MOLECULAR CHARACTERIZATION OF FIVE SELECTED BRINJAL (*Solanum melongena* L) GENOTYPES USING SSR MARKERS

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ABSTRACT

Five brinjal (*Solanum melongena* L.) genotypes were selected for characterization using Simple Sequence Repeats (SSR) markers. All the genotypes showed considerable variation in respect of morphological, anatomical and biochemical aspects. For study of relatedness, plant genomic DNA was extracted by CTAB based method using 11 randomly selected primers produced from Calgene Inc. USA. The primers developed 22 bands through PCR amplification out of which 15 from 3 primers and were polymorphic. Genetic similarities of SSR profiles were estimated based on Jaccard's coefficient value. The dendrogram generated two clusters and they were clearly distinct and separated from each other. Cluster-I consisted of genotypes TURBO and BL009; and cluster-II comprised of genotypes EG058, EG075 and ISD006. Genotype TURBO and BL009 were identified as the diverse genotype and showed a maximum of 17% dissimilarity from EG058, EG075 and ISD006. The similarity value ranged from 0.83 to 1.00 which indicated the presence of narrow range of genetic diversity at molecular level but have still a possibility of crossing among the genotypes of two clusters. The banding pattern of different genotypes could be utilized as reference for further comparisons.

Key words: Brinjal (*Solanum melongena* L.), molecular characterization, SSR markers

INTRODUCTION

Brinjal (*Solanum melongena* L.) is an important vegetable grown throughout the South Asian countries (Alam *et al.* 2003, Kumar *et al.* 2003). Many species of *Solanum* are available in nature of which most of them are wild and few are cultivated (*S. melongena* L. and *S. tuberosum* L.). Among the cultivated ones there exist numerous cultivars with considerable morphological and genetic variability, which is an opportunity for the improvement of the crop like brinjal. For sustainable improvement of this vegetable, judicious and effective use of germplasms has become must today for the improvement of agronomic characters coupled with resistant to biotic and abiotic stress factors of the crop. Molecular characterization of germplasms is one of the important and efficient technique for assessment of genetic variability; consequently for successful breeding program.

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Biochemical, morphological and genetic markers are being used now-a-days as recent innovation for characterization in a number of crops. These markers are powerful tools that can provide significant information which may enhance the scope of diversified use of germplasm in the crop improvement program. It reveals the extent of relationship among the genotypes and in the estimates of genetic diversity or relatedness. Simple Sequence Repeats (SSR) marker is the procedure developed by Bell and Ecker (1994), which is reproducible and is highly informative. Since this is stable, co-dominant and inexpensive, and therefore advantageous for genetic analysis and Marker Assisted Selection (MAS).

SSR marker was first developed for brinjal by Nunome *et al.* (2003) where they confirmed the usefulness of these markers for genetic analysis and found useful for agronomical important traits in brinjal that could facilitate marker assisted breeding. In Bangladesh, information on molecular characterization is scanty and for brinjal is a new attempt. In the present study, the selected brinjal genotypes are expected to show considerable variations in morphological, anatomical and biochemical aspects with consistently resistant to brinjal shoot and fruit borer (*Leucinodes orbonalis* Guenee), which is a major constrains of healthy production of this crop. Therefore, the present study was undertaken to know the relatedness among the selected brinjal genotypes in molecular level.

MATERIALS AND METHODS

The study on molecular characterization of selected brinjal genotypes using SSR marker technique was carried out at the Center for Plant Molecular Biology (CPMB), Tamil Nadu Agricultural University (TNAU), Coimbatore, India during June to December 2005.

Plant material used

Among the five brinjal (*Solanum* spp.) genotypes, BL009 and ISD006 were collected from Bangladesh Agricultural Research Institute (BARI) and EG058, TURBO and a susceptible check (EG075) were from Asian Vegetable Research and Development Centre (AVRDC), Taiwan. All genotypes were grown in the transgenic greenhouse of the CPMB and 20 days old seedlings were used for the study.

