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STUDIES ON MOLECULAR DIVERGENCE IN EGG PLANT *(SOLANUM MELONGENA* L.) USING RAPD MARKERS

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Abstract – The RAPD analysis with 11 primers produced 100 per cent polymorphism with an average of 4.5 polymorphic bands per primer and detected a moderate level of genetic variation among brinjal accessions with average similarity coefficient of 0.36. Accession-specific RAPD markers were detected only for few accessions such as IC112726 (OPP 17) and IC336793 (OPB 20). The UPGMA analysis grouped the accessions into two main clusters *viz.,* cluster I (forty four accessions) and cluster II (forty accessions).

INTRODUCTION

Egg plant (*Solanum melongena L),* also known as aubergine or brinjal is an important vegetable in central, southern and south-east Asia and in a number of African countries (Kalloo, 1988). Brinjal, usually referred as the poor man's crop is highly productive and is preferred and consumed by every class of people because of its nutritive value (rich in vitamin A and B) and availability at very low price.

Traditionally, morphological traits were used to assess diversity. However, because of high level of variability; morphological data can lead to ambiguous interpretations. The burgeoning field of molecular biology has now provided tools suitable for rapid and detailed genetic analysis of higher organisms. DNA markers, perhaps are the most fundamental tools in molecular biology which have wide spread application in construction of genetic maps and form the basis to determine chromosomal location of genes affecting either simple or complex traits in plants and animals. Besides these, molecular markers are particularly useful in the assessment of genetic diversity for the identification and removal of duplicates, protection of core collection, evaluating genetic relationships between taxa and selecting diverse parental genotypes required for planning an efficient breeding programme. Detailed characterization and evaluation of brinjal genepool is necessary for identification and exploitation of useful lines in crop improvement programmes and also for better conservation of genetic resources.

Of the several classes of DNA-based markers, the RAPD are short tandem repeats of DNA sequences that are dispersed in all eukaryotic genomes. These are polymerase chain reaction (PCR) - based, highly polymorphic and widely distributed in the genome. Detailed information on genetic diversity and structure of the brinjal germplasm lines suitable to coastal region of Andhra Pradesh is yet to be generated. Hence a study was proposed to characterize different accessions of brinjal collected from the National Bureau of Plant Genetic Resources, Regional Station, Hyderabad for morphological traits and also at molecular level using selected RAPD markers.

MATERIALS AND METHODS

The materials used for the study comprised of 80 germplasm lines and 4 check varieties of brinjal. The plant material was maintained at Horticultural Research Station, Venkataramannagudem.

Extraction of plant DNA

DNA was extracted by modified CTAB (Cetyl Trimethyl Ammonium Bromide) (Murray and Thompson, 1980) as detailed below.

- **a.** Healthy young leaves (5g) were collected from young plants and brought to the laboratory on ice. The leaves were surface sterilized with 70 per cent ethyl alcohol and cut into pieces and immediately used for further processing.
- **b.** Approximately 1 g of fresh leaf sample was weighed and homogenized completely with liquid Nitrogen using mortar and pestle.
- **c**. Powdered leaf material was transferred to eppendorf tubes and suspended in a pre-heated (65°C) 500 µ1 of extraction buffer (containing 2% CTAB, 100mM Tris, 20 mM EDTA, 1.4M NaCl, 1% PVP and 1% β-mercapto ethanol.
- **d**. The contents were mixed by vortexing for few minutes and the reaction mixture was incubated for 45 minutes at 65 °C in water bath.
- **e.** The tubes were centrifuged at 14,000 rpm for 10 minutes at 4 °C.
- **f.** Supernatant was collected in a separate tube to which 900 µl Chloroform: Isoamyl alcohol (24:1) mixture was added and centrifuged at 14,000 rpm for 10 minutes at 4 °C.
- **g.** To the supernatant, 0.6 volume of ice cold isopropanol was added and centrifuged at 14,000 rpm for 10 min at 4 °C.
- **h**. Pellet was collected and added with 150 µl of TE buffer to dissolve the pellet.
- **i.** To this 150 µl Phenol and 150 µl Chloroform: Isoamyl alcohol (24:1) was added and centrifuged at 14,000 rpm for 10 minutes at 4^0 C.
- **j.** To the supernatant collected equal volume of Chloroform: Isoamyl alcohol (24:1) was added and centrifuged at 14,000 rpm for 10 minutes at 4^0 C.
- **k.** Supernatant was collected in a separate tube to whcih equal volume of 95 per cent ethanol and 1/3rd volume of sodium acetate (pH 5.2) was added and left at -20 °C overnight for precipitation of DNA.
- **l.** After incubation, the tubes were spun at 14,000 rpm for 10-15 minutes. Later, the pellet obtained was washed with 70 per cent ethanol (100 µl) and centrifuged at 14,000 rpm for 5 minutes.
- **m.** The pellet obtained was air dried and was dissolved in TE buffer and stored at -20 °C for further use.

