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Molecular Fingerprinting of Lal Teer Chili Variety "Morich Super F_1"

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Abstract

Primer PP 24 & PP 53 characterized the"Morich SuperF_1", Lal Teer chili variety and its parents. The "Website" software used SSR primer development. Genomic DNA extracted from leaves and polymerase chain reaction did. Then the PCR products run on 6% polyacrylamide gel. The primer PP 24 and PP 53 showed polymorphic band to identify the Lal Teer chili Morich Super F1 and its parents. Morich Super hybridity test conducted at the Lal Teer Biotech Laboratory using these primers.

Keywords: Fingerprinting; Chili; Hybridity Test

Introduction

Chili is one of the most important spice crops in Bangladesh. It cultivates the all-round the year and can grow in any part of the country [4]. Chili (*Capsicum spp.*) originated in Central & Southern America. It belongs to the Solanaceae family having the chromosome number 2n = 2x = 24. Among the domesticated Capsicum species, *Capsicum annuum L.* is the most popular one and world-wide commercially distributed [8].

Chili has plenty of chemicals compound like unstable oil, carotenoids, nutrients, protein, fiber, and minerals. It utilizes for various purposes because of its health benefit, season, fragrance, surface, sharpness, and shading. It additionally has hostile to contagious property against parasitic species like Aspergillus and Fusarium [5]. It is a decent wellspring of Vitamin A, B, C, E and minerals like molybdenum, manganese, folate, potassium, thiamin, copper.

Lal Teer is one of the pioneer seed company in the agribusiness sector in Bangladesh. Lal Teer developed chili varieties "Morich Super", "Morich King" and "Sonic" to meet up the local market demand.

Nowadays molecular markers use in studying genetic diversity, population genetics, and genetic characterization. Primers do not influence on the environmental factors. The genetic relationship will sight accurately [2]. Molecular markers can also determine closely connected plant species. The result does not vary on the season and age of plant species [1]. Different molecular markers developed for Chili [6, 7]. Among these molecular markers, SSR markers represent highly polymorphic, reproducible, co-dominant [9]. These are used in genome mapping, gene tagging, and estimation of genetic diversity, variety identification, and marker-assisted selection [10].

From a commercial point of view, DNA fingerprinting is a useful tool for varietal protection to prove ownership or derivation of parent lines. Genetic diversity is commonly measured by genetic distance or genetic similarities [3].

The study aim is the identification of Lal Teer chili variety Morich Super F_1 through SSR markers and another aim is to hybrid determination in the laboratory.

Materials and Methods

Material

Lal Teer chili variety Morich Super F1 and its parents' seeds collected from the Seed Operation Department and Stock Seed Department respectively. Plants are grown in the field for Grow out Test. Leaf samples are collected for the molecular study at Biotech Lab., R&D, Lal Teer Seed Limited (Figure 1).

Figure 1: Morich Super F_1

Primer Development

First, searched the genome sequence of the species or related species at NCBI webpage for primer development. "WebSat" software used for micro-satellite marker development. Five chili primers are listed below with its sequences:

Sl. No.	Primer Name	Forward Sequence	Reverse Sequence
1	PP 24	AAAGCATGAAATCACCCTCC	CGGCAAGAAGATGAAAGTCA
2	PP 53	AACCTTGCAAGCTACAGGCT	CTGCCAAAGGTGCTTGTAAA
3	PP 88	AGTAGCTCCATCGCCAGTTT	TCGAAAGACAACTCCATCGT
4	PP 99	CTTTCAACCCCATCGTTGTT	CCGGTCTTTTGACCTTCAAT
5	PP 141	TTCCTCCCAATCTCTTGTCC	GTTCAAGAAGTCAGTGCCGA

DNA extraction

Fresh & tender leaves collected from 15 to 21 days of seedlings of the plants. A few milligrams of young leaf sample placed into a 1.5 ml Eppendorf tube. For every mg of tissue should added 10 µl of 0.5 N NaOH in the Eppendorf tube. Samples placed on the water-bath at 80^0 for 20 minutes. Then quickly transferred 5-10 µl of supernatant to a new tube containing 495 µl of 100 mM TrispH 8.0. The samples mixed properly by finger tapping. Two µl DNA used for a polymerase chain reaction.

