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Genetic diversity of Indian brinjal revealed by RAPD and SSR markers

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ABSTRACT

Brinjal or eggplant (*Solanum melongena* L.) is one of the most important solanaceous vegetable crop plants after tomato and potato. Through the use of PCR based markers, genetic analysis in brinjal has gained momentum during the past few years. In this study, 11 RAPD and 6 SSR primers were used to analyze the genetic variation in 29 popular Indian brinjal varieties. The 11 RAPD primers generated 64 polymorphic markers with an average of 5.81 polymorphic bands per primer. Genetic distance based on RAPD markers among all the varieties ranged from 0.07 to 0.78 with an average of 0.33. All the six SSR primer pairs were polymorphic with a total of 25 detected alleles. The number of alleles per primer ranged from 2 to 10, with a mean of 4.67. UPGMA clustering for RAPD and SSR markers grouped all the brinjal varieties into two clusters, but grouping patterns were different for each of the marker system. Based on SSR markers, the maximum genetic distance of 1 was found between Pusa Bhairav and Green Long, Green Long and KS-224, Green Long and SL-195, Green Long and KS 331 and between Pusa Kranti and SL-195 followed by 0.85 between Pusa Kranti and KS-224, and NDB-25 and Pusa Kranti. However, majority of the cultivated varieties did not cluster concordant to the collection site information or phenotypic data such as fruit shape or any other known traits. The genetic diversity of brinjal varieties reported in this study will be useful when planning future crosses amongst these varieties.

Key words: Genetic diversity analysis, RAPD, SSR, *Solanum melongena*.

INTRODUCTION

Brinjal or eggplant (*Solanum melongena* L.) is an important vegetable in central, southern and south-east Asia, and in a number of African countries. It was domesticated in India, which is the centre of diversity for this crop (Vavilov, 18). It is a good source of vitamins and minerals especially iron contributing to its nutritional importance (Kalloo, 6). Brinjal exhibits extensive variation in morphological and biochemical traits (Arivalagan *et al*., 2). DNA based molecular markers techniques have become powerful and accurate tools for cultivar identification and analysis of genetic diversity in this era (Karp *et al.*, 8; Arya *et al.,* 3). Sakata *et al.* (14) studied the taxonomic affinities between *S. melongena* and related species using the distribution of restriction fragments on chloroplast DNA.

Karihaloo *et al.* (7) analysed 52 accessions of *S. melongena* and its related wild species using RAPD markers and concluded that even though *S. melongena* and *S. insanum* were highly diverse morphologically, it was no longer appropriate to distinguish them taxonomically. Mace *et al.* (9) carried out AFLP analysis for assessing genetic relationships among 33 accessions belonging to *S. melongena* and related species. Nunome *et al.* (10) evaluated the potential of microsatellite markers for use in genetic studies in *S. melongena* and related species. A total of 37 PCR primer pairs were designed, 23 of which amplified a single or several products. Two to six alleles per primers were displayed in 11 cultivars or breeding lines of *S. melongena* and two to thirteen alleles were displayed in 11 *Solanum* relatives included in the study. Of the 23 microsatellite, seven showed polymorphism between parental lines of F_2 mapping population and could be mapped on to the linkage map constructed using microsatellite, RAPD and AFLP markers.

Simple Sequence Repeats (SSR) markers have been used for assessing genetic relationships among 96 accessions of *Solanum melongena* and four related species (Behera *et al.*, 4). They found the closely related species *S. insanum* and *S. incanum* clustered along with *S. melongena* accessions and constitute important sources of genes that can be introgressed by backcross breeding. Singh *et al.* (15) carried out RAPD analysis of 28 *S. melongena* accessions and its related wild species. They suggested that genetically distinct genotypes identified using RAPD markers could be used as potential sources of germplasm for eggplant improvement. This study was intended to assess the morphological and molecular diversity in Indian cultivated brinjal varieties as they represent an

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elite germplasm suited to open field conditions that can be used for hybrid development.