DNA extraction

Plant genomic DNA was extracted using standard methods given by Nagarajan and Kumar (2002) with appropriate modifications. Fresh 20-day-old leaves were taken, weighed and cut into small pieces and were ground in liquid nitrogen with pre-chilled pestle and mortar and the powder was transferred into centrifuge tubes carrying 25ml pre-heated (65 ^{0}C) 2% Cityl-triammonium-bromide (CTAB) extraction buffer. The tubes were incubated at 65 ^{0}C for an hour followed by addition of 15ml chloroform:isoamyl alcohol (24:1). The samples were centrifuged at 10,000 rpm for 10 minutes at room temperature. Upper aqueous phase was precipitated with 2-volumes of ice cold ethanol and 0.1 volumes of 3M sodium acetate (pH=5.2) and centrifuged at 15,000 rpm for 15 minutes. The DNA was recovered as pellet which was washed in 70% ethanol. The concentration of DNA was determined spectrophotometrically by taking UV absorbance 260 nm. The DNA concentration was rechecked by running samples in 0.80% agarose gel at 100 volt for an hour in Tris-Base Extraction (TBE) buffer along with lambda DNA standard followed by polymerase chain reaction (PCR).

Agarose gel electrophorasis

Amplification products obtained through PCR were loaded into individual channels of 1.2% agarose horizontal gel in TBE buffer (pH=8.0). Electrophorasis was carried out at 100 volts for an hour. Gels were stained with ethidium bromide (2µg/l) and were observed in a transilluminator under UV light. The λ DNA double digested with H*ind* and E*coR* I (Sigma D 9281) were used as DNA markers for comparing the molecular weights.

Data collection and analysis

Data were taken using standard scoring sheet supplied by Bangalore genee. The number of DNA bands formed by each sample (genotype) using different primers were recorded and analyzed using NTSYS-pc version 2.02 software. Principal Coordinate Analysis/Principal Component Analysis (PCA) was done as per method described by Rohlf (2001). Attribute of the SSR primers with repeat motif, primer sequence, expected length and annealing temperature used in the present study were presented in (Table 1).

RESULTS AND DISCUSSION

Randomly selected 11 primers of the micro satellite (SSR) marker (Table 2) were used for amplification of DNA bands in five brinjal genotypes. Among them, SSR114 results sub-optimal or non-distinct amplification in few lanes, whereas SSR119 produced no bands in the entire five lanes. As this primer was not consistent in all five samples, therefore, the results of amplification patterns from the primer SSR114 was not used in the statistical analysis. However, among the 10 primers, true amplifications were obtained from nine primers (SSR128, SSR131. SSR127, SSR120, SSR126, SSR116, SSR104, SSR107 and SSR117), which were consistent in all the five samples. Among them, 3-primers (SSR104, SSR107 and SSR117) produced more than one bands (4-6 bands); 6-primers (SSR128, SSR131, SSR127, SSR126, SSR120 and SSR116) produced just a single band each. A total of 22 amplification products were scored with a mean of 2.2 per primer (Table 2).

Number of amplification products obtained from gel plate were specific to each primer and ranged from 0-6 with fragment size varying from 220-1300bp. The selected primers gave a total of 22 amplification products of which 30.0% were polymorphic and 70.0% monomorphic in nature. Among 9, 8-primers has produced identical banding pattern and only a single primer SSR131 produced bands clearly in two separate axis (Plate 1), which strongly supported that 1 (BL009) and 5 (TURBO) are of same group as of 2 (EG058), 3 (EG075) and 4 (ISD006) are in another. Comparatively, a higher degree of monomorphism (70.0%) was obtained in 7-primers (SSR128, SSR131, SSR127, SSR120, SSR126, SSR116 and SSR114) (Table 2) indicating a narrow range of diversity present at DNA level among the selected brinjal genotypes.

Similarity index

The similarity index was determined using Jaccard's similarity coefficient. Banding profiles obtained with 10 primers for five brinjal genotypes were analyzed for construction of dendrogram. Jaccard's similarity coefficient value ranged from 0.83 to 1.00, which indicated narrow genotypic diversity present at molecular level among the five brinjal genotypes.

