



## GENETIC PURITY ASSESSMENT OF CASTOR HYBRIDS USING EST-SSR MARKERS

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### SUMMARY

Maintenance of genetic purity of a hybrid is essential to exploit its full potential. Genetic purity is generally assessed by conducting grow out test (GOT) where morphological and floral characters are analyzed, however results may be influenced by the environment. Further, it is expensive and time consuming. DNA based markers are rapid, reliable and cost effective for hybrid purity assessment. Several molecular markers such as RAPD, ISSR, AFLP, SSR, EST-SSR are being used for purity determination. Co-dominant markers are preferred over dominant markers as they reveal the heterozygous condition of the hybrid accurately. SSR markers are reliable markers for purity assessment. Because of inherent limitations of SSR markers, EST-SSR markers are considered as high quality markers as they give sharp, clear, and robust amplification. In this study, the parents (DPC-9, CS-1 and CS-18) of the two castor hybrids PCH-111 (DPC-9 x CS-1) and PCH-222 (DPC-9 x CS-18) were screened using 283 EST-SSR markers, nine markers showed polymorphism between DPC-9 and CS-1 and 10 between DPC-9 and CS-18 whereas 3 expressed polymorphism among 3 parents. As castor is highly cross pollinated crop and cytoplasmic male sterility system is not yet reported, the possible impurities in hybrid seed are selfing of female parent and pollination of female parent by unknown pollen parent. Selfed seed of female parent can be identified using single polymorphic marker, however contamination of female parent with unknown male parent can be detected reliably by using more number of polymorphic markers.

**Keywords:** EST-SSR, castor, genetic purity, hybrid

Manuscript received: April 26, 2013; Decision on manuscript: November 2, 2013; Manuscript accepted: December 13, 2013.  
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Communicating Editor: Akshaya K. Biswal

### INTRODUCTION

Castor (*Ricinus communis* L.) is an important non-edible oilseed crop of India. It is typically a monoecious crop and is highly cross pollinated in nature. Identification of completely pistillate plants and presence of exploitable levels of heterosis paved the way for the development of castor hybrids resulting in quantum jump in productivity. India is the pioneer in development and release of castor hybrids wherein first castor

hybrid, GCH-3 based on an exotic pistillate line TSP 10R was developed and released for general cultivation in 1968. Development of indigenous, non-shattering pistillate line, VP-1 gave new impetus to hybrid development program in the country. In course of time several hybrids viz., GAUCH-1, GCH-2, GCH-4, GCH-5, GCH-6, GCH-7, DCH-32, DCH-177, DCH-519, RCH-1, TMVCH-1, PCH-111 and PCH-222 were released with resistance to various biotic and abiotic stresses.

Castor is normally monoecious with pistillate flowers on the upper portion of the raceme and staminate flowers on the lower part. The proportion of pistillate and staminate flowers in different orders of raceme shows wide variation both within and among genotypes. In castor, so far cytoplasmic genetic male sterility (CGMS) system has not been reported. The plants with complete female flowers in different order spikes under femaleness promoting environment (post rainy season, low temperature, adequate nutrition) are used as female in hybrid seed production and such plants are called pistillate plants. Along the raceme of the pistillate plant, interspersed staminate flower (ISF) buds will be developed and these are to be removed regularly to avoid selfing in the female parent during hybrid seed production. As the crop is highly cross pollinated in nature, hybrid seed production should be taken up in non-traditional areas during off season. Isolation distance of 1000 m should be provided to avoid contamination from the other castor plants.

Maintenance of high level of genetic purity of hybrid is essential to exploit high level of heterosis. However, during hybrid seed production, delay in removal of interspersed staminate flower (ISF) buds and stray castor plants within 1000 m radius may reduce the genetic purity of hybrid seed. Therefore, one of the challenges is rapid and accurate assessment of the purity of hybrid seeds before they are supplied to farmers.

Most widely used method for determination of genetic purity is the grow out test (GOT) that involves growing of representative sample of the seed followed by analysis of several morphological and floral characters that distinguishes the hybrid. But it is a time and resource consuming exercise, and is also influenced by environmental factors (Noli *et al.*, 1999). Hence, it is essential to develop more rapid, accurate and cost effective method for assessing the genetic purity of hybrid.

