Tuijin Jishu/Journal of Propulsion Technology
ISSN: 1001-4055
Vol. 44 No. 2 (2023)

Identification of *Solanum melongena* Linn. Accessions using the RAPD method: A case study

B.S. Jyothsna,[1] Mrittika Das,[2*] Priya Deori[3]

[1] Department of Botany and PG Studies, Nrupathunga University, N.T. Road, Bangalore, Karnataka, India.

[2] Department of Life Sciences, Dibrugarh University, Tinsukia, Assam, India

[3] Department of Life Sciences, Dibrugarh University, Dhemaji, Assam, India

ABSTRACT

Brinjal (*Solanum melongena* L.) is an important solanaceous vegetable crop in many countries. It is a native of India and widely grown in Karnataka, West Bengal, Odisha, Gujarat, Madhya Pradesh, Chhattisgarh, Maharashtra, Andhra Pradesh, Haryana, Tamil Nadu, Uttar Pradesh, Jharkhand and Tamil Nadu. Arka Navneet is a popular brinjal variety known for its high yield and adaptability to different climatic conditions. Although many molecular markers have been developed for it, not much data is available for the accession of Tamil Nadu. The present study aimed to identify different accessions of Kanyakumari district, Tamil Nadu, India through RAPD analysis. A total of 15 accessions were collected from various locations and named B1 to B15. They were identified differently based on morphological and taxonomical characteristics. Molecular characterization was done by using 15 universal RAPD primers. Fifteen RAPD primers generated 1466 amplicons, of which 42 (2.86%) were monomorphic. OPL 15 could identify all fifteen accessions, so, it was used for phylogenetic analysis. The dendrogram showed a minimum distance between B3 and B9 followed by B5 and B10. Accession B11 was noted most distant from all other accessions. We conclude that RAPD technique is efficient for the characterization of *Solanum melongena* accession from Tamil Nadu, India. Even This technique was noted as efficient for phylogenetic identification and assessing diversity.

Keywords: Phylogenetic analysis, *Solanum melongena,* RAPD analysis, dendrogram, accessions.

Article History: **Received:** 12/06/23 **Revised:** 16/08/23 **Accepted:**19/08/23 **Published online:**31/08/23

INTRODUCTION

One of India's most well-liked and economically significant vegetable crops, brinjal (*Solanum melongena* L.), is grown over a 0.5 million hectare area with an annual yield of 8.4 million tonnes (Mondal *et al*., 2019). Since brinjal may be found in many different sizes, hues, and shapes across South and Southeast Asia, it is likely that this area is a significant hub for both diversity and origin. Although a lot of research has been done on the morphological characteristics of brinjal, only a small amount of it has focused on the use of molecular markers. Even in these investigations, the characterization of particular accessions has been less of a priority than the analysis of the genetic diversity and interspecific relationships of *S. melongena* (Dash and Sharma *et al*., 2019).

For effective management and exploitation of germplasm and preservation of associated intellectual property rights, genetic resource characterization and evaluation of diversity are fundamental requirements. It has been discovered that conventionally employed morphological and biochemical markers are insufficiently discriminative for the characterization of closely related genotypes, necessitating the adoption of more exacting procedures (Parida *et al*., 2020). More variation is detected by molecular markers, which are also simply inherited, unaffected by environment or growth stage. Random DNA-based markers like RAPD (Random Amplified Polymorphic DNA) and ISSR are helpful for molecular characterization since they don't require knowledge of the target genome beforehand. RAPD offers the potential for automation and minimal startup costs. The complete genome is often represented by RAPD band patterns, hence we chose to adopt this method in the current study (Srivastava *et al*., 2007).

Although many molecular markers have been developed for *Solanum melongena* worldwide. Not much data is available for accessions of Tamil Nadu. The present study aimed to identify different accessions of Dhemaji district, Tamil Nadu, India through RAPD analysis.

MATERIAL AND METHOD

2.1 Study Area: The study was conducted in southern district, Kanyakumari, of Tamil Nadu. For the present study, 15 accessions of *Solanum melongena* leaves were collected from the 20 km^2 range of the above-mentioned study site. The random plot sampling method was used with the help of a GPS map. The study area was divided into fifteen sections and 30 blocks, 2 blocks in each section. This study used one plant from each block and 5 plants from each section.

2.2 Collection and Identification of Plants: Single samples were collected from each block. The accession number was given based on plot sections. The morphological identification and verification of the collected specimens were done by consulting different taxonomic literature. Total 15 accessions were collected from various locations and named B1 to B15.

