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RESEARCH ARTICLE :

Studies on molecular divergence in egg plant (*Solanum melongena* L.) using SSR markers

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SUMMARY : In total, thirty alleles were detected using twenty two SSR primer pairs and Polymorphic Information Content (PIC) values ranged from 0.1491 (155) to 0.5293 (117). UPGMA analysis grouped the accessions into two main clusters *viz.*, cluster I (eighty two accessions) and cluster II (two accessions). Among the commercial check varieties, Bhagyamati, Shyamala and Gulabi were included in one cluster while Arka Kesav was included in another cluster. The exotic collections *viz.*, EC386589, EC316280, EC384565, EC385380, EC329327 and EC316226 were included separately in different clusters along with other indigenous collections. The SSRs were able to differentiate exotic collections and commercially grown check varieties into different groups to some extent, indicating that SSRs is a more accurate and reliable method than RAPD to study the genetic diversity in brinjal.

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KEY WORDS :
SSR marker, Primers, Cluster, Genetic diversity, Accessions

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BACKGROUND AND OBJECTIVES

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. The success of breeding programme for high yield and quality depends on the nature and magnitude of variation available in the genotypes. Genetic diversity is the primary source of variation and is the basic factor in the evolution of any species.

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. Of the several classes of DNA-based markers, the microsatellites (or simple

sequence repeat or sequence tagged microsatellites or STMS) are short tandem repeats of DNA sequences that are dispersed in all eukaryotic genomes. These are polymerase chain reaction (PCR) - based, highly polymorphic, multi-allelic, frequently co-dominant, highly reproducible and widely distributed in the genome. STMS markers are being used in a number of crop species for genome mapping, marker assisted selection, germplasm analysis and for varietal identification (Behara *et al*., 2006). 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. 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 SSR markers.

RESOURCES AND METHODS

The materials used for the study comprised of 80 germplasm lines and 4 check varieties of brinjal presented in Table A. 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) method and 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. After quantification, the DNA was diluted to a concentration of 50ng/µl for SSR diversity analysis. A total of 22 SSR primers were used for the analysis.

PCR amplification for SSR marker :

The PCR reaction was carried out in a total volume of 20 µl containing the following Table A.

PCR amplifications were performed on a Eppendorf Thermal Cycler by using the following temperature cycle.

Profile 1: 94°C for 3 minutes — Initial denaturation
Profile 2: 94°C for 30 seconds — Denaturation
Profile 3: 44-59°C for 30 seconds — Annealing
Profile 4: 72°C for 30 seconds — Extension
Later step profile 2 to 4 were repeated 30 times
Profile 5: 72°C for 12 minutes — Final extension
Profile 6: 4°C infinitive

Table A : PCR amplification for SSR marker

	Stock concentration	Volume taken	Final concentration
DNA	50ng	2.0µl	5.0 ng
Forword Primer	10p mole	2.0 µl	1 p mole
Reverse Primer	10p mole	2.0 µl	1 p mole
dNTPs	10mM	0.4 µl	0.2mM
Taq buffer	10x	2.0 µl	1x
Taq DNA polymerase	100 units	0.3 µl	1.5 Units
(M/s Bangalore Genei Pvt. Ltd., Bangalore)			
Sterile water		11.3 µl	
		20 µl	

Agarose gel electrophoresis:

Amplified products of SSR primers were separated on 1 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 20 µl of SSR PCR product and mixed well before loading into wells. 100 bp ladder was loaded in first lane for SSR markers. 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 SSRs 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) co-efficient, 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).

OBSERVATIONS AND ANALYSIS

Twenty two SSR primers were selected to analyze the inter-accession diversity in 84 accessions of brinjal and are furnished in Table 1. The annealing temperatures and PCR conditions for these SSRs were first standardized by using a PCR with temperature gradient technique, which indicated that annealing temperatures of 44 to 58 °C is optimum for obtaining scorable bands. Stuttering of bands is common with SSRs if annealing temperatures are not optimized. All the SSR primers used in the present study produced discrete, scorable and unambiguous bands. Among the twenty two SSR primers used in the analysis, only thirteen primers produced the polymorphic bands. The PCR product size obtained by the amplification of SSR primers ranged from 80bp to 590bp.

