***Original Research Article***

**SSR Markers Mediated Diversity Analysis of Brinjal *(Solanum melongena* l.)**

**ABSTRACT**

Brinjal (*Solanum melongena* L.) is an essential vegetable crop that is consumed globally, particularly in areas that are prone to drought. Due to its remarkable range of sizes and shapes, this vegetable is highly valued for both nutritional content and aesthetic appeal. Because of its high protein content, potent antioxidants, and vital minerals, brinjal is a valuable supplement to a balanced diet. Brinjal is widely grown in Bihar, India, but little is known about the genetic diversity among its genotypes, despite its importance in agriculture and the economy. In order to develop improved varieties with improved traits such as: increased yield, disease resistance, and environmental adaptability, breeding programs must have a thorough understanding of genetic variation present within the species and its wild relatives. The genetic diversity of 19 brinjal genotypes —16 inbred lines and three wild varieties —was assessed in this study using 21 SSR markers. EM107, EM114, EM133, EM140, EM145, EMB01I13, EMB01D10, and EMK03O04 were the eight polymorphic markers used in the genetic analysis. The genotypes were grouped into two major clusters using UPGMA clustering, and these clusters were further subdivided into four sub-clusters according to their genetic similarity. The degree to which the various genotypes are related to one another was revealed by this classification. The markers EMK03O04 (0.3957) and EMB01D10 (0.4139) had the highest Polymorphic Information Content (PIC) values, suggesting that they are very useful and informative in differentiating among the genetic variants found in the brinjal genotypes. To further enhance our understanding of genetic diversity, future research is encouraged to encompass a wider variety of genotypes and markers, paving the way for more comprehensive insights into this important vegetable crop. This would serve to deepen the knowledge of genetic diversity in this significant vegetable crop further, ultimately leading to improved varieties.

**Keywords**: Brinjal, inbred, molecular diversity, *Solanum melongena*, SSR markers, wild relatives.

**INTRODUCTION**

Brinjal (*Solanum melongena* L., 2n=2x=24), commonly known as eggplant in the United States, is a prominent member of the Solanaceae family (Nagar et al., 2024). It is widely recognized in India by the regional names of Bangna, Bhagwata, Badanik, and Vangi, reflecting its deep cultural and agricultural importance. India is the main source and diversity center of the brinjal and its historical records indicate that it has been cultivated for over 4,000 years (Vavilow, 1951). The crop has been widely grown in Asia and Europe (Ansari & Singh, 2024) with world production of about 54.08 billion tonnes, with Asia contributing 93 per cent of the production. China dominates global production with 45 % of Gross Domestic Products (GDP) followed by India with 24% (FAO, 2022). The main producers of brinjal in India are West Bengal, Maharashtra and Bihar states (Akhtar et al., 2019).

Nutritionally, brinjal is a valuable source of carbohydrates, fats, proteins, food fibers, essential minerals, vitamins and antioxidants. Medications are used in traditional treatments such as diabetes, leprosy, gonorrhea, cholera, cancer, bronchitis, pneumonia and asthma (Nandi et al., 2021). In addition, brinjal has important pharmacological properties, including lowering of cholesterol, spasmogenic, central nervous system inhibitor, blocking calcium channels, and hypotensive properties (Saha et al., 2023). In addition to its nutritional and medical value, brinjal has significant potential as a raw material for the pickle and dehydration industries. This multifunctional crop is thus a key element of food security, traditional medicine and agricultural industry, and its diverse role in sustainable agriculture and public health reflects this.

Brinjal's wide genetic diversity is demonstrated by the considerable variation in both its morphological and biochemical traits (Ariwatgen et al., 2013). Breeding programs that aim to improve yield, disease resistance, and stress tolerance must advance by understanding the molecular diversity and genetic relationships among native genotypes. For evaluating genetic variation both within and between populations, molecular markers like Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), and Inter-Simple Sequence Repeat (ISSR) have become essential instruments (Nunome et al., 2009; Stagel et al., 2008; Cagusat & Hautea, 2014; Fallahi et al., 2022). By providing reliable information about genetic relationships, these markers help identify agronomically valuable traits for marker-assisted selection (MAS) and enable precise evaluation of genetic diversity.