MATERIALS AND METHODS

Twenty nine indigenous brinjal genotypes were selected based on genetic as well as eco-geographical diversity (Table 1) and the investigation was carried out at the Research Farm of Division of Vegetable Science, IARI, New Delhi (latitude 28°40' North, longitude 77°12' East; altitude of 228.6 m above mean sea level). Total DNA was extracted from young healthy leaves using the CTAB (Cetyl Trimethyl Ammonium Bromide) method developed by Saghai Maroof *et al.* (13) with minor modifications. Purified DNA was subjected to amplification for RAPD in a 25 µl reaction mixture containing 100 ng DNA, 1 U *Taq* DNA polymerase (MBI Fermentas), 1x *Taq* buffer (MBI Fermentas), 3 mM $MgCl_2$, 200 µM dNTP, and 0.4 µM primer. A set of 56 primers (Operon Technologies Inc., USA) consisting of OPA, OPB, OPC, OPD and OPE series were used for the analysis. Amplification was carried out in 96 well thermocycler (Perkin Elmer, Model 9600) programmed for an initial denaturation at 94°C for 4 min., followed by 40 cycles of 94°C for 1 min., 36°C for 1 min., 72°C for 2 min. and finally a 5 min. extension at 72°C. The amplified products were resolved on a 1.5% agarose gel containing ethidium bromide at a constant voltage of 60 V for 3 h using a horizontal gel electrophoresis system (Bio-Rad). The amplified fragments were visualized and photographed under UV light using a gel documentation system.

Table 1. List of brinjal genotypes used in the study and/ their sources.

Sl. No.	Genotype	Source
1.	NDB-25	Faizabad, U.P.
2.	DBL-21	I.A.R.I., New Delhi
3.	PPC	I.A.R.I., New Delhi
4.	DBR-31	I.A.R.I., New Delhi
5.	Kt-4	I.A.R.I., New Delhi
6.	Pusa Bhairav	I.A.R.I., New Delhi
7.	Green Long	Kalayani, West Bengal
8.	Aruna	Akola, Maharastra
9.	JNDBL-1	J.N.K.V.V., Jabalpur
10.	APAU SL-2	A.P.A.U., A.P.
11.	LWC	I.A.R.I., New Delhi
12.	KS-335	Kalayanpur, U.P.
13.	PPR	I.A.R.I., New Delhi
14.	Pusa Kranti	I.A.R.I., New Delhi
15.	G-190	I.A.R.I., New Delhi
16.	DBL-11	I.A.R.I., New Delhi
17.	KS-224	Kalayanpur, U.P.
18.	DBR-8	I.A.R.I., New Delhi
19.	91-2	Pantnagar, Uttranchal
20.	SL-195	I.A.R.I., New Delhi
21.	KS-327	Kalayanpur, U.P.
22.	KS-331	Kalayanpur, U.P.
23.	SL-190-10-12	Panitpat, Hyderabad
24.	Swarna Shree	C.H.E.S., Ranchi
25.	DBSR-44	I.AR.I., New Delhi
26.	SL-71-19	Sabour, Bihar
27.	ABR-1.	Anand, Gujrat
28.	DBSR-91	I.A.R.I., New Delhi
29.	ABSR-1	Anand, Gujrat

A total of 16 primer pairs used by Nunome *et al*. (10) for diversity analysis in *Solanum* spp. were custom synthesized and used in PCR reactions. Purified DNA was subjected to amplification for SSR in a 25 µl reaction mixture containing 50 ng DNA, 1 U *Taq* DNA polymerase (MBI Fermentas), 1x *Taq* buffer (MBI Fermentas), 3 mM $MgCl_2$, 200 µM dNTP, and 0.4 µM of each primer (forward and reverse). Amplification was carried out in 96 well thermocycler (Perkin Elmer, Model 9600) programmed for an initial denaturation at 94°C for 4 min., followed by 35 cycles of 94°C for 1 min., 65°C for 1 min., 72°C for 2 min. and finally a 5 min., extension at 72°C. The amplified products were resolved on a 3% metaphor agarose gel containing ethidium bromide at a constant voltage of 60 V for 3 h using a horizontal gel electrophoresis system (BioRad). The amplified fragments were visualized and photographed under UV light using a gel documentation system. Polymorphic information content (PIC) as a measure of allele diversity at a locus was determined for each SSR primer pair (Anderson *et al.*, 1). The amplified fragments were scored manually for their presence (denoted as '1') or absence (denoted as '0') for each primer for RAPD and SSR marker systems. The binary matrix was used to estimate Jaccard's genetic similarity coefficients (Jaccard, 5) for RAPD and SSR. The similarity matrices were subjected to unweighted pair group method of arithmetic averages (UPGMA; Sneath and Sokal, 16) clustering method in order to construct the two dendrograms. The NTSYS-PC software, Version 2.1 (Exter Software, Setauket, NY, USA) was employed to carry out these analyses (Rohlf, 12).

