

## Identification of diverse genotypes in brinjal (*Solanum melongena* L.) based on RAPD markers analysis

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### ABSTRACT

Molecular characterization of germplasm is rapid and most reliable method for genetic diversity and inter-relationship analysis in crop. Assessment of genetic diversity in 30 brinjal genotypes was carried out with ten Random Amplified Polymorphic DNA (RAPD) markers. A total of thirty RAPD markers were screened out of which ten primers produced clear bands were finally selected for diversity analysis. Ten primers produced a total of 20 bands with an average 2.0 bands per primer thus generated 100% polymorphism. RAPD primers showed variable bands and were in range of 01- 4 bands. Maximum bands 04 generated by Primer-4 and minimum band 01 produced by Primer-2, Primer-3 and Primer-6. Heterozygosity varied from 0.00-0.48 while PIC was in the ranged from 0.37 to 0.49. The highest Polymorphic Information content (PIC) value (0.49) was observed for Primer -2 while lowest PIC value (0.37) was exhibited by Primer -1. Effective multiplex ratio was maximum (2.00) for the Primer-7 and minimum effective multiplex ratio (0.73) showed by Primer-10. Maximum discriminating power of marker (0.97) showed by Primer-8 while Primer-7 did not show any discriminating power of marker. Resolving power of markers varied in the range of 0.00 to 1.87 with an average of 0.820. Maximum resolving power of markers (1.87) noted with Primer-4 while Primer-2 did not exhibit any resolving power of marker. Jaccard's similarity between pairs of cultivars ranged between 0.35 and 0.95. Highest Jaccard's genetic similarity was found between the genotype of Pusa Bindu and JBH-3, Arka Nidhi and JBH-3, KKM-1B and RCMBL-1, DRNKV-02-104 and JB-69 and KKM-1B to Shyamal while the lowest Jaccard's genetic similarity was observed between genotype of VR-11 and JB-18 and genotype BCB-464 and PB-67. The dendrogram generated through RAPD revealed 2 major clusters at Jaccard's similarity coefficient (GS=0.59) which showed narrowed base of diversity among the germplasm. Molecular data produced by RAPD markers clearly indicates that genotypes like BR-11 and JB-18 and PB- 67 was found more divergent and can be used for further improvement programme.

**Key words :** *Brinjal, Solanum melongena, RAPD, Genetic diversity, Cluster analysis*

## Introduction

Brinjal is having fourth position after tomato, potato and chilli under Solanaceae family (FAO, 2017). However, Solanum is a large genus of over 1400 species, out of which several members are poisonous to humans, such as *S. dulcamara* L. (the night-shades). Brinjal (*Solanum melongena* L.) and other two underutilized eggplant species like as African eggplant (*S. macrocarpon* L.) and the scarlet eggplant (*S. aethiopicum* L.) were also cultivated with local importance where the leaves and fruits are used for food and medicinal purposes (Oladosu *et al.*, 2021). Brinjal botanical known as *Solanum melongena* L. (2n = 24) is one of the most popular vegetable crops grown in South Asia and other parts of the world. Brinjal is popularly known as eggplant and its centre of origin is India (Tsao, 2006; Doijode, 2001). The most recent and reliable sources of origin suggested by (Weese *et al.*, 2010) they mentioned that the Middle Eastern/African species of *S. incanum* L. was intentionally transported into the Indo-China region, where the true wild progenitor of *S. insanum* L. evolved from which *S. melongena* was derived. Globally, China is leading producer of brinjal (35.5 million) followed by India (12.6 million), Egypt (1.2 million), Turkey (0.8 million), Indonesia (0.5 million), Philippines (0.2 million) and Sri Lanka (0.1 million) are also important eggplant producers in Southeast Asia (FAOSTAT, 2021). Various shapes and colours in brinjal are found throughout South and Southeast Asia, suggesting this region to be an important centre of diversity. However, consider-

able work has been carried out on morphological characterization of brinjal (Kumar *et al.*, 2008, Solaimana *et al.*, 2015) and molecular markers have been used to a limited extent for this purpose (Tiwari *et al.*, 2009; Kumar *et al.*, 2014).