The first SSR markers for brinjal, showed their efficacy in genetic analysis and mapping of agronomic characteristics, which was a major advance in molecular breeding. Subsequent studies have highlighted the use of SSR markers to assess the genetic diversity of various brinjal accessions (Nunome et al., 2009; Stagel et al., 2008). Nevertheless, although its agricultural, nutritional, and economic importance is small, the Salini genome has not been studied as much as other solanaceous crops, such as tomatoes, potatoes, and peppers (Fallahi et al., 2022; Rao et al., 2022). Recent advances in next-generation sequencing (NGS) and high-throughput sequencing technologies have provided promising paths for deep genomic research and the identification of new markers related to important traits (Hirakava et al., 2014; . Gaccione et al., 2022; Prasad et al., 2024)

Given the limited comprehensive studies on brinjal’s genetic diversity, the present investigation aims to estimate the level of genetic variation among diverse brinjal genotypes using SSR markers. This approach is expected to provide valuable insights for future breeding strategies, contributing to sustainable crop improvement and resilience to biotic and abiotic stresses.

**MATERIALS AND METHODS**

**Experimental Materials:** Table 1 presents a comprehensive list of 19 genotypesutilized in the experiment,encompassing both cultivated and wild accessions of Solanum species. The genotypes arecategorized primarily asinbred lines and wildaccessions, sourced from agricultural research institutesacross India. Among thecultivated genotypes,'Sel 91-2 (PH-6male)' and 'Sel Br-112 (PH-6female)' are inbred linesprocured from IARI, NewDelhi, and maintained atBAU, Sabour. Other notable cultivated genotypes include'Haritha' from KAU, Kerala,'Swarna Mani' from CHES, Ranchi,'IIHR-563' from IIHR,Bengaluru, and 'Pant Samrat' from GBPUAT, Pantnagar,each preserved at BAU, Sabour.Additionally, genotypes such as 'Rajendra Baigan-2,' 'VR-2,' and a series of BRBL lines (BRBL-01, BRBL-02, BRBL-04, BRBL-06, BRBL-07, BRBL-10, and BRBL-11)were sourced from BAU, Sabour.The wild accessions, including'BRBWM-07' and 'BRBWM-06' (Solanum macrocarpon), and S. gilo (Solanumgilo), were collected from BCKV, Mohanpur, andsubsequently maintained at BAU, Sabour. Thisdiverse set of genotypes represents significant genetic variability, which iscritical for breedingprograms, stress tolerancestudies, and the enhancement of desirableagronomic traits. The seeds from allthe genotypes were cultivated inclean and sterilizedpetridisheswithin a plant growthchamber. A total of 19 brinjal genotypes, selected for their uniformity, viability, health, and disease and insect tolerant, were utilized to growseedlings. DNA extractionfrom these 19genotypes wasconducted under controlled conditions to facilitate diversity analysis.

**DNA Extraction:**Genomic DNA was extracted from 15-day-old brinjal seedlings using immature leaf tissue (200-300 mg) by CTAB method (Doyle & Doyle, 1990) with the necessary modifications. The tissue was homogenised with 1 ml of CTAB buffer, incubated for 45 minutes at 65 degrees Celsius and then centrifuged with chloroform:isoamyl alcohol (24:1). The aqueous phase was precipitated with cooled isopropanol and the resulting DNA pellet was washed with 70 per cent ethanol, dried in air and dissolved in 100 ml 10-triols of buffer. The quality and quantity of DNA was assessed by 0.8-percent agarose gel electrophoresis and visualised under UV light using the GelDoc system.

**SSR-Marker analysis:** For SSR analysis, the genomic DNA was diluted to 40 ng per ml using 21 primers from 19 brinjal genotypes (primer sequences available in the supplementary file). PCR reactions (10.10 ml total volume) consisted of 1.5 ml DNA, 0.30 ml each of primers upstream and downstream, 4.0 ml pre-mix (10 times buffer, dNTP, MgCl, Taq polymerase) and water free from nucleosides. The induction consisted of initial denaturation at 94 degrees Celsius for 5 min followed by 35 cycles at 94 degrees Celsius for 30 seconds, 65 degrees Celsius for 1 min, 72 degrees Celsius for 2 min and finally a final prolongation at 72 degrees Celsius for 7 min. The products were separated by means of a catalytic converter of 2 percent agarose gel at 100 V and visualised under a UV light. The 100-bp DNA ladder was used to estimate the size of the PCR fragments. The gel images were documented with the Uvitec Gel Documentation System for further analysis.

