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Analysis of Genetic Diversity of Commercial Tomato Varieties using Molecular Marker viz. RAPD

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A B S T R A C T

The present Study Analysis of genetic diversity of commercial tomato varieties using molecular marker viz. RAPD was carried out at carried out at Department of Plant Biotechnology SDMVM's College of Agricultural Biotechnology, Georai Tanda, Paithan Road, Aurangabad (M.S.), 431001 with an objectives 1.Extraction of DNA from commercial tomato varieties. 2.To analyzed genetic diversity of commercial tomato variety using molecular marker viz. RAPD. The information about the genetic diversity will be very useful for proper identification and selection of appropriate parents for breeding programmed including gene mapping, and ultimately for emphasizing the importance of marker assisted selection (MAS) in tomato improvement worldwide. PCR based molecular marker RAPD allows the rapid detection of DNA polymorphisms from many individuals samples in order to avoid erratic amplifications, good quality of genomic DNA free from contaminations and standardization of PCR reaction concentration is a perquisite for developing strategies for crop improvement programs in future. Out of those two primers, both of shows polymorphisms. In the result the OPA-11 primer shows 25% polymorphisms and the OPG-19 primer shows 66.66% polymorphisms.

Introduction

The cultivated tomato (*Solanum lycopersicum* L.) is economically one of the most important and widely grown plants of the Solanaceae family. The tomato core collection of European Solanaceae database is composed of about 7000 do-mesticated (*S. lycopersicum* L.) lines, along with representatives of wild species. The cultivated tomato is a well-studied species in terms of genetics, genomics, and breeding (Foolad 2007). It has been one of the first crop plants for which a genetic linkage map was constructed (Rick 1975). Tomato in Maharashtra is cultivated in 34000 hectares with production at about 8 lakh tons. The top five districts viz., Nasik, Pune, Nagpur, Chandrapur and Ahmad nagar contributes for nearly 75% of state's production. Nasik stands with 35% contribution. RAPD markers exhibit reasonable speed, cost and efficiency compared with other MethodsAnd RAPD can be done in a moderate laboratory. Therefore,

despite its reproducibility problem, it will probably be important until better techniques are developed in terms of cost, time and labors (N. Senthil Kumar *et al.* September 2011). The present Study Analysis of genetic diversity of commercial tomato varieties using molecular marker viz. RAPD was carried out at carried out at Department of Plant Biotechnology SDMVM's College of Agricultural Biotechnology, Georai Tanda, Paithan Road, Aurangabad (M.S.), 431001 with an objectives 1.Extraction of DNA from commercial tomato varieties. 2.To analyzed genetic diversity of commercial tomato variety using molecular marker viz. RAPD.

Materials and Methods

The present Study Analysis of genetic diversity of commercial tomato varieties using molecular marker viz. RAPD was carried out at carried out at Department of Plant Biotechnology SDMVM's College of Agricultural Biotechnology, Georai Tanda, Paithan Road, Aurangabad (M.S.), 431001.

A. DNA extraction

DNA was extracted according to Lodhi *et al.*, (1994). The method used was CTAB-based (Cetyl -Trimethyl Ammonium Bromide). DNA concentration was determined by diluting the DNA 1:5 with sterilized distilled water and loaded in 1% Agarose gel (Sambrook *et al.,* 1989) and run against DNA size marker.

Plant material

Plant sample taken from Nidhona farm (Tq. Dist. Jalna).

Fresh green leaves collected from plants. The total DNA isolated using the modifying C-TAB protocol.

Quantification of DNA

The quality and quantity of genomic DNA was estimated using NanoDropR ND-1000 spectrophotometer. Before taking sample readings, the instrument was set zero by taking 1µl autoclaved distilled water as blank. One micro litre of nucleic acid sample was measured at a wavelength of 260 nm and 280 nm and OD260/ OD280 ratios were recorded to assess the purity of DNA A ratio of 1.8 to 2.0 for OD260/OD280 indicated good quality of DNA. The quantity of DNA in the pure sample was calculated using the formula OD260= 1 is equivalent to 50 µg double stranded DNA/ µl sample.

1OD at 260 nm = 50 µg DNA/ml
Therefore OD260 × 50 gives the quantity of DNA in µ g/ml.

Gel electrophoresis

Purity of isolated DNA checked with Agarose (1%) gel electrophoresis and quality by Nano drop spectrophotometer.