The concentration and quality of DNA was estimated using Nano Drop spectrophotometer at 260 nm and verified by running sample on 1.0 per cent agarose along with 1 kb marker.

RAPD reaction mixture

The RAPD reaction mixture consisted of 60 ng of template DNA, 1 unit of Taq polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India), $MgCl_2$ - 2.5mM; dNTPs (Genei)- 0.2mM; 10 p mole of Random decamer primer (Operon, USA), Buffer (Genei) – 10X in a reaction volume of 25 µl.

Amplification conditions for RAPD primers

Amplification was carried out on eppendorf thermal cycler using 11 decamer primers.
The amplification profile was as follows:

a.	Initial denaturation temperature	94 °C	2 minute
b.	Denaturation	92 °C	1 minute
c.	Primer annealing	37 °C	1 minute
d.	Primer extension	72 °C	2 minute
Later step b to d were repeated			45 times
e.	Complete primer extension	72 °C	5 minutes
f.	Soak temperature	4 °C	Till removal

Agarose gel electrophoresis

Amplified products of RAPD primers were separated on 3 per cent agarose gel stained with Ethidium bromide (1 µg/ml of gel). The microscopic pores in the agarose gel act as a molecular sieve. TAE buffer 50 X (2M Tris-base-242g, 17.4M glacial acetic acid – 57.1 ml and 200 ml of 0.5 M EDTA, pH 8.0 per litre) was used as running buffer for electrophoresis. 5 µl of gel loading dye (Bromophenol blue) was added to 25 µl of RAPD product and mixed well before loading into wells. 1 kb ladder (Genie) was loaded in first lane as marker for RAPD primers. Electrophoresis was conducted at 100 volts for 3 hours and the gel was photographed under UV light using Syngene gel doc system.

Scoring and statistical analysis for molecular analysis

Data were entered using a matrix in which all observed bands or characters were listed. The RAPDs pattern of each accession was evaluated; assigning character state '1' to all the bands that could be reproducible and detected in the gel and '0' for the absence of band for each primer and used to calculate a genetic similarity matrix using the Jaccard (J) coefficient, which is more appropriate for dominant markers as it does not count 0/0 matches in the calculation. The genetic distance between each pair of accessions was calculated by SIMQUAL analysis of the NTSYS-pc software package version 2.1 (Rohlf, 2000).

RESULTS AND DISCUSSION

Among 11 primers screened, RAPD pattern of 10 primers were reproducible and scored for analysis (Table 2). Remaining one primer (OPA 1) did not produce polymorphic profiles with different accessions. The number of the amplified fragments varied from 1 to 7 with an average of 4.5 polymorphic fragments per primer. Similarly, a total of 45 amplicons were produced by examining the 84 accessions of brinjal with 10 RAPD primers. The number of scored polymorphic bands was also in the range of 1 to 7 in different primers with an average of 4.5 polymorphic fragments per primer. The molecular weight of PCR amplified band ranged from 120 bp to 3200 bp. A total of 45 amplified fragments were scored and all these fragments were found to be completely polymorphic. The characteristic fragments generated by an array of 10 decamer primers employed as single orbitrary primers are summarized in Table 1. The polymorphism showed by these 10 markers was 100 per cent with different brinjal accessions. The polymorphic fragments present in only one accession were considered to be unique fragments. Out of the total 45 polymorphic fragments, two were unique to particular accessions *viz;* IC112726 and IC336793 (Table 2).

Allele diversity of RAPD marker analysis

The allele frequency, gene diversity, heterozygosity and polymorphic information content (PIC) were calculated by using the Power marker 3.25 version statistical software for the RAPD markers used in this study (Table 3). The highest PIC was recorded by the RAPD marker OPP 17 (0.6605) while it was found to be the lowest for the marker OPB 19 (0.2930). Higher the PIC value, more informative is the RAPD marker and hence, the primer OPP 17 was found to be highly informative. Similarly, this marker also recorded the highest gene diversity and heterozygosity of 0.6913 and 1.0000 respectively and lowest allele frequency (0.5000).

Similarity index

Baning profiles obtained with 11 primers for 84 accessions of brinjal were analyzed on the basis of presence or absence of the band. Jaccard's similarity coefficients among these accessions were calculated to establish the genetic relationships. Genetic similarity based on Jaccard's coefficient revealed considerable level of diversity among the accessions under the study. The average genetic similarity among eighty four brinjal accessions ranged from a coefficient of 0.00 to 0.71 with an average similarity coefficient of 0.36 among the group of accessions studied.

