Genetic diversity within the genus *Solanum* (Solanaceae) as revealed by RAPD markers

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Random amplified polymorphic DNA (RAPD) technique was used as a tool for assessing genetic diversity and species relationships among 28 accessions of eggplant representing five species. Twenty-eight samples of eggplants were collected from different parts of the country. A total of 144 polymorphic amplified products were obtained from 14 decamer primers, which discriminated all the accessions. The value of Jaccard's coefficient ranged from 0.05 to 0.82. The similarity result indicates presence of high level of genetic diversity in eggplants and a dendrogram constructed by UPGMA method shows that *S. incaum* is closest to *S. melongena* followed by *S. nigrum*. Only one accession of *S. nigrum* and *S. surattense* was taken in the present study that showed grouping with each other. Genetically distinct genotypes identified using RAPD markers could be potential sources of germplasm for eggplant improvement.

Keywords: Brinjal, genetic diversity, RAPD markers, Solanaceae.

BRINJAL, eggplant or aubergine (*Solanum melongena* L.) is widely cultivated as vegetable in both temperate and tropical areas, especially in Asia. In India, it is also used for the treatment of diabetes, bronchitis, asthma, dysuria, dysentery, etc.¹ In African countries *S. aethiopicum* group *giolo* and *S. anguari* are used for the treatment of many diseases. Many other *Solanum* species are also used for medicinal purposes². For an effective breeding programme, information concerning the extent and nature of genetic diversity within a crop species is essential. It is particularly useful for characterizing individual accessions and cultivars and as a general guide in the selection of the parents for hybridization. Several workers have contributed to the characterization of the largest genus of *Solanaceae* family³. Great degree of taxonomic confusion exists as regard to genus *Solanum*⁴.

Genetic fingerprinting has been accomplished traditionally through the use of isozymes, total seed protein and more recently through various types of molecular

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markers. However, DNA-based markers provide powerful tools for discerning variations within crop germplasm and for studying evolutionary relationships. Among molecular markers, random amplified polymorphic DNA (RAPDs) have been extensively used in genetic research owing to their speed and simplicity. Most variability/taxonomic affinity studies in eggplant have focused mainly on morphology, crossability, anatomy, isozyme and chloroplast DNA diversity. Limited work has been done so far with nuclear DNA diversity. Greater DNA polymorphism has been reported in weedy *S. insanum* than in advanced cultivars of *S. melongena*. In another study AFLP was found to be an excellent tool for the determination of genetic relationship among the species of *Solanum*.

India or Indochina is the centre of eggplant diversity, but the affinities of *S. melongena* to related species are uncertain. Taxonomic uncertainties exist because phylogenetic relationships among taxa have been established considering mainly the morphological features, crossability and F₁ fertility. Establishing genetic affinities on such parameters are insufficient, as *S. melongena* makes successful cross with putative progenitors as well as distantly related species. To overcome these problems, isozyme was used as tool but met with little success. The use of molecular techniques in genetic diversity studies is supported by the finding that evolutionary forces such as natural selection and genetic drift produce divergent phylogenetic branches which can be recognized because the molecular sequences on which they are based share a common ancestor. The present study was aimed at analyzing the eggplant germplasm to RAPD markers and classifying the relationship and variability using RAPD data among the eggplant taxa with numerical taxonomic techniques.

The plant material for the study comprised 28 accessions (morphologically and geographically distinct genotypes) representing five species including cultivated eggplant (Table 1). The materials were collected from different parts of India, and maintained at Research Farm of the Indian Institute of Vegetables Research, Varanasi. Young leaves were collected from ten field grown plants for each accession listed in Table 1. Harvested leaves were immediately stored at −80°C. Total genomic DNA was extracted using the protocol of DNeasy plant mini kit (Qiagen). The DNA concentration was estimated with UV/VIS spectrophotometer (Lambda Bio20, Perkin Elmer, USA). Bulk samples were prepared by pooling equal amount of purified genomic DNA from 10 individuals of each accession and aliquot from these combined sample was used for polymerase chain reaction (PCR).

