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RESEARCH ARTICLE

GENETIC DIVERSITY ANALYSIS OF BRINJAL (*Solanum melongena* L.) ACCESSIONS USING RAPD MARKERS

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ABSTRACT

Random Amplified polymorphic DNA (RAPD) analysis was applied to brinjal genotypes in order to assess the degree of polymorphism among genotypes. Three RAPD Primers (OPB-07,OPL-04 and OPC-07) were used to estimate genetic diversity in five brinjal cultivars. A total of 30 bands were scored corresponding to an average of 10 bands per primer with 10 bands showing polymorphism (33.33%).One out of three primers (OPB-07) gave 50% polymorphism. Dice`s similarity coefficient ranged from 0.857-0.980.Similarity matrix computed with dice`s coefficient reveals maximum similarity between cultivars Swarna mani (S1) and Punjab-70 (S2), Arka kranti (S4) and Swarna abhilamb (S5) i.e, 0.980 and 0.923 respectively while distantly related cultivars were Swarna mani (S1) and Swarna abhilamb (S5) with similarity coefficient 0.857.A dendogram constructed based on UPGMA clustering method revealed two major clusters-cluster-1 and cluster-2.The cluster – 1 comprises of Swarna mani (S1) and Punjab-70 (S2) and cluster -2 comprises of Arka kranti (S4) and Swarna abhilamb (S5), while as Arka shree (S3) occupies a distinct place as revealed in dendogram.

INTRODUCTION

Eggplant (*Solanum melongena* L.) (2n = 24), commonly known as aubergine, guinea squash or brinjal, is an important vegetable crop of tropical and temperate regions of the world (Daunay *et al*., 2001b). According to FAO, in 2010 production of eggplant is highly concentrated, with 90 percent of output coming from five countries. China is the top producer (58% of world output) and India is second (25%), followed by Egypt, Iran and Turkey. More than 4,000,000 acres (1,600,000 ha) are devoted to the cultivation of eggplant in the world (FAOSTAT, 2012). It is a good source of vitamins and minerals, especially iron,making its total nutritional value comparable with that of tomato (Kalloo, 1993). Besides being used as an important vegetable, eggplant has been exploited extensively in traditional medicines (Khan, 1979). The evaluation of genetic resources is crucial for breeders to produce new cultivars or to further improve the existing ones, according to changing consumer demands or challenges during growth conditions such as resistance attributes. So far, eggplant genotypes have been collected only in some European and Asian countries, but there is no comprehensive collection dedicated to the germplasm of eggplants available worldwide. It has been reported that gene banks for eggplant and related species have been generated by the EGGNET project in Europe.

Determination of genetic diversity of any given crop species is a suitable precursor for improvement of the crop because it generates baseline data to guide selection of parental lines and design of a breeding scheme (Vand dar Maesen LIG, 1990). Randomly amplified polymorphic DNA (RAPD) markers have been used for numerous applications in plant molecular genetics research despite having disadvantages of poor reproducibility and not generally being associated with gene regions (Welsh and McClelland, 1990). RAPD techniques are a quick and effective method for producing species-specific fingerprints (Williams *et al*., 1990; Cipriani *et al*., 1996). The RAPD analysis has been used extensively in phylogenetic studies of bacteria, fungi and plants. RAPD analysis also has the advantage of requiring very small amount of genetic DNA without the need for blotting and radioactive detection and is moderately reproducible (Atienzar *et al*., 2000; Rogers and Bendich, 1988). Genetic variability of brinjal has been studied using several other genetic markers such as RFLP, AFLP, micro satellite markers and diversity array technology. This paper reports assessment of genetic diversity among 5 brinjal cultivars with 3 RAPD primers. The purpose of the present study was to characterize *Solanum melongena* lines at molecular level. Such analysis, which are not previously available to these *solanum* lines can be utilized for selecting better parents for breeding programs. For this purpose, 5 brinjal lines were analyzed at molecular level using random amplified polymorphic DNA (RAPD) primers.

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MATERIALS AND METHODS

Plant Material

Five brinjal cultivars viz. Swarna mani(S1), Punjab-70(S2), Arka shree(S3), Arka kranti(S4) and Swarna abhilamb(S5), used for present investigation were collected from Indian Institute Of Vegetable and Research, Varanasi (U.P), India. Seeds of each cultivar were sown separately in pots and leaf samples pooled from all plants of each cultivar were collected into labeled bags and stored in -96°C in liquid N2 prior to genomic DNA Isolation.

Genomic DNA Isolation

Genomic DNA was isolated from fresh young leaves using CTAB method (Murray and Thompson, 1980), analyzed and quantified by standard methods.