In the present study, IC112750, IC112741, IC345740, IC354651, Shyamala, IC249358, IC112322, IC427008, IC089890, IC354517, IC090942, IC090785, EC329327, IC427007, IC111387, IC280957, IC374867, IC427017, IC089912, IC354564, IC279555, IC281092, IC112997, Bhagyamati, IC354135, IC467274, IC413648, IC421194, IC090938 and IC281104 were found to be most genetically similar (0.15). On contrary, IC249358 and IC112909 were found to be genetically diverse (0.68) among all the germplasm lines.

The exotic collection EC386589 had exhibited a high average similarity with IC397299 with a similarity coefficient value of 0.46. Among the commercially grown check varieties Bhagyamati

Table 1. List of Random primers used for DNA amplification in brinjal

S. No.	Primer code	Total number of bands	Poly morphic bands	Polymorphism (%)	Range of Fragment size (bp)
1	OPA – 09	5	5	100	150-2600
2	OPB – 18	4	4	100	130-1400
3	OPB – 19	1	1	100	600
4	OPB – 20	2	2	100	500-700
5	OPC – 5	4	4	100	150-2100
6	OPC – 9	5	5	100	120-3200
7	OPC – 14	7	7	100	220- 1500
8	OPO – 03	5	5	100	350- 800
9	OPP – 17	7	7	100	150-1250
10	OPV – 14	5	5	100	120-700
	Total	45	45		
	Average	4.5	4.5		

and Shyamala had exhibited a high average similarity with a similarity coefficient value of 0.15, ascertaining the existence of high genetic similarity between these cultivars. Among the exotic collections, EC316280 exhibited an average similarity coefficient value of 0.43 with EC384565 indicating the existence of high genetic similarity between these two accessions.

Table 2. Details of unique RAPD fragments associated with specific brinjal accessions

S. No.	Name of the accession	Primer revealing unique RAPD	No. of base pairs of fragment
1	IC112726	OPP 17	1250 bp
2	IC336793	OPB 20	700 bp

Cluster analysis

The genetic relationships of brinjal accessions were further evaluated by UPGMA cluster analysis, on RAPD data set, using a minimum variance algorithm (Fig. 1). It resulted in two main clusters at a genetic similarity coefficient of 0.71 with EC 386589 and IC112909 at the extreme ends of the dendrogram. All the 84 accessions were grouped into two clusters of which 44 accessions grouped in cluster I and 40 accessions grouped in cluster II. Accessions in these two clusters diverged at similarity value of 0.71. Cluster I was further divided into sub cluster IA with 43 accessions and sub cluster IB with one accession (IC249358) at similarity coefficient value of 0.67 as shown in the dendrogram. The sub cluster IA further sub divided into two groups IA-a (IC112909) and IA-b (42 accessions) at similarity index of 0.60 in the dendrogram. The accessions in IA-b were further divided into two groups. 1A-b1 (6 accessions) and 1A-b2 (36 accessions) at similarity index of 0.43 in the dendrogram. In 1A-b1 group of cluster I, the accessions IC467271 and IC305131 were straddling the extremes while in 1A-b2 group the accessions IC112750 and IC090026 were spanning the extremes. Cluster II was further divided into sub cluster IIA with 3 accessions (IC218975, IC261772 and Arka Kesav) and sub cluster IIB with 37 accessions at similarity coefficient value of 0.61 as shown in the dendrogram. The sub cluster IIB further sub divided into five groups IIB-a (11 accessions), IIB-b (3 accessions), IIB-c (3 accessions), IIB-d (14 accessions) and IIB-e (6 accessions) at similarity index of 0.45 in the dendrogram. In sub cluster IIB, IC350885and IC354563 accessions were spanning the extremes in IIB-a group while IC089949-B and IC272927 accessions in IIB-b group; IC298633 and EC384565 accessions in IIB-c group; IC354528 and IC336793 accessions in IIB-d group and EC386589 and IC112747 accessions in IIB-e group were spanning the extremes. Among the exotic collections viz., EC386589 was grouped in IIB-e, EC316280 and EC384565 were grouped in IIB-c group, EC385380 in IIB-d group and EC329327 and EC316226 were grouped in IA-b2 group along with other indigenous collections. Among commercially grown check varieties Bhagyamati, Gulabi and Shyamala were grouped in IA-b2 group with similarity index of 0.15 and Arka Kesav was grouped in IIA group with similarity index of 0.49.