Table 1. Attribute of the brinjal simple sequence repeats (SSR)

EM no.	Repeat Motif	Primer Sequence (5'-3')	Expected length (bp)	Ann. temp. (C)
104	(TC)9(AC)38(AT)19	TGGATCTGCAAAGAAAAGGAGAAAG CGCAAAATCGCGTAGACTTTCGAT	246	60
107	(AC)13(AT)7	GGCCCTAGACTGAGCTGAAATGTT TGCTACAACCAACACAACCCTCAA	214	65
114	(AC)13	AGCCTAAACTTGGTTGGTTTTTGC GAAGCTTTAAGAGCCTTCTATGCAG	221	65
116	(AC)12(AT)8	TTAGAAATTTCGGAACAAAGAGA CCACATGAAACTTGGACCAATGAG	246	60
117	(AC)19(AT)11	GATCATCACTGGTTTGGGCTACAA AGGGGAGAGGAAACTTGATTGGAC	123	65
119	(GGAGG)5(AT)8(GT)3AT(GT)14	CCCCACCCCATTTGTGTTATGTT ACCCGAGAGCTATGGAGTGTTCTG	201	65
120	(AC)16	GGATCAACTGAAGAGCTGGTGGTT CAGAGCTTCAATGTTCCATTCACA	160	65
126	(AT)7(GT)18	GCATAGCTTATGAGTCAGGTGGCTTT GCTCATCAAACCATCACATTCAAG	209	65
127	(AC)13(AT)13	CAGACACAACTGCTGAGCCAAAAT CGGTTTTAATCATAGCGGTGACCTT	213	65
128	(CA)26(TA)19	TCTGGGACACCAAGTGAAAAATCA TGGCTTTTTGGCTCCTCTATGAAT	295	60
131	(AT)5(AC)3A(AC)14(AT)7GTA(TG)5(TA)3	GCGGATCACCTGCAGTTACATTAC TCCTTTGACCTATAGTGGCACGTAGT	213	65

Source: Nunome *et al.* (2003)

Table 2. Number bands formed by SSR markers in brinjal genotypes

Primers' Code no.	Primers' name	Number of bands formed
1	SSR128	1
2	SSR131	1
3	SSR127	1
4	SSR120	1
5	SSR126	1
6	SSR116	1
7	SSR119	0
8	SSR114	1
9	SSR104	4
10	SSR107	6
11	SSR117	5
Total		**22**
Mean		**2.2**