Selection of the primer

The three decamer primers, OPB 07(5' GGT GAC GCA G 3'), OPL 04(5' GAC TGC ACA C 3') and OPC 05 (5' GAT GAC CGC C 3') used for RAPD analysis in *Solanum melongena* L. accessions were selected on the basis of prior knowledge. The primers revealed a high

degree of polymorphism in the earlier studies also as revealed by Demir *et al.,* 2010.

Polymerase Chain Reaction

PCR is a technique used to selectively amplify in-vitro a specific segment of the total genomic DNA a billon fold (Saiki *et al*., 1985, Mullis *et al.,* 1986). The most essential requirement of PCR is the availability of a pair of short (typically 20-25 nt) oligonucleotide called primers having sequences complementary to either end of the target DNA segment to be synthesized in large amounts. DNA amplification was carried out in a sterile 0.2ml thin- wall PCR tubes containing sterile water 18µl, Buffer 2.5µl, dNTPs composed of 0.5µl (200 µm) each, RAPD Primers (1µl,300 ng), Enzyme (1 unit) and DNA template 2 µl (100ng) (Table 2.) A positive control was prepared by adding the vector DNA carrying the gene construct to the reaction mixture instead of the target genomic DNA (presently DNA of the transgenic plant). Negative control tubes were also prepared by adding DNA from the transformed control plant to one tube and no DNA to another tube containing all other PCR components. As the number of PCR cycle increases the amount of the target DNA synthesized increases exponentially. The PCR involves three basic steps which constitute single cycle. Thermo cycling of DNA was carried out for 39 cycles.1.5% agarose gel was prepared in 0.5 x TE buffer. 2.5 µl gel loading dye was added to each tube containing the amplified DNA. Samples were loaded and electrophoresed at 50 v for 3 hours. The gel was stained with ethidium bromide solution (0.5 µg/ml) then after the gel was visualized under U.V light, band position of amplified DNA were compared with that of the positive control. Then the gel was photographed.

Table 1. PCR Thermal Profile

Step	Temperature	Duration
Hot Start	94^0C	5 min
Denaturations	94^0C	1 min
Primer annealing	35^0C	2 min
Extension	72^0C	2 min
Final extension	72^0C	10 min

Table 2. PCR Reagents

Sterilized water	18 µl
Buffer $mgCl_2$	2.5 µl
DNTPs	0.5µl (200 µM) each
Primer	1µl (300 ng) each
Enzymes	1 unit
Template DNA	2 µl (200 ng) each
Total	To a final volume of 25 µl each

Data Analysis

The amplification products were scored for each accession because of presence and absence of band .i,e use of binary code 1 and 0 for the presence and absence of band, respectively. Molecular size (bp) of amplified DNA fragment was determined by 100- 3000bp ladder. All statistical analysis was performed with aid NTSYS-PC computer genotypes with cluster analysis using the SHAN module of NTSY sp. version 2.0. (Rohlf, 1998).

RESULTS AND DISCUSSION

Random Amplified Polymorphic DNAs (RAPDs) analyses are widely used for detecting genetic polymorphism among genotypes at molecular levels in many crop species.

Keeping this in view, the selected experimental accessions were subjected to RAPD analysis using 3 randomly selected decamer primers - OPB-07, OPL-04, and OPC-05 (Fig. 2). A total of 30 bands were scored for 3 RAPD primers ranging from 8-12 corresponding to an average of 10 bands per primer. Out of these 10 bands were polymorphic with a polymorphism of about 33.33%. Pair wise comparison between the tested genotypes were used to calculate the genetic similarity. The similarity matrix computed with dice`s coefficient revealed the maximum similarity between accessions S1 (Swarna mani) with S2 (Punjab-70) and S4 (Arka kranti) with S5 (Swarna abhilamb) i.e, 0.980 and 0.923 respectively, while distantly related varieties were S1 (Swarna mani) and S5 (Swarna abhilamb) with similarity coefficient 0.857. Polymorphic bands for each primer ranged from 25% - 50 % as shown in Table: 3. Primer OPB-07 generated a total of eight bands out of which four bands were scored as polymorphic. Similarly OPL-04 and OPC-05 generated at total of 12 bands and 10 bands repectively, out of which 3 bands each were scored as polymorphic.

For individual RAPD primers, higher level of genetic polymorphism among selected accessions was found in OPB-07., where higher level of genetic diversity were observed among different comparison, indicating its power for the identification of individual genotypes. The Bivariate 1-0 data matrix generated dendrogram shows that the genotypes analyzed on DNA basis belong to two clusters (Fig. 3). Cluster -I includes two accessions S1 (Swarna mani) and S2 (Punjab-70). The major cluster-II comprises of Arka kranti (S4) and Swarna abhilamb (S5), while as Arka shree (S3) occupies a distinct place as revealed in dendrogram. The results obtained during the present investigation are in agreement with previous report by Demir *et al*., (2010) who reported a total of 100 amplified bands by using 11 RAPD primers out of which 29 bands were polymorphic indicating 29 % polymorphism in case of eggplant accessions, while Biswas *et al*., (2009) also demonstrated RAPD markers as a potential tool for eggplant improvement and found 57.89 % polymorphism among 10 eggplant accessions using four decamer RAPD primers.

