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Assessment of Genetic Purity of commercially cultivated Hybrid vegetable crops of solanaceae Family using SSR Molecular Markers

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Abstract - *The economically important vegetable crops of solanaceae family are Chilli, tomato and brinjal. The ultimate identity of any variety is of its genetic composition, achieving and maintaining genetic purity in crops is of importance for both agronomy and plant breeding programs. The present study was carried out to identify polymorphic SSR primers for one variety of each crop of Chilli, Tomato and Brinjal, that can distinguish between the hybrids & parental lines and further utilized for assessment of genetic purity of the hybrids. Ten SSR primers for each crop was used in this study , out of which 3 primers of chilli, 4 primers of tomato and 2 primers of brinjal were able to distinguish the hybrid parental lines among which only one prominent polymorphic primer were used to test the genetic purity. A total of 93 hybrid individuals of each crop were analyzed. The results of the SSR marker analysis showed that 1 out of 93 F1 plants of chilli, 4 out of 93 F1 plants of tomato and 55 out of 93 F1 plants of brinjal are genetically impure and the overall genetic purity of the three F1 hybrid seed lots were 98.9%, 95.7% and 40.2% respectively. This study showed that a single appropriate SSR marker could be used as an efficient tool in quality control of commercial hybrid seeds for assessing hybrid seed purity over the conventional Grow out test.*

Key Words: **Solanaceae, Grow-Out-Test, Genetic purity, SSR, Efficient tool**

1. INTRODUCTION

The family of solanaceae has more than 90 genera and nearly 3,000 species distributed throughout the world. Chilli (Capsicum annuum) is an important spice cum vegetable crop belonging to solanaceae family grown in the tropical, subtropical as well as temperate regions. India is the largest producer and consumer of chillies in the world with the annual production of around 1.1 million tonnes [7]. Tomato (Solanum lycopersicum L., formerly Lycopersicum esculentum Mill.) ranks second to potato in the worldwide commercial distribution and is a significant vegetable crop of special economic importance [3]. Brinjal or eggplant (Solanum melongena L.) is one of the important solanaceous vegetable crops after potato and tomato in India, which attributes 8.23% of the total vegetable production (Government of India, 2014).

At present, hybrids are of much importance because of their high yield and resistant to pest and diseases. Hence in seed industries, hybrid seed production has become dominant and the most important aspect is the hybrid seed purity, which is been checked by grow-out-test (GOT). However, it is environment dependent and may vary the expression of specific morphological or physiological traits. Apart from that, major limitation factor of GOT will be the time and space required for assessing a large number of seed lots. Thus, an alternative and reliable method to evaluate the genetic purity of the seed samples is required to benefit the seed industries to assure high quality standards.

The availability of different molecular markers like RAPD, ISSR, RFLP, SSR etc which are based on DNA sequence variation would provide unbiased means for identification of crop varieties. Among all these currently available DNA-based markers, the microsatellite (SSR) markers are most widely used for rapid genetic purity assessment of parental lines and its hybrids due to its co-dominant nature and their abundance and uniform distribution throughout the genome [1], [4], [5], [8].

DNA molecular marker techniques exhibit great potential for determining genetic purity of F1 hybrids. In this present study, the molecular marker system of SSR was employed for identifying three hybrids of chilli, tomato and brinjal and its respective parental lines for their utility in assessing the genetic purity. In addition, these markers were validated for the purpose of testing the genetic purity of the commercial seed lots. An efficient, reliable and accurate method was established for rapid testing of genetic purity of commercial hybrids compared to the grow-out test.

2. MATERIALS AND MATHODS

2.1 Plant Materials

The leaf material of all the plant samples used in this study was collected from young plantlets of only 15 to 20 days old. Three commercial hybrids of chilli, tomato, brinjal and its respective parental lines were used for the purpose of molecular identification (Table 1) and a random sample of 93 seeds, representing the commercial F1 hybrid seed lot was used for testing their genetic purity using specific SSR marker.

Table -1: Details of the hybrids and their parental lines used in this study

Sl. No	Crop	Hybrid line	Parental line (Female)	Parental line (Male)
1	Chilli (Capsicum annuum)	CA-Hy-01	CA-01-F	CA-01-M
2	Tomato (Solanum lycopersicum)	LE-Hy-02	LE-02-F	LE-02-M
3	Brinjal (Solanum melongena)	SM-Hy-03	SM-03-F	SM-03-M

2.2 DNA Extraction

The F1 seeds were germinated in the trays for molecular analysis. DNA of the parental lines and individual F1 leaf samples were extracted by a simple protocol according to Wang et al., 1993 [2] for PCR amplification.