Molecular Markers are reliable tools to characterize the DNA profile of plant genotypes and study the genetic diversity in plants (Williams *et al.*, 1990). Among the molecular markers, Randomly Amplified Polymorphic DNA (RAPD) markers are simple and cost effective for the evaluation of genetic diversity in crop plants as compared to other molecular markers (Rafalski *et al.*, 1999). In recent years, one of the important problems faced by brinjal breeder is a narrowing of the genetic base. Therefore, assessment of genetic variation is required for identification of diverse parents for further breeding programme. Keeping in view the above facts, the present study have been carried to identification of diverse parents by using RAPD profile.

## Materials and Methods

### Plant materials

Thirty genotypes of brinjal were collected from IIVR, Varanasi (Table 1). The genotypes were raised at Horticultural Research Centre (HRC), Department of Horticulture, SVPUA and T, Meerut, U.P. India 250110

### Isolation of DNA

Fresh and young leaves were collected and stored at -80°C until further use. The genomic DNA was ex-

**Table 1.** List of 30 brinjal genotypes used for the present study

Genotype	Source	Genotype	Source
1. Aruna	I.I.V.R., Varanasi	16. ARKA NIDHI	I.I.V.R., Varanasi
2. RCMBL 04 04 04	I.I.V.R., Varanasi	17. A. KUSAMAKAR	I.I.V.R., Varanasi
3. JB-67	I.I.V.R., Varanasi	18. BCB-469	I.I.V.R., Varanasi
4. JB-18	I.I.V.R., Varanasi	19. UTKAL JYOTI	I.I.V.R., Varanasi
5. Bhagaymati	I.I.V.R., Varanasi	20. KS-331	I.I.V.R., Varanasi
6. J.B.H-3	I.I.V.R., Varanasi	21. DBL-24	I.I.V.R., Varanasi
7. SWEATA	I.I.V.R., Varanasi	22. A. ABHILOMB	I.I.V.R., Varanasi
8. DRNKV-02-026	I.I.V.R., Varanasi	23. A. PRATIBHA	I.I.V.R., Varanasi
9. UTKAL MADHURI	I.I.V.R., Varanasi	24. JB-6	I.I.V.R., Varanasi
10. JAWHAR BRINJAL	I.I.V.R., Varanasi	25. PUSA BINDU	I.I.V.R., Varanasi
11. PB-67	I.I.V.R., Varanasi	26. PANJAB SHREE	I.I.V.R., Varanasi
12. GREEN LONG	I.I.V.R., Varanasi	27. DRNKV 02 104	I.I.V.R., Varanasi
13. RCMBL-1	I.I.V.R., Varanasi	28. PUSA SHAYAMAL	I.I.V.R., Varanasi
14. PLR-1	I.I.V.R., Varanasi	29. K K M-1B	I.I.V.R., Varanasi
15. JB-69	I.I.V.R., Varanasi	30. VR-11	I.I.V.R., Varanasi

tracted using modified CTAB method (Doyle and Doyle, 1987). The quality and quantity of DNA was analyzed using gel electrophoresis and spectrophotometer (Thermo Scientific, New Delhi). The DNA concentration of samples were adjusted to 25ng/ $\mu$ l for RAPD analysis and stored at -20°C until further use.

### RAPD Markers

A total of 30 RAPD makers were synthesized (Macflow Engineering Pvt. Ltd) among them ten primers produced reproducible bands were finally selected for PCR amplification (Table 2). The PCR products were visualized in EtBr staining and documented by using of gel documentation system. Clear and reproducible bands were scored as present (1) and absence (0) at respective base pair.