**Statistical analysis of SSR data:** The expected size of the amplified bands for each SSR marker was determined by comparing their migration patterns with the 100-bp DNA ladder. Clear, distinct bands were visually scored as present (1) or absent (0) for eight polymorphic SSR markers in 19 brinjal genotypes. Polymorphic information content (PIC) and heterozygosity was calculated to evaluate the marker's ability to detect genetic variation. Binary data was used to construct a dendrogram based on a matrix of similarities generated by the Jaccard standard (Panwar et al., 2010). Clustering was performed using UPGMA (Unweighted paired-group method with arithmetic mean) to illustrate the genetic relations between genotypes.

**RESULTS AND DISCUSSION**

Nineteen brinjal genotypes were evaluated for allelic diversity using 21 SSR markers. Amplified products, resolved on 2 % agarose gel, were compared against a 100 bp DNA ladder to estimate fragment sizes. Among the 21 markers, eight were polymorphic, while thirteen were monomorphic, yielding 18 alleles. Polymorphic Information Content (PIC) values ranged from 0.1706 to 0.4139, with EMB01D10 (0.4139) and EM140 (0.3126) being the most informative markers.Genetic diversity was analyzed using UPGMA clustering and Jaccard's coefficient, with genetic distances ranging from 0.091 (e.g., PH-6 male with Mukta Keshi, IIHR-563, BRBL-04, BRBL-07, BRBL-10) to 0.875 (BRBL-11 with S. gilo) and averaging 0.401. Complete genetic similarity (0% dissimilarity) was observed among several genotype pairs, including PH-6 male, PH-6 female, Swarna Mani, and RB-2, as well as Mukta Keshi, IIHR-563, BRBL-01, BRBL-04, BRBL-07, and BRBL-10 **(Fig.1)**.

The genetic relationships among 19 brinjal genotypes were elucidated through phylogenetic analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm implemented in DARwin 5.0 software. The analysis incorporated allelic variation data obtained from eight polymorphic SSR markers, revealing distinct clustering patterns in the resultant dendrogram (Fig. 2). The UPGMA tree topology segregated the germplasm into two major clades with significant bootstrap support. Clade I exclusively contained wild accessions, forming a monophyletic group that further bifurcated into two well-supported subclades comprising S. macrocarpon (BRBWM-07 and BRBWM-06) and S. gilo genotypes. Clade II encompassed all cultivated inbred lines, demonstrating clear genetic differentiation from wild relatives, and was resolved into two distinct subclusters. These subclusters systematically grouped the genotypes based on their breeding history and pedigree relationships, including PH-6 derived lines (male and female parents), improved cultivars (Harita, Mukta Keshi, Swarna Mani, IIHR-563, Pant Samrat), and advanced breeding lines (RB-2, VR-R2, and the BRBL series from BRBL-01 to BRBL-11). The observed clustering pattern provides molecular evidence for the genetic divergence between cultivated and wild Solanum germplasm, while revealing finer-scale relationships among improved varieties and breeding lines.