RESULTS AND DISCUSSION

In the present study, 56 RAPD and 6 SSR primers were used for genetic diversity analysis of 29 brinjal genotypes. Eleven RAPD and six SSR primers showed clear bands and polymorphism on profiling (Tables 2&3). The usefulness of RAPD markers in brinjal genotype identification and analysis of genetic relatedness has been demonstrated by Karihaloo *et al.* (7). The 11 RAPD primers produced 124 bands, 64 (51.16%) of these were found to be polymorphic. The degree of RAPD polymorphism was slightly higher than reported by Tiwari (17) who observed 47.88% of polymorphism in brinjal. The size of the amplified products varied from approximately 260 (OPC20) to 3300 bp (OPD03). The total number of bands per primer ranged from 6 (OPA20) to 18 (OPD10) with an average of 11.27 bands per primer. Maximum numbers of polymorphic bands (9) were obtained with the primer OPD10 followed by primers OPA15 and OPD03 which produced 8 polymorphic bands. The average number of polymorphic bands per primer was 5.81. The percent of polymorphic bands ranged from 41.66% (OPD12) to 61.54% (OPA15). Singh *et al.* (15) analysed genetic diversity in brinjal and found a similar average number of bands per primer (10.28), while Tiwari (17) reported a lower average number of polymorphic bands per primer (3.4) than the present study. Genetic distance among 29 genotypes ranged from 0.07 (DBL-21 vs. SL-195) to 0.78 (DBR-8 vs. KS-331) with an average of 0.33 for the 29 genotypes. UPGMA based cluster analysis for RAPD showed that 29 genotypes were grouped in one major group at similarity coefficient

Table 2. Sequence, number of bands, percentage polymorphism of RAPD primers.

Name	Sequence	Total bands	No. of polymorphic bands	% of polymorphic bands
OPA03	5'-GTCGCCGTCA-3'	12	6	50.00
OPA13	5'-CAGCACCCAC-3'	7	4	57.14
OPA15	5'-TTCCGAACCC-3'	13	8	61.54
0PA20	5'-GTTGCGATCC-3'	6	3	50.00
OPC10	5'-GGTCTACACC-3'	11	6	54.54
OPC20	5'-ACTTCGCCAC-3'	11	6	54.54
OPD03	5'-GTCGCCGTCA-3'	15	8	53.33
OPD10	5'-GGTCATACACC-3'	18	9	50.00
OPD12	5'-CACCGTATCC-3'	12	5	41.66
OPD18	5'-GAGAGCCAAC-3'	10	6	60.00
OPE01	5'-CCCAAGGTCC-3'	9	5	55.55
Total		124	64	51.16

Table 3. Repeat motif, sequence, allele number and PIC of SSR markers used for diversity analysis.

EM No.	Repeat motif	Primer sequence (5'-3')	Allele number	PIC
133	$(AC)_{13}(AT)_6$	GCGGATCACCTGCAGTTACATTAC TCCTTTGACCTATAGTGGCACGTAGT	3	0.179
135	$(CA)_{11}$ $(GA)_{20}$	ATCCTGTTGCTGCTCATTTTCCTC AGGAGGATCCAAGAGGTTTGTTGA	3	0.530
140	$(AC)_4GC(AC)_5T(AC)_3ATGC(AC)_4AT(AC)_6(AT)_5G(TA)_{13}$	CCAAAACAATTTCCAGTGACTGTGC GACCAGAATGCCCCTCAAATTAAA	10	0.700
141	$(AT)_{16}(GT)_{19}$	TCTGCATCGAATGTCTACACTGTGC AAAAGCGCTTGCACTACACTGAAT	2	0.450
145	$(TACA)_4TA(TACA)_4(CA)_{37}$	CAGTGCTACATAAATTGAGACAAGAGG GGAGGTACAACGGATTTTCATATGGT	4	0.814
155	$(CT)_{38}$	CAAAAGATAAAAAGCTGCCGGATG CATGCGTGAGTTTTGGAGAGAGAG	3	0.629

of 0.44. The main group consisted of one large sub-cluster (9 genotypes, *viz*., NDB-25, DBL-21, SL-195, Green Long, KS-327, SL-91-2, KS-331, PPC and Pusa Kranti) and 6 small sub-clusters (2-3 genotypes). It was observed from the dendrogram (Fig. 1) that DBR-31 is genetically diverse from the rest of the 28 genotypes. High degree of variation reported in the present study could be due to the fact that our analysis included genotypes from India where greatest diversity is reported. Interestingly collections originating from various parts of the country did not form well defined distinct groups and were interspersed with each other indicating no association between RAPD pattern and geographic origin of accessions.