PCR amplification and polyacrylamide gel run

10 µl reaction mixtures prepared which contained 5 µl master mixes (Promega), 3 µl of nuclease-free water, 1 µl of forward and reverse primers and 2 µl of DNA. For the PCR reaction, the initial temperature was 95^0 for 4 minutes. The denaturation temperature was 95^0 for 30 seconds and followed by 32 amplification cycles. Finally, the annealing temperature was 55^0 for 30 seconds and the extension temperature was 72^0 for 10 minutes. The PCR products run on a 6% polyacrylamide gel.

Gel Analysis

Elpha Ease FC 4.0 software used for molecular band analysis.

Results and Discussion

Molecular Fingerprinting

Two SSR primers PP 53 & PP 24 used for amplification of DNA. In the case of Primer PP 53, the female parent (P_1) showed amplification at 264 bp whereas the male parent (P_2) showed the amplification at 228 bp on the 6% polyacrylamide gel. The hybrid showed both female and male band in particular positions. Primer PP 24 also showed polymorphism to the parents and its hybrid. In the case of PP 24 primer, the P_1 and P_2 showed polymorphism at 50 bp, 100 bp and 96 bp, respectably. The hybrid Morich Super showed both bands the same as male and female. Fifty base pair (bp) DNA marker used. The banding pattern of the hybrid compared with its respective parents, Morich Super F_1 identified. P_1, P_2, and F_1 run on polyacrylamide gel several times for confirmation of the result (Figure 2).

Figure 2: DNA Amplification of P_1 (Female), P_2 (Male) and F_1 (Hybrid) with the primer PP 53 & PP 24 and visualized by 6% Polyacrylamide gel

Hybridity Test

In the (figure 3), Morich Super F_1 and its parents are clearly identified by primer PP 53. Male, female and reference F_1 plants are showed at the left then Morich Super F_1 samples are on the right.

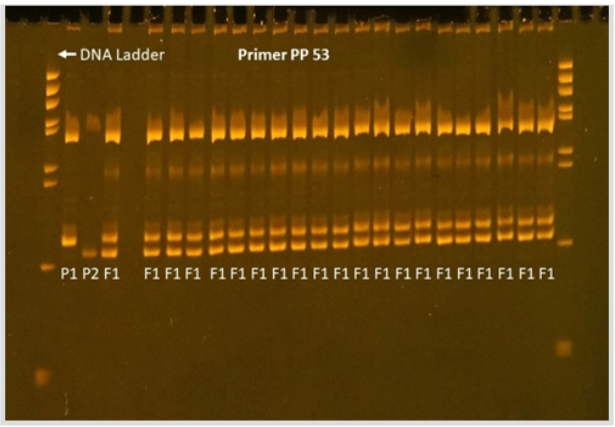

Figure 3: Hybridity test of Morich Super F_1 using primer PP 53

In (figure 4), Morich Super F_1 is identified by primer PP 24. Male, female and reference F_1 plants are showed at the left then Morich Super F_1 samples are on the right. Missing samples did not show any bands.

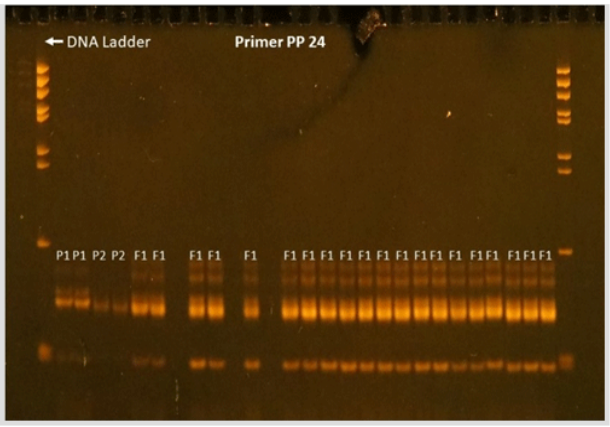

Figure 4: Hybridity test of Morich Super F_1 using primer PP 24

Conclusion

Primer PP 24 and PP 53 able to detect Lal Teer chili variety Morich Super F_1 and its parents. These primers could be used for molecular identification of Lal Teer chili variety Morich Super F_1. Grow-out test result varies on environmental factors but the molecular result is reliable for the plant detection and hybridity test. Lal Teer does hybridity test in the lab for confirmation of the variety. On the other hand, the grow-out test is time-consuming. Molecular test result provides the exact result within a short time. Molecular test especially is done in Lal Teer Seed Limited for exporting seed lot.

Conflicts of interest

Authors have declared no conflicts of interest.

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