Numerous studies have employed various molecular marker systems to assess genetic diversity in brinjal (*Solanum melongena* L.), demonstrating the critical role of DNA-based technologies in facilitating selective breeding through parental diversity characterization and germplasm enhancement (Fu et al., 2010). Extensive molecular characterization has consistently revealed substantial genetic variation among eggplant cultivar groups (Nunome et al., 2009; Stagel et al., 2008, Tumbilien et al., 2011 Cericola et al., 2018; Gramazio et al., 2018). While Random Amplified Polymorphic DNA (RAPD) markers have been traditionally predominant in brinjal diversity studies, showing greater efficiency than Inter-Simple Sequence Repeat (ISSR) markers (Tiwan et al., 2009; Thakkar et al., 2014). Comparative analyses have established the superior discriminatory power of Simple Sequence Repeat (SSR) markers (Demir et al, 2010 and Akhther et al, 2021). SSR-based studies have particularly demonstrated robust phylogenetic relationships within the Solanum complex (including *S. viarum*, *S. melongena*, and *S. aethiopicum* from the Aculeatum group), confirming their utility as ideal markers for genetic variation analysis ( Stagel et al., 2008, Numone et al., 2009; Musa et al., 2024). Quantitative evaluation of polymorphism information content (PIC) has revealed moderate genetic diversity levels, with PIC values ranging from 0.1706 to 0.4139, where markers EMB01D10 (PIC=0.4139) and EM140 (PIC=0.3126) emerged as particularly informative for germplasm characterization (Rajan et al, 2023). These collective findings underscore the progressive refinement of molecular tools for eggplant genetic studies, with SSR markers currently representing the most robust system for comprehensive diversity assessment in breeding programs.

SSR markers has been employed to assess genetic diversity among various eggplant varieties (Younas et al., 2022; Lipio et al, 2024). A range of allelic variations and clustering patterns were identified that reflected the genetic relationships among different genotypes. The cluster analysis revealed that several genotypes grouped closely together, with both the PH-6 male and PH-6 female lines—two widely cultivated varieties in India—showing minimal genetic variation. This lack of variation is likely due to their extensive use in heterosis breeding programs.

These results are consistent with findings from other researchers, who reported a high pairwise similarity index among closely related brinjal genotypes, indicating a narrow genetic base at the molecular level (Fallahi et al., 2022; Akhther et al, 2021). The brinjal genome, estimated to be 1,155.8 Mb in size, comprises approximately 70.1% repetitive sequences (Li et al., 2021) which complicates the detection of polymorphism using a limited number of SSR markers.

Recent studies has also reported restricted allelic variation in brinjal due to the limited number of primers used for diversity analysis (Musa et al, 2024 and Oladosu et al, 2021). Therefore, there is a need for a greater number of polymorphic markers and advanced genomic tools for more comprehensive analysis.

**CONCLUSIONS**

The usefulness of SSR markers in determining the genetic diversity among 19 brinjal genotypes—a critical step for successful breeding and conservation strategies—is highlighted in the current study. The potential of the 21 SSR markers used for molecular diversity analysis was demonstrated by the observation that eight of them (EM107, EM114, EM133, EM140, EM145, EMB01I13, EMB01D10, and EMK03O04) were polymorphic. The genotypes were effectively grouped using UPGMA-based cluster analysis into two major clusters and four sub-clusters, with inbred lines making up Cluster II and wild varieties making up Cluster I. The PIC values (0.4139 for EMB01D10 and 0.3957 for EMK03O04) and the greatest genetic distance (200–300 bp) demonstrate the dependability and informativeness of these markers for genetic differentiation. The study determines the degree of genetic diversity, groups genotypes into clusters, and assesses the informativeness of particular genotypes by analyzing 19 genotypes—including inbred lines and wild varieties—using SSR markers.

Disclaimer (Artificial intelligence): Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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**CONFLICT OF INTEREST**

We hereby certify that there is no conflict of interest among the authors in submission of the research article.

**AUTHOUR'S CONTRIBUTION**

ZQ, TK, TR and RRK: conceptualization, conducted the study and writing; RSS and AKP: Data analysis ; SA, BK and KR: Designing and proof reading

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**Table 1** List of the genotypes that used in experiment