The six SSR primers produced a total of 28 alleles. The size of amplified alleles for SSR markers varied from approximately 160 (EM No. 133) to 369 bp (EM No. 145). The number of alleles for each primer ranged from two (EM No. 141) to ten (EM No. 140) with an average of 4.67 per primer. Nunome *et al.* (10) reported 16 polymorphic STMS primers out of 23 primers, average number of alleles per primer was 3.1, and Behera *et al.* (2) reported 4.4 alleles per primer. In the present study 6 primers showed 2-10 alleles with an average of 4.6. The average genetic distance based on SSR markers for the 29 genotypes was 0.47. The PIC values varied from 0.18 for primer EM No. 133 to 0.81 for primer EM No. 145.

UPGMA based cluster analysis for SSR markers (Fig. 2) showed that 29 genotypes were grouped into two major groups at the similarity coefficient of 0.38. Group I had 25 genotypes except Green Long, Pusa Kranti, Aruna and JNDBL-1. Group I could be further divided into two sub-groups at a similarity coefficient of 0.48. The subgroup I consisted of 4 genotypes, *viz*., Pusa Purple Cluster, DBSR-91, DBSR-44 and ABSR-1. The subgroup II was further divided into two clusters at similarity coefficient of 0.51. The small cluster contains 7 genotypes represented by NDB-25, DBL-11, Kt-4, SL-71-19, G-190, SL-190-10-12 and Swarna Shree. The large cluster consisted of 14 genotypes which are represented by DBL-21, KS-327, Pusa Bhairav, Pusa Purple Round, KS-224, KS-335, KS-331, DBR-31, Long White Cluster, DBR-8 and SL-91-2. Minimum genetic distance of 0 was found between DBR-8 and SL-91-2, and Aruna and JNDBL-1 followed by 0.08 between Pusa Bhairav and Pusa Purple Round, Pusa Bhairav and KS-224 and between SL-190-10-12 and Swana Shree. The maximum genetic distance of 1 was found between Pusa Bhairav and Green Long, Green Long and KS-224, Green Long and SL-195, Green Long and KS 331 and between Pusa Kranti and SL-195 followed by 0.85 between Pusa Kranti and KS-224, and NDB-25 and Pusa Kranti.

These results reveal that the genotypes taken in the study are genetically diverse. The present results are similar to those obtained by Nunome *et al.* (10) and Behera *et al.* (2). The genetically divergent varieties identified in the present study can be utilized in the