PCR conditions were optimized by varying concentrations of template DNA, *Taq* DNA polymerase and *Mg*²⁺ ion. Initial screening was done with 10-mer primers (Operon Technologies Inc., USA) using DNA from four accessions. The primers that gave reproducible and scorable amplifications were used in the analysis of all the 28 genotypes (Table 2). Total reaction volume for DNA amplification was 25 μl. A reaction mixture contained 1X PCR buffer (10 mM Tris HCl (pH 8.8), 50 mM KCl and 15 mM *MgCl*₂), 3 mM *MgCl*₂, 300 μM each dNTPs, 0.5 μM primer, 1 unit of *Taq* DNA polymerase and 60 ng of genomic DNA. The reaction mixture was overlaid with one drop of mineral oil to prevent evaporation. DNA amplification was performed (Thermal Cycler, Perkin Elmer, Cetus) as follows: 1 cycle of 4 min at 94°C (initial strand separation) followed by 40 cycles of 1 min at 94°C (denaturation), 1 min at 36°C (annealing) and 2 min at 72°C (primer extension). After amplification, PCR product was stored at 4°C till electrophoresis. Reactions products were mixed with 2.5 μl of 10X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a micro centrifuge before loading. PCR products were resolved by electrophoresis at 1.4% agarose gel, 60 V for 3 h followed by staining with ethidium bromide. Gel was photographed in Gel Documentation System (UVP, PDS-8000).

Data were scored as discrete variables, using 1 to indicate presence and 0 to indicate absence of the band. A pairwise different matrix between genotypes was determined using Jaccards similarity coefficient and the average taxonomic distance. The statistical analysis was carried out using NTSYS software (Version 2.11) and Winboot Soft-

### Table 1. Accessions of eggplant and its related species analysed for RAPDs

<table>
<thead>
<tr>
<th>Material</th>
<th>Taxon</th>
<th>Code</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB-40</td>
<td><em>S. melongena</em></td>
<td>BB</td>
<td>Bhuvneshwar</td>
</tr>
<tr>
<td>KS-331</td>
<td><em>S. melongena</em></td>
<td>KSA</td>
<td>Kanpur</td>
</tr>
<tr>
<td>Uttara</td>
<td><em>S. melongena</em></td>
<td>UTR</td>
<td>Bangladesh</td>
</tr>
<tr>
<td>Faizabadi</td>
<td><em>S. melongena</em></td>
<td>FZB</td>
<td>Faizabad</td>
</tr>
<tr>
<td>BR-112</td>
<td><em>S. melongena</em></td>
<td>BR</td>
<td>Kanpur</td>
</tr>
<tr>
<td>Baramasi</td>
<td><em>S. melongena</em></td>
<td>VNS</td>
<td>Varanasi</td>
</tr>
<tr>
<td>Punt Ritiraj</td>
<td><em>S. melongena</em></td>
<td>RTJ</td>
<td>Pattanagar</td>
</tr>
<tr>
<td>CHBR-2</td>
<td><em>S. melongena</em></td>
<td>CBR-2</td>
<td>Ranchi</td>
</tr>
<tr>
<td>DBSR-44</td>
<td><em>S. melongena</em></td>
<td>DRR-2</td>
<td>Delhi</td>
</tr>
<tr>
<td>KS-326</td>
<td><em>S. melongena</em></td>
<td>KSB</td>
<td>Kanpur</td>
</tr>
<tr>
<td>Pusa Purple Long</td>
<td><em>S. melongena</em></td>
<td>PPL</td>
<td>Delhi</td>
</tr>
<tr>
<td>Ramnagar Giant</td>
<td><em>S. melongena</em></td>
<td>RNZ</td>
<td>Ramnagar</td>
</tr>
<tr>
<td>CHBR-1</td>
<td><em>S. melongena</em></td>
<td>CBR-1</td>
<td>Ranchi</td>
</tr>
<tr>
<td>JB-80</td>
<td><em>S. melongena</em></td>
<td>JB</td>
<td>Jabalpur</td>
</tr>
<tr>
<td>Aruna</td>
<td><em>S. melongena</em></td>
<td>AR</td>
<td>Akola</td>
</tr>
<tr>
<td>KT-4</td>
<td><em>S. melongena</em></td>
<td>KT</td>
<td>Katrain</td>
</tr>
<tr>
<td>IC-114274</td>
<td><em>S. incamum</em></td>
<td>INCA</td>
<td>NBPRG*</td>
</tr>
<tr>
<td>IC-11427</td>
<td><em>S. incamum</em></td>
<td>INCB</td>
<td>NBPRG*</td>
</tr>
<tr>
<td>NIC-18360</td>
<td><em>S. incamum</em></td>
<td>INCC</td>
<td>NBPRG*</td>
</tr>
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<td>NBPRG*</td>
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</tr>
<tr>
<td>IC-135913</td>
<td><em>S. incamum</em></td>
<td>INCF</td>
<td>NBPRG*</td>
</tr>
<tr>
<td>NJ-94-3</td>
<td><em>S. viaram</em></td>
<td>VRMA</td>
<td>NBPRG*</td>
</tr>
<tr>
<td>VRV-1</td>
<td><em>S. viaram</em></td>
<td>VRMB</td>
<td>Assam</td>
</tr>
<tr>
<td>V-4532</td>
<td><em>S. viaram</em></td>
<td>VRMC</td>
<td>NBPRG*</td>
</tr>
<tr>
<td>IC-135922</td>
<td><em>S. viaram</em></td>
<td>VRMD</td>
<td>NBPRG*</td>
</tr>
<tr>
<td>IC-135932</td>
<td><em>S. suratense</em></td>
<td>SRT</td>
<td>Varanasi</td>
</tr>
<tr>
<td>VRN-91-0-196</td>
<td><em>S. nigrum</em></td>
<td>NGR</td>
<td>Varanasi</td>
</tr>
</tbody>
</table>

