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RAPD Based Genetic Diversity Analysis within the Genus *Solanum* (Solanaceae)

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Abstract

The advances in molecular biology techniques have led to identification of large number of highly informative DNA markers that are useful for the identification of genetic polymorphism. In the present study, Random Amplified Polymorphic DNA (RAPD) technique was used as a tool for assessing genetic diversity and species relationship among the thirteen accessions of eggplants. Thirteen seed samples of eggplants were collected from different parts of the country. A total of 116 polymorphic amplified products were obtained from seven decamer primers, which discriminated all the accessions. The similarity results indicate presence of high levels of genetic diversity in eggplants and a dendrogram constructed by TFPGA methods shows 86.5% polymorphism. Genetically distinct genotypes identified using RAPD markers could be a potential source of germplasm for eggplant's improvement.

Keywords: Brinjal, Genetic diversity, RAPD markers, Solanaceae.

Introduction

Eggplant *(Solanum melongena* L.) is a vegetable crop of the family Solanaceae, grown in the sub-tropics and tropics. It is one of the most popular vegetables in many parts of the world including India. The crop is cultivated on small family farms and considered to be an important source of nutrition and cash income for many resource poor farmers (1). Eggplants can be cultivated and grown round the year but the productivity and quality of this crop suffers due to its susceptibility to a number of diseases and insect pests (2). In India, it is also used for the treatment of diabetes, bronchitis, dysuria and dysentery (3). Many other *Solanum* species are also used for medicinal purposes (4). For an effective breeding programme, information concerning the extent and nature of genetic diversity within a crop species is essential. It is particularly useful for characterizing individual accessions and cultivars and as a general guide in the selection of the parents for hybridization. Several workers have contributed to the characterization of the largest genus, *Solanum,* of Solanaceae family (5-9). Great degree of taxonomic confusion exists as regards to genus *Solanum* (10). India or Indochina is the center of eggplants diversity (11), but the affinities of S. melongena to related species are uncertain (9). Genetic fingerprinting has been accomplished traditionally through the use of isozymes, total seed protein and more recently through various types of molecular markers. However, DNA based markers provide powerful tool for discerning variations within crop germplasm and

for studying evolutionary relationships (9). Among molecular markers, random amplified polymorphic DNAs (RAPDs) have been extensively used in genetic research owing to their speed and simplicity (12, 13). The use of molecular techniques in genetic diversity studies is supported by the finding that evolutionary forces such as natural selection and genetic drift produce divergent phylogenetic branchings which can be recognized because the molecular sequences on which they are based share a common ancestor. The present study was aimed at analyzing the eggplant germplasm with RAPD markers and classifying the relationship and variability using RAPD data among the eggplant taxa with numerical taxonomic techniques.

Materials and Methods

***Plant material*:** The plant material for the study comprised 13 accessions (morphologically and geographically distinct genotypes), representing twelve species of cultivated and one species of wild eggplant (Table-1). The materials were collected from different parts of India, grown and maintained at our green house. Young leaves were collected form thirteen samples grown in the green house and immediately stored at -80^{0}C.

***Genomic DNA isolation*:** Genomic DNA from thirteen cultivars of eggplants was isolated by CTAB method (14) with certain modifications. Five grams of young leaves frozen in liquid nitrogen were ground to fine powder and transferred to 15 ml pre-warmed DNA extraction buffer [10mM, Tris-HCl (pH-8.0), 20mM, EDTA, 1.4M, NaCl, 2%, CTAB (cetyl trimethyl ammonium bromide)] and 0.2%, ß-mercaptoethanol. The samples were incubated at 60^{0}C for 1 h with occasional mixing by gentle shaking. To this, 15 ml of chloroform isoamyl alcohol (24:1 v/v) was added, mixed gently by inversion and kept for 20 min, followed by centrifugation at 15,000 rpm for 10 min at room temperature. The aqueous phase was recovered and mixed with 2/3 volume of isopropanol to precipitate DNA, which was recovered with a glass rod, washed with 70% ethanol and dried overnight. DNA was finally suspended in 2 ml of TE buffer {10mM, Tris-HCl (pH-8.0), 0.1mM, EDTA}. DNA was extracted from all the samples and ten samples of each cultivar from different vines were pooled together for further steps.

***DNA purification*:** The isolated DNA was further purified to remove RNA, proteins, polysaccharides and phenols using RNase, proteinase, phenol: chloroform: isoamyl alcohol (25:24:1), phenol: chloroform (24:1), chloroform: isoamyl alcohol and sodium acetate treatments.

***DNA quantification*:** The purified DNA was quantified following the protocol given by Pharmacia Biotech DyNA Quant TM 200 flurometer instruction manual. Finally the isolated DNA was diluted to 25ng/µl.