The set of SSR markers used in this study substantially revealed amplified products, which is in accordance with the earlier reports of high level of conservation of SSR primers across brinjal species and related genera (Williams *et al*., 1990 and Nunome *et al*., 2003). The total number of alleles found was thirty nine for the twenty two microsatellite loci and the average number of alleles per locus was 1.77. This is in accordance with the findings of Nunome *et al*. (2003); Behara *et al*. (2006); Munoz-Falcon *et al*. (2009a) and Sunseri *et al*. (2010) in brinjal.

Allele diversity of SSR 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 SSR markers used in this study (Table 2). The highest PIC was recorded by the SSR marker 117 (0.5293), while it was found to be lowest for the marker 155 (0.1491). Similarly, this marker also recorded the highest gene diversity and heterozygosity of 0.6004 and 0.9351, respectively and the lowest allele frequency (0.5325). The PIC was calculated for each primer to evaluate usefulness of each primer to detect polymorphic loci. The PIC value ranged from 0.1491 (155) to 0.5293 (117) and the mean PIC recorded was 0.3062. All SSR markers detected thirty microsatellite loci, in total thirteen alleles were obtained in all accessions

Table 1: List of SSR markers used for DNA amplification in brinjal

Sr. No.	Primer	Allele number	No. of poly- morphic alleles	Polymorphism (%)	Allele size
1.	117	3	3	100	85-105
2.	119	2	2	100	140-160
3.	120	2	2	100	120-180
4.	126	1	0	0	90
5.	128	1	0	0	280
6.	131	2	2	100	195-225
7.	133	3	3	100	80-100
8.	134	4	4	100	180-220
9.	139	2	2	100	245-265
10.	141	2	2	100	190-220
11.	145	1	0	0	390
12.	146	2	2	100	95-110
13.	151	2	2	100	145-190
14.	155	2	2	100	290-300
15.	162	2	2	100	185-210
16.	RyMP2U5	1	0	0	350
17.	437_2	1	0	0	210
18.	3017_4	1	0	0	300
19.	2875_4	2	2	100	95-115
20.	241_2	1	0	0	200
21.	PMC125238P1	1	0	0	560
22.	PMC106412P1	1	0	0	590

investigated at twenty two SSR loci. All studied accessions were found to have unique allelic profiles with SSR loci.

The 22 selected SSR primers amplified alleles across the 84 accessions with varying degrees of polymorphism. High level of polymorphism was observed with as many as 4 alleles per locus with 134 primer. Nine primers *viz.*, 126, 128, 145, Rymp2U5, 437_2, 3017_4, 241_2, PMC125238P1, PMC106412P1 yielded only monomorphic bands with all the accessions. Further, null alleles were also observed in accessions with the SSR primer pairs. This might be due to template DNA concentration and purity, primer selection, primer design, amplicon size, PCR conditions and sensitivity of product detection. On a practical level, improper primer design along with artifacts/problems in the accuracy and reliability of DNA quantification and variation in the purity of the extracted DNA may affect the amplification of the alleles (Gilbert *et al.*, 1999).

The high levels of polymorphism obtained with thirteen SSRs in the present study is consistent with their known characteristics- that they are more variable and reveal greater diversity than RFLPs or RAPDs (Powell *et al.*, 1996). The high polymorphism associated with SSR is to be expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Tautz and Renz, 1984 and Tautz *et al*., 1986) rather than only by single nucleotide mutations and insertions or deletions.

Similarity index:

The banding pattern of SSR markers scored in the form of binary data was used for computing Jaccard's similarity index values obtained for each pair wise comparison among the 84 accessions. The similarity coefficient based on 22 SSR markers ranged from 0 to 0.77. Among the 84 accessions studied, the highest similarity index (0.076) was recorded between IC112738, IC112750, IC090938 and IC397557 accessions. The accessions IC354651 and IC112993 recorded the lowest similarity index value (0.77) with rest of the germplasm lines.