*National Bureau of Plant Genetic Resources, India.
Figure 1. a, b. RAPD profiles for *Solanum* species generated by primer OPC-09 (a) and primer OPC-14 (b) Lane 1: BB-40, Lane 2: KS-331, Lane 3: Uttara, Lane 4: Faiabad, Lane 5: BR-112, Lane 6: Baramasi, Lane 7: Pant Rituraj, Lane 8: CHBR-2, Lane 9: DBSR-44, Lane 10: KS-326, Lane 11: Pusa purple long, Lane 12: Ramnagar Giant, Lane 13: CHBR-1, Lane 14: JB-30, Lane 15: M.W. Marker (1 kb), Lane 16: Aruna, Lane 17: KT-4, Lane 18: IC-1114574, Lane 19: IC-111427, Lane 20: NIC-18360, Lane 21: IC-111311, Lane 22: IC-135912, Lane 23: IC-135913, Lane 24: NL/94-3, Lane 25: VRV-, Lane 26: 1 V-4532, Lane 27: IC-1359922, Lane 28: IC-135992, Lane 29: VRN-91-40-196, Lane 30: M.W. Marker (1 kb).

Table 2. Selected primers along with their sequence and some characteristics of amplification products in accessions analysed

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5'</th>
<th>Number of bands</th>
<th>Mol. wt. range (Kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA 01</td>
<td>CAGGCCCTTC</td>
<td>9</td>
<td>0.15–3.5</td>
</tr>
<tr>
<td>OPA 09</td>
<td>GGATACGCC</td>
<td>9</td>
<td>0.13–3.8</td>
</tr>
<tr>
<td>OPA 10</td>
<td>GTGATCGCAT</td>
<td>9</td>
<td>0.14–2.1</td>
</tr>
<tr>
<td>OPA 16</td>
<td>AGCCAGCGGA</td>
<td>10</td>
<td>0.14–3.8</td>
</tr>
<tr>
<td>OPB 12</td>
<td>GTGATCGCAG</td>
<td>11</td>
<td>0.13–3.2</td>
</tr>
<tr>
<td>OPB 18</td>
<td>CCACAGCAGT</td>
<td>10</td>
<td>0.12–3.3</td>
</tr>
<tr>
<td>OPB 19</td>
<td>ACCCCGAAG</td>
<td>12</td>
<td>0.13–3.1</td>
</tr>
<tr>
<td>OPB 20</td>
<td>GACCGCTTAC</td>
<td>9</td>
<td>0.14–2.5</td>
</tr>
<tr>
<td>OPC 05</td>
<td>GATGACCGCC</td>
<td>13</td>
<td>0.12–2.4</td>
</tr>
<tr>
<td>OPC 07</td>
<td>GTCCCGAGCA</td>
<td>13</td>
<td>0.12–3.3</td>
</tr>
<tr>
<td>OPC 09</td>
<td>CTCACCGTCC</td>
<td>9</td>
<td>0.13–2.8</td>
</tr>
<tr>
<td>OPC 10</td>
<td>TGCTGGTCGT</td>
<td>8</td>
<td>0.11–2.1</td>
</tr>
<tr>
<td>OPC 14</td>
<td>TGCGGTCTTG</td>
<td>12</td>
<td>0.12–4.5</td>
</tr>
<tr>
<td>OPC 17</td>
<td>TTCCCCCAG</td>
<td>10</td>
<td>0.10–4.3</td>
</tr>
</tbody>
</table>

A dendrogram was constructed by UPGMA method to measure the resulting phenotypic groups, the original matrix was bootstrapped 1000 times by employing Winboot to group the accessions into discrete clusters.

Varying the concentration of DNA from 20 to 90 ng revealed that 60 ng of DNA gave the maximum number of reproducible bands; therefore 60 ng was used in all the analyses. Different concentrations of *Taq* DNA polymerase, MgCl₂, and primer were used to optimize the RAPD reaction. One unit of *Taq* DNA polymerase, 3.0 mM MgCl₂ and 0.5 μM of primer were found best for all subsequent PCR reaction. A higher or lower concentration resulted in either sub-optimal or complete lack of PCR amplification. The RAPD analysis carried out on all accessions produced a large number of distinct fragments for each primer. Eighty selected arbitrary primers generated a total of 44 scorable
