

# DETERMINING GENETIC PURITY OF COMMERCIAL HYBRIDS BELONGING TO CUCURBITACEOUS FAMILY USING MICROSATELLITE MARKERS

<sup>1</sup>Kiruthika .S, <sup>2</sup>Padmanabha B.V

<sup>1</sup>Research Associate, <sup>2</sup>Chief Scientific Officer

<sup>1</sup>Department of Research and Development,

<sup>1</sup>OrrisLife Sciences Private Limited, Bangalore, India

**Abstract:** The cucurbitaceae family ranks highest among all the plant families for their number and percentage of species used as human food. It is important for any seed companies to examine the genetic purity before selling. This assures the uniformity and stability of the field performance. Grow out test (GOT) for genetic purity is been traditionally conducted by the many seed companies but lacks accuracy and time consuming. Advanced molecular tools such as microsatellite markers can be used for such purity test. In the present study, PCR based SSR molecular marker technology was utilized in order to determine the genetic purity of the cucurbits (cucumber, musk melon and bitter gourd) F1 seeds. The results clearly indicated that, among Fifteen SSR primers analyzed for each variety of the crops, one pair of primer showed polymorphism with single alleles in the parental lines and both the alleles of parents in the hybrids, indicating the heterozygosity. Among 93 F1 seeds of each crop that were analyzed using the polymorphic SSR primer 27 of cucumber, 10 of musk melon and 19 of bitter gourd were known to be off-types resulting in 70.7%, 89.2% and 79.6% of genetic purity respectively. This study showed that PCR based microsatellite marker technology is as an efficient tool in determining seed purity of the F1 hybrids over the conventional Grow out test.

**Index Terms - Genetic purity, Microsatellite markers, Cucurbits, F1 Hybrids**

## I. INTRODUCTION

The Cucurbitaceae, also called cucurbits and gourds are the plant family consisting of about 965 species in around 95 genera. Cucurbita (gourds), Citrullus (water melon and others), Cucumis (cucumber and other melons) are most important among the cucurbitaceae and are grown around the tropics and in temperate regions. Cucumber (*cucumis sativus*) is a widely cultivated plant in the gourd family and is the fourth most important vegetable worldwide (Tatlioglu T., 1993). Muskmelon (*Cucumis melo*) is a species of melon that has been developed into many cultivated varieties. It is one of the most nutritive and commercially important cucurbit in the world with United States as the world's largest consumer (Boriss *et al.*, 2014). Bitter gourd or bitter melon (*Momordica charantia*) is widely grown in Asia, Africa and the Caribbean for its edible fruit. Among the cultivated cucurbits, bitter gourd has been identified as one of the potent vegetables for export by Agricultural Processed Food Products and Export Development Authority (APEDA).

It is mandatory for the commercialization of hybrids to examine the genetic purity, to assure uniformity and stability of field performance and yield before exporting or selling into the market. Maintaining purity at the genetic level is of utmost importance that helps to exploit the full potential of the hybrids. When the seeds are passed from one generation to another, genetic contamination may occur and if it is unnoticed in the population it may lead to genetic deterioration of the variety. Therefore, a dependable method to differentiate between the cultivars as well as to determine genetic purity of the seed samples will enable seed producers to observe and maintain adequate levels of genetic purity at each generation of seed production and multiplication, which would ultimately ensure high quality seeds.

Currently, the conventional method of grow-out test is being used for assessing the genetic purity which requires lots of man power, resources and time. Thus an alternative way to overcome limitations of GOT and to speed up the testing procedures is to use DNA markers which are environmental independent, quick and reliable (Ali *et al.*, 2008 and Rakshit *et al.*, 2010). DNA markers are becoming more popular and effective for genetic diversity characterization, diversity assessment for choosing right parental lines and for determining genetic purity of the variety.

Among the available DNA markers like RAPD, RFLP, ISSR, AFLP etc, microsatellites or SSR (Simple Sequence Repeat) markers have gained considerable importance in plant genetics and breeding due to their co-dominant, multi-allelic nature, wide genome coverage and ease of detection by Polymerase Chain reaction (PCR). SSR are known to have high heterozygosity values and are of more informative than dominant DNA markers. They are widely used for genetic purity analysis of hybrids and their parents (Li OJ. *et al.*, 2015 and Ai cheng *et al.*, 2006).