The exotic collections *viz.*, EC386589, EC316280, EC384565, EC385380, EC329327 and EC316226 were

Table 2: Major allele frequency, gene diversity, heterozygosity and polymorphic information content of SSR primers used for DNA amplification in brinjal

Sr. No.	Marker	Major Allele Frequency	Gene Diversity	Hetero zygosity	PIC
1.	117	0.5325	0.6004	0.9351	0.5293
2.	126	1.0000	0.0000	0.0000	0.0000
3.	120	0.8013	0.3185	0.3974	0.2678
4.	119	0.6813	0.4343	0.6375	0.3400
5.	241	1.0000	0.0000	0.0000	0.0000
6.	162	0.8359	0.2743	0.3281	0.2367
7.	155	0.9110	0.1622	0.1781	0.1491
8.	131	0.8692	0.2273	0.2615	0.2015
9.	128	1.0000	0.0000	0.0000	0.0000
10.	133	0.5985	0.5261	0.8030	0.4421
11.	139	0.7541	0.3709	0.4918	0.3021
12.	141	0.8590	0.2423	0.2821	0.2129
13.	145	1.0000	0.0000	0.0000	0.0000
14.	146	0.7818	0.3412	0.4364	0.2830
15.	134	0.6694	0.5150	0.6613	0.4809
16.	151	0.7045	0.4163	0.5909	0.3297
17.	2875	0.8651	0.2334	0.2698	0.2062
18.	3017	1.0000	0.0000	0.0000	0.0000
19.	PMC106412P1	1.0000	0.0000	0.0000	0.0000
20.	PMC125238P1	1.0000	0.0000	0.0000	0.0000
21.	RyMP2U5	1.0000	0.0000	0.0000	0.0000
22.	Mean	0.8506	0.2220	0.2987	0.3062

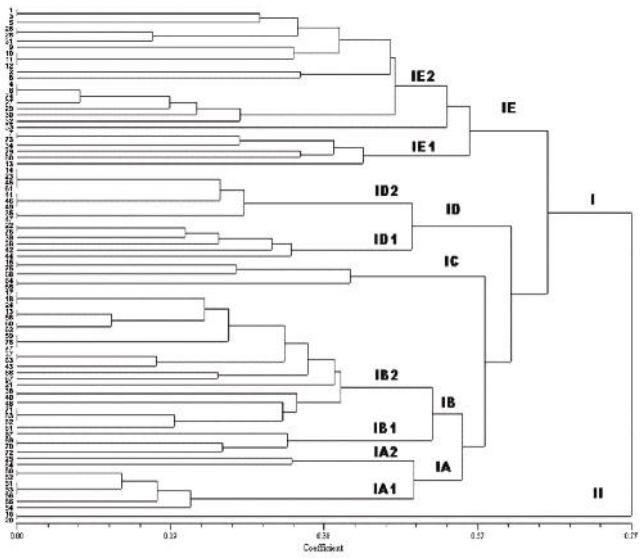
grouped in different clusters along with other indigenous collections. Among these EC386589 had exhibited a high average similarity with IC089949-B and IC354528 with a similarity co-efficient value of 0.29. EC316280 exhibited a high average similarity with IC090942 and IC398820 with an average similarity co-efficient value of 0.23 while

Table 3 :Principal component analysis on the contribution of SSR markers among brinjal accessions

