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Analysis of Hybrid Purity in Watermelon using Microsatellite marker in comparison with field GOT

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Abstract - *Traditionally genetic Purity of F1 hybrids are been assed at field level based on the morphological markers. Later with the improvement in molecular marker technology, assessing the genetic purity was much more accurate at molecular level using microsatellite as a marker. In the present study, watermelon hybrid variety "CL-Hy-01" and its parental inbred lines "CL-01-F" and "CL-01-M" were used to study the percent accuracy of genetic purity at molecular level in comparison with field Grow-Out-Test (GOT). One pair of polymorphic microsatellite (SSR) primer was identified out of 23 pairs, which was later subjected on 98 F1 seeds for accessing the genetic purity, similarly, 100 F1 seeds were sown at field for accessing the genetic purity based on morphological markers. The SSR primer "WMU4702" amplified one allele from each of the parental inbred lines and two alleles from F1 hybrid variety corresponding to those of its parental line, resulting in 96.9% purity. Similarly, at field level the purity was observed to be 97%, indicating that microsatellite can be successfully employed for testing purity of hybrids in watermelons.*

***Key Words*: Hybrid Purity Test, Grow Out Test, Watermelon, Microsatellite markers**

1. INTRODUCTION

The agricultural paradigm shift in the recent years has moved the focus from self-sufficiency of the traditional model to scale up production of modern farming. One such prominent and prevailing technology that has made this possible is Hybrid Seed Technology. It is a method to improve the quality of germplasm using breeding techniques to improve the crop quality and quantity. Breeding techniques are prone to error due to human and environmental factors and not always consistent. Various follow up tests with stringent quality check tests are required to ensure successful crossing. While the conventional methods of Grow out Test (GOT) so far used have been reliable but is time consuming so, implementation of genetic marker techniques for various applications would be less time consuming, simple, and reliable in the hybrid industry.

Over few decades, the use of molecular markers, revealing polymorphism at DNA level, has been playing an important role in plant biotechnology and their genetic studies. Progressive development of new and specific types of markers makes their significance in understanding the genomic variability and the diversity between the same as well as different species of the plant [6]. At present, the DNA markers have become the marker of choice for the study of crop genetic diversity to revolutionize plant biotechnology and plant breeding. Amongst the currently available molecular markers, Microsatellites offers a vital DNA marker system for testing purity of hybrids because of their co-dominant inheritance, relative abundance, reproducibility, robustness, multi-allelic nature and good genome coverage [7], [3].

The present study was aimed at identification and validation of polymorphic SSR (Simple Sequence Repeats) marker for the watermelon hybrid and its parental lines, which can be used for the purpose to assess the genetic purity of the commercial seed lot in short time compared to the conventional Grow out test.

2. MATERIALS AND MATHODS

2.1 Plant Materials

Two inbred lines CL-01-F and CL-01-M as the female and male parents respectively were used for the purpose of molecular identification and a random sample of 200 seeds, representing the commercial F1 hybrid seed lot was used for testing their genetic purity. Out of 200 seeds, half of the randomly drawn F1 seeds were used for watermelon specific SSR marker analysis and the rest were used for Grow-out-Test.

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 [5] for PCR amplification.

2.3 PCR amplification and gel electrophoresis

23 sets of watermelon specific SSR markers have been used for the study and their primer sequences were obtained from Cucurbit Genomics Database (http://cucurbitgenomics.org/). For SSR analysis, 20µL of PCR reaction for individual samples were set with 3 µL of crude DNA lysate, 10µL of 2X 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 and the conditions used for PCR amplification 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. 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.

2.4 Grow-out trials

The parental lines CL-01-F and CL-01-M and their respective commercial F1 seeds were grown in 10 × 10 Grow-out matrix in R&D field trail plot. All the agronomic practices and plant protection measures were implemented as per recommended practices for raising healthy crops. Genetic purity was visually evaluated based on the important Phenotype/morphological characters though out the vegetative and reproduction period of growth.

3. RESULTS AND DISCUSSION

3.1 Microsatellite marker analysis

Assessment of genetic purity testing is crucial for the successful hybrid seed production and for its commercial use because there is always a possibility of contamination in the hybrid seed production plot due to pollen shedders, out crossing and physical mixture due to the human error on handling the harvested material [9]. Reliability on hybrid purity testing depends on the Co-dominant DNA markers like SSRs which produces unambiguous banding patterns that are useful for hybridity.

The watermelon hybrid "CL-Hy-01" and its parental lines were analyzed using SSR markers. The parental polymorphic screening indicated that out of 23 SSR markers used, only one marker WMU4702 showed polymorphism between the parental lines. The polymorphic band size of female and male parental line was found to be 200bp and 180 bp respectively. The banding pattern of the hybrid corresponds to both the bands, of size 200bp and 180bp. Off types showed only one band of size 200bp corresponding to one of the parental line (i.e., female) in the present study. Any other mixtures in the lot may show differential banding pattern than the parental bands.

