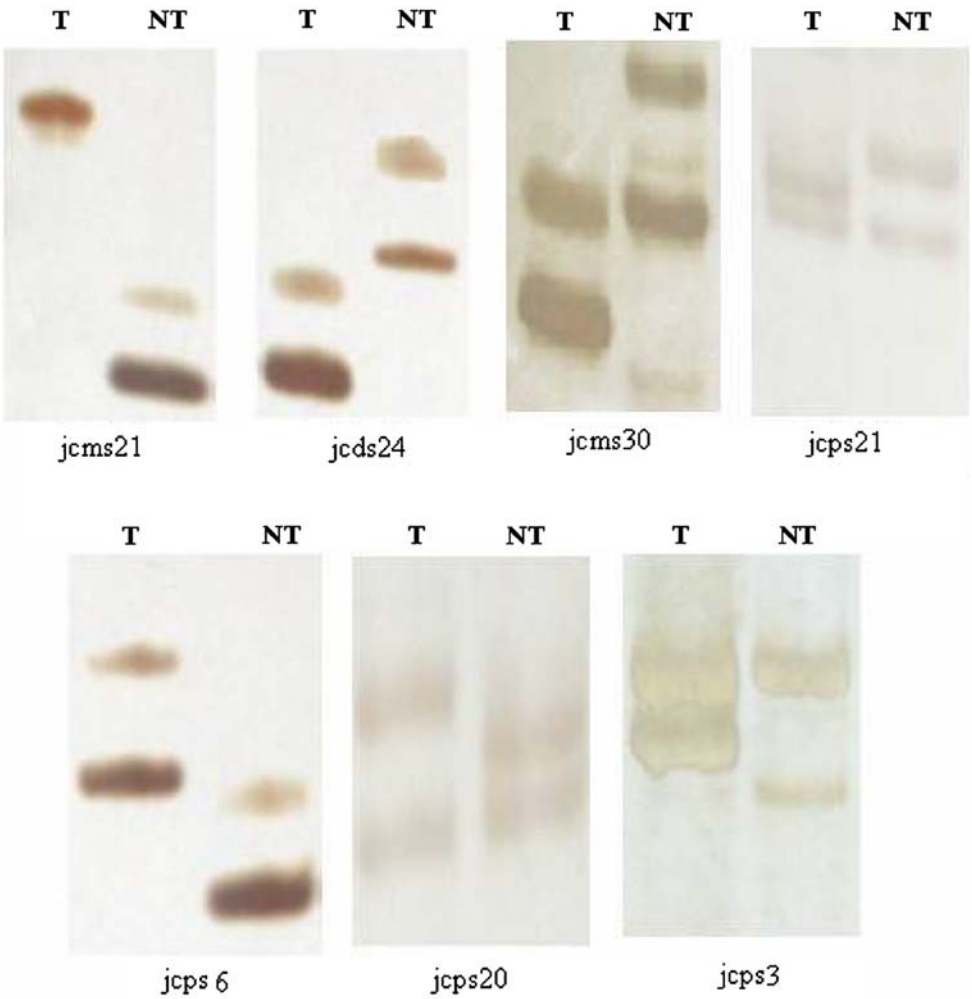


**Fig. 4** Size polymorphism SSRs in toxic (T) and non-toxic (NT) variety of *J. curcas* (jcms21, jcds24, jdms30, jcps21, jcps6, jcps20 and jcps3)

polymorphic SSR markers can be exploited in Marker Assisted Selection (MAS) QTL analysis and for other molecular breeding studies.

