Theor Appl Genet (1995) 90:767–770

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Random amplified polymorphic DNA variation in the eggplant, *Solanum melongena* L. (Solanaceae)

Received: 16 April 1994 / Accepted: 22 September 1994

Abstract RAPD analysis was carried out on 52 accessions of *Solanum melongena* (eggplant) and related weedy forms known as "insanum". Twenty-two primers amplified 130 fragments. *Solanum melongena* exhibited 117 of the fragments, all of which were also present in insanum. Insanum displayed an additional 13 fragments not found in *S. melongena*. Overall, the insanum accessions were more diverse than those of *S. melongena*. The calculated similarity between them was 0.947. The RAPD results were closely concordant with the results of an electrophoretic isozyme survey performed on the same accessions. The concordance of the results shows that even though *S. melongena* and insanum are highly diverse morphologically, it is no longer appropriate to distinguish them taxonomically.

Key words *Solanum melongena* · Insanum · RAPD · Interrelationships

Introduction

Enzyme electrophoretic studies that were presented in an earlier paper (Karihaloo and Gottlieb 1995) revealed high genetic identity ($\bar{I} = 0.963$) and low genetic polymorphism between *Solanum melongena* L. (eggplant) cultivars and a diverse group of weedy forms previously designated as *S. insanum* or *S. melongena* var '*insanum*' (Prain). This was initially surprising because both *S. melongena* and "insanum" (tentative nomenclature given to the weedy forms as detailed in the earlier article) are morphologically highly diverse (Martin and Rhodes 1979; personal observation of JLK), and the diversity was well-represented in our materials. Consequently, we examined the relationship between cultivated *S. melongena* and the weedy insanum at the genomic DNA level by the random amplified polymorphic DNA (RAPD) technique (Welsh and McClelland 1990; Williams et al. 1990). Because we were able to analyse a much larger number of 'loci', RAPD analysis was expected to provide a more thorough assessment of the variability of these plants and, more importantly, provide an independent test.

RAPD analysis may not be suitable for general systematic studies primarily because the homology of the amplified fragments is uncertain in the absence of specific tests and because errors in scoring may be unavoidable, especially if poorly amplified fragments are included (Weeden et al. 1992). However, our concern in both the electrophoretic and DNA studies was not, in general, to ascertain if Taxon A is more closely related to Taxon B than to Taxon C. The studies were, instead, aimed at determining if a group of morphologically highly diverse plants from South Asia, the centre of their diversity, which had not been intensively examined and yet have had a variety of taxonomic names applied to them, was actually diverse from a genetic standpoint and represented more than a single species.

Communicated by G. E. Hart

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Materials and methods

Fifty-two accessions, comprising 27 cultivars of *S. melongena* and 25 collections of insanum, were used in the present analysis (Figs. 1,2). These same accessions formed the bulk of the materials of the *S. melongena* complex that had been analysed earlier for allozyme variability (Karihaloo and Gottlieb 1995). The *S. melongena* accessions included nine named cultivars and 18 landraces. The accessions of insanum represented the entire range of morphological diversity available in the previous study.

Genomic DNA was extracted from 2-week-old seedlings using the method of Doyle and Doyle (1987). For RAPD polymerase chain

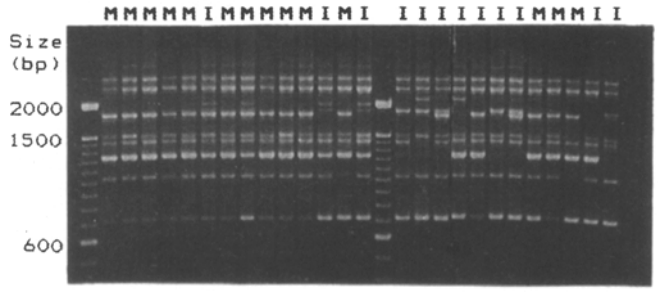

Fig. 1 Genomic DNA fragments of *Solanum melongena* (*M*) and insanum (*I*) accessions amplified with primer CO6. The two unnamed lanes are size markers

