10. Juwarkar, A. S. and Juwarkar, Asha, A case study on the use of sewage for crop irrigation – Indian experience, Food and Agriculture Organization, Rome, 1987.
11. Juwarkar, A. S., Thawale, P. R., Jambulkar, H. P. and Juwarkar, A. A., Management of wastewater through crop irrigation – an ecofriendly approach. Ecotechnology for Pollution Control and Environment Management, Enviro Media, 1998, pp. 25–48.
12. Overcash, M. R. and Pal, D., *Design of Land Treatment Systems for Industrial Waste, Theory and Practices*, Ann. Arbor Science Publication, 1979.
13. Juwarkar, A. S., Thawale, P. R., Juwarkar, A. A. and Singh, S. K., An ecofriendly approach treatment and disposal of pulp and paper mill wastewater through land management: A case study. Souvenier and Abstract, IAEM National Conference, New Delhi, 2003, p. 21.
14. Chabra, R. S., *Sewage Water Utilization through Forestry*, Central Soil Salinity Research Institute, Karnal, 1989.
15. APHA, *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington DC, USA, 1995, 19th edn.
16. Black, C. A., *Methods for Soil Analysis*, Am. Soc. Agro. Inc., Madison, Wisconsin, USA, 1965.
17. Blaney, H. F. and Criddle, W. D., Determining water requirements in irrigated areas from climatological and irrigation data. US Dept of Agric., Soil Conservation Service Tech. Paper 96, 1950.
18. Juwarkar, A. S., Moghe, M. and Thawale, P. R., Sewage treatment and disposal through high rate transpiration system. National Seminar on Ecotechnological Wastewater Management through Service System of Nature, Puri, 1994.
19. Levin, P. E., Crites, R. W. and Olson, J. V., Soil chemistry changes at rapid infiltration site. *J. Environ. Eng. Div., ASCE*, 1980, **106**, 869–883.
20. Gupta, S. K. and Nema, P., Conservation and renovation of wastewater. National Meet on S&T Issue to Water Resource Management, IIT, New Delhi, 1997, pp. 133–144.
21. Sepp, The use of sewage for irrigation: A literature review. California State Department of Public Health, Bureau of Sanitary Engineering, 1971.
22. Lance, J. C. and Whisler, F. D., Nitrogen balance in soil columns intermittently flooded with secondary effluent. *J. Environ. Qual.*, 1972, **1**, 180–186.
23. Lance, J. C. and Whisler, F. D., Stimulation of denitrification in soil columns by adding organic carbon to wastewater, *J. Water Pollut. Control Fed.*, 1976, **48**, 346–356.
24. Lance, J. C., Whisler, F. D. and Rice, R. C., Maximising denitrification during soil filtration of sewage water. *J. Environ. Qual.*, 1976, **5**, 102–107.
25. Kaur, S. and Singh, M., Soil aquifer treatment system: A case study. *Indian J. Environ. Health*, 2002, **44**, 244–246.
26. Igbounamba, O., Exchangeable reserve potassium and other cation reserves in agodi and ado soils. *Soil Sci.*, 1972, **113**, 394–409.
27. Bose, T. K., Basu, S. and Basu, R. N., Changes in rooting on cutting of Baugainvillea and Hibiscus. *Indian J. Plant Physiol.*, 1973, **16**, 127–139.
28. Patra, S. K. and Behera, S., Effect of paper mill sludge on mineralization of soil nitrogen. *Agra Univ. J. Res.*, 1974, **4**, 111–113.
29. Hansen, E. A., Dawson, H. D. and Tolsted, N. D., Irrigation of intensively cultured plantation with paper mill effluent. *Tappi*, 1980, **63**, 139.
30. Thawale, P. R., Juwarkar, A. S., Kulkarni, A. B. and Juwarkar, A. A., Lysimeter studies for evaluation of changes in soil properties and crop yield using wastewater. *Int. J. Trop. Agric.*, 1999, **17**, 231–244.
31. Hongve, D., Van Hees, P. A. W. and Lundstrom, U. S., Dissolved components in precipitation water percolated through forest litter. *Eur. J. Soil Sci.*, 2000, **51**, 667–677.