2.3 SSR Molecular Analysis

10 sets of SSR markers specific for each crop of chilli, tomato and brinjal has been used for the study and their primer sequences were obtained from Solanaceae genome network (SGN; http://www.sgn.cornell.edu) and Nunome et al., 2009 [6].

2.4 PCR amplification and gel electrophoresis

For SSR analysis, 20µL of PCR reaction for individual samples were set with 3 µL of crude DNA lysate, 10µL of 2 X ampliqon PCR master mix, 0.5µL of each forward and reverse primer at 10pmol concentration and for the remaining, 6µL of autoclaved HPLC grade water was added to the reaction.

The PCR was carried out in 2720 Thermal Cycler of Applied Bio systems by Life technologies. The conditions used for PCR amplification of chilli and tomato samples were: initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 25 sec, annealing at 52°C for 25 sec, extension at 72 °C for 30 sec and final extension at 72°C for 5 min while for the brinjal samples the annealing temperature was set at 60°C and all other parameters remains the same as like chilli and tomato samples. PCR amplified products were run on 3.5% agarose gel at 150 V in 1X SB buffer for 60 min and stained with Ethidium bromide for gel documentation.

3. RESULTS AND DISCUSSION

3.1 SSR Analysis

A total of 30 SSR primers, with 10 primers for each crop were employed for testing polymorphic loci between three hybrid cultivars CA-Hy-01, LE-Hy-02, SM-Hy-03 and their corresponding parental lines. Out of these three primers for chilli (Set 6, 7 & 8), four primers for tomato (Set 3, 6, 9 & 10) and two primers for brinjal (Set 3 & 8) were able to distinguish between the parental lines (Fig 1). Out to which only one prominent SSR marker per crop was fixed and that was used for further studies of genetic purity assessment of the commercial hybrid lots. Upon subjecting these fixed primers on hybrids, it was observed that the CAMS647, AI491173 and emb01M15 was able to differentiate both the parents with its respective hybrids CA-Hy-01, LE-Hy-02 and SM-Hy-03 (Table 2).

Table -2: SSR marker specific for the hybrids in the study

SSR Marker	Primer Sequence 5'-3'	Size of allele (bp)	
		F	M
CAMS647	F-CGGATTCGGTTGAGTCGATA R-GTGCTTTGGTTCGGTCTTTC	190	240
AI491173	F-GCACGAGCACATATAGAAGAGAATCA R-CCATTTCATCATATCTCTCAGCTTGC	200	250
Emb01M15	F-GCAAGGCTCAAAGTCACAAGTCAA R-GGCTCTGCCCCTAACATCTACAAA	230	270

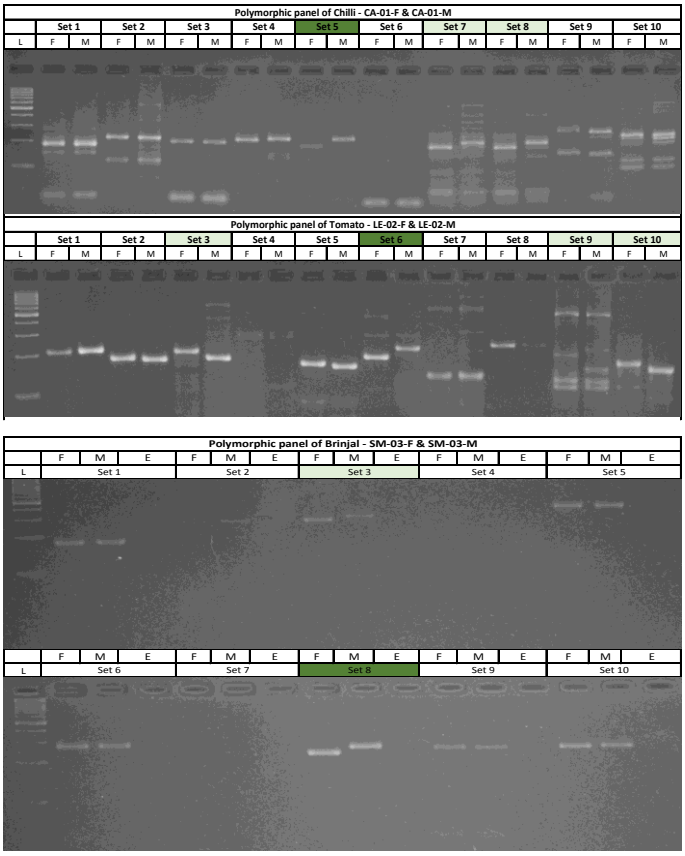

Fig - 1: Polymorphism profile between parental lines of hybrids using SSR markers