reaction (PCR), the DNAs were diluted 1:9 with distilled water. The PCR was initially performed by 32 arbitrary decamer primers (A01–A16 and C01–C16 primers from Operon Technologies, Alameda, Calif.). A detailed survey was made from 22 of these, A02, A04–A07, A09–A11, C01, C02, C04–C16, which yielded a consistent amplification of DNAs resulting in reproducible bands. Amplification was performed in 25 µl of reaction mixture comprising 10 m*M* Tris-HCl (pH 9.0), 50 m*M* KCl, 0.1% Triton-X-100, 3 m*M* $MgCl_2$, 0.2 µ*M* each of dATP, dCTP, dGTP and dTTP, 5 p*M* primer, with approximately 25 ng DNA template and 1 unit *Taq* polymerase. The reaction was incubated in a DNA thermocycler (MJ Research, model PTC-100) programmed for 1 cycle of 4 min at 94 °C; 40 cycles of 1 min at 94 °C, 1 min at 35 °C and 2 min at 72 °C; followed by one cycle of 5 min at 72 °C. The DNA fragments were resolved by electrophoresis in a gel made up of 1.5% agarose in 1 × TBE. Electrophoresis was performed with a 125-V power supply for about 4 h, until the bromophenol blue front had migrated to 11.5 cm. Molecular sizes of the amplification products were estimated by a 100-base pair (100 bp-2.0 kb) ladder (Gibco BRL). Gels were stained in ethidium bromide and photographed with no. 667 black and white Polaroid film. All bands were scored as either present or absent. Polymorphisms at most of the loci were confirmed by repeating tests on some of the relevent accessions. A conservative approach to scoring of the amplified fragments was adopted; all photographs were scored independently by two researchers, and only consensus bands were included for the final analysis.

Data were analysed with a number of computer software packages. An overall comparison between *S. melongena* and insanum was made from the pooled data using the similarity equation followed by Kresovich et al. (1992) $(S = (2N_{ab})/N_a + N_b]$ where N_a is the number of scored fragments of taxon 'a', N_b is the number of scored fragments of taxon 'b' and N_{ab} is the number of shared fragments between 'a' and 'b'. Pairwise comparisons between accessions were made using Jaccard's similarity coefficient. The matrix of similarities was used to construct a dendrogram according to the unweighted paired group method of Sneath and Sokal (1973). In addition, the proportion of fragments that were different in pairwise comparisons of accessions were also calculated, and a UPGM dendrogram generated from these.

Results

The 22 primers amplified 130 fragments in the 52 accessions of *S. melongena* and insanum (Table 1, Fig. 1). The number of fragments per primer varied from 1 to 11, with a mean of 5.9. The *S. melongena* accessions exhibited 117 fragments, all of which were also present in insanum. Insanum had an additional 13 fragments not observed in *S. melongena*. All of these 13 fragments were polymorphic in the sense that each was present or absent in one or more of insanum accessions. Of the 117 fragments present in both *S. melongena* and insanum, 93 were monomorphic in the former and 75 in the latter.

Table 1 Distribution of amplified fragments in *Solanum melongena* and insanum

Total number of fragments	130
Number of fragments in *S. melongena*	117
Number of fragments in insanum	130
Number of fragments shared	117
Number of fragments in insanum only	13
Shared fragments:	
Number of fragments monomorphic	71
Number of fragments monomorphic in *S. melongena* but polymorphic in insanum	22
Number of fragments polymorphic in *S. melongena* but monomorphic in insanum	4
Number of fragments polymorphic	20
Fragments unique to insanum:	
Number of fragments monomorphic	0
Number of fragments polymorphic	13

A pairwise comparison of *S. melongena* cultivars showed that they differed by 0–13 fragments, with an average difference of 3.6. The insanum accessions were more diverse, with a pairwise difference ranging from 3 to 35 fragments, and an average difference of 16.9. The calculated similarity between the two was 0.947.

The UPGM dendrogram generated from the matrix of Jaccard's similarity values is presented in Fig. 2. The 52 accessions were grouped into two clusters, one comprising only 3 insanum accessions and the other including the remaining 22 insanum accessions and all of the 27 *S. melongena* accessions. The latter group exhibited a large number of subclusters, with nearly all *S. melongena* accessions separated from nearly all insanum accessions even though the similarity values were high overall.

The two groups did not separate completely because insanum IC-90061 clustered with *S. melongena* 'Baingan Lamba', and *S. melongena* IC-89823 clustered closely with insanum accessions (Fig. 2). But the differences in similarity values separating these accessions from their larger groups were extremely small. Within the *S. melongena* subcluster, the named cultivars and landraces were well interspersed.

