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Research Paper

Assessment of Genetic Purity of Maize Hybrid Varieties MI Maize Hy 03, MI Maize Hy 04 and MI Maize Hy 05 Using SSR Markers

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Abstract

Genetic purity is an important criterion for quality assurance in hybrid seed production. Grow-out-test (GOT) based on morphological traits is usually practiced to confirm hybrid purity. The morphological trait-based analysis is time-consuming and highly influenced by the environment. In contrast, molecular markers, are capable of distinguishing parents and F_1 hybrids. In this study, the purity of three locally developed maize hybrids namely, MI Maize Hy 03, MI Maize Hy 04, and MI Maize Hy 05 were tested with their parental lines. Eight SSR markers were used to identify parental polymorphism of the hybrids. Of these SSR markers, three markers, designated as phi057, UMC1066, and UMC 1997 were found to be effective in determining the genetic purity of MI Maize Hy 03, MI Maize Hy 04, and MI Maize Hy 05. Maize hybrid MI Maize Hy 03 and MI Maize Hy 04 showed a hybridization percentage of 100 % whereas MI Maize Hy 05 showed a purity of 97.5%.These findings are more reliable and can be efficiently utilized for hybridity testing. Further, these findings will assist to maintain genetic purity and quality seed certification of the tested maize hybrid varieties. In addition, the results of the present study will help breeders and growers to identify the purity of commercially produced seeds of MI Maize Hy 03 MI Maize Hy 04 and MI Maize Hy 05.

Keywords: Genetic purity, Hybridity testing, Molecular markers, *Zea mays* L.

Introduction

Maize (*Zea mays* L.) occupies the second largest of the total cultivated extent of cereals in Sri Lanka, where about 95% is rice, 4% is maize and the rest by millets (Kumari *et al*., 2013). In the recent years, exotic hybrid varieties have become popular among farmers due to their high yield potential. Uniform growth, ability to provide few extra grains per each ear harvested and high plant vigor due to increased metabolic activities are the attributes contributed to the growing interest in hybrid maize among farmers (Karunarathne, 2001). The maize hybrid development program in Sri Lanka was initiated 2000 at the Field Crops Research & Development Institute, Mahailuppallama (anonymous, 2000). The first local maize hybrid "Sampath" was released in 2004 (VRC, 2004). The local maize hybrid development program was collaborated by CYMMYT (International Maize & Wheat Improvement Center). The quality protein maize (QPM) hybrid "MI Maize hybrid 01" was released in 2013 by using QPM inbred lines developed by CYMMYT (Kumari *et al*., 2013). The "MI Maize hybrid 02" with an average yield of 5.5 t ha^{-1} was released in year 2016. Then the drought tolerant maize hybrids MI Maize Hy 03, MI Maize Hy 04 & MI Maize Hy 05 with higher realizable yield potential 7.5-8.0 t ha^{-1} were released in 2019. (Kumari *et al*., 2019).

Due to the high yield performance farmers are tempted to grow locally developed maize hybrids. These contribute to increase the maize productivity, by reducing it's importation. Production and distribution of high-quality seeds are important for yield enhancement and the genetic purity testing of seeds contributes to overall seed quality (George *et al*., 2004). Genetic purity is one of the quality criteria required for successful hybrid seed production and identification of genetically distant parental combinations (Elci & Hancer 2015). Conventionally, characterization of hybrids is done based on specific morphological and floral characters of plants at the maturity stage through grow out test (GOT), which is normally practiced by the Seed Certification Service of the Department of Agriculture, Sri Lanka. However, this grow out test is time consuming, restricted to a few characteristics and influenced by environmental factors (Tiwari, 2020).

Molecular marker approach detects variation in the genotype directly at the DNA level and Simple Sequence Repeat (SSR) markers are ideal for genotyping since they are co-dominant, highly abundant and polymorphic, independent of the environment, reproducible, expressed at all developmental stages, in known positions in the genome and linked to traits of interest. Gowda *et al.*, (2017) reported molecular marker-based seed tests are more efficient, economical, user-friendly, reliable and quick, and help to determine the genetic purity. Microsatellite markers have been proven to be the preferred markers for purity identification, due to their high efficiency, reproducibility and simplicity (Bhat *et al*., 2017). Further, Chaudhary *et al*., (2018) stated that co-dominant SSRs are preferred for genotyping because of their reproducibility,

abundance and amenability to high throughput screening. Establishing a proper genetic purity testing system is a fundamental requirement for all level of seed producers as well as for seed certification services of a country.