2.3 DNA isolation: Genomic DNA isolation is done with the help of CTAB Method (Doyle and Doyle, 1991). The concentration of DNA was determined with Hoefer Quant DNA 200 flourimeter using Hoechst 33258 as dye and calf thymus DNA as standard.

2.4 RAPD assays: PCR amplification was carried out using 15 random decamer primers (Table 1). Monomorphic bands and No. of accession distinguished were noted manually.

Table 1. Band statistics and analysis of data from RAPD primers used in the study

S. No	Primer	Sequence (5' to 3')	Total bands	Monomorphic bands	No. of accession distinguished
1	OPW 11	CTGATGCGTG	180	2	2
2	OPS 11	AGTCGGGTGG	90	3	1
3	OPL 15	AAGAGAGGGC	162	5	15
4	OPX 16	CTCTGTTCGG	70	2	0
5	OPU 09	CCACATCGGT	97	4	3
6	OPM 04	GGCGGTTGTC	76	3	1
7	OPX 07	GAGCGAGGCT	111	4	3
8	OPB 10	CTGCTGGGAC	101	5	0
9	OPN 05	ACTGAACGCC	93	2	4
10	OPW03	GTCCGGAGTG	78	4	1
11	OPY 04	GGCTGCAATG	84	3	3
12	OPA 15	TTCCGAACCC	59	1	0
13	OPQ 20	TCGCCCAGTC	68	0	0
14	OPA 14	TCTGTGCTGG	89	2	2
15	OPT 06	CAAGGGCAGA	108	2	1

2.5 Gel electrophoresis and band scoring — Electrophoresis of amplification products was performed in a 1.5% agarose 0.5x Tris-Borate-EDTA gel that included ethidium bromide (3l per 100 ml) at 60V for 4-5 hours. Under UV illumination, the amplification products were seen, and Polaroid film was used to take pictures. With the assumption that each band reflects a different genetic locus, differences in the banding patterns were subjectively assessed from gel photos for the presence (1) and absence (0) of bands. From 300 bp to 3000 bp, obvious, unambiguous amplicons were scored. By comparing the band sizes to a 100 bp DNA ladder, the band sizes were identified.

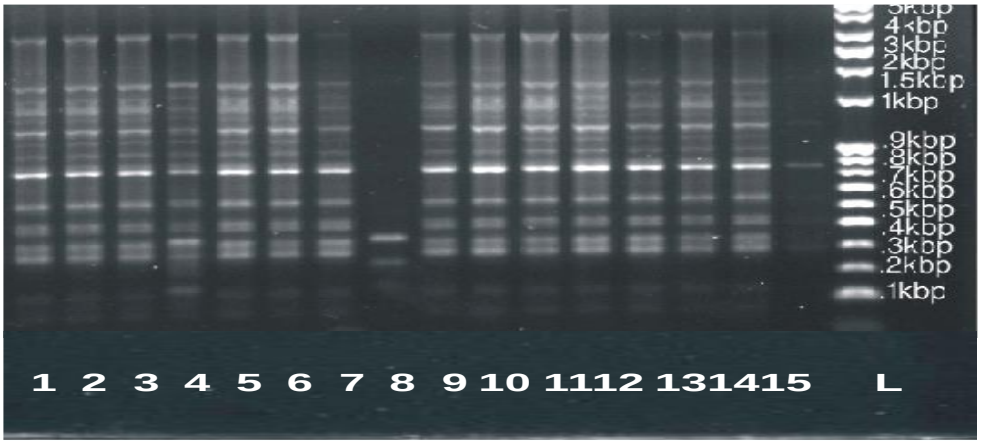
2.6 Data analysis: It was utilized to identify cultivars by determining whether a single band or a collection of bands were present or absent. To assess the effectiveness of these factors in choosing primers that might best discriminate the cultivars, Rp and PIC of primers were determined.

A pair-wise comparison was used to assess binary data that depended on whether or not bands were present. In order to analyze how closely related the cultivars were to one another, a dendrogram was created using UPGMA's cluster analysis on the similarity matrix that was thus acquired. Control sequence was taken from NCBI.