Discussion

The results from the RAPD analysis of *S. melongena* and insanum were closely similar to those obtained by isozyme electrophoretic analysis and described in the earlier article (Karihaloo and Gottlieb 1995). The isozyme analysis showed that *S. melongena* has very little genetic polymorphism, and even that little present is confined to a small number of accessions. Insanum is more variable, and two-thirds of the sampled accessions exhibited polymorphism. The two groups were monomorphic for the same allele at 19 loci and showed the same high frequency alleles at the other loci, giving them a Nei genetic identity of $\bar{I} = 0.963$. Thus, the isozyme

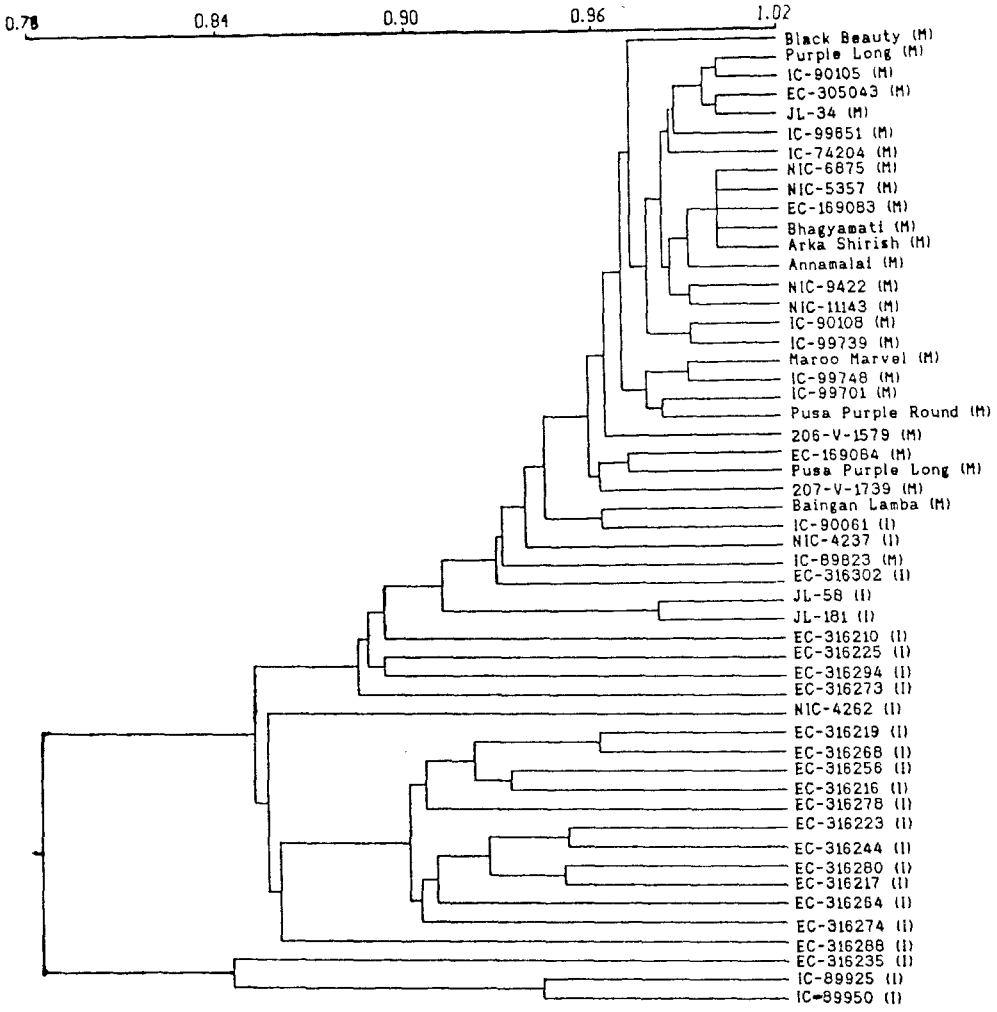

Fig. 2 UPGM dendrogram of *Solanum melongena* (*M*) and insanum (*I*) accessions based on Jaccard's similarity measure of the RAPD data. *Horizontal axis* is Jaccard's similarity value

analysis suggested that even though *S. melongena* and insanum are morphologically highly diverse, from a genetic standpoint (at least at isozyme loci) they are very similar and that there is no evidence to regard them as separate species.

The RAPD results are concordant. *S. melongena* is less variable and every RAPD fragment observed was also present in insanum which, itself, showed only about 10% additional fragments. The overall similarity between them based on RAPD fragments was 0.947, which is of the same high order as their genetic identity calculated from the isozyme data. We believe the concordance between the independent data sets makes possible a much simpler view of the diversity of eggplant and the associated weedy forms and sets the stage for clarifying their taxonomy.