The objective of the present study was to identify SSR markers for detecting hybridity and genetic purity percentage of MI Maize Hy 03, MI Maize Hy 04 and MI Maize Hy 05.

Materials and Methods

Planting materials

The study was carried out at biotechnology laboratory at the Field Crops Research & Development Institute (FCRDI), Mahailuppallama Sri Lanka during the seasons 2020/2021 *Maha*, 2021 *Yala*, 2021/2022 *Maha*.

Genomic DNA extraction

Seeds of MI Maize Hy 03, MI Maize Hy 04, MI Maize Hy 05 hybrids and their parental lines were planted in the field (Table 1) and leaf samples from 2-3 weeks old seedlings were collected from hybrid parents and F_1 populations of MI Maize Hy 03, MI Maize Hy 04 & MI Maize Hy 05. Collected leaf samples (100 mg) were stored in a (-) 80 °C freezer for future use. Samples were ground separately using chilled mortars and pestles. DNA extraction was done for these leaf samples using a modified CTAB method (Allen *et al*., 2006). Isolated DNA was treated with RNase (Promega, USA) to remove RNA. DNA isolation was done from 50 individual plants of the MI Maize Hy 03 MI Maize Hy 04 and MI Maize Hy 05 hybrids, as well as their corresponding parents and PCR was carried out using selected SSR markers.

Testing of quality and quantity of DNA

The DNA yield was measured by using a UV-Visible spectrophotometer (Jenway, Genova Nano, UK) at 260 nm. DNA purity was determined by calculating the absorbance ratio A260/280. Further, to assess the quality and yield, gel electrophoresis was done for all the DNA samples using a 0.8 % agarose gel and stained with ethidium bromide (0.5 µg ml^{-1}). The amplified products were observed in a gel documentation system (Vilber-Quantum, France).

PCR and Gel visualization

The PCR amplification reaction was carried out in a 15 μl reaction volume for each DNA sample, containing 2X Go Taq (Promega, USA), 10 mM of each forwarded and Reverse primer and 50 ng of template DNA using ABS Verity thermal cycler (Applied Bio Systems, USA). The primer sequence information was obtained from the maize genetics and genomics database (MaizeGDB, (2021), Table 2). The 35 cycles of PCR consisted of initial denaturation at 94 °C for 5 min, denaturation at 94 ^{0}C for 30 s, annealing temperatures at 54-60 °C and extension at 72 °C for 2 min. The final extension was carried out at 72 °C for 7 min. The amplified product selection was done using polyacrylamide gel electrophoresis (PAGE (8% =acrylamide 29: 1 bis acrylamide ratio).

Table 1. Maize varieties used for hybridity testing

MI Maize hybrids	Parental lines
MI Maize Hy 03	CAL 147 / CML 451
MI Maize Hy 04	CAL 1426 /CLO 2450
MI Maize Hy 05	CAL 1471 / CLO 2450

Analysis Scoring and analysis of data

Amplified products were compared with 100bp DNA ladder and their accurate sizes were determined using Vilber Bio Vision Software (Vilber Lourmat, France). The banding pattern of gel was manually scored by visual observations to select polymorphic molecular markers. Hybrid purity was evaluated using the heterozygous banding pattern (co dominance), male specific and female specific alleles. Hybrid percentages were calculated considering total number of individuals with their allelic behavior.