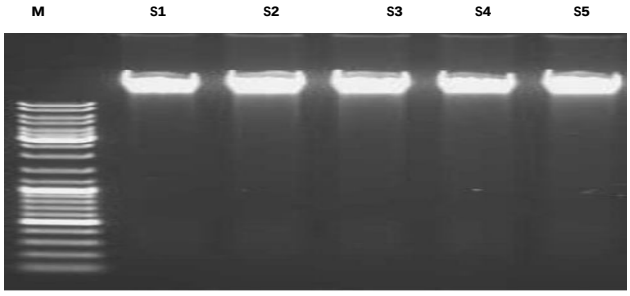

FIG.1. GENOMIC DNA OF FIVE ACCESSIONS OF SOLANUM MELONGENA

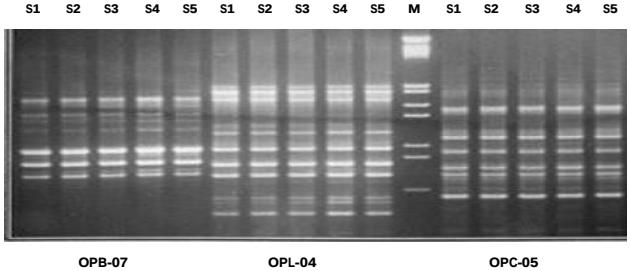

FIG.2. RAPD PROFILE OF FIVE BRINJAL ACCESSIONS USING 3 RAPD PRIMERS (0PB-07,OPL-04 AND OPC-05).

Table 3. Total Number of Amplified Bands and Number of Polymorphic Bands Generated By PCR Using Three Randomly Selected Primers

NAME OF PRIMER	Nucleotide Sequence 5`-3`	Polymorphic Bands	Monomorphic Bands	% G+C Content	Total Bands	% POLYMORPHISM
OPB-07	GGTGACGCAG	4	4	70%	8	50%
OPL-04	GACTGCACAC	3	9	60%	12	25%
OPC-05	GATGACCGCC	3	7	70%	10	30%
TOTAL		10	20		30	
AVERAGE/PRIMER					10	33.33%

Table 4. Similarity Matrix Computed with Dice`s Coefficient

	S1	S2	S3	S4	S5
S1	1.000				
S2	0.980	1.000			
S3	0.894	0.913	1.000		
S4	0.868	0.885	0.880	1.000	
S5	0.857	0.875	0.913	0.923	1.000

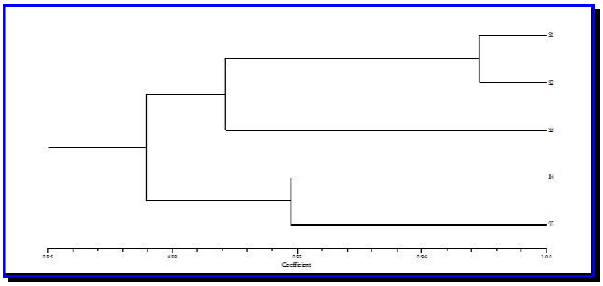

Figure 3. UPGMA-based dendrogram showing genetic relationship among five Brinjal genotypes based on Dice's Similarity, estimates for RAPD data

Conclusion

Although there has been great advancement in the marker technology with the advent of different DNA markers like Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Single Nucleotide Polymorphism (SNPs) and Diversity arrays technology (DArt), still RAPD is quite convenient to apply provided the problem of reproducibility is minimized. The only option left over is to validate by using maximum number of primers for the samples provided. The preliminary work carried out with three random primers selected from literature revealing the genetic diversity among 5 brinjal cultivars could be exploited further by increasing the number of random primers and validating it with other available DNA markers. Furthermore the results concluded that more molecular data is required to elaborate the complete profile of the genetic diversity of brinjal and select more promising lines for the improvement of the crop. Such information may be useful for selecting the diverse parents and monitoring the genetic diversity periodically in the breeder's collection of brinjal.

List of Abbreviations

CTAB: Cetyltrimethylammonium Bromide
RAPD: Randomly Amplified Polymorphic DNA
AFLP: Amplified Fragment Length Polymorphism
SSR: Simple Sequence Repeats
UPGMA: Unweighted Paired Group Method Using Arithmetic Averages

Competing Interests

The authors declare that they have no competing interests.

Author's Contributions

The work is original and it has been carried out by MAK for M.Phil. degree under the supervision of RDK at Bundelkhand University, Jhansi (U.P.) India, and at Indian Grassland of Fodder Research Institute (IGFRI), Jhansi (U.P.) India.

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