High levels of marker monomorphism, similar to that existing among *S. melongena* cultivars, have been recorded in some of the other crop species analysed for RAPDs. In *Apium graveolens* cultivars, Yang and Quiros (1993) recorded 91.7% bands to be monomorphic. In the wild and cultivated accessions of *Lycopersicon esculentum*, the frequency of monomorphic markers was 62.8% (Williams and St. Clair 1993). A similar low level of RAPD polymorphism was recorded by Halward et al. (1991) in cultivated peanut though among the wild species polymorphism was abundant. *Brassica oleracea* cultivars seem to be an exception in having as many as 117 of the over 140 amplified bands polymorphic (Kresovich et al. 1992).

The UPGM dendrogram (Fig. 2), besides reflecting the above discussed overall pattern of diversity, provides a more critical comparison among the accessions. The majority of *S. melongena* cultivars are of Indian origin, 'Black Beauty' and 'Purple Long' are from the USA, EC-305043 from Bangladesh and EC-169084 from Japan. Except for 2 accessions, all of those investigated grouped into a single subcluster, a reflection of their genetic homogeneity. More interestingly, the named cultivars and the landraces were interspersed with each other. Identical band profiles were seen for 'Purple Long' and IC-90105, and among NIC-6875, NIC-5357, EC-169083, 'Bhagyamati' and 'Arka Shirish'. These results indicate that the named cultivars are very similar if not genetically identical with the landraces. This is consistent with the fact that the former have been bred from local materials, mostly through simple selection.

The greater polymorphism of insanum is evident from the wide range in similarity values obtained be-

tween pairs of its accessions/groups of accessions (Fig. 2). Taxonomists have treated weedy eggplants either under a single taxon (*S. melongena* var '*insanum*' or *S. insanum*) or as distinct groups of separate origins (Roxburgh 1832; Prain 1903; Duthie 1911; Hepper 1987; Lester and Hasan 1991). Lester and Hasan (1991) distinguished Group E (*S. insanum*), comprising suberect, highly prickly forms, from the erect and less prickly types designated as Group F. RAPD data do not support this classification since typical Group E accessions, IC-89925, IC-89950, EC-316274, EC-316288, JL-58 and JL-181, are distributed throughout the insanum section of the dendrogram. The obvious conclusion is that these Group E forms have no greater genetic similarity with each other than with the rest of the insanums. In fact, preliminary morphological observations on 65 insanum accessions from South Asia (personal observations of JLK) have revealed considerable overlapping of characters among the different morphoforms and the consequent impracticability of clearly separating them. It is possible that a more critical morphological evaluation may yield a classification showing greater concordance with the present RAPD clustering.

The existence of a close genetic relationship between *S. melongena* and insanum was emphasised earlier in this article. Figure 2 further reveals that *S. melongena* is not uniformly related with all of the components of insanum. Thus, while on the one hand, the cluster of 3 insanums, EC-316235, IC-89925 and IC-89950, was quite distinct from the *S. melongena* subcluster, cv 'Baingan Lamba' and the weedy accession IC-90061, on the other hand, had greater similarity with each other than with any other accessions of their respective taxa. *S. melongena* accession IC-89823 is interesting because it had greater band conformity with some insanums than with any other cultivar. Morphologically too, *S. melongena* and insanum range from being distinct from each other to almost indistinguishable. Some cultivars, including IC-89823, possess several weedy characters: a thin stem and branches, small leaves and small green fruits. Conversely, some of the weedy forms, due to their sparse or no prickles, moderately-sized leaves and medium- sized coloured fruits, bear considerable similarity to the cultivars. In fact, the only feature that distinctly distinguishes the cultivated and the weedy forms of similar morphologies is the edibility of the fruit, being bitter and inedible in insanum. The present studies clearly show that it is no longer appropriate to distinguish taxonomically the weedy and cultivated eggplants.

Acknowledgements JLK thanks Dr. R. S. Rana, Director, N.B.P.G.R. and Dr. K. P. S. Chandel Jt. Director N.F.P.T.C.R. for their keen interest and encouragement. The major part of this work was carried out at the University of California, Davis under a grant from the Indo-USAID Plant Germplasm project. We are grateful to OICD, Washington and FERRO, New Delhi for their support in executing the project.

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