Received 29 April 2005; revised accepted 22 November 2005

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. incanum* 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. aetheopicum* group *gilo* and *S. anguri* are used for the treatment of many diseases. Many other *Solanum* species are also used for medicinal purposes[2]. 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 characterrization of the largest genus of *Solanaceae* family[3–7]. Great degree of taxonomic confusion exists as regard to genus *Solanum*[8].

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[9]. Among molecular markers, random amplified polymorphic DNAs (RAPDs) have been extensively used in genetic research owing to their speed and simplicity[10,11]. Most variability/taxonomic affinity studies in eggplant have focused mainly on morphology[12,13], crossability[14], anatomy[15], isozyme[12,16,17] and chloroplast DNA diversity[18–20]. 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 eggplant[13]. In another study AFLP was found to be an excellent tool for the determination of genetic relationship among the species of *Solanum*[21].

India or Indochina is the centre of eggplant diversity[16,22] but the affinities of *S. melongena* to related species are uncertain[7]. Taxonomic uncertainties exist because phylogenetic relationships among taxa have been established considering mainly the morphological features, crossability and F_1 fertility[16,23]. Establishing genetic affinities on such parameters are insufficient, as *S. melongena* makes successful cross with putative progenitors as well as distantly related species[24]. To overcome these problems[12,16], 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 branchings which can be recognized because the molecular sequences on which they are based share a common ancestor. The present study was aimed at analysing 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, grown and maintained at Research Farm of the Indian Institute of Vegetable 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 (denatuartaion), 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[25]. 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[26] 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

Material	Taxon	Code	Source
BB-40	*S. melongena*	BB	Bhubneshwar
KS-331	*S. melongena*	KSA	Kanpur
Uttara	*S. melongena*	UTR	Bangladesh
Faizabadi	*S. melongena*	FZB	Faizabad
BR-112	*S. melongena*	BR	Kanpur
Baramasi	*S. melongena*	VNS	Varanasi
Pant Rituraj	*S. melongena*	RTJ	Pantnagar
CHBR-2	*S. melongena*	CBR-2	Ranchi
DBSR-44	*S. melongena*	DBR-2	Delhi
KS-326	*S. melongena*	KSB	Kanpur
Pusa Purple Long	*S. melongena*	PPL	Delhi
Ramnagar Giant	*S. melongena*	RNZ	Ramnagar
CHBR-1	*S. melongena*	CBR-1	Ranchi
JB-80	*S. melongena*	JB	Jabalpur
Aruna	*S. melongena*	AR	Akola
KT-4	*S. melongena*	KT	Katrain
IC-1114274	*S. incanum*	INCA	NBPGR*
IC-111427	*S. incanum*	INCB	NBPGR
NIC-18360	*S. incanum*	INCC	NBPGR
IC-111311	*S. incanum*	INCD	NBPGR
IC-135912	*S. incanum*	INCE	NBPGR
IC-135913	*S. incanum*	INCF	NBPGR
NL/94-3	*S. viarum*	VRMA	NBPGR
VRV-1	*S. viarum*	VRMB	Assam
V-4532	*S. viarum*	VRMC	NBPGR
IC-135922	*S. viarum*	VRMD	NBPGR
IC-135932	*S. surattense*	SRT	Varanasi
VRN-91-0-196	*S. nigrum*	NGR	Varanasi

*National Bureau of Plant Genetic Resources, India.