Polymerase chain reaction (PCR) conditions: PCR amplification reactions were carried out as described by Williams (12) with minor modifications. Reaction mixture (25µl) contained genomic DNA (50ng), Tris-HCl (10mM), $MgCl_2$ (1.9mM) and 100µM DNTPS, primer (0.4mM) and 0.5 units of AmpliTaq DNA polymerase. The tubes were centrifuged for few seconds and placed in a thermocycler for cyclic amplification using the following parameters: 1 cycle of 5 min at 94^{0}C followed by 35 cycles of 1 min each at 94^{0}C for denaturation, 1 min at 38^{0}C for primer annealing and a 2 min extension at 72^{0}C followed by a 5 min cycle at 72^{0}C and finally the machine was held at 4^{0}C till analysis. Amplification products were analyzed by gel electrophoresis on 1% agarose gel incorporated with 1.0 µl/ml of ethidium bromide in 0.5 X TBE buffer. Gels were visualized on a UV

transilluminator and photographs were taken with the help of a Polaroid camera.

***Data analysis*:** For RAPD analysis, the bands with same molecular weight and mobility were treated as identical fragments. The data matrices were analyzed by TFPGA program of dendrogram (Version 0.3) and similarities between cultivars were estimated using Nei's co-efficient method to develop a dendrogram by UPGMA.

Results and Discussion

A total of 134 bands were amplified with 7 primers and 13 cultivars, and these were in the size ranges of 0.1 to 5.0 kbp. The individual primers produced between 7 (OPF-01) and 14 (OPF-4, OPF-8) bands. Out of 134 bands, 116 (86.5%) were found polymorphic for one or more accessions. Polymorphism was observed with four primers OPF-3, 4, 8 and 9 which are shown in Plate-1. The primer of OPF-01 amplified minimum number of unique accession-specific bands, whereas OPF-4 amplified maximum number of polymorphic bands. The results show an average of 86.5% polymorphic bands per primer. The remaining 18 of the 134 bands (13.4%) were monomorphic i.e. they were observed in all the 13 cultivars. Fourteen bands were identified as unique to a particular accession which made it distinct from all the other accessions. Out of all the primers, OPF-04 gave the maximum number of 6 unique bands with four different accessions (Table-2; Plate-1).

The pair wise Nei's co-efficient (1972) for the genetic similarities among the 13 accessions are presented in Table-2. The cluster analysis of the distribution of 134 RAPD bands is shown as a dendrogram (Fig-1). This analysis clearly distinguished all the 13 accessions from each other, the accessions Debgiri and wild brinjal were grouped together as they were separated from the remaining accessions with only 25% similarity. Mangiri Gutta and Covai Vari Kathiri were separated from the other members of its cluster with only 40% similarity. The accessions Surachi selection-10 and Padma were grouped together with a maximum similarity of 90% followed by Purple round cluster and Kranthi which showed a similarity of 85%. No duplicates were found among the 13 accessions.

In the present study, a dendrogram was constructed based on the PCR (RAPD) markers

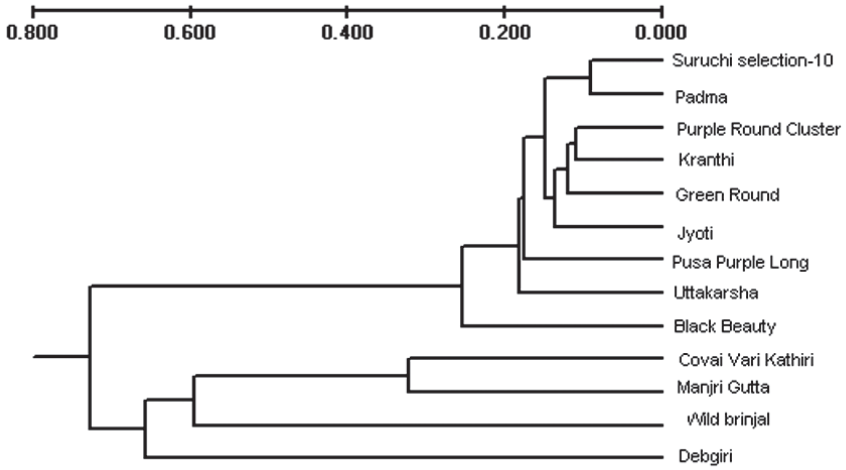

Fig. 1. Genetic relatedness among thirteen cultivars of *Solanum* Sps. based on all seven primers.