bands of which 14 were polymorphic, with an average of 10.28 amplicons per primer. Figure 1a and b shows the RAPD profile for the 28 accessions yielded by OPC-09 and OPC-14. The numbers of amplification products obtained were in the range 8–13 with the primer OPC-10 producing the minimum number of (8) bands whereas primers OPC-5

Figure 2. UPGMA cluster analysis-based dendrogram showing genetic relationship among genus *Solanum*. 

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and OPC-7 producing the maximum number of bands (13). On an average 10.28 bands were obtained per primer with range 0.15–3.50 kb (Table 2). Jaccard’s similarity coefficient showed a wide range (0.050 to 0.821) of variability. With combination of suitable primers, all accessions could be identified by having accession-specific band, or by lacking a band that was present in all other accessions.

Jaccard’s similarity coefficient matrix was used to generate a dendrogram using UPGMA clustering method using NTSYS and later it was bootstrapped (10,000 replicates) to confirm the grouping (Figure 2). Not much variation in the dendrogram grouping pattern between UPGMA and Bootstrapped version was observed. The Bootstrap method showed that only 24 of 28 accessions grouped together in seven clusters (Figure 2). BB-40 (S. melongena), VRV-1 (S. viarum), DBSR-44 (S. melongena), IC-135922 (S. viarum) and CHBR-2 (S. melongena) did not group with any cluster. Cluster 2 was the largest group with six represented by two accessions each from S. melongena, S. incanum and S. viarum. Clusters 4 and 7 were represented by four accessions each represented by two each of S. melongena and S. incanum. Cluster 5 contained only three accessions represented by S. melongena. Clusters 1, 3 and 6 are the smallest clusters represented by only two accessions. In cluster 6, S. surattance and S. nigrum are grouped together whereas in clusters 1 and 3 only S. melongena accessions were present. The grouping pattern clearly indicates that in all the clusters S. incanum is always grouped with S. melongena indicating closeness between S. melongena and S. incanum. Whereas, S. viarum grouped with S. melongena only in cluster 2, indicated some similarity with S. melongena, S. surattance and S. nigrum were grouped with each other indicated that they are only distantly related to cultivated type (S. melongena) and are more closely related to each other.