In the present study, the PCR based DNA marker system of microsatellite (SSR) was employed for identifying three hybrids of cucumber, muskmelon and bitter gourd and its respective parents for their utility in determining the genetic purity. These markers were also validated for the purpose of testing the genetic purity of the commercial F1 seed lots. An efficient, reliable and accurate method was established for rapid testing of genetic purity of commercial hybrids when compared to the GOT.

## II. RESEARCH METHODOLOGY

### 2.1 Materials and Methods

#### 2.1.1 Plant Materials

The leaves of all the plant samples used in this study were collected from plantlets of 15 to 20 days old. Three commercial hybrids of Cucumber, Musk melon, Bitter gourd belonging to cucurbitaceous family and their respective parental lines were used for the purpose of molecular analysis (Table 1) and a random sample of 93 seeds from each crop, representing the commercial F1 hybrid seed lot was used for genetic purity analysis using specific microsatellite 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	Cucumber ( <i>Cucumis sativus</i> )	CS-Hy-01	CS-01-F	CS-01-M
2	Musk melon ( <i>Cucumis melo</i> )	CM-Hy-02	CM-02-F	CM-02-M
3	Bitter gourd ( <i>Momordica charantia</i> )	MC-Hy-03	MC-03-F	MC-03-M

#### 2.1.2 DNA Extraction

The random samples of F1 seeds were germinated under sterile conditions in the trays for molecular analysis. A simple DNA extraction procedure according to Wang *et al.*, 1993 was carried out to extract DNA of the parental lines and the F1 samples for PCR amplification.

#### 2.1.3 Microsatellite Marker Analysis

15 sets of microsatellite markers specific for each crop of Cucumber, Musk melon and Bitter gourd have been used for the study and their primer sequences were obtained from Cucurbits genomic database (<http://cucurbitgenomics.org/>), Swathi saxena *et al.*, 2014 and Lv J *et al.*, 2012.

#### 2.1.4 PCR amplification and gel electrophoresis

For SSR molecular analysis, 20µL of PCR reaction for individual samples were set with 3 µL of crude DNA lysate, 10µL of 2 X ampliçon 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 cucumber, bitter gourd and musk melon samples were: initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 25 sec, annealing at 55°C for 25 sec, extension at 72 °C for 30 sec and final extension at 72°C for 5 min. PCR amplified products were resolved on 3.5% agarose gel at 150 V in 1X SB buffer for 60 min and stained with Ethidium bromide for gel documentation.

## III. RESULTS AND DISCUSSION

### 3.1 SSR Analysis

A total of 45 SSR primers, with 15 primers for each crop were utilized for analyzing polymorphic loci between three hybrid cultivars CS-Hy-01, CM-Hy-02, MC-Hy-03 and their respective parental lines. Out of 45 SSR primers tested, SSR16695, DM0173 and McSSR\_20 were able to differentiate both the parents and its hybrids CS-Hy-01, CM-Hy-02 and MC-Hy-03 respectively (Table 2) and (Fig 1). These polymorphic SSR markers were used for future studies. In this study, SSR markers amplified single allele in the parental lines and both the alleles corresponding to the parents in the hybrids.

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

Crop	Hybrid	SSR Marker	Primer Sequence 5'-3'	Size of allele (bp)	
				Female	Male
Cucumber ( <i>Cucumis sativus</i> )	CS-Hy-01	SSR16695	F-GGACTAGAAACACAATCCCACG R-GTTTGGTTTGTCTCAAGTAGGTTT	170	200
Musk Melon ( <i>Cucumis melo</i> )	CM-Hy-02	DM0173	F-ATCGTCAGTCACCTTTTTTC R-AAGGAGGAGTTTGTGAAG	250	280
Bitter gourd ( <i>Momordica charantia</i> )	MC-Hy-03	McSSR_20	F-GGAATTCAGGTGAACCTGACG R-CCAGGAGGAAGAGGAAGTGC	200	210