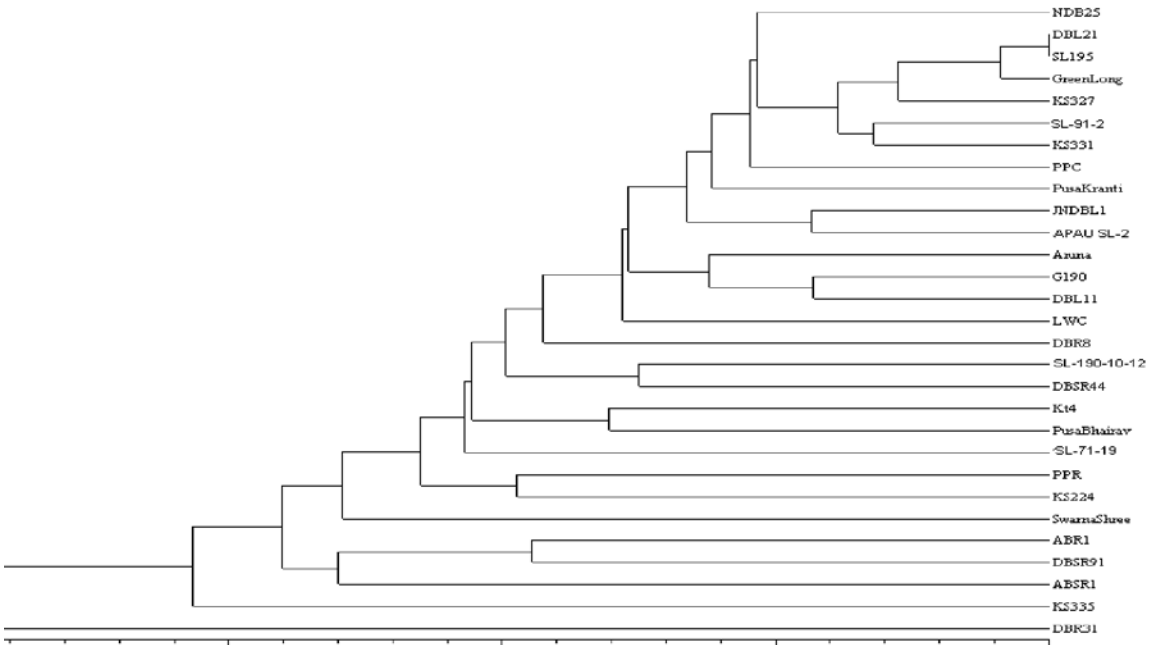

Fig. 1. UPGMA dendrogram based on RAPD analysis of the 29 brinjal genotypes.

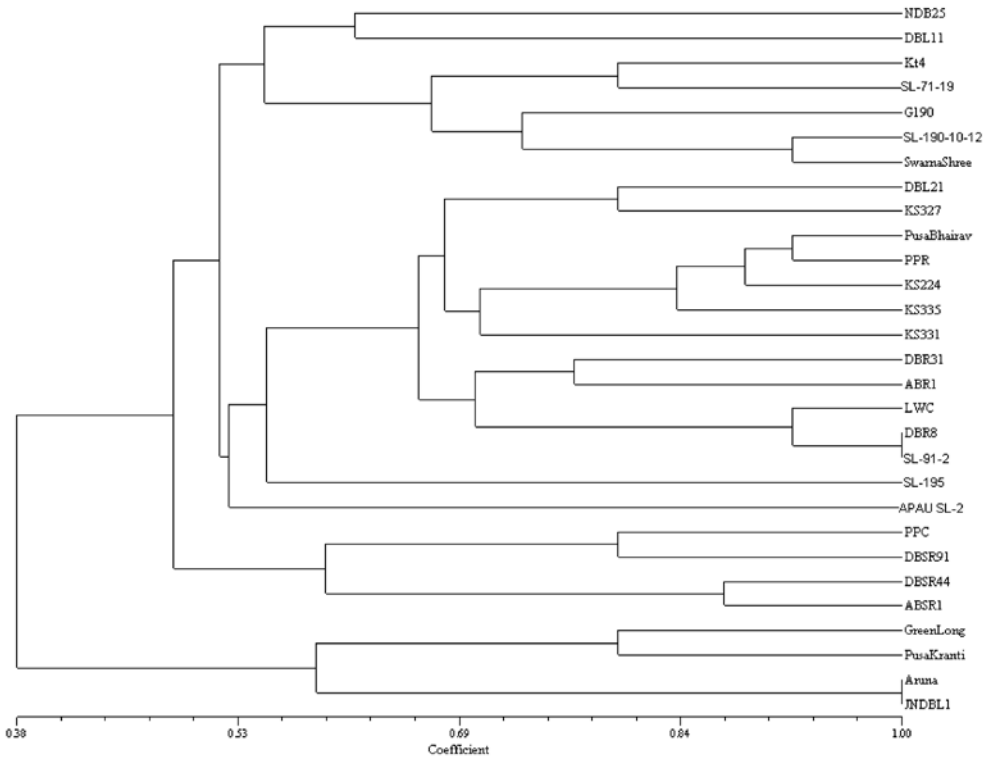

Fig. 2. UPGMA dendrogram based on SSR analysis of the 29 brinjal genotypes.

hybridization programmes. The average similarity coefficient by RAPD analysis is 0.67 and by SSR is 0.53. Hence, even though numbers of polymorphic markers used were less in SSR they have revealed more overall diversity in the genotypes taken for study. UPGMA clustering for both RAPD and SSR marker system grouped all the brinjal varieties into two clusters, but grouping patterns were different for each of the marker system. However, majority of the cultivated varieties did not cluster concordant to the collection site information or phenotypic data such as fruit shape or any other known trait (Rathi *et al*., 11). The genetic diversity of brinjal varieties reported in this study will be useful when planning future crosses amongst these varieties.

From this study it may be concluded that, a wide range of variation for almost all the economically important traits are present in this crop so there is a vast scope for improvement through different breeding procedure. The diverse genotypes can be utilized for hybridization programme based on the clustering pattern adopted through different procedures.

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