Table 2. List of SSR primers used in the study

Primer code	*Primer sequence -Forward (5´- 3´)*	*Primer sequence -Reverse (5´- 3´)*	*Annealing temperature*	*References*
phi057	CTCATCAGTGCCGTCGTCCAT-3	CAGTCGCAAGAAACCGTTGCC -3	54 ^{0}C	Shinde *et al*., 2021
UMC 1746	ACACGAGCATCCTACATCCTCCTA	ACCTTGCCTGTCCTTCTTTCTCTT	60 ^{0}C	Shinde *et al*., 2021
UMC 1040	CATTCACTCTCTTGCCAACTTGA	AGTAAGAGTGGGATATTCTGGGAGTT	56 ^{0}C	Shinde *et al*., 2021
UMC 1997	GTTCCAATGAGATGAGACGATGTG	CTCCAAAAAGGCCGTCGTAGTA	56 ^{0}C	Ubesekara *et al*., 2020
UMC 1260	CTTAAGCAGAGCTCAAAAACTGCC	TAAATTGTCAAGCGAGGTTTGGAT	60 ^{0}C	Ubesekara *et al*., 2020
PHI 076	TTCTTCCGCGGCTTCAATTTGACC	GCATCAGGACCCGCAGAGTC	60 ^{0}C	Ubesekara *et al*., 2020
PHI 402893	GCCAAGCTCAGGGTCAAG	CACGAGCGTTATTCGCTGT	60 ^{0}C	Ubesekara *et al*., 2020
UMC 1066	ATGGAGCACGTCATCTCAATGG	AGCAGCAGCAACGTCTATGACACT	58 ^{0}C	Shinde *et al*., 2021

Results and Discussion

Marker validation to select SSR makers for genetic purity testing

PCR amplification results of molecular marker phi057 showed polymorphism for MI Maize Hy 03 parents (CAL 147/CML 451) figure 1 and molecular marker UMC1066 clearly showed polymorphism for MI Maize Hy 04 parents (Figure 2). However, parents of the MI Maize Hy 05 did not show proper polymorphism for aforementioned SSR markers. Therefore, parents of the MI Maize Hy 05 were subjected to PCR analysis with another 6 pairs of markers. PCR analysis results revealed that molecular markers UCM 1997, UMC1746, UMC1260, PHI076 and PHI 404893 showed proper polymorphism. For genetic purity analysis of MI Maize Hy 05, the molecular marker UMC 1997 was selected. The banding pattern of gel was manually scored by visual observation to select polymorphic molecular markers. Hybrid purity of MI Maize Hy 03, MI Maize Hy 05 was evaluated using the heterozygous banding pattern (co dominance), of male specific and female specific alleles.

The use of SSR markers for genetic purity testing has already been demonstrated in maize (Daniel *et al*., 2012; Mrutu, 2015; Wu *et al*., 2010; Sudharani *et al*., 2013), rice (Bora *et al*., 2016, Cai *et al*., 2020), watermelon (Lu *et al*., 2018), barley (Romdhane *et al*., 2018) and capsicum (Mongkolporn *et al*., 2004). Production & distribution of high-quality seed is an important pre requisite to improve hybrid maize productivity and production. Gowda *et al*., 2017 also, stated that improved maize productivity, will be beneficial food security and enhanced income and livelihoods of farmers if the seed meets the high standards of genetic and physical purity, besides physiological and seed health standards.

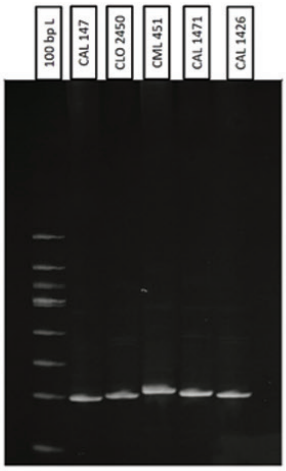

Figure 1. Identification of polymorphism of SSR marker phio57 for parents of MI Maize Hy 3 (CAL 147, CML 451) (Band Size - 200bp)

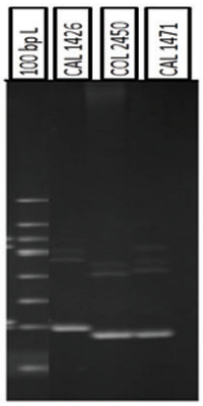

Figure 2. Identification of polymorphism of SSR marker UMC 1066 for parents of MI Maize Hy 4 (CAL 1426, CLO 2450) (Band Size-180bp)

