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Identification of SSR Markers for Testing of Hybridity and Seed Genetic Purity in Maize (*Zea Mays* L.)

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Abstract: ***The present investigation was undertaken to identify specific marker associated with the maize hybrid DMH-117 and its parental lines, hybrid purity testing as well as identify the off-types from the seed lot. The hybrid DHM-117 could be clearly identified by using the SSR loci Umc1600 based on the banding pattern resolved on agarose gel (3%). The complementary banding pattern of the male and female parents made a way to identify the hybrid. The SSR loci Umc1600 amplified a specific allele size of 180bp in seed parent (BML-6) and 195bp was specific to pollen parent BML-7. These two bands of allele size 180bp and 195bp were found in hybrid DHM-117, there by confirming that this hybrid was produced from the cross combination of BML-6 and BML-7.***

Key words: Genetic purity, Maize hybrids, SSR markers

1. Introduction

Maize (*Zea mays* L.) is an important cereal crop next to wheat. Leading maize producing countries are USA, Brazil, South Africa and India. A quantum jump in the yields of corn could be achieved with the introduction of hybrids. Higher genetic purity is an essential pre–requisite for commercialization of any hybrid. Further, identification of hybrids, parental lines and determination of their genetic relatedness are very important for variety registration system, DUS testing and Protection of Plant variety and Breeder's rights [2]. So, clear cut identification of elite crop varieties/hybrids and corresponding parental lines is essential for protection and prevention of unauthorized commercial usage [7].

Conventionally, characterization of hybrids is being done based on specific morphological and floral characters in plants grown to maturity through grow out test (GOT), which is time consuming, restricted to a few characteristics and influenced by environmental conditions [1]. Further, morphological and biochemical markers are very few and their ability to distinguish the cultivar is poor. DNA based molecular markers are reliable and less time consuming, also not stage or tissue specific and not affected by the environment. From late 1980s, different electrophoresis [19], [13] and reversed phase high performance liquid chromatography (RP- HPLC) [9] of seed storage proteins have been well established and often considered effective method of cultivar identification. Currently, simple sequence repeat (SSR) technique is most widely used [10], [5], [18] for rapid assessment of hybrid and parental line seed purity [17], [12]. Therefore, the present investigation was conducted with the purpose of finding out a specific SSR marker to distinguish maize hybrid DHM-117 from its parental lines and also to replace GOT. In maize, several studies yielded a significant correlation between genetic distance obtained by molecular markers and the coefficient of co-ancestry [11], [4]. RAPD markers [15] have been widely used for genotype identification in maize. With the objective of replacing GOT with DNA based arrays (which are available in plenty, highly heritable) the parent investigation was carried out at Biotechnology laboratory of Seed Research & Technology centre, Rajendranagar, Hyderabad during the year 2011-12.

2. Methodology

A random sample of maize hybrid namely, DHM-117 along with parents (BML - 6 and BML -7) was collected from Maize Research Centre, Rajendranagar, Hyderabad. DNA was extracted from fresh leaves of 10 days old seedling by Cetyl-Tri-methyl Ammonium Bromide (CTAB) method [6] and quantification was done by Nanodrop spectrophotometer (Thermo). DNA was diluted in 0.1 T.E buffer to a concentration of 50 ng/micro liter for PCR analysis.

Twelve SSR markers *viz.*, Phi053, Umc1600, Bnlg 1043, Umc 1659, Bnlg 1867, Umc2258, Phi126, Bnlg 1867, Bnlg 1257, Umc2071, Bnlg 1647, Umc2101 and Umc2265 distributed in different chromosomes of maize were selected for the study of parental polymorphism. The primer sequence information was obtained from the published sequence data (www.Gramene.org) and presented in Table1. PCR plates were taken and 3 µl of template DNA was pipetted into each of the PCR tubes after proper labeling and the PCR plate was kept at 4°C. Then the master mix was prepared by taking 0.5 µl of each primer (both forward and reverse primers), 0.5 µl of 2.5 mM deoxyribonucleotides (dNTPs), 1 µl of Genei 10X assay buffer (10 mM Tris-Cl (pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin) and 0.2 µl of 3 U/µl Taq DNA polymerase (Bangalore Genei Private Limited, Bangalore). Usually minor errors occur while pipetting. To avoid such errors, a little excess quantity of master mix was prepared to ensure that adequate quantity is available for all the samples. Empirically, for every 10 reactions, one extra reaction was taken. The temperature profile used for PCR amplification comprised of 94^0C for 5min, followed by 35 cycles of 94^0C for 1min, 58^0C for 1min, 72^0C for final extension. The annealing temperature was adjusted based on the specific requirement of each primer combination. The PCR products were exposed to

electrophoresis in 3% agarose gel at 120V and observed under gel documentation system (Syngene make).