PCR amplification

The application of polymerase chain reaction (PCR) based markers such as RAPD is a powerful measure for the detection of polymorphism in tomato (Foolad and Lin, 2001) RAPD markers were used to identify polymorphism between the four genotypes under study as it was used earlier with tomato genomic DNA by Klein-Lankbrust *et al.* (1992); Foolad *et al.* (1993) and Lin *et al.* (2006). PCR was performed with genomic DNA of commercial cultivated tomato variety by using OPA-11 and OPG-19.

Results and Discussion

The results of the present Study Analysis of genetic diversity of commercial tomato

varieties using molecular marker viz. RAPD was carried out at carried out at Department of Plant Biotechnology SDMVM's College of Agricultural Biotechnology, Georai Tanda, Paithan Road, Aurangabad (M.S.), 431001, are presented in this chapter under different sub headings.The sample taken into NIDHONA village (Tq. Dist. JALNA) and check out the tomato variation i.e. genetic diversity.

A) Source of explants

Eight tomato samples are collected from Nidhona village farm in Jalna district. We analyzed the 8 sample for genetic variation. We screening all above sample and after PCR, We are check out the genetic variation in tomato by using RAPD primers.

Using RAPD primer,

1. OPA - 11
2. OPG - 19

DNA confirmation

The DNA extraction was followed by loading the sample in 1 % Agarose gel for determining the concentration. After performing PCR randomly chosen PCR product were loaded on 1 % gel to check the amplification followed by which restriction digestion was carried out.

PCR amplification

PCR performed with genomic DNA of commercial cultivated Tomato variety by using primer for the present investigation.

Annealing temperature varied from primer to primer.

After completion of the cycles keep the samples at 4°C till electrophoresis.

Binary data

The RAPD markers as viewed from the gels after electrophoresis and staining were converted in to a matrix of binary data, where the presence of the band corresponded to value 1 and the absence to value 0.

The eight tomato varieties and 2 RAPD primer used for Binary data are following:

Table.1 Preparation of 50x TAE buffer

Sr.no.	Chemical	Quantity
1.	Tris Base	242 gm
2.	Glacial Acetic Acid	57.1 ml
3.	EDTA	100 ml
4.	SDW	To setup 1 liter
5.	TOTAL	1000 ml/ 1 Liter

Table.3 Primer sequence

Sr. no.	Primer	Primer Sequence	Temperature
1	OPA – 11	5' CAATCGCCGT 3'	36.7
2	OPG – 19	5' CCCGACTGCC 3'	42.1

Table.2 DNA Concentration in Nano gram (ng)

Sr.no.	Samples	Concentration (ng)
1	W 2819	445
2	W27872790/4366/Sheikh-1	212
3	312F-Ty 2	601
4	W 2848	627
5	W 2703	592
6	US-1/W-2738	257
7	UAB-2/4551/W2786	302
8	2737/near to US-1	490

Table.4 OPA - 11

VARITIES								
W 2819	**W27872790/ 4366/Sheikh-1**	**312F-Ty 2**	**W 2848**	**W 2703**	**US-1/W-2738**	**UAB-2/4551/ W2786**	**2737/ near to US-1**	**TOTAL**
1	1	1	1	1	0	1	1	7
1	1	1	1	1	1	1	1	8
1	1	1	1	1	1	1	1	8
1	1	1	1	1	1	1	1	8
								31

Table.5 OPG-19

VARITIES								
W 2819	**W27872790/ 4366/Sheikh-1**	**312F-Ty 2**	**W 2848**	**W 2703**	**US-1/W-2738**	**UAB-2/4551/ W2786**	**2737/ near to US-1**	**TOTAL**
0	0	1	1	1	0	0	0	3
0	0	1	1	1	1	1	0	5
1	1	1	1	1	1	1	1	8
1	1	1	1	1	0	1	0	6
1	1	1	1	1	1	1	1	8
0	1	1	1	1	1	0	0	5
								35

Table.6 List of RAPD (primer) marker, their sequence and details of amplified fragments of Tomato

Sr. no.	RAPD Primers	Primer Sequence	Monomorphic Bands	Polymorphic bands	Total band	Percentage of polymorphic bands (%)
1	OPA-11	5' CAATCGCCGT 3'	3	1	4	**25 %**
2	OPG-19	5' CCCGACTGCC 3'	2	4	6	**66.66 %**