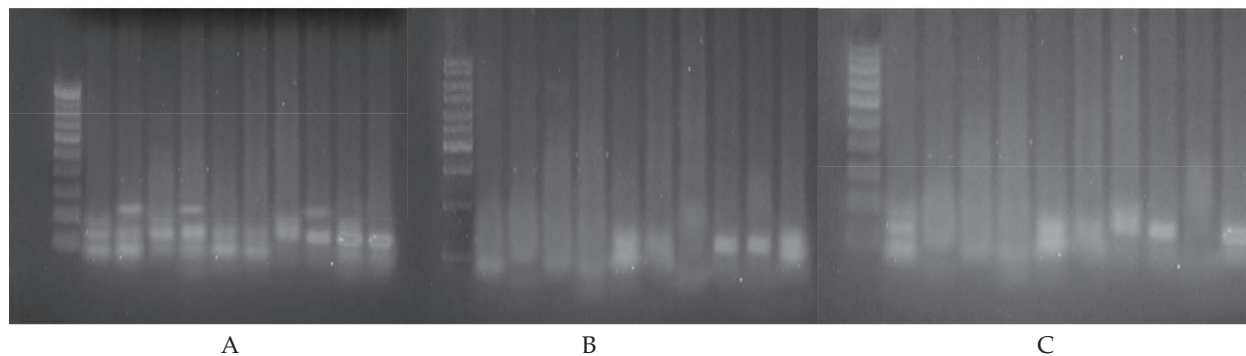
### Molecular analysis

Molecular analysis was done by online Marker Efficiency Calculator (*iMEC* software) as described by (Amiryousefi *et al.*, 2018) which is a simple computation of seven basic measures polymorphism indices for individual markers such as, *iMEC* calculator, H- Heterozygosity index, PIC- Polymorphic Information Content, EMR- Effective multiplex ratio, DP- Discriminating power, RP- Resolving power. The source code used to develop *iMEC* is available on GitHub (<https://github.com/Limpfrog/iMEC>). *iMEC* software is also available at <https://irscope.shinyapps.io/iMEC/>. Similarity matrix and a dendrogram was constructed with UPGMA (unweighted pair-group method with arithmetic mean) (Sneath and So-kal, 1973), using the NTSYS-*pc* software (Numerical Taxonomy and Multiware Analysis System) (Version 2.0) (Rohlf, 1988).

## Results and Discussion

### Markers efficiency

DNA based molecular markers have been proved as potential tool for assessment of genetic diversity and parental analysis of horticultural crops (Ahmad and Anjum, 2018; Kumar *et al.*, 2018, Kumar *et al.*, 2019, Ahmad *et al.*, 2021). Polymorphic genetic markers have wide potential applications in plant improvement programmes as a means for varietal and parentage identification, evaluation of polymorphic genetic loci affecting quantitative economic traits, and genetic mapping of plants (Baliyan *et al.*, 2014). In the present study diversity analysis based on RAPD fingerprinting showed by number of bands detected through 10 different RAPD primers ranged from 01- 04 bands with an average of 2.0 bands per primer (Table 3 and Fig. 1). There was a total of 20 polymorphic bands were produced by 10 primers, which generated 100% polymorphism (Table-3). Our results are superior to other studies carried out by Tiwari *et al.*, (2009) they obtained 27.5% polymorphism by RAPD and 18.73% polymorphism with ISSR when examine 19 advanced cultivars and landraces of brinjal. Thakkar *et al.*, (2014) reported 83.43 and 81.03 percent polymorphisms when brinjal genotypes analysis with RAPD and ISSR markers respectively. Maximum number of bands 04 generated by Primer-4 and minimum band 01 produced by Primer-2, Primer-3 and Primer-6. According to Amiryousefi *et al.*, (2018), there are two major dimensions of genomic marker polymorphism efficiency and informativeness as heterozygosity (H) and the polymorphic information content (PIC). These indices were measured based on data gained from RAPD primers using the *iMEC* (online



**Fig. 1.** Representative gel image depicting PCR amplifications using primers -4 primer in 30 selected genotypes of brinjal (ABC each gel containing 10 genotypes DNA profiling).