3. Results and Discussion

Among the 12 microsatellite markers tested, loci Umc1600 and Phi053 were able to generate complementary banding pattern between hybrid DHM-117 and its corresponding parental lines BML-6 (Female) and BML-7 (Male) (Fig. 1). The loci Umc1600 amplified a specific allele size of 180bp in F_1 hybrid and seed parent (BML-6) but not in its pollen parent (BML-7). Further, Umc1600 also amplified allele size of 195bp in pollen parent BML-7, which restored the fertility in male sterile parent. The same allele size of 195bp was also expressed in F_1 hybrid, but not in the female parent (BML-6). These two bands of allele size 180bp and 195bp were found in hybrid DHM-117, thus confirming that this hybrid was produced from the cross combination of BML-6 and BML-7 (Fig. 2).

Similarly, Phi053 loci amplified allele size of 170bp in female parent, F_1 hybrid and it was absent in pollen parent. On the other hand, the pollen parent (BML-7) generated an amplicon at 190bp, which was absent in the female parent. However, F_1 hybrid showed the allele of both parents confirming the heterozygosity by generating two bands at 170 and 190bp. Thus, it could be confirmed that these SSR marker alleles, Umc1600 and Phi053 can be identified as molecular tags for distinguishing maize hybrid DHM-117 from its parental genotypes. The use of selective primers for discrimination of hybrids and analysis for purity was also indicated by several workers. [16] developed RAPD markers for *Lilium* species characterization and hybrid detection. Earlier studies have shown that SSR markers are useful in identification of hybrid and assessment of respective parents of plant to plant variation within parental lines and testing the genetic purity of rice hybrids [17], [7].

3.1 Testing the Genetic Purity of Hybrid Seed

A sample of 400 seeds were collected randomly from seed lots of hybrid as well as parental lines of DHM-117 and were grown in 20x20 matrix (20 rows and 20 column). DNA isolation was done from the randomly selected individual plants from the hybrid DHM-117 as well as from its corresponding female (BML-6) and male (BML-7) parents and subjected to PCR analysis using microsatellite markers Umc1600, which has been identified for characterization of the hybrid. The study of DNA profile through gel pictures revealed that in female line (BML-6), out of 20 randomly selected plants, 4th, 10th, 12th, 15th and 19th plants showed both the parental bands indicating the contamination of female line with hybrid seed material. (Fig. 3), where as in the DNA profile of 20 randomly selected plants from male parent (BML-7), it was indicated that amplification pattern of 1st, 4th, 10th,12th,15th and 19thplant was different and they were considered as off types (Fig. 4). The genetic purity testing of 40 randomly selected plants of hybrid DHM-117 in two sets (20 each) with Umc 1600 showed 100 per cent purity in the selected plants in the first set (Fig-5). However, DNA profiling of hybrid in the second set of 20 individual plants specified 1st, 3rd, 5th, 7thand 9thplants (Fig. 6) as off types. Earlier researchers [14] and [7] also demonstrated the use of SSR marker for genetic purity testing in maize and rice respectively. [8] also opined that SSR markers have an advantage of co-dominance inheritance easy scoring of allele, reproducibility and accessibility to laboratories and hence are useful in genetic purity testing.

4. Conclusion

The maize hybrid DHM-117was found to be clearly distinguished from its parental lines; using SSR marker Umc1600 and the same can be used for identification of DHM-117 besides seed purity testing of hybrid without any ambiguity. The SSR marker is currently the preferred molecular marker for purity identification in many crops due to its high efficiency and simplicity.

Table 1: Primer sequence information of primers used in the study

S. No.	*Primer*	*Sequence (5'-3')*
1	Phi053	F: CTGCCTCTCAGATTCAGAGATTGAC R: AACCCAACGTACTCCGG
2	Umc1600	F: CGATCAGTGCGTGGAGAGTA R: TAGGCATGCATTGTCCATTG
3	Bnlg 1043	F: TTTGCTCTAAGGTCCCCATG R: CATACCCACATCCCGGATAA
4	Umc1659	F: CAAGCTTGCTACTGTGATTTCTGG R: AACTTCTCGGTGATCTTGTCCATC
5	Bnlg 1867	F: CCACCACCATCGTAGGAGTT R:CAGTACACAGCAGGCAGCTC
6	Umc2258	F: AAGATTGTATAAATGGCAGCCACG R:GAATAAGACCAGACAGCACCGAAC
7	Phi126	F:TCCTGCTTATTGCTTTCGTCAT R: GAGCTTGCATATTTCTTGTGGACA
8	Bnlg 1257	F: CGGACGATCTTAGCAAACA R:ACGGTCTGCGACAGGATATT
9	Umc2071	F: ATACACGCAGTTACCCGAAGGTT R: ACTGATGGTGTTCTTGGGTGTTTT
10	Bnlg 1647	F: CGTCGTCTGTGGACGTACTG R:AGAAGCTCACAAGCCTGCTC
11	Umc2101	F: CTAGCTAGTTTGGTGCGTGGTGAT R:CCCGGCTAGAGCTATAAAGCAAGT
12	Umc2265	F: CTGGACGTGGACTCAGACACC R: AAGACGGTCCCGAAGAAAGC