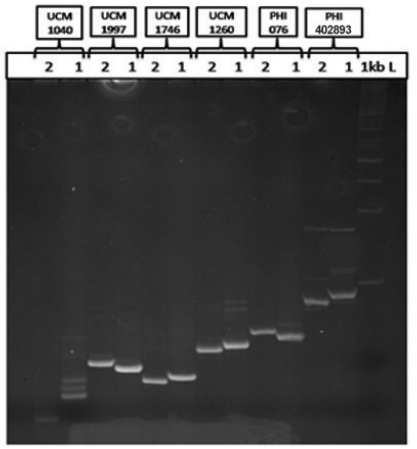

Figure 3. Identification of polymorphic SSR marker UMC 1040, UMC1997, UMC1746, UMC1260, PHI076 & PHI402893 for parent of MI Maize Hy 5 Parents of MI Maize Hy 5, (1-CLO 2450, 2- CAL 1471)

Fifty lines from each MI Maize Hy 03 & MI Maize Hy 04 were screened with SSR marker phi057 & UMC1066 respectively. All the tested lines from each hybrid were heterozygous for both two alleles (Figure 4, Figure 5).

Further, out of 40 samples tested in MI Maize Hy 05 with SSR marker UMC1997 (197-200bp), only one sample (no. 15) exhibited homozygous banding pattern for its parental inbred line CLO 2450 (Figure 6) and all other 39 lines tested were heterozygous for both two alleles (Figure 6). Genetic purity percentage was 100 % in MI Maize Hy 03 and MI Maize Hy 04 while MI Maize Hy 05 showed 97.5 %. SSR markers detected both alleles of the parental lines in pure hybrids,-proving their heterozygosity whereas impurities were identified by the presence of only one sample from MI Maize Hy 05 because it' behaved as it's parental line allele of CLO2450 and samples of all other two hybrids MI Maze Hy 03 and MI Maze Hy 04 were heterozygotes for both two alleles. Zhang *et al.,* (2014) indicated that confirmation of hybrid purity with a single polymorphic marker is sufficient for detection of contaminations of tested soybean hybrids from their parents.

According to the results SSR marker, phi057, UMC1066 and UMC 1997 can be used to detect genetic purity of commercial grown maize hybrids MI Maize Hy 03, MI Maize Hy 04 & MI Maize Hy 05.

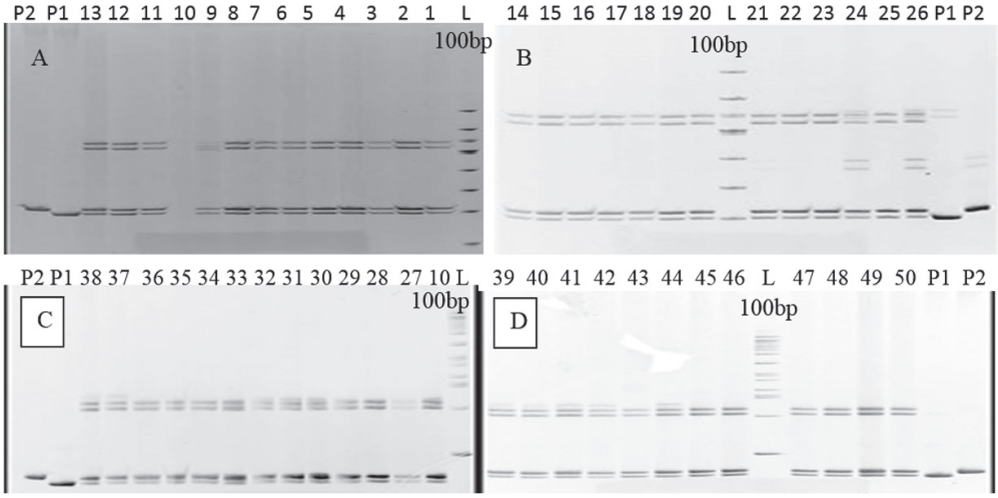

Figure 4. A, B.C.D Molecular screened MI Maize Hy 03 samples (1-50) and parental inbred lines P1- CAL 147 & P2- CML 451, SSR molecular marker phi057, 100bp L