Sr. No.	Accession	Eigen value	% variarion explained	Cumulative variation	Sr. No.	Accession	Eigen value	% variarion explained	Cumulative variation
1.	EC386589	22.23	26.46	26.46	43.	IC281112	-0.01	-0.01	133.05
2.	IC249358	15.81	18.82	45.28	44.	IC112818	-0.01	-0.01	133.04
3.	IC089949-B	12.25	14.59	59.87	45.	IC090785	-0.03	-0.03	133.01
4.	IC112738	10.26	12.22	72.09	46.	EC329327	-0.03	-0.03	132.97
5.	IC354528	8.71	10.37	82.46	47.	IC545948	-0.03	-0.04	132.94
6.	IC112750	6.67	7.94	90.40	48.	IC218975	-0.04	-0.05	132.88
7.	IC090915	5.97	7.10	97.50	49.	IC345747	-0.05	-0.05	132.83
8.	IC298633	4.55	5.42	102.92	50.	IC261899	-0.06	-0.07	132.76
9.	IC112909	3.80	4.52	107.44	51.	IC090987	-0.07	-0.08	132.68
10.	IC374892	3.59	4.28	111.71	52.	IC427007	-0.08	-0.09	132.59
11.	IC312984	3.10	3.69	115.41	53.	IC272927	-0.08	-0.10	132.49
12.	IC397299	2.56	3.04	118.45	54.	IC111387	-0.09	-0.10	132.39
13.	IC112997	2.33	2.78	121.23	55.	IC354135	-0.11	-0.13	132.26
14.	IC112741	1.97	2.35	123.58	56.	IC074239	-0.12	-0.15	132.12
15.	IC345740	1.63	1.94	125.51	57.	IC104083	-0.15	-0.18	131.93
16.	IC354651	1.58	1.88	127.39	58.	IC280957	-0.18	-0.21	131.72
17.	IC467271	1.42	1.69	129.08	59.	IC374867	-0.18	-0.21	131.51
18.	IC090905	0.84	1.00	130.08	60.	IC545919	-0.21	-0.25	131.26
19.	IC336472	0.59	0.70	130.78	61.	IC427017	-0.24	-0.29	130.98
20.	IC112993	0.35	0.42	131.20	62.	IC305048	-0.26	-0.31	130.67
21.	IC089989	0.29	0.35	131.55	63.	IC354564	-0.29	-0.35	130.32
22.	EC316280	0.24	0.28	131.84	64.	IC089912	-0.32	-0.39	129.94
23.	IC545937	0.18	0.21	132.05	65.	DBT/098	-0.36	-0.43	129.50
24.	IC279555	0.14	0.16	132.21	66.	IC112350	-0.38	-0.46	129.05
25.	IC281092	0.13	0.15	132.36	67.	IC354597	-0.49	-0.58	128.47
26.	IC545844	0.11	0.13	132.49	68.	IC354563	-0.56	-0.67	127.80
27.	IC397557	0.09	0.11	132.60	69.	IC261772	-0.64	-0.76	127.05
28.	IC350885	0.07	0.09	132.69	70.	IC305131	-0.72	-0.86	126.18
29.	EC384565	0.06	0.08	132.77	71.	IC467274	-0.80	-0.95	125.23
30.	IC332508	0.05	0.07	132.83	72.	IC413648	-0.88	-1.05	124.18
31.	IC304072	0.05	0.06	132.89	73.	IC421194	-0.98	-1.17	123.01
32.	IC099676	0.03	0.04	132.93	74.	IC090938	-1.10	-1.31	121.70
33.	IC336793	0.03	0.04	132.97	75.	IC281104	-1.45	-1.72	119.98
34.	IC112726	0.03	0.03	133.00	76.	IC090942	-1.73	-2.06	117.92
35.	IC112322	0.02	0.02	133.03	77.	IC112747	-1.81	-2.16	115.76
36.	IC374912	0.01	0.02	133.04	78.	IC089890	-2.19	-2.61	113.16
37.	EC385380	0.01	0.01	133.05	79.	IC427008	-2.80	-3.34	109.82
38.	IC354612	0.00	0.01	133.06	80.	EC316226	-3.36	-4.00	105.82
39.	IC398820	0.00	0.00	133.06	81.	Bhagyamati	-4.89	-5.82	100.00
40.	IC344646	0.00	0.00	133.06	82.	Gulabi	0.00	0.00	100.00
41.	IC354517	0.00	0.00	133.06	83.	Shyamala	0.00	0.00	100.00
42.	IC090026	0.00	0.00	133.06	84.	Arkakeshav	0.00	0.00	100.00

EC384565 exhibited a high average similarity with IC397557, IC090938, IC112750 and IC112738 with an average similarity co-efficient value of 0.26 EC385380 exhibited a high average similarity with IC354564, IC281112 accessions and EC316226 exhibited a high average similarity with IC427008 with an average similarity co-efficient value of 0.18 and 0.36, respectively. Whereas EC329327 exhibited 100% similarity with IC354517, IC345747 and high average similarity with IC112741, IC545937, IC090785 and IC427017 accessions with an average similarity co-efficient value of 0.23.