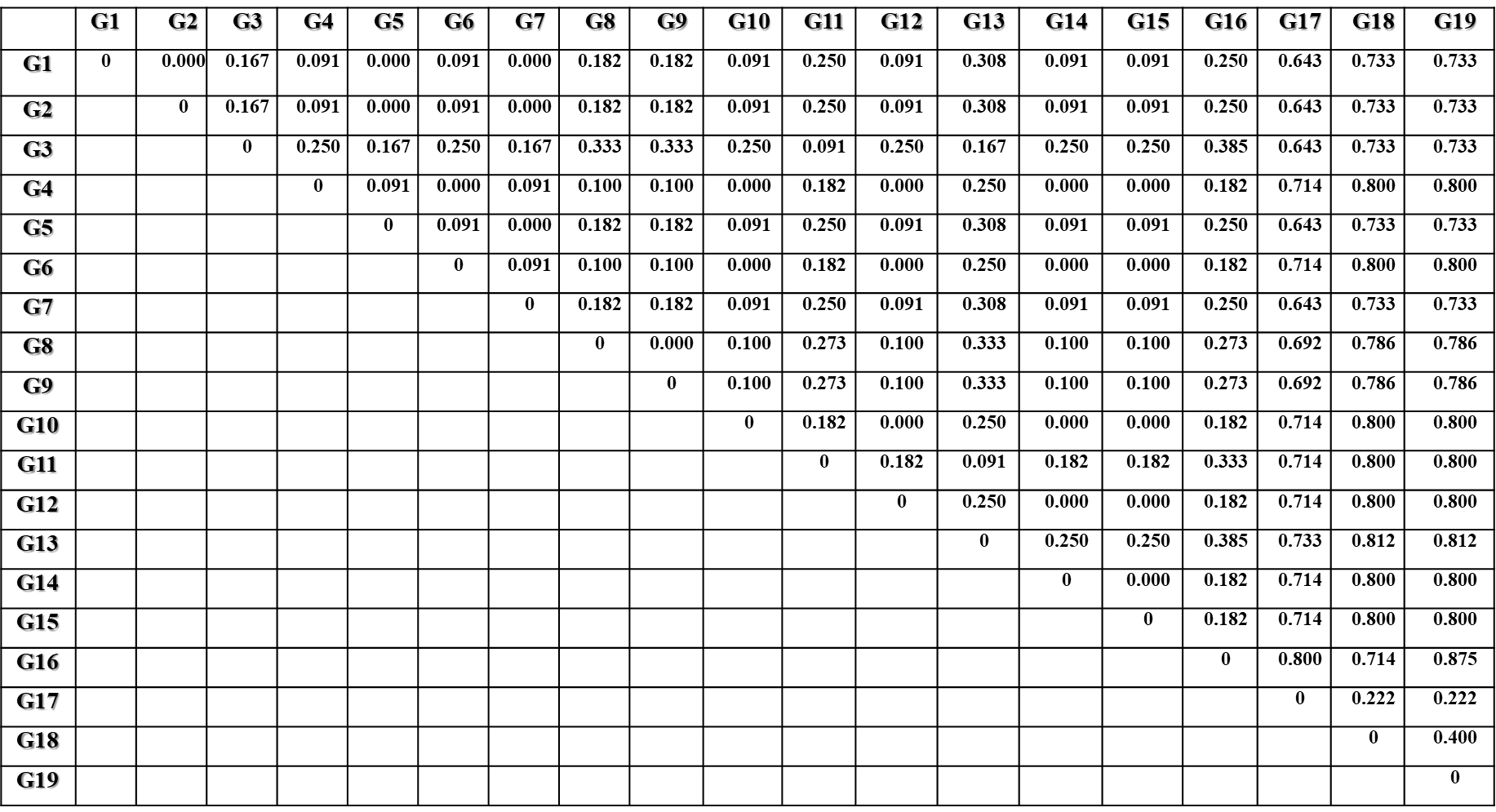
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SN** | **Genotypes** | **Types** | **Sources** | **Code** |
| 1 | Sel 91-2 (PH-6 male) | Inbred line | Collected from IARI, New Delhi maintained at BAU, Sabour | G1 |
| 2 | Sel Br-112 (PH-6 female) | Inbred line | Collected from IARI, New Delhi maintained at BAU, Sabour | G2 |
| 3 | Haritha | Inbred line | Collected from KAU, Kerala maintained at BAU, Sabour | G3 |
| 4 | Muktakeshi | Inbred line | BAU, Sabour | G4 |
| 5 | Swarna Mani | Inbred line | Collected from CHES, Ranchi maintained at BAU, Sabour | G5 |
| 6 | IIHR-563 | Inbred line | Collected from IIHR, Bengaluru maintained at BAU, Sabour | G6 |
| 7 | Rajendra Baigan-2 | Inbred line | BAU, Sabour | G7 |
| 8 | VR-2 | Inbred line | Collected from IIVR, Varanasi maintained at BAU, Sabour | G8 |
| 9 | Pant Samrat | Inbred line | Collected from GBPUAT, Pantnagar maintained at BAU, Sabour | G9 |
| 10 | BRBL-01 | Inbred line | BAU, Sabour | G10 |
| 11 | BRBL-02 | Inbred line | BAU, Sabour | G11 |
| 12 | BRBL-04 | Inbred line | BAU, Sabour | G12 |
| 13 | BRBL-06 | Inbred line | BAU, Sabour | G13 |
| 14 | BRBL-07 | Inbred line | BAU, Sabour | G14 |
| 15 | BRBL-10 | Inbred line | BAU, Sabour | G15 |
| 16 | BRBL-11 | Inbred line | BAU, Sabour | G16 |
| 17 | BRBWM-07 | Wild accession of *Solanum macrocarpon* | Collected from BCKV, Mohanpur, maintained at BAU, Sabour | G17 |
| 18 | BRBWM-06 | Wild accession of *Solanum macrocarpon* | Collected from BCKV, Mohanpur, maintained at BAU, Sabour | G18 |
| 19 | *S gilo* | Wild accession of *Solanumgilo* | Collected from BCKV, Mohanpur, maintained at BAU, Sabour | G19 |