The dendrogram showed two close knit clusters (Figure 1). However, the number of genotypes in each cluster varied from 2-3 (2 in cluster-I and 3 in cluster-II). Cluster-I consist of genotypes TURBO and BL009; whereas cluster-II comprised of genotypes EG058, EG075 and ISD006. Genotype TURBO and BL009 were identified as the diverse genotype and showed a maximum of 17% dissimilarity with EG058, EG075 and ISD006 as clearly evident from the dendrogram.
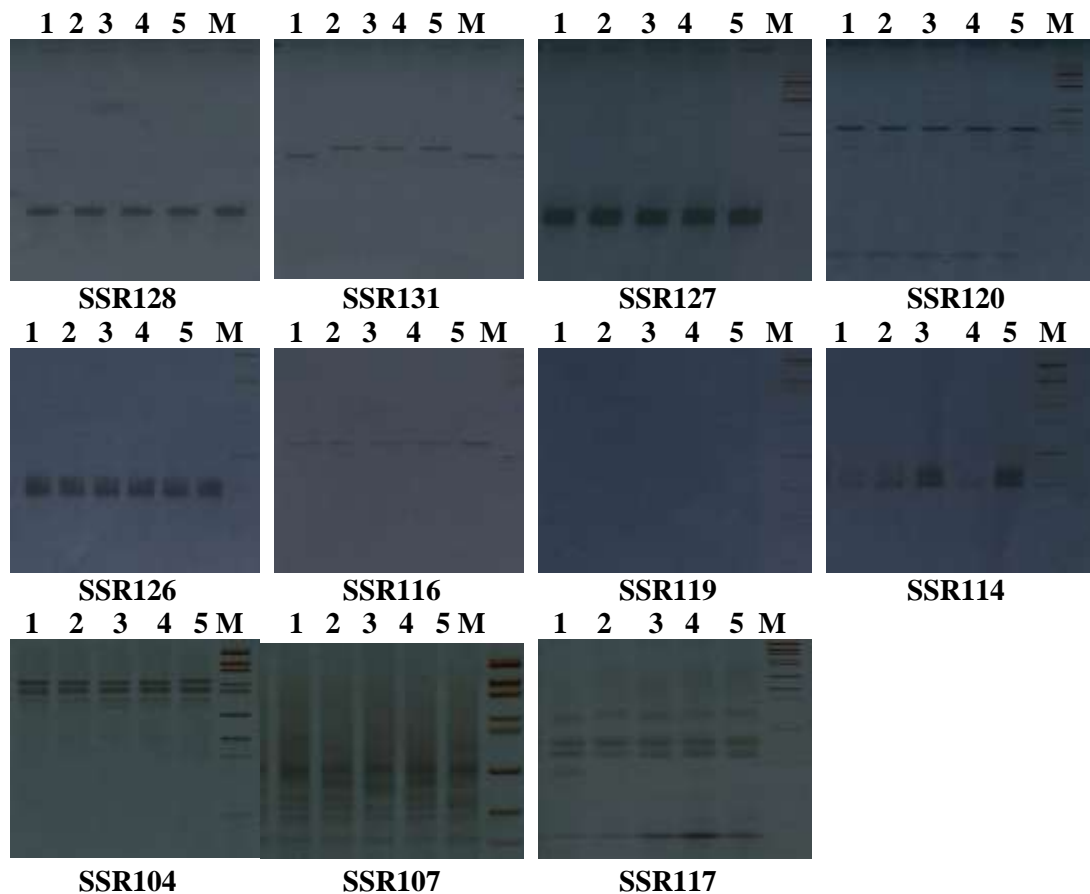
Plate 1. Banding patterns (Close view) obtained from five brinjal genotypes using SSR marker. 1=BL009, 2=EG058, 3=EG075, 4=ISD006, 5=TURBO and M=Marker.
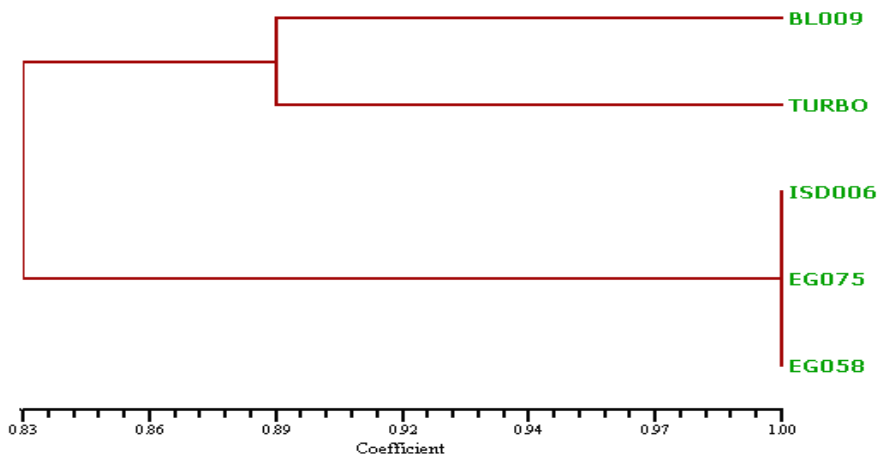

Figure 1. Dendrogram showing clusters of five brinjal genotypes with degree of relatedness and coefficient value.

Sometimes, few primers produced suspicious bands that might be due to the primers used in the study were not appropriate for the detection of genetic diversity or the primers used were a few to respond the real results. Besides, impurities of the extracted DNA might have influenced the result of PCR products, for instance production of undesired products or even failure to produce. But it has been reported that SSR markers were ideal markers for constructing high resolution genetic maps in order to identify similarity between different species within a single genus (Provan *et al.* 1999). Nunome *et al.* (2003) evaluated primer SSR for *S. melongena* and its related species and found them most suitable for brinjal (Anon. 2007). It is revealed from the results that, the morphological variability is one of the primary factors, which contribute to the level of genetic or molecular diversity available in the genotypes. In the present investigation, five brinjal genotypes were analyzed through different randomly selected primers and represented low genetic variability. Therefore, a higher level of crossing possibility is prevailing between among genotypes of two clusters.

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