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## References

1. Ghosh A, Chaudhary DR, Reddy MP, Rao SN, Chikara J, Pandya JB et al (2007) Prospects for *Jatropha* methyl ester (biodiesel) in India. Int J Environ Stud 64:659–674. doi:10.1080/00207230701766499
2. Takeda Y (1982) Development study on *Jatropha curcas* (Sabudum) oil as a substitute for diesel engine oil in Thailand. J Agric Assoc China 120:1–8
3. Mandpe S, Kadlaskar S, Degen W, Keppeler S (2005) On road testing of advanced common rail diesel vehicles with biodiesel from the *Jatropha curcas* plant. Soc of Automotive Eng Inc 26:356–364
4. Jones N, Miller JH (1991) *Jatropha curcas* a multipurpose species for problematic sites. Land Resour Ser 1:1–12
5. Heller J (1996) Physic nut- *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. International Plant Genetic Resources Institute, Rome, Italy
6. Gubitz GM, Mittelbach M, Trabi M (1999) Exploitation of the tropical oil seed plant *Jatropha curcas* L. Bioresour Technol 67:73–82. doi:10.1016/S0960-8524(99)00069-3
7. Francis G, Edingger R, Becker K (2005) A concept for simultaneous wasteland reclamation fuel production and socio economic development in degraded areas in India need potential & perspectives of *Jatropha* plantation. Nat Resour Forum 29:12–24. doi:10.1111/j.1477-8947.2005.00109.x
8. Makkar HPS, Becker K (1997) Potential of *Jatropha* seed cake as protein supplement in livestock feed and constraints to its utilization. In Proceedings of *Jatropha* 97: International Symposium on Biofuel and Industrial Products from *Jatropha curcas* and Other Tropical Oilseed Plants. Managua/Nicaragua, Mexico, pp 23–27
9. Makkar HPS, Aderibigbe AO, Becker K (1998) Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chem 62:207–215. doi:10.1016/S0308-8146(97)00183-0

10. Martinez-Herrera J, Sibdhiraju S, Francis G, Davila-Ortiz G, Becker K (2006) Chemical composition, toxic/antimetabolic constituents and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. Food Chem 96:80–89. doi:10.1016/j.foodchem.2005.01.059
11. Ginwal HS, Rawat PS, Srivastava RL (2004) Seed source variation in growth performance and oil yield of *Jatropha curcas* Linn in central India. Silve Senetica 53:186–192
12. Sujatha M, Makkar HPS, Becker K (2005) Shoot bud proliferation from axillary nodes and leaf sections of non-toxic *Jatropha curcas* L. Plant Growth Regul 47:83–90. doi:10.1007/s10725-005-0859-0
13. Becker K, Makkar HPS (1998) Effects of phorbol esters in carp (*Cyprinus carpio* L.). Vet Hum Toxicol 40:82–89
14. Sudheer Pamidiamarri DVN, Nirali P, Reddy MP, Radhakrishnan T (2008) Comparative study of interspecific genetic divergence and phylogenic analysis of genus *Jatropha* by RAPD and AFLP. Mol Biol Rep. doi:10.1007/s11033–008–9261-0
15. Williams JG, Kubelik AR, Livak J, Rafalski JA, Tingey SV (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535. doi:10.1093/nar/18.22.6531
16. Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414. doi:10.1093/nar/23.21.4407
17. Makkar HPS, Becker K, Sporer F, Wink M (1997) Studies on nutritive potential and toxic constituents of different provenance of *Jatropha curcas*. J Agric Food Chem 45:3152–3157. doi:10.1021/jf970036j
18. Basha SD, Sujatha M (2007) Inter and intra-population variability of *Jatropha curcas* (L.) characterized by RAPD and ISSR markers and development of population-specific SCAR markers. Euphytica 156:375–386. doi:10.1007/s10681-007-9387-5
19. Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401
20. Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 17:6463–6471. doi:10.1093/nar/17.16.6463
21. Weber JL, Wong C (1993) Mutation of human short tandem repeats. Hum Mol Genet 2:1123–1128. doi:10.1093/hmg/2.8.1123
22. Schuler GD, Boguski MS, Stewart EA (1996) A gene map of the human genome. Science 274:540–546. doi:10.1126/science.274.5287.540
23. Knapik EW, Goodman A, Ekker M (1998) A microsatellite genetic linkage map for zebrafish (*Danio rerio*). Nat Genet 18:338–343. doi:10.1038/ng0498-338
24. Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Mol Ecol 11:1–16. doi:10.1046/j.0962-1083.2001.01418.x