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Hybrid Purity Test in Chilli (*Capsicum annum L.)* by Using SSR Marker

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A B S T R A C T

The present investigation entitled hybrid purity test in chilli (*Capsicum annum L.)* by using SSR marker was carried out at Department of Plant Biotechnology SDMVM's College of Agricultural Biotechnology, Georai Tanda, Paithan Road, Aurangabad (M.S.), 431001. To evaluating the hybrid purity for Disha X Jalwa.4 Polymorphic primer were tested. Primer I showed 100% Heterozygocity (H) such as binding pattern looking like P1 and P2 primer II and III also showed 100% hybrid purity. Primer IV showed 99% hybrid purity. Genetic purity or genuiness of the cultivar is tested by means of heritable characters (morphological, physiological, or chemical) of fruits of chilly or plants. Morphological evaluation of fruits of chilli used for purity and variety distinctness is time consuming and expensive. This experiment set to evaluate the usefulness of SSR markers to determine genetic purity of commercial hybrids and their inbreed lines.

Introduction

Capsicum genus originated from tropical and humid zone of central and southern America and belongs to the *Solanaceae* family. Chilly is one of the most important commercial crops of India. It is grown almost throughout the country. There are more than 400 different varieties of chillies found all over the world. In Indian subcontinent, chillies are produced throughout the year. Two crops are produced in kharif and rabi seasons in country . Chilli grows best at 20℃-30℃. Growth and yields suffer when temperatures exceed 30℃ or drops below 15℃ for extended periods. India is the world largest producer, consumer and exporter of Chilly peppers. Among which the city of Guntur in Andhra Pradesh produces 30% of all the Chillies produced in India, and the state of Andhra Pradesh contributes to 75% of all the chilly exports from India. Deoxyribonucleic acid (DNA) Polymorphism provides a powerful tool for quantifying the existing level of genetic variation in plant germplasm, either cereal or vegetable. Molecular marker can provide an effective tool for efficient selection of desired agronomic trait because they are based on the plant genotype and thus are independent of environment variation. Now a day, several molecular markers are developed, of which Simple Sequence repeats (SSR) or microsatellites are the most widely use types. Simple sequence repeats (SSR) are the most

widely used marker system for plant variety characterization and diversity analysis especially in cultivated species which have low level of polymorphism.

Various marker techniques have been applied either individually or in combination to study the comparative study of various plant species. Among the wide variety of available marker system, Simple Sequence repeat (SSRs, also called microsatellite), which are short random repeat units of between 1 and 6 bp in length (Tautz, 1989), offer a number of advantages which have made them increasingly popular in plant and animal studies. SSRs are highly polymorphic due to variation in repeat number and are co-dominantly inherited (Rafalski *et al.*, 1996). Most SSRs are single - locus markers.

The presence of SSRs in the transcripts of genes suggests that they might have a role in gene expression or function; however, it remain to be whether any unusual phenotypic variation might be associated with the length of SSRs in coding region. Varshney *et al.*, (2005) **s**tandardized the protocol for DNA isolation by CTAB method.

Materials and Methods

The present investigation entitled 'genetic/hybrid purity test in *Capsicum annum spp*. By SSR marker' was carried out at Department of Plant Biotechnology SDMVM's College of Agricultural Biotechnology, Georai Tanda, Paithan Road, Aurangabad (M.S.), 431001 during Jan. 2014 to May 2014.

The details of the material used and methods fallowed for the present investigation are given below. There was crossing between two hybrid variety i.e., DISHA x JALNA for hybrid purity testing of F1 generation.

Isolation of DNA by SDS method (Sodium dodacyle sulphate)

Reagents

Extraction buffer consist of following reagents (PH-8.0)

Tris HCl 100 mM -0.1 M
NaCl 250 mM -0.25 M
EDTA 250 mM -0.05 M
Glucose 15% -3.75M

Ammonium acetate - 7.5M

Suspension buffer - 400µl

Isopropanol - 2 /3 drops

T E buffer (10mM Tris and 1mM EDTA, pH 8.0).