marker efficiency calculator). The range of H and PIC value for a binary or dominant marker is maximum as 0 (monomorphic) to 0.5 (highly judicial, with multiple alleles in an identical frequency) due to assume of two alleles per locus and both are influenced by the number and frequency of alleles (Ahmad *et al.*, 2018). In the present study, the ranges of PIC and H values indicate the moderate discriminatory capacity of markers. Heterozygosity varied from 0.00-0.48 while PIC was in ranged from 0.37 to 0.49. Highest Polymorphic Information content (PIC) value (0.49) was observed for Primer -2 while lowest PIC value (0.37) exhibited by Primer -1. Effective multiplex ratio was maximum (2.00) for the Primer-7 and minimum effective multiplex ratio (0.73) showed by Primer-10. Maximum resolving

power of markers (1.87) noted with Primer-4 while Primer-2 did not exhibit resolving power of marker. In the present study, PIC values, derived from allelic diversity and frequency among genotypes were not uniform for all of the RAPD primers. The PIC value in present study was higher than the previous studies as conducted by Tiwari *et al.* (2009) but lower than other studies as conducted by Thakkar *et al.* (2014) in brinjal. Markers with PIC value of 0.5 or higher are known to be highly informative for genetic studies and extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (Botstein *et al.*, (1980). The greater value of the resolving power (RP) and marker index (MI) indices refer to the greater efficacy of the respective primer (Zarei and Erfani-Moghadam, 2021). Maximum dis-

**Table 2.** List of RAPD primer Sequence and annealing temperature

RAPD PRIMERS			
S/No	Primer code	Sequence	Annealing temperature (°C)
1.	Primer-1	TTCGAGCCAG	32
2.	Primer-2	GTGAGGCGTC	34
3.	Primer-3	GGGGGTCTTT	32
4.	Primer-4	CCGCATCTAC	32
5.	Primer-5	GATGACCGCC	34
6.	Primer-6	GAACGGACTC	32
7.	Primer-7	GTCCCGACGA	34
8.	Primer-8	CTGCTGGGAC	34
9.	Primer-9	GTAGACCCGT	32
10.	Primer-10	CCTTGACGCA	32

**Table 3.** Primer name, total bands, polymorphic bands, monomorphic bands, and polymorphism indices of RAPD primers

S. No.	Primer	TA	PB	Polymorphic content %	H	PIC	EMR	DP	RP
1	Primer-1	3	3	100	0.48	0.37	1.83	0.63	1.53
2	Primer-2	1	1	100	0.00	0.49	1.00	0.00	0.00
3	Primer-3	1	1	100	0.06	0.48	0.97	0.07	0.07
4	Primer-4	4	4	100	0.46	0.38	1.47	0.87	1.87
5	Primer-5	2	2	100	0.34	0.43	1.57	0.39	0.87
6	Primer-6	1	1	100	0.06	0.48	0.97	0.07	0.07
7	Primer-7	2	2	100	0.00	0.49	2.00	0.00	0.00
8	Primer-8	2	2	100	0.30	0.44	0.37	0.97	0.73
9	Primer-9	2	2	100	0.48	0.37	0.80	0.84	1.60
10	Primer-10	2	2	100	0.46	0.38	0.73	0.87	1.47
	TOTAL	20	20	1000	2.64	4.31	11.71	4.71	8.21
	Avg	2	2	100	0.264	0.431	1.17	0.471	0.820

TA- Total number of alleles, PB- Polymorphic Bands, H- Heterozygosity index, PIC- Polymorphic Information Content, EMR- Effective multiplex ratio, DP- Discriminating power, RP- Resolving power