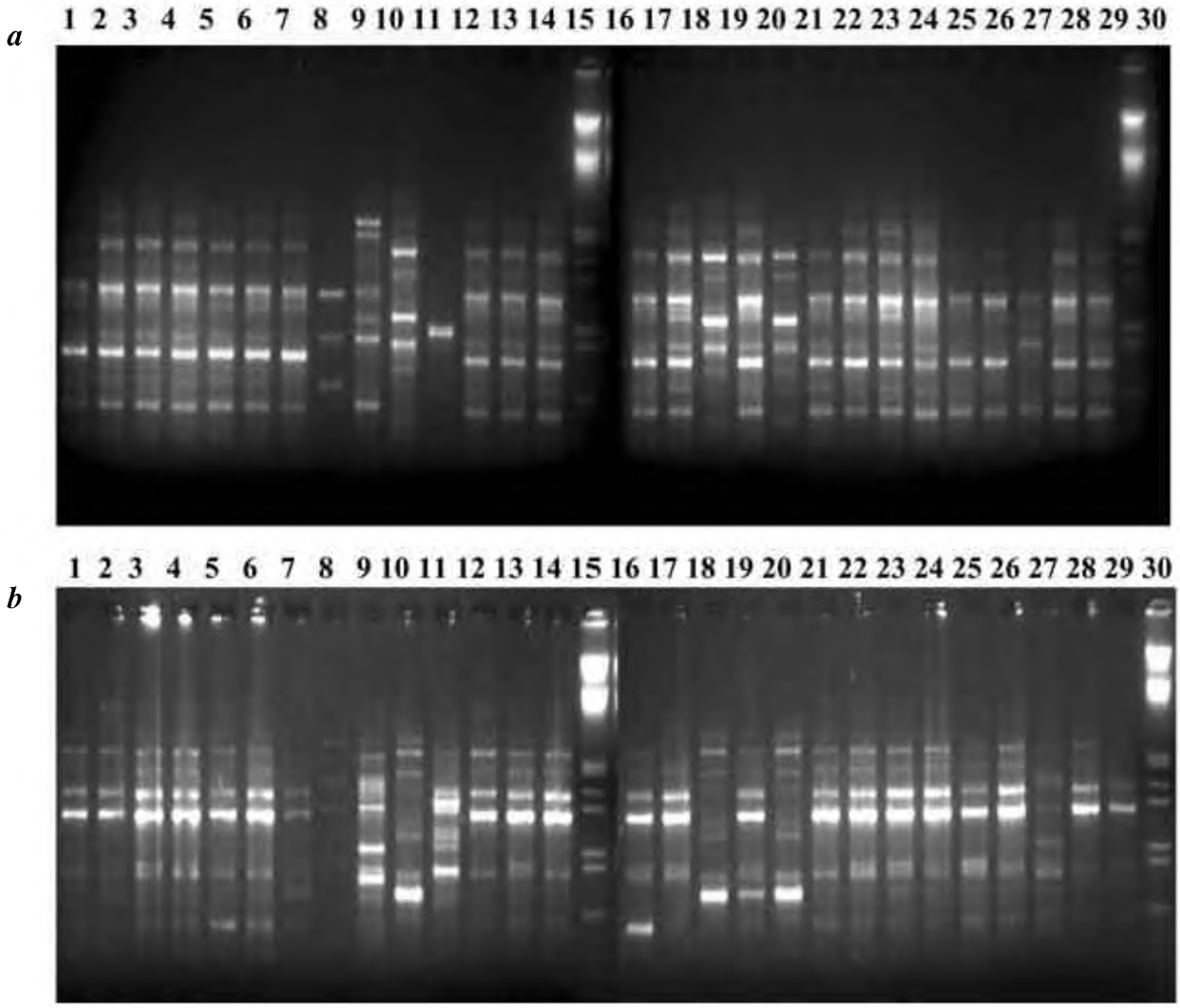

Figure 1. ***a, b.*** RAPD profiles for *Solanum* species generated by primer OPC-09 (***a***) and primer OPC-14 (***b***) Lane 1: BB-40, Lane 2: KS-331, Lane 3: Uttara, Lane 4: Faizabadi, Lane 5: BR-112, Lane 6: Baramasi, Lane 7: Pant Rituraj, Lane 8: CHBR-2, Lane 9: DBSR-44, Lane 10: KS-326, Lane 11: Pusa purple long, Lane 12: Ramnagar Giant, Lane 13: CHBR-1, Lane 14: JB-80, Lane 15: M.W. Marker (1 kb), Lane 16: Aruna, Lane 17: KT-4, Lane 18: IC-1114274, 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-135922, Lane 28: IC-135932, Lane 29: VRN-91-0-196, Lane 30: M.W. Marker (1 kb).