Methodology for SSR marker analysis

Genomic DNA was isolated from each of the parents and individual genotype of each of the backcross population using a modified CTAB method (Sharma *et al.*, 2008).

Sample collection for DNA extraction

Fresh tissue leaf sample were collected from three weeks old seedling.

DNA was extracted from each of the parents and using cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle *et al.*, 2001)

PCR amplification

Preparation of reaction mixture for PCR

The PCR was carried but in small reaction tubes containing a reaction volume typically of 25 µ that was inserted into a PCR machine. For PCR reaction, master mix was prepared first.

Procedure for PCR reaction

i) Sterile micro centrifugation tubes were numbered and placed on PCR tube stand.
ii) 2µl of DNA was added to each PCR tube fallowed by 19.99µl master mix.
iii) 1.5µl of each forward and reverse primers was added in each PCR tube.
iv) The samples were mixed by brief centrifugation to bring down the content to tube.
v) PCR was run on the programmable thermal cycler with the following programme.

Results and Discussion

To evaluating the hybrid purity for Disha X Jalwa.4 Polymorphic primer were tested. Primer I showed 100% Heterozygocity (H) such as binding pattern looking like P1 and P2 primer II and III also showed 100% hybrid purity. Primer IV showed 99% hybrid purity.

Plant number 10 did not show any binding pattern so it was scored "O" (OFFLINE).

- Total 20 plants of hybrid were scored in the study total 87 marker data point generated. 4 primer were used for evaluating hybrid purity and showed 99% purity.

Genetic purity or genuiness of the cultivar is tested by means of heritable characters (morphological, physiological, or chemical) of fruits of chilly or plants. Morphological evaluation of fruits of chilli used for purity and variety distinctness is time consuming and expensive. This experiment set to evaluate the usefulness of SSR markers to determine genetic purity of commercial hybrids and their inbreed lines. There was crossing between 2 varieties of chilli of some crop and then hybrid variety of chilli was formed, By using some technique and some protocol for purity testing of chilli the hybrid purity was checked and their morphological characters were studied (Table 1–4 and Fig. 1).

Table.2 Master mix for 1X of 25 µl reaction

MASTER MIX	1X
10X Taq polymerase	2.5µl
$Mgcl_2$ (25mM)	1.3µl
dNTPs(10mM)	o.27µl
Taq polymerase (15U/µl)	0.10µl
Sterile distilled water	15.65µl
PCR Reaction	
Master mix volume	2oµl
Primer **1.Riverse primer** **2.forward primer**	 1.3µl 1.3µl
Template DNA (30ng)	6µl
Total Reaction volume	25µl

Table.1 List of SSR primer used with their sequence

SR.NO.	PRIMER CODE	PRIMER SEQUENCE	ANNEALING TEMP	PRODUCT SIZE(bp)
1	SSR1	TGTATCCTGGTGGACCAATG	57^0C	240-300
2	SSR2	TAGTGCAGCGGGATTAAGAG	57^0C	280-350
3	SSR3	AATTCGGTGCACCGTTATCC	57^0C	150-250
4	SSR4	GGGTTACCTTCAATAGACCC	57^0C	170-250