Simple sequence repeats (SSR), is a class of genetic marker that has proven to be abundant and well distributed throughout the genome of plants (Wu and Tanksley, 1993; Chin *et al.*, 1996 and Wu *et al.*, 2006). They are co-dominant, detect high levels of allelic diversity, and are assayed easily by the polymerase chain

reaction (PCR), they have been used for the assessing purity of F<sub>1</sub> hybrid in rice (Yashitola *et al.*, 2002; Nandakumar *et al.*, 2004; Sundaram *et al.*, 2008), maize (Wu *et al.*, 2006), sunflower (Antonova *et al.*, 2006) and horticultural crops like tomato (Smith and Register 1998), cabbage (Liu *et al.*, 2007) and melon (Jianli *et al.*, 2006). However, development of SSR markers is costly, time consuming and labour intensive. Availability of EST (expressed Sequence tags) in public databases, the development of EST based SSR markers through data mining has become a fast, efficient and relatively inexpensive method compared with the development of genomic SSRs (Gupta *et al.*, 2003). EST-SSR markers have been developed in several crops such as wheat, maize, rice, barley, sugarcane, sorghum, pearl millet, cotton, citrus, coffee, sunflower, safflower, soyabean, castor etc. In the present study EST-SSR markers of castor were utilized for the assessment of genetic purity of two castor hybrids PCH-111 and PCH-222.

## MATERIALS AND METHODS

### Plant material

Two high yielding wilt resistant castor hybrids PCH-111 (DPC-9 x CS-1) and PCH-222 (DPC-9 x CS-18) were developed and released from Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Palem, Andhra Pradesh, India during 2010 and 2012, respectively. Female parent is common for both the hybrids. The hybrids are under cultivation in different parts of India.

### DNA Extraction

Fifty seedlings from each parental line (DPC-9, CS-1 and CS-18) and 100 seedlings of hybrids (PCH-111 and PCH-222) were raised and genomic DNA was isolated from the young tender leaves following the standard CTAB method protocol with minor modifications (Doyle and Doyle, 1987). The isolated DNA quantification was done by using a Nanodrop Spectrophotometer as well as by agarose gel

electrophoresis using known amount of lambda DNA as standards.

### EST-SSR marker polymorphism

A total 283 EST SSR markers developed at Directorate of Oilseeds Research (DOR), Hyderabad were used for polymorphism survey of three parental lines. The link to the source of EST data base is (<http://www.ncbi.nlm.nih.gov/sites/entrez>).

### PCR amplification

PCR was performed with 10 µl reactions containing 1.0 µl of 10X reaction buffer containing 2.0 mM MgCl<sub>2</sub>, 0.1 mM dNTP, 10 pmoles of each forward and reverse primer, 50 ng/µl of template DNA and 1.0 U of *Taq* DNA polymerase. The thermal profile followed was 94 °C for 4 min (initial denaturation), followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, extension at 72 °C for 1 min, and a final extension of 5 min at 72 °C. All PCR products were separated on 3% (w/v) agarose gels (Lonza), stained with ethidium bromide and visualized under UV.

## RESULTS AND DISCUSSION

First commercial castor hybrid in the world GCH-3 was released in India in 1968. Due to high level heterosis in the hybrids, there is a large demand for quality hybrid seed. Involvement of public and private sectors in large scale hybrid seed production demands quality control in both parental and hybrid seed production stages. As castor is a highly cross pollinated crop, pollen of unknown parent may contaminate the female parent during hybrid seed production, leading to decline in genetic purity. Apart from this, selfing of female parent is another source of contamination since cytoplasmic genetic male sterility system (CGMS) is not yet reported in castor. Removal of male bud from the female parent before opening is the crucial aspect in quality seed production. Genetic purity up to 85% is essential for certified seed (hybrid) as per the certification standards in India. Genetic purity of castor

hybrid is assessed by grow out test i.e., raising the representative samples up to 60 days. The important morphological features taken into consideration are stem color (red and green), internodal length (elongated and condensed), bloom (zero, single, double and triple) and spike nature (spiny, semi-spiny and non-spiny). Interestingly, all these characters are governed by single genes with independent assortment (Gourishankar *et al.*, 2010).

After seed production, produce has to be kept for 60 days for quality assessment which is laborious, expensive and time consuming. Considering the disadvantages of grow out test, rapid and reliable methods using molecular markers are getting attention for genetic purity testing. Now several molecular markers i.e., RAPD, ISSR, AFLP, SSR etc. are being used. SSR markers are extensively used because of their co-dominant nature however, these are species specific and development of genomic SSRs is expensive, skill oriented and time consuming. Availability of ESTs in public databases, the development of EST based SSR markers through data mining has become a fast, efficient and relatively inexpensive method compared with the development of genomic SSRs.