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

Primer	Sequence 5′3′	Number of bands	Mol. wt. range (Kb)
OPA 01	CAGGCCCTTC	9	0.15–3.5
OPA 09	GGGTAACGCC	9	0.13–3.8
OPA 10	GTGATCGCAT	9	0.14–2.1
OPA 16	AGCCAGCGAA	10	0.14–3.8
OPB 12	GTGATCGCAG	11	0.13–3.2
OPB 18	CCACAGCAGT	10	0.12–3.3
OPB 19	ACCCCCGAAG	12	0.13–3.1
OPB 20	GGACCCTTAC	9	0.14–2.5
OPC 05	GATGACCGCC	13	0.13–2.4
OPC 07	GTCCCGACGA	13	0.12–3.3
OPC 09	CTCACCGTCC	9	0.13–2.8
OPC 10	TGTCTGGGTG	8	0.11–2.1
OPC 14	TGCGTGCTTG	12	0.12–4.5
OPC 17	TTCCCCCCAG	10	0.10–4.3

ware. 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, $MgCl_2$, and primer were used to optimize the RAPD reaction. One unit of *Taq* DNA polymerase, 3.0 mM $MgCl_2$ 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

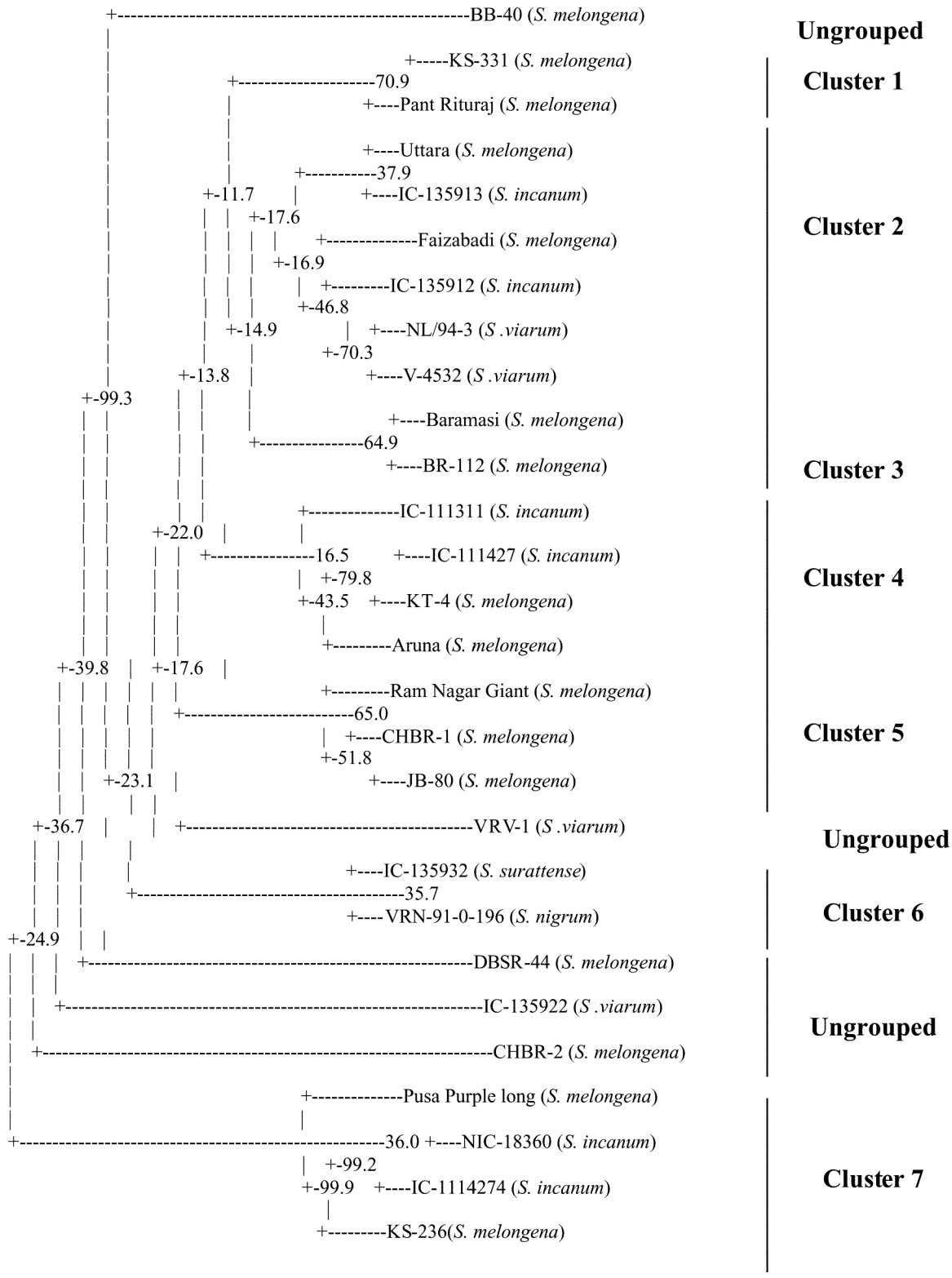

Figure 2. UPGMA cluster analysis-based dendrogram showing genetic relationship among genus *Solanum*.