3. RESULTS AND DISCUSSION

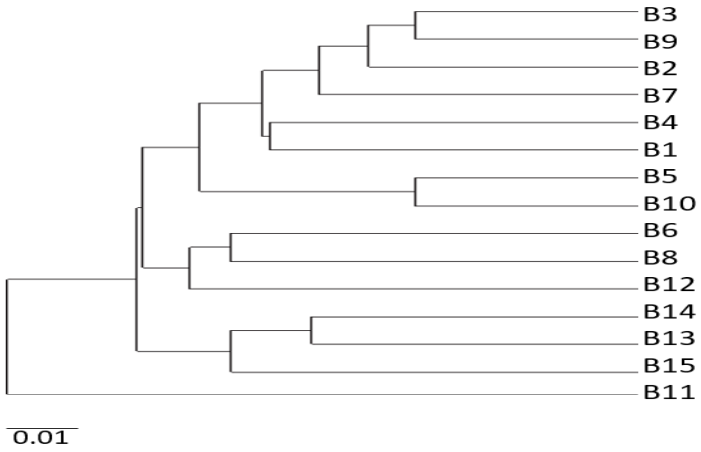
3.1 Band statistics — DNA was extracted and the quality check was done for all samples. Good quality DNA for each accession was amplification by Standard RAPD primers (Table 1). Fifteen RAPD primers generated 1466 amplicons, of which 42 (2.86%) were monomorphic (Table 1). The number of total bands produced per primer ranged from 59 (OPA 15) to a maximum 180 (OPW 11). The number of monomorphic bands produced per primer ranged from 0 to a maximum of 15 (OPL 15). Bands were counted manually looking into Figure 1.

Figure 1: DNA amplification of different accession (B1- B15) using RAPD primer OPL 15



3.2 Accession identification using phylogenetic analysis — Fifteen accessions could be identified by the unique presence or absence of a single RAPD markers. OPL 15 could identify all fifteen accessions, so, it was used for phylogenetic analysis. In the present study, mutants were identified based on the similarity, addition, or deletion of monomorphic bands in amplification banding patterns, which reflects the genetic similarities and dissimilarities among themselves. The dendrogram showed a minimum distance between B3 and B9 followed by B5 and B10. Accession B11 was noted most distant from all other accessions (Figure 2). RAPD methods were noted as successful in the identification of *S. melongena* by Karihaloo *et al*., (1995), Tiwari *et al*., (2009) and Ali *et al*., (2011) and many more researchers.

Figure 2: Phylogenetic distance between different accessions of *S. melongena* using OPL 15 RAPD marker



CONCLUSION: RAPD analysis was noted efficient technique for the characterization of *Solanum melongena* accession. Even This technique was noted as efficient for phylogenetic identification and assessing diversity.

CONFLICT OF INTEREST: No conflicts of interest were reported by the authors.

REFERENCES

[1] Ali Z, Xu ZL, Zhang DY, He XL, Bahadur S, Yi JX. Molecular diversity analysis of eggplant (Solanum melongena) genetic resources. Genetics and Molecular Research. 2011 Jun 14;10(2):1141-55.
[2] Dash SP, Singh J, Sharma D. Morphological characterization of brinjal (*Solanum melongena* L.) germplasm. Journal of Pharmacognosy and Phytochemistry. 2019;8(2):1574-8.
[3] Doyle J. DNA protocols for plants. In Molecular techniques in taxonomy 1991 (pp. 283-293). Berlin, Heidelberg: Springer Berlin Heidelberg.
[4] Karihaloo JL, Brauner S, Gottlieb LD. Random amplified polymorphic DNA variation in the eggplant, *Solanum melongena* L.(Solanaceae). Theoretical and Applied Genetics. 1995 May;90:767-70.
[5] Mondal M, Hasrat Ali and Bimal Kumar Bera. 2019. An Economic Analysis of Brinjal (*Solanum melongena* L.) Cultivation in Nadia District of West Bengal, India. Int.J.Curr.Microbiol.App.Sci. 8(11): 210-216.
[6] Parida H, Mandal J, Mohanta S. A note on morphological characterization of brinjal (*Solanum melongena* L.) genotypes. J. Crop. Weed. 2020;16:250-5.
[7] Srivastava S, Gupta PS, Saxena VK, Srivastava HM. Genetic diversity analysis in sugarbeet (Beta vulgaris L.) using isozymes, RAPD and ISSR markers. Cytologia. 2007;72(3):265-74.
[8] Tiwari SK, Karihaloo JL, Hameed N, Gaikwad AB. Molecular characterization of brinjal (*Solanum melongena* L) cultivars using RAPD and ISSR markers. Journal of Plant Biochemistry and Biotechnology. 2009 Jul;18:189-95.