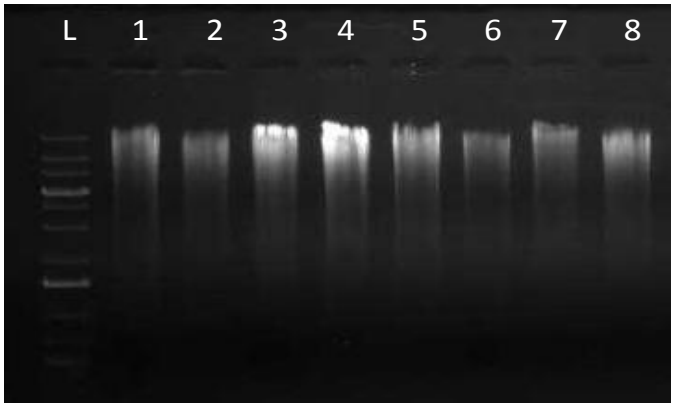

Fig. Quantifications of DNA

A.

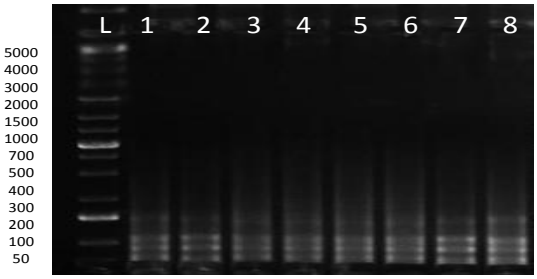

Fig. Primer OPA-11

B.

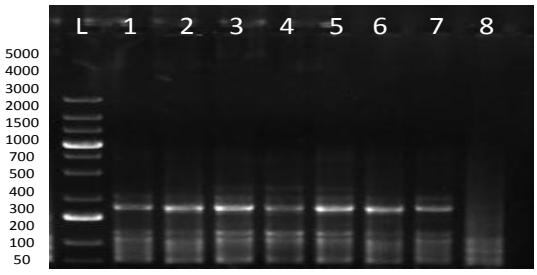

Fig. Primer OPG-19

DNA bands were scored from the photographs as bands present in all lanes (monomorphic bands) or bands absent from one or more lanes (polymorphic bands). Four of the primers used (OPA-15, OPU-03, OPU-14 and OPA-14) did not provide any evaluable bands. The polymorphic bands obtained with the other primers: OPC-08, OPC-09, OPB-17, OPB-18, OPV-19 and OPG-17, were scored as 1 for presence or 0 for absence and imported into SPSS. A band was considered polymorphic if it was present or absent in at least 3 of the 19 accessions tested. A total of 26 scorables bands were obtained from 19 cultivated tomato accessions (Saida sharifova *et al*., 2013). Polymorphism percentage for each primer was calculated for the 16 primers. Number of amplification bands per primer varied between 6 and 14 for the 16 tested primers. The total amplified fragments were 155 bands, 61 of them were polymorphic. The 16 primers gave polymorphism percentage for each single primer range between 0 – 83% with a total polymorphism percentage reaching 39%. Primers OPB-0 2, OPA- 10 and OPB- 20 gave the highest polymorphism percentage in a range of 71 - 83% while OPC-07 did not give any polymorphic fragments (Aida, A. Elsharief *et al*., 2015). According above reference in our present study we used 2 primers (OPA-11 and OPG-19) which gives evaluable bands. The polymorphic bands obtained with these two primers, we scored as 1 for presence or 0 for absence are checked out the Genetic variation in Tomato used by primer OPA-11 and OPG-19. Variations are clearly seen in tomato samples by using these primers.

In conclusion, the result indicated that the RAPD markers are dominant in nature, therefore heterozygous individuals cannot be distinguished from homozygous. The information about the genetic diversity will be very useful for proper identification and selection of appropriate parents for breeding programmed including gene mapping, and ultimately for emphasizing the importance of marker assisted selection (MAS) in tomato improvement worldwide. PCR based molecular marker RAPD allows the rapid detection of DNA polymorphisms from many individuals samples in order to avoid erratic amplifications, good quality of genomic DNA free from contaminations and standardization of PCR reaction concentration is a perquisite for developing strategies for crop improvement programs in future. Out of those two primers, both of shows polymorphisms. In the result the OPA-11 primer shows 25% polymorphisms and the OPG-19 primer shows 66.66% polymorphisms.

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