Principal component analysis for RAPD analysis

The Principal Component Analysis was based on molecular data of 10 RAPD markers to visualize the genetic relatedness among the brinjal accessions in detail. The description of the data using three

Table 3. Major allele frequency, gene diversity, Heterozygosity and polymorphic information content of random primers used for DNA amplification in brinjal

S.No.	Marker	Major Allele Frequency	Gene Diversity	Hetero zygosity	PIC
1	OPP17	0.5000	0.6913	1.0000	0.6605
2	OPV14	0.5060	0.6531	0.9881	0.6044
3	OPC 9	0.5000	0.6732	1.0000	0.6315
4	OPC 5	0.6012	0.5753	0.7976	0.5275
5	OPO 3	0.5595	0.6042	0.8810	0.5508
6	OPB 18	0.5000	0.6414	1.0000	0.5846
7	OPA 19	0.5060	0.6536	0.9881	0.6054
8	OPB20	0.5060	0.6215	0.9881	0.5514
9	OPB19	0.7679	0.3565	0.4643	0.2930
10	OPC 14	0.5000	0.6916	1.0000	0.6604
	Mean	0.5446	0.6162	0.9107	0.5669**T**

dimensional pictorial graph and the same is presented in Fig. 2. It is evident that the brinjal accessions were dispersed on the PC plot, which is a reflection of variation among the accessions. The results of PCA showed a clear cut separation. However, as depicted in figure, some of the accessions appear to be overlapping with each other depicting high similarity in these accessions.

Genetic diversity and relatedness among the brinjal accessions were estimated by 11 RAPD markers amplified bands which ranged from 120 bp to 3200 bp (Table 1). This was comparable with results generated by polymorphic bands ranging from 150 bp to 3500 bp (Singh *et al.*, 2006) in brinjal, 200 bp to 3000 bp (Dey *et al.*, 2006) in bitter gourd, 250 bp to 1400 bp (Veerendra *et al.*, 2007) in ash gourd. In the present study, a total number of 45 polymorphic bands were produced by the use of 10