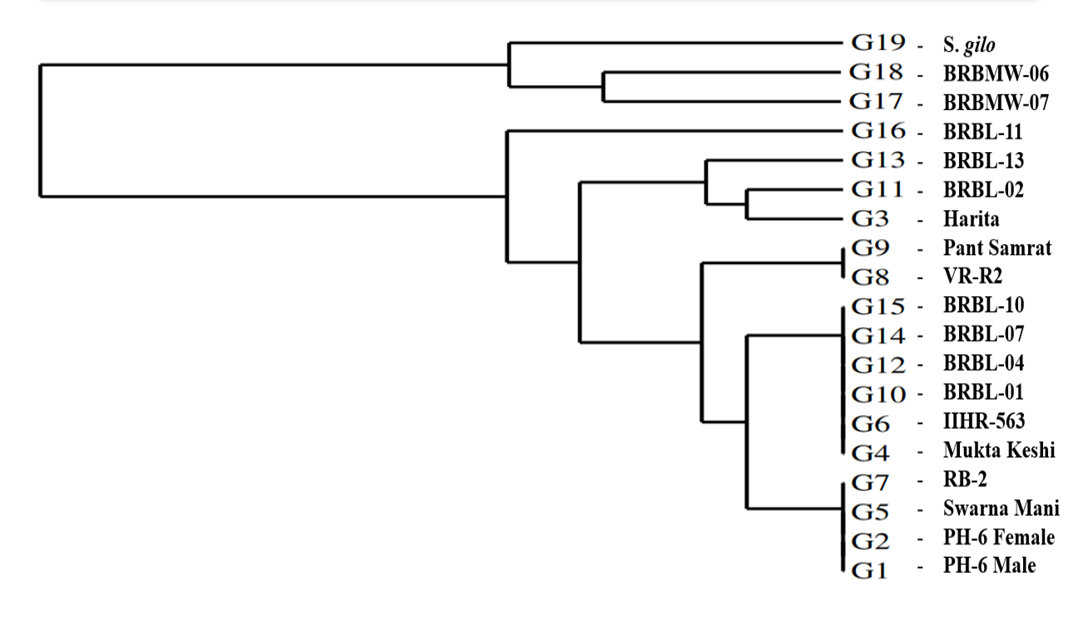
**Table 2: List of brinjal SSR primers used for diversity analysis**