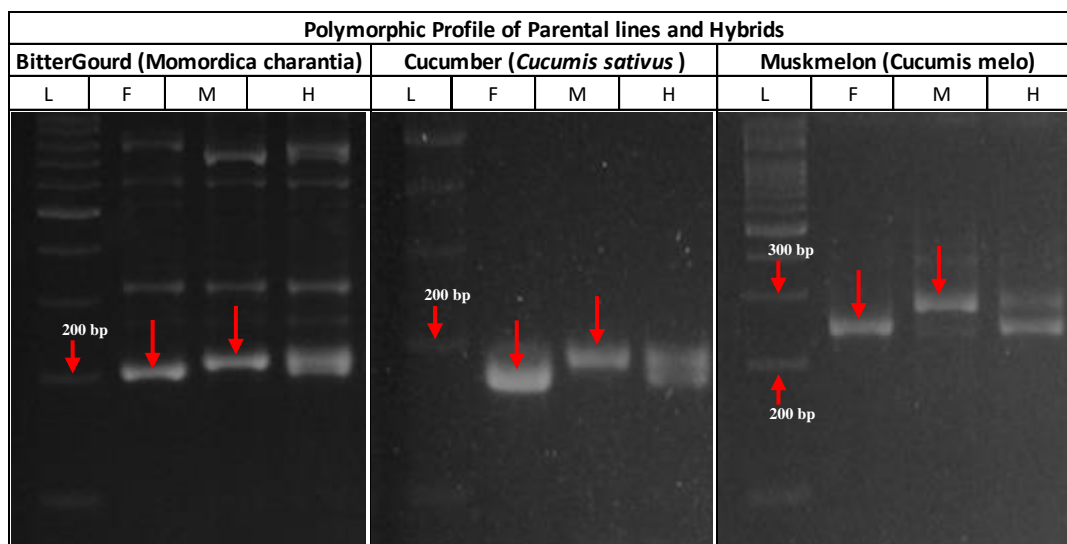


Figure 1: Polymorphism profile between parental lines and their hybrids using SSR markers

Note: Lane L – 100bp DNA ladder; Lane F – Female parent; Lane M – Male parent; Lane H – Hybrid

### 3.2 Assessing Genetic purity of the hybrids:

To analyze genetic purity of the hybrids, the primers namely SSR16695, DM0173 and McSSR\_20 were subjected on 93 individual F1 plant samples of CS-Hy-01, CM-Hy-02 and MC-Hy-03 respectively. Out of 93 F1 plants of CS-Hy-01 analyzed, 65 plants were heterozygous with the primer SSR16695 amplifying both amplicons corresponding to the parents, one individual was not amplified, two individuals were homozygous with a female specific allele and 25 individuals were homozygous to male allele (Fig 2). Out of 93 F1 plants of CM-Hy-02 tested, 83 plants were heterozygous with the primer DM0173 and 10 individuals were homozygous with a female specific allele (Fig 3), while out of 93 F1 samples of MC-Hy-03 plants assessed, 18 individuals were homozygous with a female specific allele, only one individual was homozygous with a male specific allele and the remaining 74 individuals were heterozygous with the primer McSSR\_20 amplifying both the bands corresponding to the parents (Fig 4) and (Table 3).

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

Crop	Hybrid	SSR marker	No. of Female off-type	No. of Male off-type	No. of Putative Hybrid	Genetic Purity %
Cucumber	CS-Hy-01	SSR16695	2	25	65	70.7
Muskmelon	CM-Hy-02	DM0173	10	0	83	89.2
Bitter gourd	MC-Hy-03	McSSR_20	18	1	74	79.6

The percentage of genetic purity of the hybrids CS-Hy-01, CM-Hy-02 and MC-Hy-03 were determined to be 70.7%, 89.2% and 79.6% respectively which were observed to be in accordance with the percentage of purity by grow-out test in the field. Thus, the genetic identity and purity of the hybrids and parental lines were successfully ensured by utilizing SSR based molecular marker system in the study. The high percentage of off-types observed in the commercial lots may be due to mixing by handling error or due to improper emasculating of flowers in the female parental line that result in selfed seeds.