Table 1. Accessions of eggplant and its related species analyzed for RAPDs

Sl. No	Materials	Taxon	Source
1	Covai Vari Kathiri	*Solanum melongena*	Bangalore
2	Suruchi selection-10	*Solanum melongena*	Jalna
3	Padma	*Solanum melongena*	Secunderabad
4	Debgiri	*Solanum melongena*	Kolkata
5	Manjri Gutta	*Solanum melongena*	Jalna
6	Pusa Purple Long	*Solanum melongena*	Delhi
7	Black Beauty	*Solanum melongena*	Delhi
8	Green Round	*Solanum melongena*	Hyderabad
9	Purple Round Cluster	*Solanum melongena*	Bangalore
10	Jyoti	*Solanum melongena*	Hyderabad
11	Kranthi	*Solanum melongena*	Secunderabad
12	Uttakarsha	*Solanum melongena*	Hyderabad
13	Wild Brinjal	*Solanum violaceum*	Gulbarga

Table 2. Selected primers along with their sequence and some characteristics of amplification products in accessions analyzed

Sl. No.	Primers	Sequence 5' to 3'	Maximum Number of bands	Number of polymorphic bands	% of polymorphic bands	Mol. Wt. Range (kb)
1	OPF-01	ACGGATCCTG	14	12	85.71	0.2-2.0
2	OPF-02	GAGGATCCCT	16	15	93.75	0.1-1.0
3	OPF-03	CCTGATCACC	19	17	89.47	0.2-1.0
4	OPF-04	GGTGATCAGG	24	22	91.66	0.1-0.5
5	OPF-06	GGGAATTCGG	18	14	77.77	0.075-0.7
6	OPF-08	GGGATATCGG	21	18	85.71	0.1-0.5
7	OPF-09	CCAAGCTTCC	22	18	81.81	0.1-1.0

which showed that 86.5% of the bands observed were polymorphic between the 13 brinjal accessions. This seems to be relatively high when compared to the reports of other RAPD studies, as in the case of sweet potato (15, 16), tomato (17, 18), chilli (19) and other species of Solanaceae. One of the reasons for this high level of polymorphism could be that the interspecific variation in brinjal is extensive. Even though the number of genotypes tested here has come from similar locations, duplicates were not observed among the 13 brinjal accessions. This indicates the level of genetic variability among the local accessions which would be useful for selection of parents in the development of scented brinjal varieties. It will be worth to investigate specific traits in the wild species and they may be introgressed by sexual crossing or somatic

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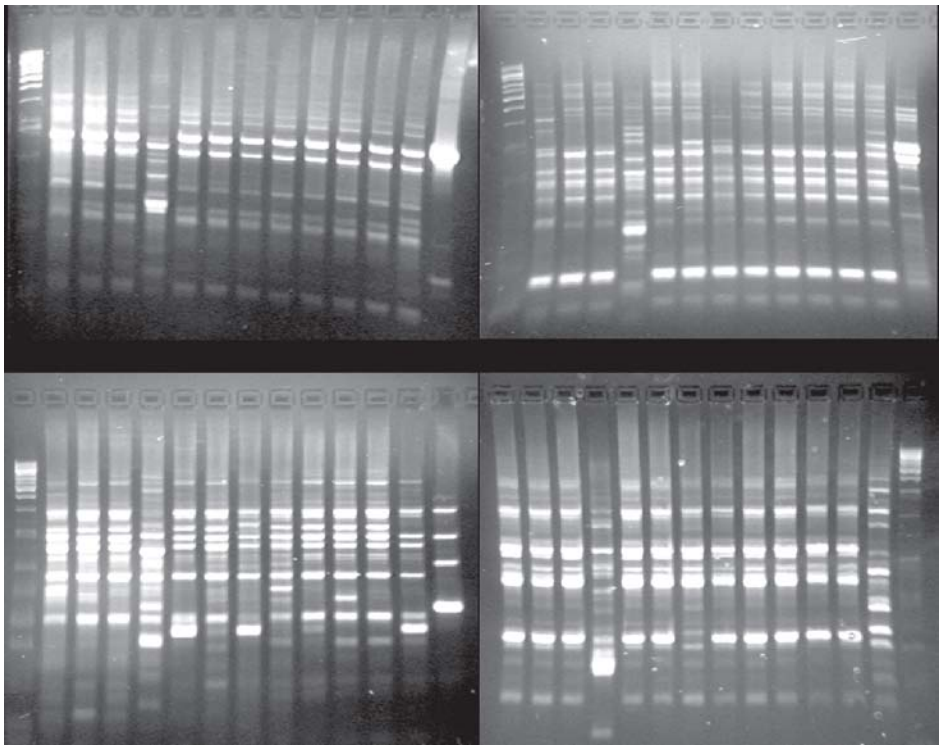

Plate-1. Amplification products from genomic DNAs of 13 accession of Indian Brinjal using a) primer OPF-3, b) OPF-4, c) OPF-8 and d) OPF-9. The lanes represents M- molecular weight 1) Covai vari kathiri, 2) Suruchi selection-10, 3) Padma, 4) Debgiri, 5) Manjri, 6) Pusa Purple Long, 7) Black beauty, 8) Green round, 9) Purple round cluster, 10) Jyothi, 11) Kranthi, 12) Uttakarsha and 13) Wild Brinjal

hybridization into commercial varieties of S. melongena. It is strongly believed that RAPD markers can be applied to other *Solanum* species to assess the genetic relationship among them and assist in the introgression of genes.

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