SSR markers have been successfully used for varietal identification and seed purity test of hybrid varieties of many crops [1], [8], [4], [2]. In the present study, to test the genetic purity of hybrids, WMU4702 marker was used which gave significant results in identifying the hybrids and Off-types (Fig 1). DNA from 98 plant samples of cross CL-01-F/CL-01-M was isolated and WMU4702 SSR marker was used along with the parental lines to assess the purity. The results showed that out of 98 plant samples, 95 samples had both the parental bands while 3 samples namely 25,36 and 66 had only one band of size 200bp corresponding to the female parent. Two samples namely 70 and 93 had faint amplification of both the bands which may be due to the presence of lesser quantity of DNA in the sample lysate taken for assay. The hybrid purity of the lot tested by SSR marker was observed to be 96.9%.

3.2 GOT Field trials of watermelon hybrids

In the Grow-out field trials, genetic purity evaluation was conducted based on morphological characters including plant length, internodal length, leaf size and shape, days to maturity, fruit color and its shape. The characters of few individuals that showed variation from the standard morphological traits were recognized as off-type and they were comparable to those of the female parental type showing 97% purity, which was also supported by the molecular marker testing, concluding that the results of the field grow-out test (GOT) and SSR marker test were comparable.

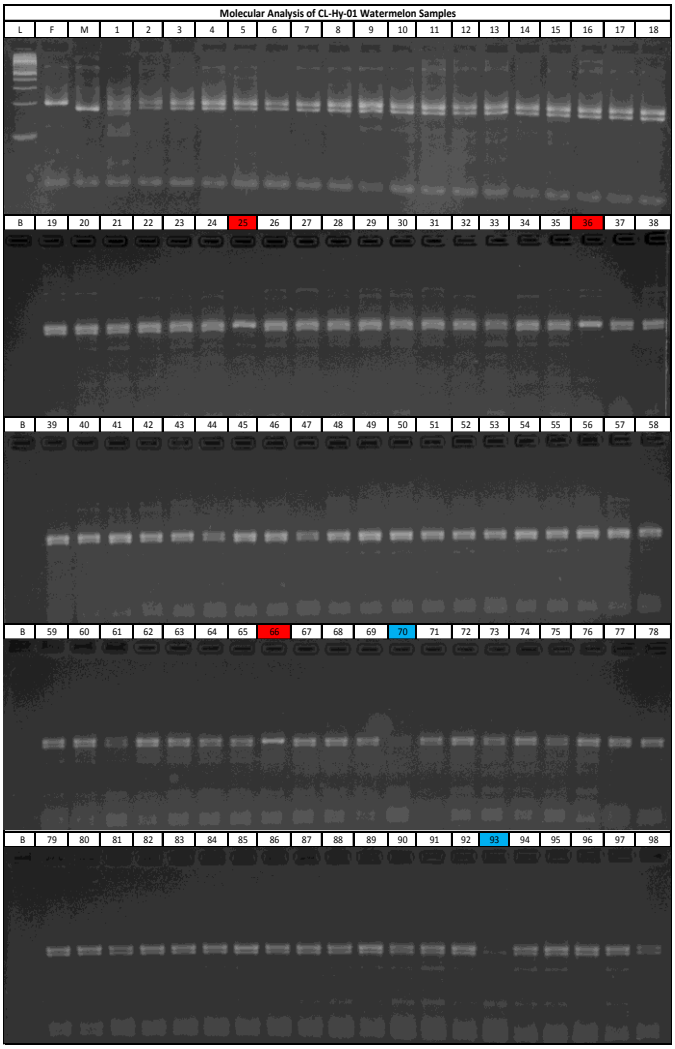

Fig-1: Molecular analysis of watermelon hybrid samples along with parental lines using WMU4702 SSR marker. Lane L – 100bp DNA ladder (GeneDirex), Lane F – Female parental line (CL-01-F), Lane M – Male parental line (CL-01-M) and Lane B – Blank with no samples.

4. CONCLUSION

Assessing genetic purity of the hybrids is most important for characterizing good quality seeds and is the first and foremost requirement before selling into commercial market for cultivation in the field. The maintenance of genetic purity of hybrid seeds at high level is thus vital to exploit heterosis, which were conventionally analyzed by Grow out test (GOT) involving representative samples of the lots. The GOT is basically based on the morphological marker that depends on phenotypic uniformity which is highly influenced by the environmental factors, and also time consuming and incurs high cost on maintenance and man power. These limitations of the morphological markers on hybrid purity analysis can be overcome with the advancement of molecular markers. The stability, reproducibility and feasibility of microsatellite/SSR markers make them the marker of choice for assessment of genetic purity of the hybrid varieties.

In the present study, one polymorphic SSR marker clearly distinguished the parental lines and was able to identify the off types from the hybrids. The validated set of SSR marker will be beneficial to facilitate hybrid breeding programmes in watermelon and for assessing genetic purity after the production of hybrid seeds for commercial use rather than the conventional field Grow-out-test.

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