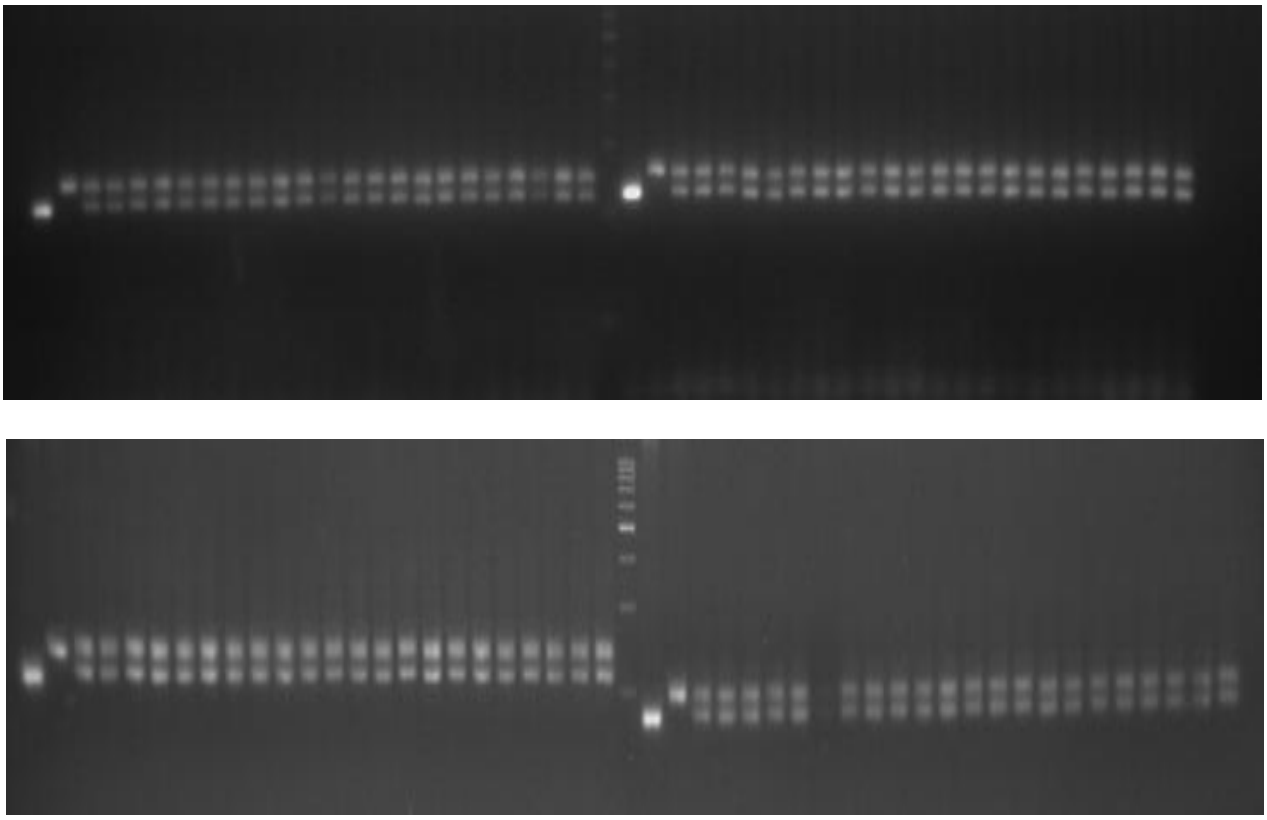
Table.3 The score sheet indicating genotype status

PLANT NO.	PRIMER			
	SSR1	SSR2	SSR3	SSR4
Plant no. 1	H	H	H	H
Plant no. 2	H	H	H	H
Plant no. 3	H	H	H	H
Plant no. 4	H	H	H	H
Plant no. 5	H	H	H	H
Plant no. 6	H	H	H	H
Plant no. 7	H	H	H	H
Plant no. 8	H	H	H	H
Plant no. 9	H	H	H	H
Plant no. 10	H	H	H	O
Plant no. 11	H	H	H	H
Plant no. 12	H	H	H	H
Plant no. 13	H	H	H	H
Plant no. 14	H	H	H	H
Plant no. 15	H	H	H	H
Plant no. 16	H	H	H	H
Plant no. 17	H	H	H	H
Plant no. 18	H	H	H	H
Plant no. 19	H	H	H	H
Plant no.20	H	H	H	H

Table.4 Summer statistics of hybrid purity testing for DISHA x JALWA

SR. NO.	Particular	Quantity
1.	No. of plants screened	20
2.	No. polymorphic band	4
3.	Scorable marker data	87
4.	Percentage of heterozygous	99%

Fig.1 Banding pattern of DNA by SSR marker in chilli



In conclusion, there was crossing between DISHA x JALWA. After crossing, total 20 plants of chilly variety tested hybrid purity. Only one hybrid plant was failure to show heterozygocity i.e., it was show the offline. This was outcome of the hybrid purity test in hybrid variety of chilly. Out of total 3extraction buffer only one is able to standardized the protocol of DNA isolation. Extraction buffer 3 shows the perfect bands of DNA.

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