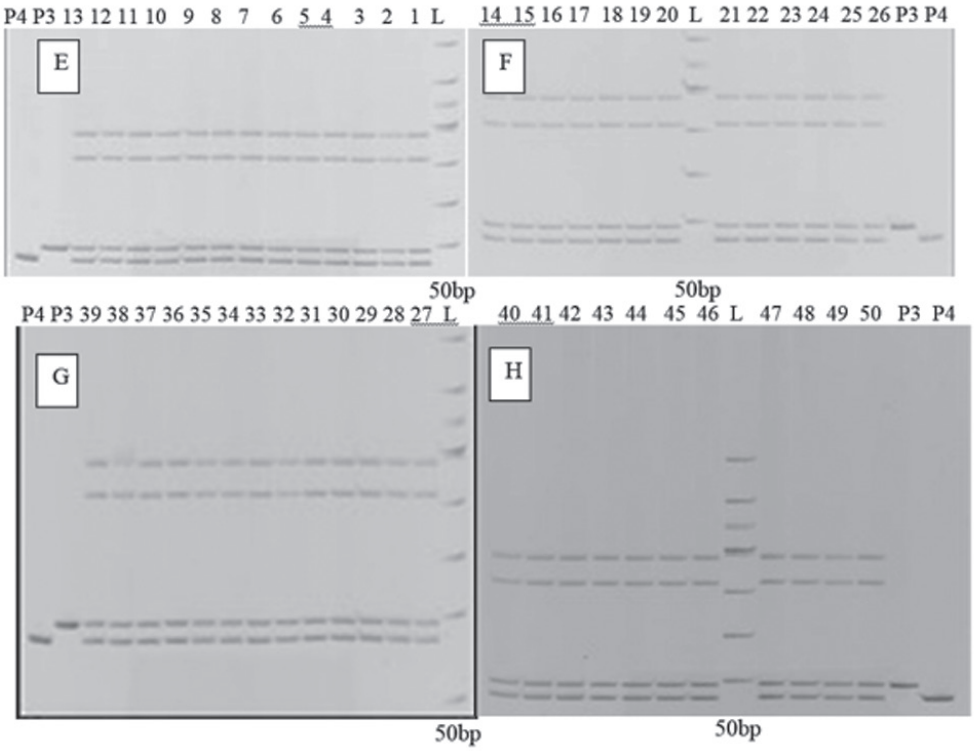

Figure 5. E,F,G,H PCR amplification results of MI maize Hy 04 samples (1-50) with primer UMC1066, parental lines P3-1426, P4- CLO 2450, L-50bp.

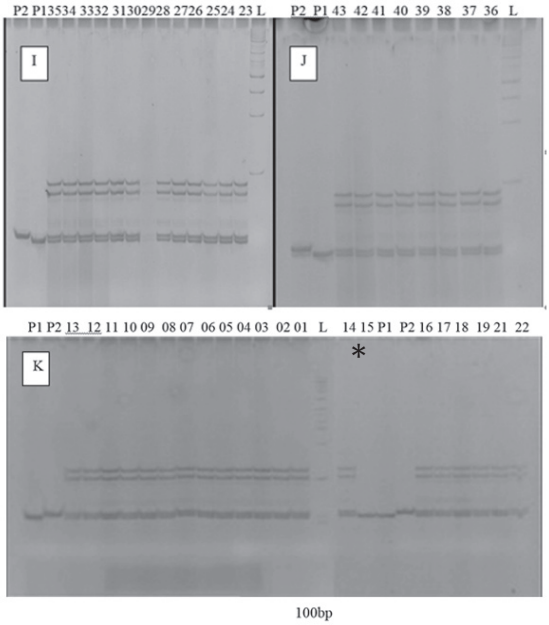

Figure 6. I, J,K PCR amplification results of MI Maize Hy 05 samples (1-40) with primer UMC1997 P1- CLO2450, P2-CAL1471, Sample no 15 * 100 bp ladder

Conclusion

SSR marker phi 057 could be effectively applied to test the genetic identity and purity of MI Maize Hy 03 and its genetic purity percentage was reported as 100 %. Genetic identity and purity of MI Maize Hy 04 can be assessed using SSR marker UMC1066. Further, SSR marker UMC 1997 can be used to assess genetic purity of MI Maize Hy 05, These findings will assist to maintain genetic purity of MI Maize Hy 03, MI Maize Hy 04 and MI Maize Hy 05. These findings could be applied to seed certification service of department of agriculture to certify the genetic purity of MI Maize Hy 03, MI Maize Hy 04 and MI Maize Hy 05.

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