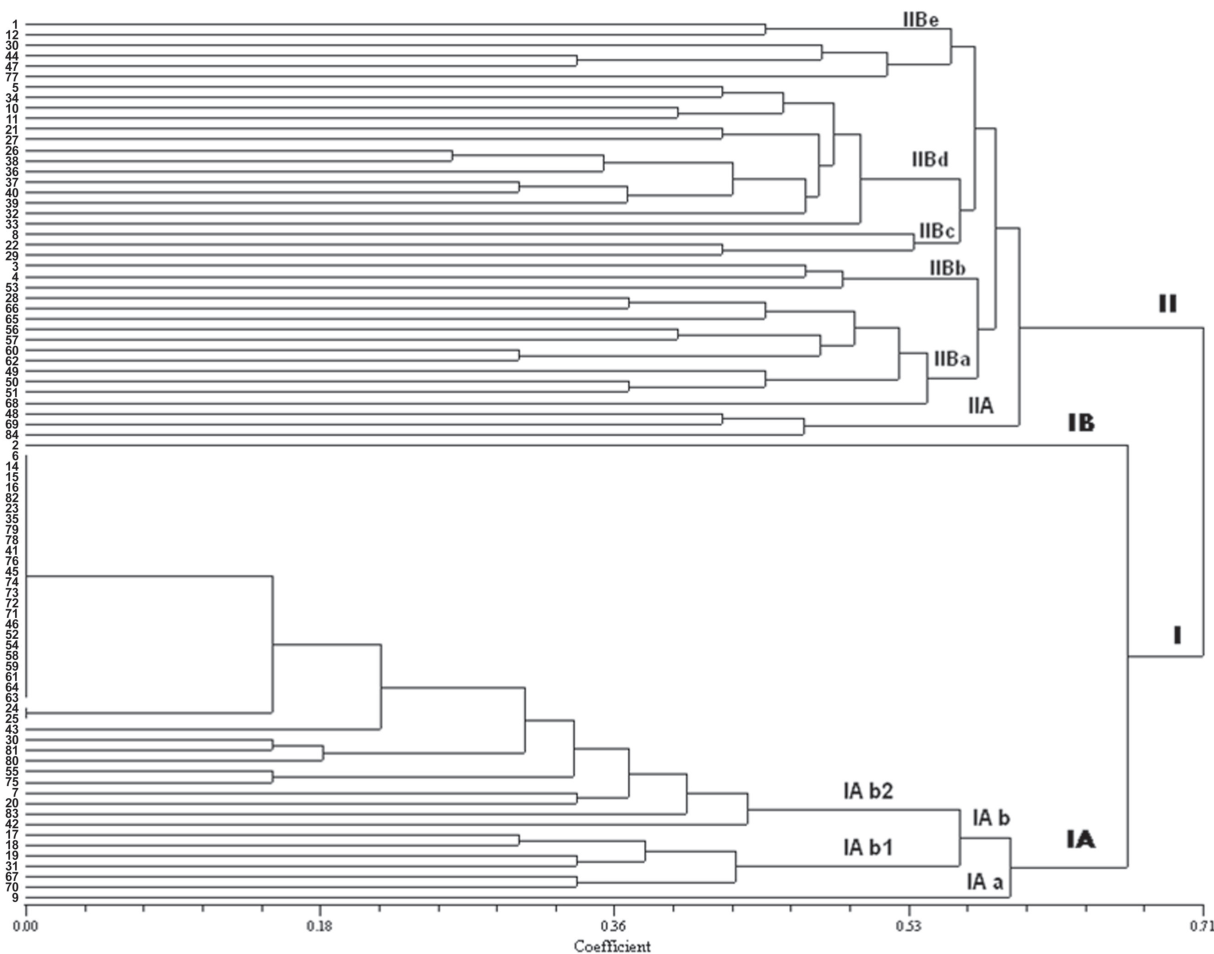

Fig. 1. Dendrogram of brinjal accessions based on RAPD markers

1. EC386589	2. IC249358	3. IC089949-B	4. IC112738	5. IC354528	6. IC112750
7. IC090915	8. IC298633	9. IC112909	10. IC374892	11.IC312984	12. IC397299
13. IC112997	14. IC112741	15. IC345740	16. IC354651	17. IC467271	18. IC090905
19. IC336472	20. IC112993	21. IC089989	22. EC316280	23. IC545937	24. IC279555
25. IC281092	26. IC545844	27. IC397557	28.IC350885	29. EC384565	30. IC332508
31. IC304072	32. IC099676	33. IC336793	34. IC112726	35. IC112322	36. IC374912
37. EC385380	38. IC354612	39. IC398820	40. IC344646	41. IC354517	42. IC090026
43. IC281112	44. IC112818	45. IC090785	46. EC329327	47. IC545948	48. IC218975
49. IC345747	50. IC261899	51. IC090987	52. IC427007	53. IC272927	54. IC111387
55. IC354135	56. IC074239	57. IC104083	58. IC280957	59. IC374867	60. IC545919
61. IC427017	62. IC305048	63. IC354564	64. IC089912	65. DBT/098	66. IC112350
67. IC354597	68. IC354563	69. IC261772	70. IC305131	71. IC467274	72. IC413648
73.IC421194	74. IC090938	75. IC281104	76. IC090942	77. IC112747	78. IC089890
79. IC427008	80. EC316226	81. Bhagyamati	82.Shyamala	83. Gulabi	84.Arkakeshav

RAPD markers which amplifies 4.5 bands per primer and appears to be adequate to make meaningful statements about the diversity or relatedness among the different brinjal accessions. Nunome (2005), Mao WeiHai *et al.* (2006) and Singh *et al.* (2006) reported amplification of 1.11, 9.67, 10.28 bands per primer respectively in brinjal. The highest percentage of polymorphism (100%) was observed with all the RAPD primers. The total number of amplified fragments generated per primer had no correlation with proportion of polymorphic bands. Similar pattern was observed by Williams *et al.*