Eggplant germplasm of the Indian subcontinent is very diverse. Wide variation in the desirable genotypes/agronomy types in different regions substantiates the high level of genetic variability observed. High degree of diversity of species belonging to Solanum may be attributable to the fact that it is an ancient plant. RAPD and other discontinuous markers can serve as a means of genetic distances to establish phylogenetic relationships among taxa. Detection of genetic differences and discrimination of genetic relationship between Solanum species are for utilization of plant genetic resources.

The level of polymorphism observed in the present study was fairly high (0.05–0.82), indicating a wide and diverse genetic base. The present results and those obtained by others are not in agreement with the earlier workers who studied variation among the cultivated and weedy taxa of S. melongena by allozymes and RAPD analysis. These authors observed little genetic polymorphism among the genotypes studied and suggested the existence of a very small gene pool from which the cultivated forms arose. The explanation for the observed high degree of variation could be due to the fact that our analysis of DNA variability is based on RAPD markers, which proved more informative than allozymes and that most of the accessions of the eggplant analysed in our study were collected from India, where the greatest diversity is reported. A high degree of variation has also been reported by using AFLP technology for S. melongena with weedy relative of the cultivated eggplant.

Interestingly collections originating from various parts of the country did not form well-defined distinct groups and were interspersed with each other, indicating no association between RAPD pattern and the geographic origin of accessions. The varieties and landraces were interspersed with each other. These results indicate that the improved cultivars studied here are very similar to the landraces used in this study. This finding is supported by others. This may be due to the fact that most of the cultivars have been bred from local material through simple selection. At the species level S. melongena (cultivable type) is more closely related to S. incanum followed by S. viarum whereas S. surattance and S. nigrum showed a closer association among themselves in comparison with the cultivated type. Wild forms of S. incanum are regarded as belonging to the same species as S. melongena.

Hybridization examples with eggplant show that S. melongena is crossable with several species and to a certain degree, also with other sections. Even when the hybrids are partially or completely sterile, their existence indicates some degree of relationship. All these wild species represent the natural gene pool available for the genetic improvement of the cultivated eggplant. For example, interspecific hybrid between S. incanum (female) and S. melongena has shown resistance against Fusarium oxysporum and Lucinodor orbinalis (brinjal fruit and shoot borer), which are the serious biotic stresses in this crop. It will be worth to investigate specific traits in the wild species and they may be introgressed by sexual crossing or somatic hybridization into commercial varieties of S. melongena. RAPD markers can be applied to other Solanum species to assess the genetic relationship among them and assist in the introgression of genes.

Reproductive biology of *Crataeva religiosa* Forst.

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*Crataeva religiosa* flowers profusely during March to May, when it sheds all its leaves. The flowers are large, hermaphrodite, actinomorphic and complete. Flowers open in the evening between 1900 and 2300 h followed by anther dehiscence at 1930–2100 h. Flowers offer pollen and nectar to the visitors, which include honey bees, moths, butterflies, bugs and birds. The plant is self-incompatible and obligate out-crosser. Fruit-set is restricted to only 22%. The beauty of the flowers as well as fruit production are adversely affected by the formation of floral galls induced by the insect, *Neolasioptera crataeae* Mani, order Diptera.

Keywords: *Crataeva religiosa*, floral galls, *Neolasioptera crataeae*, reproductive biology.

*Crataeva religiosa* Forst. (family Capparidaceae) is a large tree distributed in the tropical zone and is common throughout India, Myanmar and Sri Lanka, either wild or cultivated. It is cultivated in the gardens for its ornamental as well as medicinal value. Despite its usefulness, less

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