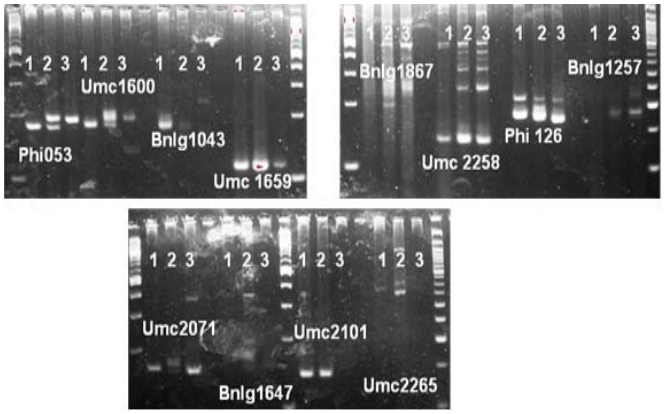

Figure 1: DNA polymorphism observed in maize hybrid (DHM-117) and parental lines with SSR markers (Phi053 and Umc1600)

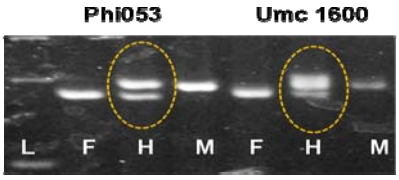

Figure 2: DNA Polymorphism Observed In Maize Hybrid (DHM-117) And Parental Lines Using SSR Markers Phi053 And Umc1600

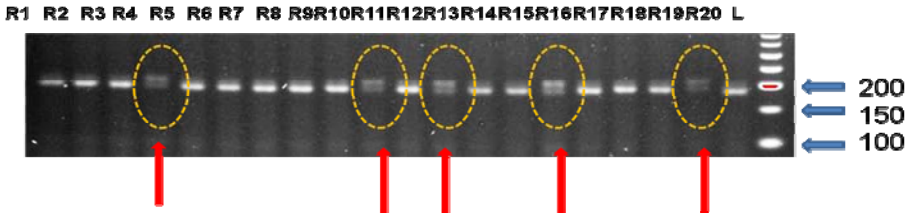

Figure 3: Testing the genetic purity of 20 randomly selected plants from BML 6, representing the off types

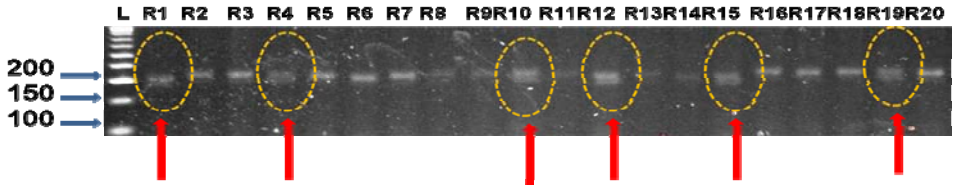

Figure 4: Testing the genetic purity of 20 randomly selected plants from BML 7, representing the off types

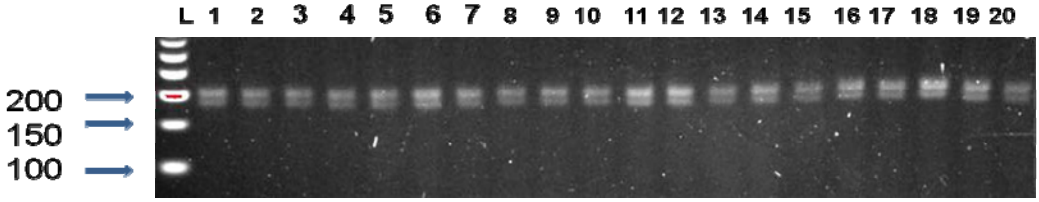

Figure 5: Testing the genetic purity of 20 randomly selected plants from DHM 117 showing 100 per cent genetic purity

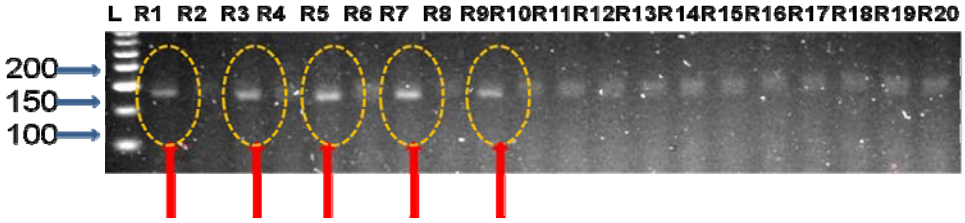

Figure 6: Testing the genetic purity 20 randomly selected plants from in DHM 117, representing the off types

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