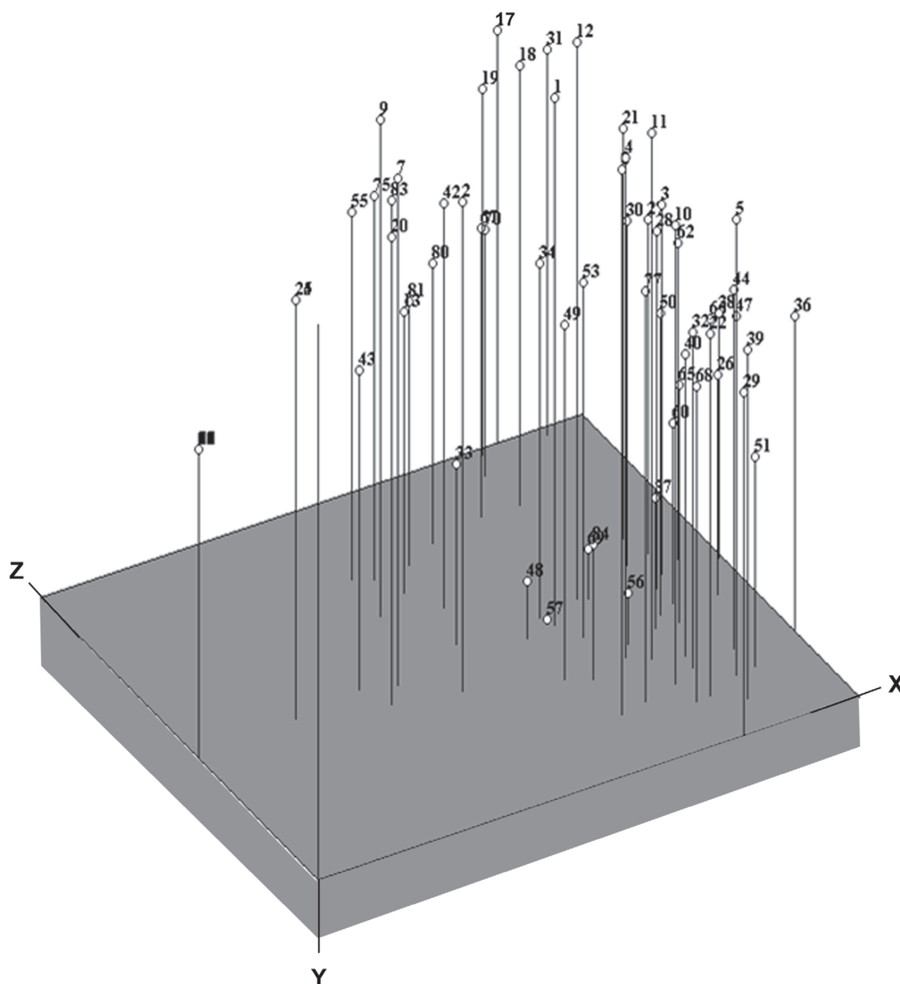

Fig. 2. The relative position of brinjal accessions based on RAPD markers (Three dimensional)

Name of the accessions:

1. EC386589	2. IC249358	3. IC089949-B	4. IC112738	5. IC354528	6. IC112750
7. IC090915	8. IC298633	9. IC112909	10. IC374892	11.IC312984	12. IC397299
13. IC112997	14. IC112741	15. IC345740	16. IC354651	17. IC467271	18. IC090905
19. IC336472	20. IC112993	21. IC089989	22. EC316280	23. IC545937	24. IC279555
25. IC281092	26. IC545844	27. IC397557	28.IC350885	29. EC384565	30. IC332508
31. IC304072	32. IC099676	33. IC336793	34. IC112726	35. IC112322	36. IC374912
37. EC385380	38. IC354612	39. IC398820	40. IC344646	41. IC354517	42. IC090026
43. IC281112	44. IC112818	45. IC090785	46. EC329327	47. IC545948	48. IC218975
49. IC345747	50. IC261899	51. IC090987	52. IC427007	53. IC272927	54. IC111387
55. IC354135	56. IC074239	57. IC104083	58. IC280957	59. IC374867	60. IC545919
61. IC427017	62. IC305048	63. IC354564	64. IC089912	65. DBT/098	66. IC112350
67. IC354597	68. IC354563	69. IC261772	70. IC305131	71. IC467274	72. IC413648
73.IC421194	74. IC090938	75. IC281104	76. IC090942	77. IC112747	78. IC089890
79. IC427008	80. EC316226	81. Bhagyamati	82.Shyamala	83. Gulabi	84.Arkakeshav

(1993). RAPD profiles illustrate that typical level of polymorphism was present in brinjal accessions. Almost all RAPDs were common to many groups. When the identification of cultivars is the purpose of the finger prints, the most important function of a primer is to discriminate as many cultivars as possible. Accession-specific RAPD markers were detected only for few accessions such as IC112726 (OPP 17) and IC336793 (OPB 20) which could be exploited for DNA fingerprinting of these accessions by converting RAPD markers into STS (Sequence Tagged Site) and this is useful for detecting mixes between cultivars (Fernandez *et al.,* 2002 and Chimote *et al.* 2007).

The data confirmed that RAPD methods are useful tools for identifying closely related accessions. Their advantage principally lies in detecting simultaneously many randomly distributed loci within the genome, in a simple, cost-effective manner, requiring no previous knowledge of genome sequence as other methods like SSRs and also RAPD primers quickly scan the whole genome detecting insertion and deletion events (Milbourne *et al.*, 1997). However, some doubts have been expressed regarding the reproducibility of the RAPD technique and the suitability of the RAPD markers for genetic diversity studies (Rafalski *et al.,* 1991; Wilkie *et al.,* 1993 and Hallden *et al.,* 1996). But the reproducibility of RAPDs can be achieved by using optimized PCR conditions and by scoring only reproducible bands as opined Aliyev *et al.,* 2007. According to the measurements of genetic similarity, there are no redundant accessions in the collection, indicating the importance of the maintenance of all accessions analyzed. The clustering pattern using RAPD data resulted in two major groups (Fig. 1).