Note: Lane L – 100bp DNA ladder; Lane F – Female parent; Lane M – Male parent; Lane E – Empty lane

3.2 Assessing Genetic purity of the hybrids:

To test the genetic purity of the hybrids, the identified primers, CAMS647, AI491173 and Emb01M15 were subjected on 93 F1 plant samples of CA-Hy-01, LE-Hy-02 and SM-Hy-03 respectively. Out of which 92 plants of CA-Hy-01 were heterozygous with the primer CAMS647 amplifying both amplicons corresponding to the parents and only one individual was homozygous with a female specific allele (Fig 2), 89 plants of LE-Hy-02 were heterozygous with the primer AI491173 and four individuals were homozygous with a female specific allele (Fig 3), while 53 individuals of SM-Hy-03 were homozygous with a female specific allele, 2 individuals were homozygous with a male specific allele and the remaining 37 individuals were heterozygous with the primer Emb01M15 (Fig 4) and (Table 3).

Table - 3: Genetic purity of hybrids determined by identified SSR markers

Hybrid	No. of Female off-type	No. of Male off-type	No. of Putative Hybrid	Genetic Purity %
CA-Hy-01	1	0	92	98.9
LE-Hy-02	4	0	89	95.7
SM-Hy-03	53	2	37	40.2

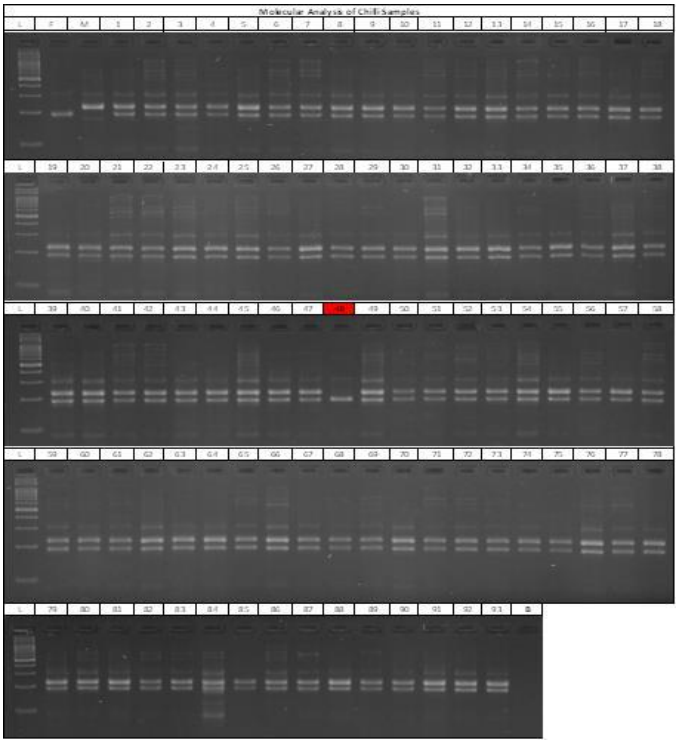

Fig - 2: SSR analysis of CA-Hy-01 individuals with CAMS647 primer

Note: Lane L – 100bp DNA Ladder; Lane F – Female parent; Lane M – Male parent; Lane B – Blank (negative control); Lane 1-93 – F1 individual samples of CA-Hy-01 and lanes marked red are female off-types.