bands of which 14 were polymorphic, with an average of 10.28 amplicons per primer. Figure 1 *a* 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

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. surattanse* 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[27]. RAPD and other discontinuous markers can serve as a means of genetic distances to establish phylogenetic relationships among taxa[13,28,29]. 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[7] are not in agreement with the earlier workers[13] 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 allonyms 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[21].

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. surattence* 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*[30].

Hybridization examples with eggplant show that *S. melongena* is crossable with several species and to a certain degree, also with other sections[31]. Even when the hybrids are partially or completely sterile, their existence indicates some degree of relationship[7]. 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 *Lucinod orbonalis* (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*[7]. RAPD markers can be applied to other *Solanum* species to assess the genetic relationship among them and assist in the introgression of genes.

1. Daunay, M. C., Lester, R. N., Hennart, J. W. and Duranton, C., Eggplants: present and future. *Capsicum Eggplant Newslett.*, 2000, **19**, 11–18.
2. Bukenya, Z. R. and Carasco, J. F., Ethnobotanicals aspects of *Solanum* L. (Solanaceae) in Uganda. In *Solanaceae IV. Advances in Biology and Utilization* (eds Nei, M. M., Symons, D. E., Lester, R. N. and Jessop, J. P.), Royal Botanic Gardens, Kew, UK, 1999, pp. 345–360.
3. Correll, D. S., The potato and its wild relatives. Contribution of Texas Research Foundation, *Bot. Stud.*, 1962, **S4**, 1–606.
4. Seithe, A. and Anderson, G. J., Hair morphology and the relationships of species in *Solanum* sect. Basarthun. *Plant. Sits. Evol.*, 1982, **139**, 229–256.
5. Whalen, M. D., Conspectus of the species group in *Solanum* subgenus *Leptostemonum*. *Genet. Herb.*, 1984, **12**, 179–282.