The dendrogram of similarity coefficients indicated that accessions EC 386589 of the group IIB-e and genotype IC112909 of the group IA-a were clustered at the two extremes with a genetic similarity coefficient of 0.71, while the remaining accessions distributed in between them. This clearly indicates diversity is mainly because of inherent genetic differences at DNA level. The accession, EC329327 and EC316226 were grouped in IA-b2 and genetically similar with Bhagyamati, Shyamala, IC112750, IC112741, IC345740, IC354651, IC249358, IC112322, IC427008, IC089890, IC354517, IC090942, IC090785, EC329327, IC427007, IC111387, IC280957, IC374867, IC427017, IC089912, IC354564, IC279555, IC281092, IC112997, IC354135, IC467274, IC413648, IC421194, IC090938 and IC281104 indicating that commercially grown brinjal varieties of Andhra Pradesh *viz*., Bhagyamati, Shyamala are genetically similar to the exotic collections. Thus, the tendency of accessions occurring in clusters irrespective of geographic boundaries demonstrated that geographical isolation is not the only factor causing genetic diversity. These results corroborate with those reported by Liu (1996) in field bean who observed that the existing variation in cultivated materials has no geographic basis. The first group comprising of 2 sub-clusters (IA, IB), had a similarity coefficient value of 0.67. The sub cluster IA further sub divided into two groups IA-a (IC112909) and IA-b (42 accessions) at similarity index of 0.60 in the dendrogram. The accessions in IA-b were further divided into two groups. 1A-b1 (6 accessions) and 1A-b2 (36 accessions) at similarity index of 0.43 in the dendrogram. Cluster II was further divided into sub cluster IIA with 3 accessions (IC218975, IC261772 and Arka Kesav) and sub cluster IIB with 37 accessions at similarity coefficient value of 0.61 as shown in the dendrogram. The sub cluster IIB further sub divided into five groups IIB-a (11 accessions), IIB-b (3 accessions), IIB-c (3 accessions), IIB-d (14 accessions) and IIB-e (6 accessions) at similarity index of 0.45 in the dendrogram. Sub-clustering pattern indicated low variation within the cluster and high variation between the clusters. Sharing of genetic similarity, among the accessions originating from widely divergent locations was also evident as exotic accessions grouped together with other local selections in cluster IA-b2. Though they differed morphologically, they were similar at the DNA level. This would suggest a distinct genetic identity and rather large genetic divergence from most plant selections of the geographically closer ones (Das *et al.,* 2004). The other possible reason might be sharing a similar gene pool before their geographical separation. This could be a reason for less genetic distance similarity observed between exotic accessions in this study. The low level of genetic variability among the local selections which yielded very similar patterns with RAPD, would have caused the accessions to be grouped together suggesting that either they might have originated from a common ancestor or that the technique was not able to detect cultivar variation, such as point mutations which cannot be detected by RAPD (Dettori and Palambi, 2000). The dendrogram (Fig.1) based on RAPD analysis showed that most of the

accessions of brinjal were closely clustered; Although the accessions have been clustered into different small sub groups, the genetic similarity among 84 accessions studied was relatively high (0.36) and it could differentiate the individuals in each group, suggesting that the genetic base of domesticated brinjal germplasm is quite diverse. Variation in brinjal had taken place mainly due to hybridization with frequent recombination of genes in sexual reproduction (Frost, 1943). Hence, the utilization of available local germplasm resources in hybridization programme is advocated to evolve high yielding cultivars of brinjal. Similar pattern of high genetic diversity with RAPD markers were observed by Singh *et al.*, (2006). On contrary, little genetic polymorphism in brinjal was reported by Karihaloo *et al.* (2005).

The Principal Component Analysis based on molecular data of 10 RAPD markers to visualize the genetic relatedness among the brinjal accessions in detail. The description of the data using three dimensional pictorial graph and the same is presented in Fig. 2. It is evident that the brinjal accessions were dispersed on the PC plot, which is a reflection of variation among the accessions. The results of PCA showed a clear cut separation. However, as depicted in figure, some of the accessions appear to be overlapping with each other depicting high similarity in these accessions. It was clear from the analysis that the results obtained from the dendrogram were repeated in the PCA, which strengthened the ability and accuracy of the RAPD analysis applied to brinjal accessions in the present study. From the foregoing discussion, it is concluded that RAPD markers could be employed for finger printing and characterization of genotypes, assessment of molecular genetic divergence and relatedness among brinjal genotypes. This information can be used successfully for cultivar identification and for assessing the genetic diversity among brinjal accessions. They are effective, precise and more efficient than morphological markers.

REFERENCES

Aliyev, R. T., Abbasov, M.A. and Mammadov, A.C. 2007. Genetic identification of Diploid and Tetraploid Wheat Species with RAPD Markers. *Turk J Biol*. 31 : 137-180.