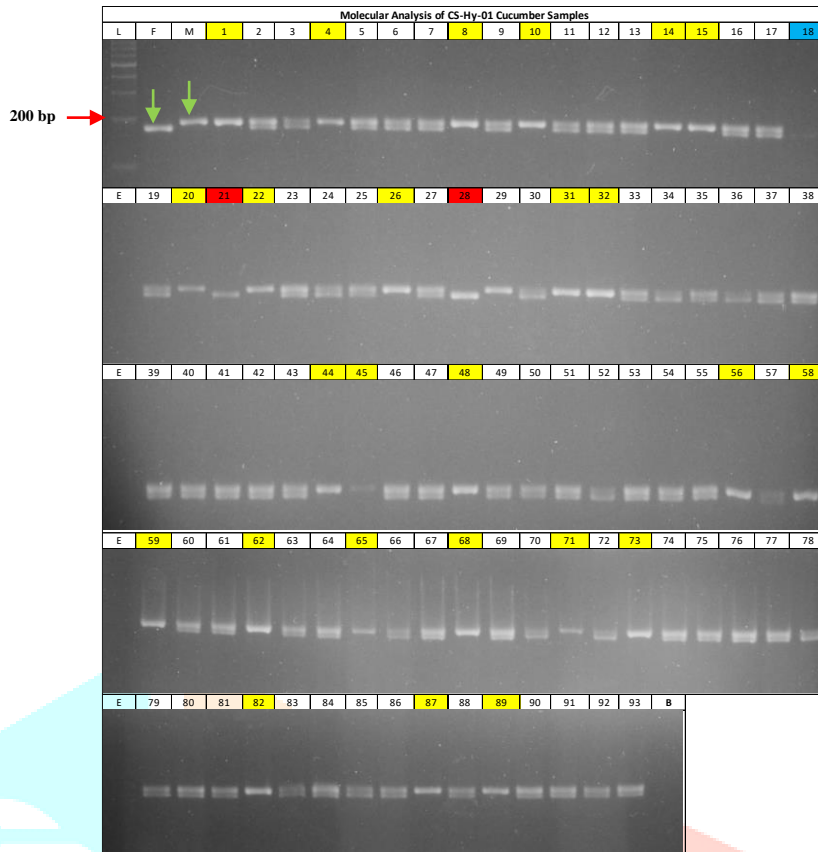


Figure 2: SSR analysis of CS-Hy-01 individuals with SSR16695 primer

Note: Lane L – 100bp DNA Ladder; Lane F – Female parent; Lane M – Male parent; Lane B – Blank (negative control); Lane E – Empty lanes; Lane 1-93 – F1 individual samples of CS-Hy-01 and lanes marked red are female off-types, yellow are male off-types and blue are non-amplified samples.