6. Bohs, L., Cyphomandra (Solanaceae). *Flora Neotropica Monograph*, 1999, **63**, New York Botanical Garden.
7. Furini, A. and Wunder, J., Analysis of eggplant (*Solanum melongena*) related germplasm: morphological and AFLP data contribute to phylogenetic interpretations and germplasm utilization. *Theor. Appl. Genet.*, 2004, **108**, 197–208.
8. Daunay, M. C. and Lester, R. N., The usefulness of taxonomy for Solanaceae breeders, with special reference to the genus *Solanum* and to *Solanum melongena* L. (eggplant). *Capsicum Newslett.*, 1988, **7**, 70–79.
9. Gepts, P., The use of molecular and biochemical markers in crop evolution studies. *Evol. Biol.*, 1993, **27**, 51–94.
10. Williams, J. G. K A. R., Kubelik, K. J., Livak, J. A., Rafalski and Tingey, S. V., DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 1990, **18**, 6531–6535.
11. Welsh, J. and McClelland, M., Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.*, 1990, **18**, 7213–7218.
12. Deb, D. B., *Solanum melongena* versus *S. incanum* (Solanaceae). *Taxon*, 1989, **38**, 138–139.
13. Karihaloo, J. L. and Gottlieb, L. D., Random amplified polymorphic variation in the eggplant, *Solanum melongena* L. (Solanaceae). *Theor. Appl. Genet.*, 1995, **90**, 767–770.
14. Hasan, S. M. Z. and Lester, R. N., Crosability relationship and *in vitro* germination of F1 hybrids between *S. melongena* L. × *S. panduriforma* E. Meyer (*S. incanum* L. Sensu ampl.). *SABRAO J.*, 1990, **22**, 65–72.
15. Hasan, S. M. Z. and Lester, R. N., Comparative micromorphology of the seed surface of *Solanum melongena* L. (eggplant) and allied species. *Pertanika*, 1990, **13**, 1–8.
16. Lester, R. N. and Hasan, S. M. Z., Origin and domestication of the brinjal eggplant *Solanum melongena* from *S. incanum* in Africa and Asia. In *Solanaceae III. Taxonomy, Chemistry, Evolution* (eds Hawakes, J. G., Lester, R. N., Nee, M. and Estrada, N.), The Royal Botanic Gardens, Kew, London, 1991, pp. 369–388.
17. Isshiki, S., Okubo, H. and Fuziada, K., Phylogeny of eggplant and related *Solanum* species constructed by allozyme variation. *Sci. Hort.*, 1994, **59**, 171–176.
18. Sakata, Y., Nishio, T. and Mathews, P. J., Chloroplast DNA analysis of eggplant (*Solanum melongena*) and related species for their taxonomic affinity. *Euphytica*, 1991, **55**, 21–26.
19. Sakata, Y. and Lester, R. N., Chloroplast DNA diversity in eggplant (*Solanum melongena*) and its related species *S. incanum* and *S. marginatum*. *Euphytica*, 1994, **80**, 1–4.
20. Sakata, Y. and Lester, R. N., Chloroplast DNA diversity in eggplant (*Solanum melongena*) and related species. *Euphytica*, 1997, **97**, 295–301.
21. Mace, E. S., Lester, R. N. and Gebhardt, C. G., AFLP analysis of genetic relationship among the cultivated eggplant. *Solanum melongena*; and its wild relatives (Solanaceae). *Theor. Appl. Genet.*, 1999, **99**, 626–633.
22. Vavilov, N. I., The origin, variation, immunity and breeding of cultivated plants. *Chron. Bot.*, 1951, **13**, 1–364.
23. Baksh, S., Cytogenetic studies of the F1 hybrid of *Solanum incanum* L. × *S. melongena* L. variety Giant of Banaras. *Euphytica*, 1979, **28**, 793–800.
24. Daunay, M. C., Lester, R. N. and Laterrot, H., The use of wild species for the genetic improvement of brinjal (*Solanum melongeana*) and tomato (*Lycopersicon esculentum*). In *Solanaceae III: Taxonomy, Chemistry, Evolution* (eds Hawkes, J. G., Lester, R. N., Nee, M. and Estrader, N.), Royal Botanic Garden, Kew, Richmond, UK, 1991, pp. 380–412.
25. Sambrook, J. E., Fritch, F. and Maniatis, T., *Molecular Cloning – A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, USA, 2nd edn, 1989.
26. Jaccard, P., Nouvelles recherches sur la distribution florale. *Bull. Soc.Vaud. Sci. Nat.*, 1908, **44**, 23–270.
27. Whalen, M. D., Speciation in Solanum, section Androceras. In *The Biology and Taxonomy of the Solanaceae* (eds Hawkes, J. G., Lester, R. N. and Skelding, A. D.), Linnean Society Symposium Series, London Academic Press, 1979, vol. 7, pp. 581–596.
28. Rodriguez, J. M., Berke, T., Engle, L. and Nienhuis, J., Variation among and within *Capsicum* species revealed by RAPD markers. *Theor. Appl. Genet.*, 1999, **99**, 147–156.
29. El, Rabey, H. A., Badr, A., Schafer-Pregl, R. and Martin Salamini, W., Speciation and species separation in *Hordeum* L. (Poaceae) resolved by discontinuous markers. *Plant Biol.*, 2002, **4**, 1–9.
30. Karihaloo, J. L., Brauner, S. and Gottlieb, L. D., Allozyme variation in the eggplant. *Solanum melongena* L. (Solanaceae). *Theor. Appl. Genet.*, 1994, **90**, 578–883.
31. Decondolle, A., *Origin of cultivated plants*, London, 1904.

ACKNOWLEDGEMENTS. We thank the Indian Council of Agricultural Research for financial support. We acknowledge IIVR for providing necessary facilities.

Received 16 August 2005; revised accepted 19 December 2005

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 2030 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 crataevae* Mani, order Diptera.**

Keywords: *Crataeva religiosa*, floral galls, *Neolasioptera crataevae*, 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[1]. It is cultivated in the gardens for its ornamental as well as medicinal value[2]. Despite its usefulness, less

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