Among the commercially grown check varieties Bhagyamati, Shyamala and Gulabi were grouped in single cluster and different from the Arka Kesav with a similarity co-efficient value of 0.267 further asserting the existence of high genetic similarity among these cultivars. The Jaccard's similarity co-efficient for the SSR data set varied from 0 to 1.00 with average similarity of 0.50. This range showed high diversity among accessions. This did not reveal any specific patterns though some geographic associations were indicated. Some of the accessions having the same geographic origin were distributed in different sub clusters suggesting their genetic diversity.



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. IC374012
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. IC104088	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

Fig. 1 : Dendrogram of brinjal accessions based on SSR markers

Cluster analysis:

The similarity matrix was computed using SSR markers on Jaccard's co-efficient following the UPGMA method using SAHN programme of NTSYS-pc version 2.1. The similarity values obtained for each pair wise comparison of SSR markers in all the 84 accessions of brinjal were used to construct dendrogram based on hierarchical clustering and the results are presented in Fig. 1.

The dendrogram demonstrated the distribution of the studied accessions into two main clusters at 0.77 similarity co-efficient value, on the basis of their similarity calculated as proportion of shared alleles. EC386589 of the cluster I and IC112993 of Cluster II were the two extremes in the dendrogram between which the rest of the accessions were distributed. The dendrogram derived from the UPGMA cluster analysis revealed one major cluster with 82 accessions grouped into several sub clusters (Fig. 1). The accessions IC354651and IC112993 grouped separately from the other accessions.

Cluster I was further divided into sub cluster IA with 9 accessions, IB with 27 accessions, IC with 5 accessions, ID with 15 accessions and IE with 26 accessions at similarity co-efficient value of 0.67 as shown in the dendrogram. The cluster IA further divided into sub cluster IA-1 containing 7 accessions in which IC261899, IC427007 and IC090987, IC272927, IC074239 were identical having common alleles among them and IA-2 containing only 2 accessions (IC281092 and Arka Kesav). The cluster IB further divided into sub cluster IB-1 containing 4 accessions, in which IC261772 and IC305131 were identical. IB-2 contained 23 accessions, which includes 3 out of 4 check varieties namely Bhagyamati, Gulabi and Shyamala. The cluster IC comprised of 5 accessions in which IC345740, IC281104 and IC089912 and DBT/098 were showed 100% similarity having common alleles among them. The cluster ID further divided into sub cluster ID-1 containing 6 accessions of which EC316280 and IC090942 were identical and ID-2 containing 9 accessions of which IC112741, IC545937, IC090785 and IC427017 accessions were grouped into one cluster and were identical.

Similarly IC354517, EC329327, IC345747 and IC112322, EC385380 accessions formed into two groups and were identical as shown in dendrogram. The cluster IE further divided into sub cluster IE-1 with 6 accessions and IE-2 with 20 accessions of which EC386589, IC089949-B; IC545844, IC350885; IC374892, IC312984, IC397299 and IC112738, IC112750, IC090938 were identical having common alleles among them as shown in dendrogram.

The results of the 22 SSRs used in the present investigation revealed that nine pairs of accessions IC261899, IC427007 accessions in sub cluster IA-1; IC281092 and Arka Kesav accessions in sub cluster IA-2; IC261772 and IC305131 accessions in sub cluster IB-1; IC345740, IC281104 and IC089912 and DBT/098 accessions in cluster IC; EC316280 and IC090942 accessions in sub cluster ID-1; IC112322 and EC385380 accessions in sub cluster ID-2; EC386589 and IC089949-B accessions in sub cluster IE-1; IC545844 and IC350885 accessions in sub cluster IE-2, four sets of three accessions IC090987, IC272927 and IC074239 in sub cluster IA- 1; IC354517, EC329327 and IC345747 accessions in sub cluster ID-2; IC374892, IC312984 and IC397299 accessions in sub cluster IE-1 and IC112738, IC112750 and IC090938 accessions in sub cluster IE-2 and one set of four accessions IC112741, IC545937, IC090785 and IC427017 in sub cluster ID-2 had a similarity co-efficient of 1, indicating identical allelic patterns probably due to duplication of the accessions. Similar findings were reported by Munoz-Falcon *et al.* (2009b), who observed reported less diversity among commercial varieties in brinjal. Identical microsatellite profiles in the studied microsatellite loci, suggested that the observed morphological differences between these two cultivars may be associated with somatic mutations, which were not detectable with the used SSR markers. Hence, analysis of additional loci is necessary to identify and discriminate further the investigated accessions that differ in their phenotypic characteristics.