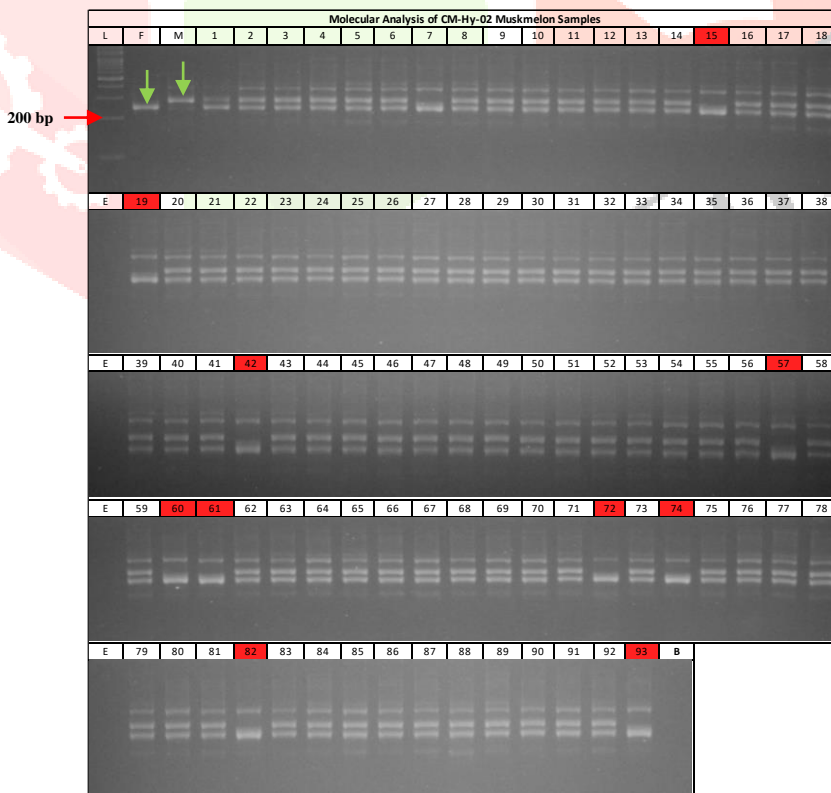


Figure 3: SSR analysis of CM-Hy-02 individuals with DM0173 primer

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 CM-Hy-02 and lanes marked red are female off-types.

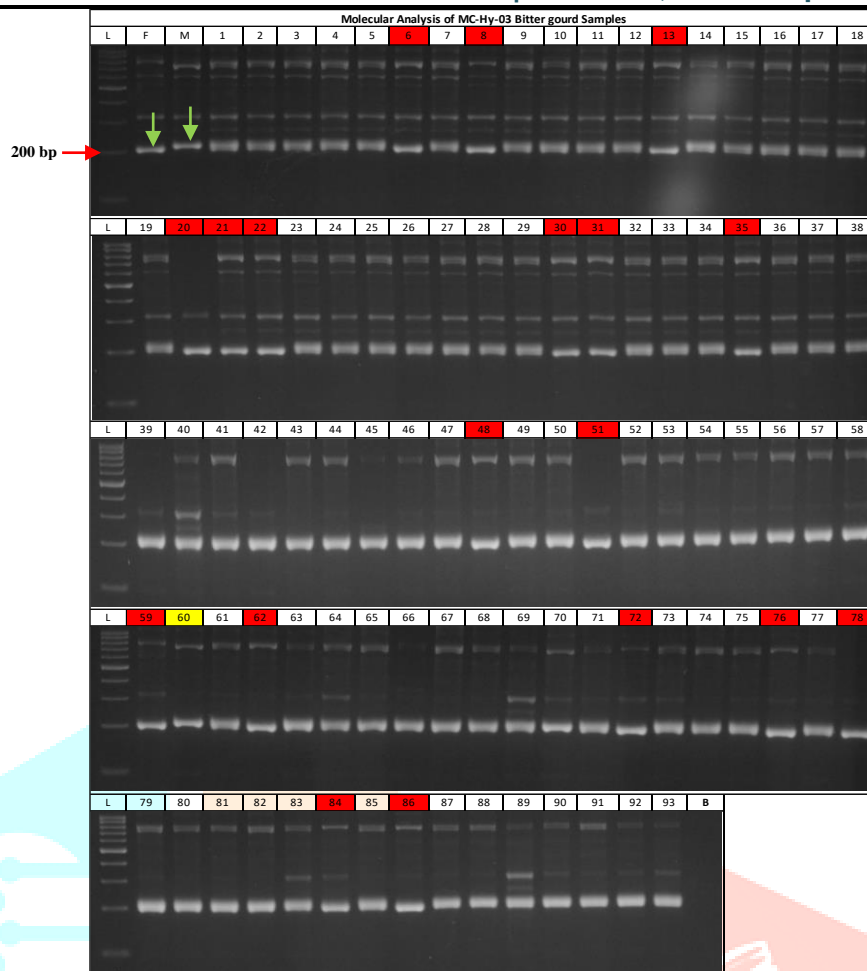


Figure 4: SSR analysis of MC-Hy-03 individuals with McSSR\_20 primer

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

#### IV. CONCLUSION

In the present study, to effectively analyze the genetic identity and purity of three hybrids of cucumber, musk melon and bitter melon studied, three microsatellite (SSR) markers were identified. For single hybrid, identifying multiple markers could be added advantage to reveal further more information on genetic level and increases the reliability of molecular marker based results. 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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