|  |  |  |
| --- | --- | --- |
| **SN** | **SSR Primer** | **Sequence (5`→3`)** |
|
|  | EM107 | **FP**- GGCCCTAGACTGAGCCTGAAATGTT  **RP**- TGGTACAACCAACACAACCCTCAA |
|  | EM133 | **FP-** GCGGATCACCTGCAGTTACATTAC  **RP-** TCCTTTGACCTATAGTGGCACGTAGT |
|  | EM140 | **FP-** CCAAAACAATTTCCAGTGACTGTGC  **RP-** GACCAGAATGCCCCTCAAATTAAA |
|  | EM145 | **FP-** TGATTTGGCCCTTAAGCCTAAGTATG  **RP-** GACTCCTCAAGCCTTTACCTCCAA |
|  | *ecm*031 | **FP-** ACCAAAGGAGAAGGAGATGTGCTA  **RP-** GTTTGCTTCCCTTCCAAAATGACAAG |
|  | emf21N03 | **FP-** ACCAGAGGAGCAAAGGGAAAAATA  **RP-** GTTTACGCTACTGGACCAAACCAACAAT |
|  | eme08D09 | **FP-** ATGGATTAGCATGTGGAGGACTGAA  **RP-** GTTTCATGGTAGGTGGAGACAGAACCA |
|  | *emf*21H22 | **FP-** CACAAGATGAACAAGACTAAGGAGTGC  **RP-** CTTCTTCAACCTGTCTTTAGCCCA |
|  | SMSSR11 | **FP-** AAACAAACTGAAACCCATGT  **RP-** AAACAAACTGAAACCCATGT |
|  | SMSSR14 | **FP-** ATACCACATCAATCCAAAGC  **RP-** CATCATCATCTTCACAGTGG |
|  | EMB01D10 | **FP-** AAGAATCGGTCCTCTTTGCATTGT  **RP-** TGCTTTTCACCTCTCCGCTATCTC |
|  | EMB01I13 | **FP-** AGTCGTGTAGGTCAAAGCAACTGA  **RP-** GTTTAGCTACGTTGGTTTGGTGCTGAA |
|  | EMK03O04 | **FP-** ATGATTTGGGCAGCCACTTTTGTA  **RP-** GTTTGGAACCAACTAAACTTAGGGCA |
|  | *emf*21C11 | **FP-** TGGTTGGAGCCATGATTACTTGAA  **RP-** ATGCTACCTATCAAACAGGCGGAA |
|  | *emh*11O01 | **FP-** GATGTGTCGATGAGATTTTGGTCA  **RP-** TAGCTACGTTGGTTTGGTGCTGAA |
|  | SMSSR01 | **FP-** GTGACTACGGTTTCACTGGT  **RP-** GATGACGACGACGATAATAGA |
|  | EMB01L13 | **FP-** TCAAAAGACTTGAAACCCGATGGT  **RP-** GTTTATCAGGTTTTTGATCACCGGACA |
|  | EM114 | **FP-** AGCCTAAACTTGGTTGGTTTTTGC  **RP-** GAAGCTTTAAGAGCCTTCTATGCAG |
|  | SMSSR03 | **FP-** ATTGAAAGTTGCTCTGCTTC  **RP-** GATCGAACCCACATCATC |
|  | EM139 | **FP-** TGC TAA GTC GTC ATC CAACAA GAA  **RP-** GAT TTT GGC TCC TTG ACC ATT TTG |
|  | *eme*03A05 | **FP-** ATTTTATGCTGCTCCTCGGATTGAT  **RP-** GTTTGTGCATCTCTTGTTGGTAGGAGC |

**Table 3**: Summary of polymorphic markers

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **SN** | **Primer** | **Total Numberof alleles** | **Total Number of Polymorphic alleles** | **Range** | **Heterozygosity** | **PIC Value** |
| **1** | EM107 | 2 | 2 | 210-190 | 02659 | 02306 |
| **2** | EM114 | 2 | 2 | 210-200 | 00997 | 00948 |
| **3** | EM133 | 2 | 2 | 190-180 | 02659 | 02306 |
| **4** | EM140 | 2 | 2 | 280-210 | 03878 | 03126 |
| **5** | EM145 | 2 | 2 | 190-180 | 01884 | 01706 |
| **6** | EMB01D10 | 3 | 3 | 300-200 | 05088 | 04139 |
| **7** | EMB01I13 | 2 | 2 | 210-200 | 02659 | 02306 |
| **8** | EMK03O04 | 3 | 3 | 200-130 | 04383 | 03957 |

**Figure 1:** Distance matrix based on Jaccard coefficient (UPGMA)



**Figure 2: clustering of brinjal genotypes by UPGMA using molecular data**