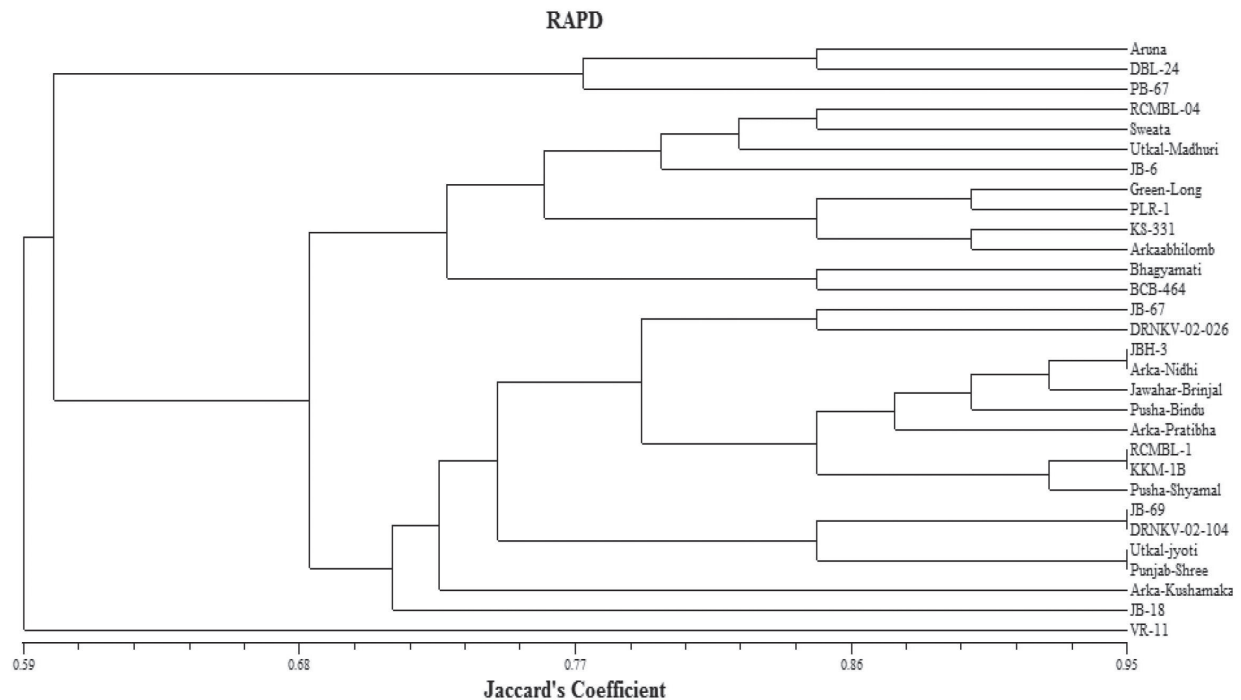


Fig. 2. Dendrogram showing clustering of 30 brinjal genotypes constructed using UPGMA based on Jaccard's similarity coefficient obtained from RAPDs analysis

(GS=0.75) consists of 3 genotypes from Aruna to PB-67 and group 2 at genetic similarity coefficient (GS=0.80) further sub divided in to seven sub cluster (2a, 2b, 2c, 2d, 2e, 2f & 2g), sub cluster 2a (GS=0.50) consisted of four brinjal genotypes from RCMBL-24 to JB-6 and group 2b at (GS=0.90) containing of four genotypes from Green Long to Arkaabhilomb, cluster 2c at similarity co-efficient (GS=0.85) had only two genotypes like Bhagyamati and BCB-464, cluster 2d at (GS=0.80) consists ten genotypes from JB-67 to Pusa Shyamal, cluster 2e at genetic similarity co-efficient (GS=0.65) consisted of four genotypes from JB-69 to Punjab Shree, cluster 2f at (GS=0.65) and cluster 2g at genetic similarity co-efficient (GS=0.55) consisted only one genotype each like Arka Khusamakar and JB-18 respectively. Similar results were also reported by Tiwari *et al* (2009) when characterization of brinjal germplasm by using RAPD and ISSR markers.

## Conclusion

Based on molecular analysis of RAPD, it may be concluded that RAPD analysis was extremely useful for identification of diverse genotypes in brinjal.

Genotypes BR-11 and JB-18 and PB-67 showed great diversity from the other genotypes. These diverse genotypes may be further use to planning of breeding strategies for development of desirable type genotypes.

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