Chimote, V. P., Pattanayak, D. and Naik, P.S. 2007. Molecular and morphological divergence studies in Indian potato varieties. *India Journal of Biotechnology*. 6: 216-223.

Das, A., Mondal, B., Sarkar, J. and Sarkar, S. 2004. RAPD profiling of some elite clones of mandarin orange (*Citrus reticulata* Blanco) in the Northern Eastern Himalayan Region of India. *The Journal of Horticultural Science and Biotechnology*. 79(6): 850-854.

Dettori, M. T. and Palombi, M. A. 2000. Identification of *Feijoa selllowiana* Berg accessions by RAPD markers. *Scientia Horticulturae*. 86 : 279-290.

Dey, S. S., Singh, A. K., Chandel, D. and Behera, T.K. 2006. Genetic diversity of bitter gourd (*Momordica charantia L.*) genotypes revealed by RAPD markers and agronomic traits. *Scientia Horticulturae*. 109: 21-28.

Fernandez, M., Figueiras, A. and Benito, C. 2002. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theor. Appl. Genet*. 104 : 845-851.

Frost, H. B. 1943. Breeding to produce superior varieties. In: Webber H J and Batchelor L D (eds.). *The Citrus Industry*. 1: 886-914.

Hallden, C., Hansen, M., Nilson, N.O., Hjerdin, A. and Sall, T. 1996.Competition as a source of errors in RAPD analysis. *Theor. Appl. Genet*. 93 : 1185-112.

Kalloo, G. 1988. *Vegetable Breeding*. Vol. III. CRC Press, Boca Raton, FL.

Karihaloo, J.L., Brauner, S. and Gottlieb, L.D. 2005. Random amplified polymorphic DNA variation in the eggplant, *Solanum melongena* L. (Solanaceae). *Theoretical and Applied Genetics*. 90 (6) : 767-770.

Liu, C. J. 1996. Genetic diversity and relationships among *Lablab purpureus* genotypes evaluated using RAPD as markers. *Euphytica.* 90(1) : 115-119.

Mao Wei Hai, Du LiMing Bao, ChongLai, Hu TianHua, Zhu QinMei, Hu HaiJiao. 2006. Genetic diversity and genetic relative's analysis of southern long-eggplant germplasm based on inter-simple sequence repeat (ISSR). *Acta Horticulturae Sinica*. 33(5) : 1109-1112.

Milbourne, D., Meyer, R., Bradshaw, J., Baird, E., Bonar, N., Provan, J., Powell, W. and Waught, R. 1997. Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. *Mol. Breed*. 3 : 127-136.

Murray, M. and Thompson, W. 1980. The isolation of high weight plant DNA. *Nucleic Acids Research*. 8 : 4321-4325.

Nunome, T. 2005. Studies on the development of DNA markers and the genetics of several traits in eggplant. *Bulletin of the National Institute of Vegetable and Tea Science*. 4 : 39-69.

Rafalski, J.A., Tingey, S.V. and Williams, J.G.K. 1991. RAPD markers-a new technology for genetic mapping and plant breeding. *Ag Biotech News Info.* 3: 645-648.

Rohlf, F. J. 2000. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.1 Exceter Software, New York, USA.

Singh, A. K., Ahmed, N. and Narayan, S. 2006. Genetic divergence studies in brinjal under temperate conditions. *Indian Journal of Horticulture*. 63(4): 407-409.

Singh, A.K., Major Singh, A.K., Rakesh Singh, Sanjeev Kumar, and Kalloo, G. 2006. Genetic diversity within the genus *Solanum* (Solanaceae) as revealed by RAPD markers. *Current Science*. 90(5) : 711-716.

Thomas, G., Mohapatra, T., Rao, A.R. and Sharma, R. P. 2006. Distinguishing Indian commercial wheat varieties using RAPD based DNA fingerprints. *Indian Journal of Biotechnology*. l6 : 200-206.

Veerendra verma, K., Behera, T. K., Munshi Swarup, A. D., Parida, K. and Mohapatra, T. 2007. Genetic diversity of ash gourd [*Benincasa hispida (Thunb.) Cogn]* inbred lines based on RAPD and ISSR markers and their hybrid performance. *Scientia Horticulturae*. 113 : 231-237

Wilkie, S. E., Isaac, P. G. and Slaten, R. J. 1993. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in Allium. *Theor. Appl. Genet*. 86 : 497-504.

Williams, J. G. K., Hanafey, M. K., Rafalski, J. A. and Tingey, S. V. 1993. Genetic analysis using randomly amplified polymorphic DNA markers. *Methods in Enzymology.* 218: 704-741.