In the present study genomic DNA was extracted from the hybrids PCH-111 and PCH-222, and their parents DPC-9, CS-1 and CS-18. Two hundred and eighty three EST-SSR markers were screened with the DNA of parental lines. Since EST-SSR markers are co-dominant, only one allele was detected in a hybrid when the parents were monomorphic for a particular microsatellite locus and two alleles (one allele per parent) were present in a hybrid when polymorphism was detected between the male parent and female parent. Out of 283 EST-SSR markers tested nine markers (RCE SSR 16, 32, 59, 82, 132, 192, 206, 256 and 278) showed polymorphism between DPC-9 and CS-1, and 10 between DPC-9 and CS-18 (RCE SSR 24, 32, 43, 59, 68, 110, 132, 156, 178 and 234) whereas 3 (RCE SSR 32, 59 and 132) expressed polymorphism among 3 parents (Table 1). In order to test the reliability of EST-SSR marker, 16 markers were confirmed using DNA from 50 individual plants of the parents. These polymorphic EST-SSRs were then assessed

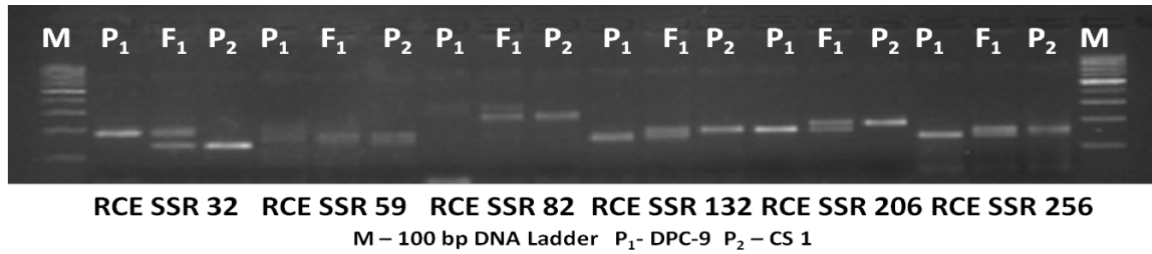
using bulk DNA of both hybrids and parents. For all the markers, parents showed single allele whereas hybrid showed both the parental alleles indicating the heterozygosity of the hybrid. The markers were further tested with DNA of 100 individual plants to assess their utility in hybrid purity assessment. All the individual hybrid plants showed both the alleles specific to the parents confirming the utility of these markers in genetic purity assessment of the hybrids (Figures 1 to 4).

Single polymorphic marker should be sufficient to ascertain the genetic purity of the

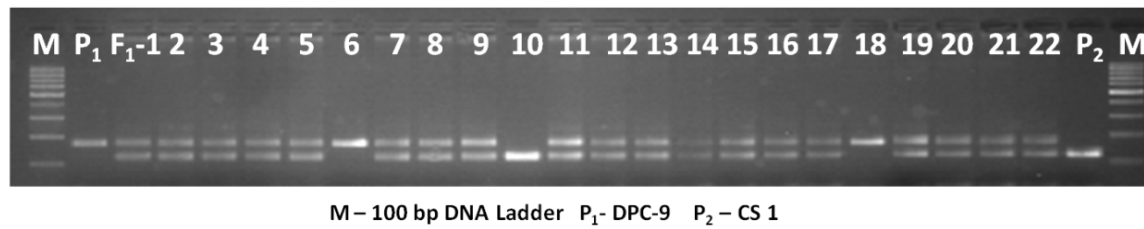
hybrids. But in cross pollinated crops like castor, more polymorphic markers should be used. Naresh *et al.* (2009) reported that in safflower two markers SES-30 and SES-33 showed similar banding pattern for both the hybrids NH-15 and NH-1 and thus cannot be used for distinguishing the two hybrids in case they contaminate each other. Admixtures of the parental lines can be identified using single polymorphic marker, however contamination of female parent with unknown male parent can be detected reliably by using more number of polymorphic markers.