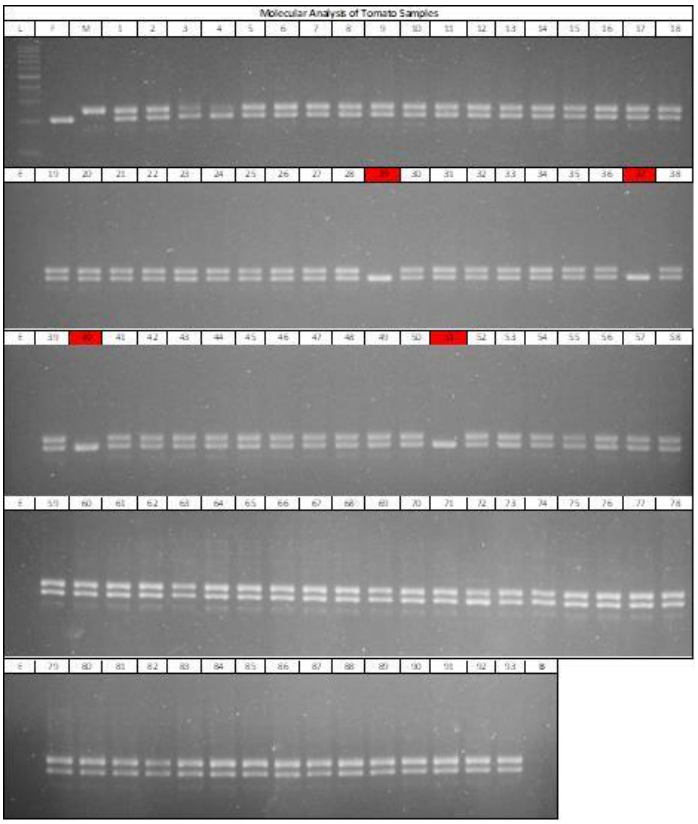

Fig - 3: SSR analysis of LE-Hy-02 individuals with AI491173 primer

Note: Lane L – 100bp DNA Ladder; Lane F – Female parent; Lane M – Male parent; Lane E – Empty; Lane B – Blank (negative control); Lane 1-93 – F1 individual samples of LE-Hy-02 and lanes marked red are female off-types.

The genetic purity percentage of CA-Hy-01, LE-Hy-02 and SM-Hy-03 hybrids were observed to be 98.9%, 95.7% and 40.2% respectively, which were in accordance with the purity observed in the field grow-out test. Thus the present study successfully established that the utility of SSR molecular marker system are effective for ensuring genetic identity and purity of the hybrids and its parental lines. The high percentage of off-types observed in the commercial lots may be due to mixing by handling error or due to missed emasculation of flowers in the female parental line that result in selfed seeds.

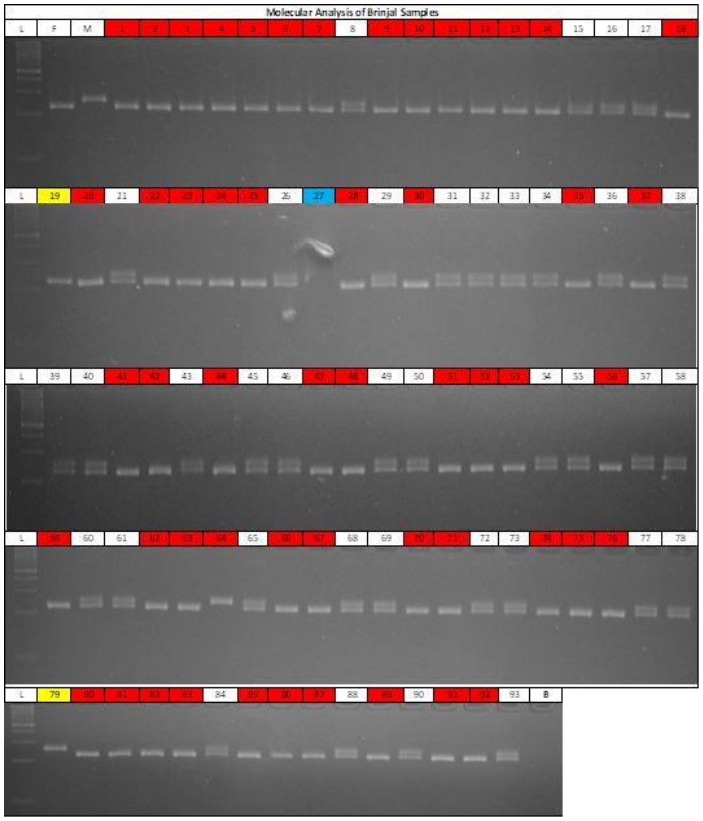

Fig - 4: SSR analysis of SM-Hy-03 individuals with Emb01M15 primer

Note: Lane L – 100bp DNA Ladder; Lane F – Female parent; Lane M – Male parent; Lane B – Blank (negative control); Lane 1-93 – F1 individual samples of SM-Hy-03 and lanes marked red are female off-types; lanes marked yellow are male off-types and lanes marked blue are error in run.

4. CONCLUSION

In the present study three SSR markers were identified that could be effectively used to test the genetic identity and purity of three hybrids of chilli, tomato and brinjal studied. Multiple markers identified for single hybrid could be useful to reveal more information on genetic level and increases the reliability of results obtained by the molecular markers. Genetic purity of commercial hybrid seeds assessed by SSR marker revealed the contamination percentage of female and male off-types in the lots, that would be useful for further stringency in performing hybrid seed production procedures to ensure the seed purity.

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