Among the commercially grown check varieties Bhagyamati, Shyamala and Gulabi were included in one cluster while Arka Kesav was included in another cluster. The exotic collections *viz.*, EC386589, EC316280, EC384565, EC385380, EC329327and EC316226 were included separately in different clusters along with other indigenous collections. It showed the differential clustering of commercial and exotic accessions and local selections into different sub clusters. The exotic accession, EC385380 grouped with local commercial varieties *viz*., Bhagyamati, Gulabi and Shyamala in sub cluster IB-2, exotic accession EC386589 grouped with IC089949-B in sub cluster IE-1, in the sub cluster ID-1 the exotic accession EC329327 shared common alleles with IC354517, IC345747 and EC385380 with IC112322 and other selections from same geographic region were grouped separately in different sub clusters. However, the absence of significant geographical associations suggests that movement of accessions from one region to another renders the historical derivations of genebank accessions inaccurate. The existence of duplicates may be due to multiple donations of the same seed source but from different collections. Therefore, diverse geographic origins among accessions may not always be a reliable indicator for the sampling of genetically diverse materials. From the conservation point of view, the identification and elimination of duplicates in a collection can save time and resources both financial and human in germplasm maintenance due to reduced number of accessions. Further, it could also be concluded that they are in fact different accessions derived from somatic mutations that were not detected by the molecular markers used in this study.

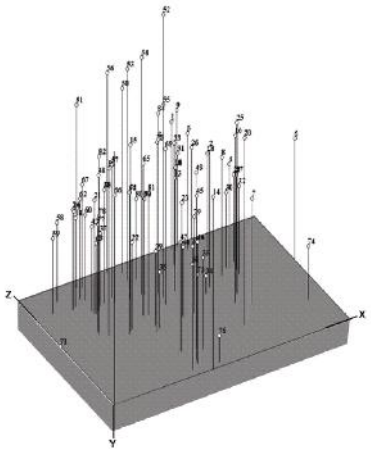
Although the development of microsatellites is considered laborious and expensive when compared to other markers (Nunome *et al*., 2003), data from other crops showed that they were extremely efficient as reproducible and highly informative genetic markers (Zane *et al*., 2002 and He *et al*., 2003). The present study of use of microsatellite markers in characterization of efficient to distinguish important accessions and will certainly be useful for purposes such as the certification of varieties, for identification of pest and disease resistant lines and there of superior plants developed from controlled crosses between brinjal accessions.

The SSR technique is very expensive compared to other marker techniques, hence a few primers were used in this preliminary study. A few amplification products could be effectively used as markers for differentiation of a part of the studied brinjal accessions. Hence this could lead to a non informative or biased analysis. Further a finer molecular analysis of brinjal accessions is required with more number of SSR markers as far as possible in order to detect and identify unique as well as fine resolution of molecular polymorphism between different identical genotypes. The use of a larger number of SSRs

with greater genome coverage could help to reveal genetic diversity more accurately and also help to unambiguously differentiate those accessions with identical allelic patterns as revealed by the set of primers used in this study. Further, by increasing the number of accessions from the different regions along with the use of a higher number of polymorphic SSR markers, a better assessment of genetic diversity could be carried out.

Principal component analysis for SSR analysis:

Principal Component Analysis was done based on the Jaccard's similarity matrix. The First component accounted for 26.46% of all changes and second component accounted for 18.82% of principal changes. In this manner, totally 7 principal components accounted for 97.5% of changes. The Principal Component Analysis was performed based on molecular data of 22 SSR markers to visualize the genetic relatedness among the brinjal accessions in detail. The description of the data was done using three dimensional pictorial graph and is represented in Fig. 2. From the graph, it is evident that the brinjal accessions were dispersed on the PC plot, which is a reflection of its genetic base. 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 PCA were in agreement with the dendrogram generated by UPGMA cluster analysis. This strengthened the ability and accuracy of the SSR analysis applied to brinjal accessions in the present study.



Name of the accessions:

1. EC386589	2. IC249358	3. IC089949-B	4. IC112738	5. IC354528	6. IC112750
7. IC090915	8. IC298033	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. IC374812
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

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

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