**Table 1.** Details of the polymorphic EST-SSR markers identified in the study.

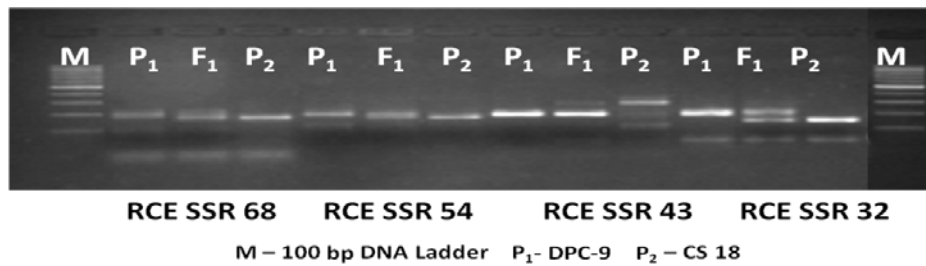
S. No	Marker	Repeat	Forward Primer	Reverse Primer	Product Size
1	RCE SSR 16	(GGT) <sub>5</sub>	AAGTGATCCAGAAAAGCGTG	TGCACTTCGAGCAGATAACA	275
2	RCE SSR 24	(GCCATA) <sub>4</sub>	ACTGCCTAAGATGAGCGATTAG	GTTCTCGTGGATCTTATCATGG	127
3	RCE SSR 32	(TGA) <sub>7</sub>	GAGTCTTCCACAAAACAAAAGG	CCTCACTCTCAGAATCACCTTC	196
4	RCE SSR 43	(TC) <sub>5</sub> -(CT) <sub>5</sub>	AATGGCGGCTTCTTCTTC	GACGACATAGCAGAGACCGT	247
5	RCE SSR 59	(AAC) <sub>8</sub>	AAACTGCACAACAACAAAGG	CTCTCTCACTGATTGATATTGC	179
6	RCE SSR 68	(CAC) <sub>6</sub>	CCCCCACAGGTACAAGATTA	AGAGGGCCCACCACTATAAC	261
7	RCE SSR 82	(AGC) <sub>8</sub>	AGTAAAAGAGACGTTGCATACC	GGAGGAAATATACCTGACTGC	281
8	RCE SSR 110	(ACC) <sub>7</sub>	AAACATCAGTTTCCAGAACGAC	CCCTTTAAGCACTGTTTTATCG	230
9	RCE SSR 132	(TGC) <sub>8</sub>	CCAACACTTCTCAGCTATATCC	GATATTATGGATACGCACAAGG	171
10	RCE SSR 156	(CAG) <sub>5</sub>	CACCTCCATCGTTTCACTTC	ATTGTCTGGCCTATTCTCC	129
11	RCE SSR 178	(AT) <sub>11</sub>	CATCTTCTTCATCATCTTTCCC	GTTAGGGTTAAAGATTCATGCG	195
12	RCE SSR 192	(GGGGC) <sub>4</sub>	AATCTGCCCTCCATCTTAC	TTGCTATAACCTCCACCAATTC	394
13	RCE SSR 206	(GTG) <sub>5</sub> -(GAG) <sub>6</sub>	CTCCTCTTCCATAAACCGAT	CCCTACCTCTGCTAATGCAA	193
14	RCE SSR 234	(TGGAGG) <sub>6</sub>	GTAGCCCTAACAATTATCGTGC	GTTTTTGACCAGATAGAGGCTG	231
15	RCE SSR 256	(CGG) <sub>7</sub>	TCTACAAGCCTATAATGCCACC	CTTCCACGTTTCTCTTAATTG	181
16	RCE SSR 278	(TTCAGA) <sub>4</sub>	AATCCTCCTCACTCTTTATCCG	TGAATTTGAAGGAGCGTAGG	217



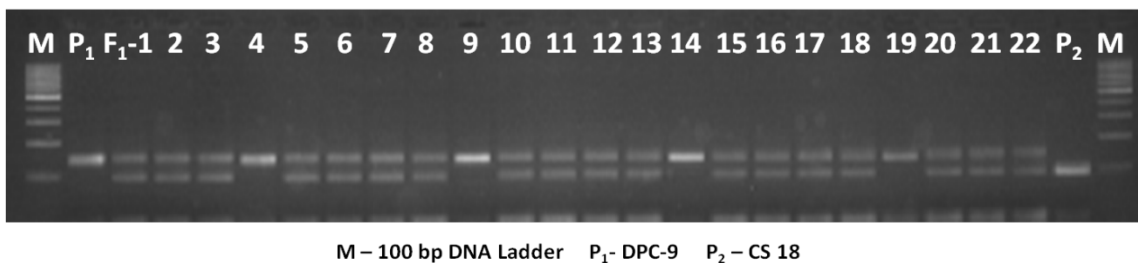
**Figure 1.** Polymorphic EST SSR markers between two parents of the castor hybrid PCH-111.



**Figure 2.** Single seedling assay for detecting genetic purity of the castor hybrid PCH-111 using RCE SSR 32.



**Figure 3.** Polymorphic EST SSR markers between two parents of the castor hybrid PCH-222.



**Figure 4.** Single seedling assay for detecting genetic purity of the castor hybrid PCH-222 using RCE SSR 32.

## ACKNOWLEDGEMENTS

The work was supported by Rashtriya Krishi Vikas Yojana (RKVY), Government of India.

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