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AFLP analysis of genetic relationships among the cultivated eggplant, *Solanum melongena* L., and wild relatives (Solanaceae)

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Abstract The AFLP technique of DNA analysis was evaluated as a tool for assessing genetic relationships among the cultivated eggplant, *S. melongena*, and related species [*Solanum* L. subgenus *Leptostemonum* (Dunal) Bitter, section *Melongena* (Mill.) Dunal, series *Incaniformia* Bitter]. Genetic distances based on the AFLP data were estimated for 49 samples of 36 distinct accessions. Phenetic trees were constructed using Jaccard's coefficient and UPGMA, and other clustering methods: they all had very high co-phenetic correlation values, and were found to be consistent with previous trees based on other data types, in particular ITS-1 sequences, isozymes and morphology, carried out on the same accessions. These results indicated that the AFLP technique is both an efficient and effective tool for determining genetic relationships among species of *Solanum*. A new classification is proposed for series *Incaniformia*.

Key words DNA · AFLP · Solanaceae ·*Solanum* · Eggplant · Phenetic analysis

Introduction

Solanum melongena L., the cultivated Brinjal eggplant or aubergine, is well known and easily recognised. However the distinction between its cultivated, and its wild and weedy forms, and between these and *S. incanum* L., is less clear (Deb 1989; Lester and Hasan 1990; Karihaloo and Gottlieb 1995). This has led to the description of an eggplant complex (Pearce and Lester 1979), which is still widely referred to today (e.g. Sakata and Lester 1997; Samuels 1996). The eggplant complex belongs to the tribe Solaneae, of the subfamily Solanoideae, family Solanaceae. Within the eggplant complex, the *Solanum incanum* aggregate (also known as *S. incanum sensu lato)*, is used to encompass some wild relatives of the cultivated eggplant. The *S. incanum* aggregate is a collection of species grouped together by various taxonomists (e.g. Whalen 1984; Jaeger and Hepper 1986; Lester and Hasan 1991) on the basis of their close relationships, and is composed of up to 27 species and 81 taxa (Bitter 1923), which are found mainly in East Africa. It belongs to *Solanum* subgenus *Leptostemonum* (Dunal) Bitter, section *Melongena* (Miller) Dunal, series *Incaniformia* Bitter, subseries *Campylacantha* Bitter and subseries *Euincana* Bitter. The cultivated eggplant itself, and related weedy species, belong to subseries *Melongena* of series *Incaniformia*. All these species are andromonoecious but self-compatible.

The classification of *Solanum* began with Linnaeus who, in his 'Species Plantarum' (1753), described the two species which are the corner stones of the eggplant complex, i.e. *S. incanum* and *S. melongena*. However in later work his concepts of these species changed quite dramatically and this has led to considerable confusion surrounding the exact delimitation of these species. A major cause of Linnaeus' confusion was the high degree of morphological plasticity shown by these species. This high level of variation led Dunal (1852), a Victorian taxonomist who was preoccupied with fine details and minute differences, to treble the number of *Solanum* species described from Africa. Later, Dammer (1915) increased not only the number (to 200) but also the confusion surrounding these species. Subsequently, Bitter (1923) began to unravel this confusion surrounding the African *Solanum* species partly by his use of the concept of species-aggregates, which indicates a close relation-

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Table 1 Informal group classification of *Solanum incanum* and *S. melongena* used by Lester and Hasan (1991), modified here

Wild taxa of *S. incanum sensu lato*, from Africa		
group A	*S. campylacanthum* etc.	eastern & southern Africa
group B	*S. panduriforme*	southern Africa
group C	*S. incanum*	northern Africa, Arabia
group D	*S. lichtensteinii*	southern Africa
Weedy and cultivated taxa of *S. melongena*, from Asia		
group E	*S. melongena* (*S. insanum*)	India
group F	*S. melongena* (*S. cumingii*)	S.E. Asia
group G	*S. melongena* (*S. ovigerum*)	S.E. Asia
group H	*S. melongena* (*S. melongena*)	worldwide

ship between a group of species but does not force premature nomenclatural decisions for that group. In particular, he circumscribed three species-aggregates in his series *Incaniformia*, which encompassed the majority of his 81 taxa of the *S. incanum* aggregate as it is referred to today: namely, *S. campylacanthum* (Hochst.) *sensu ampliore* Bitter, S. *bojeri* (Dunal) *sens. ampl*. Bitter, and *S. incanum* L. *sens. ampl*. Bitter. However, Bitter's work was generally ignored, and most of his taxa were indiscriminately lumped together and treated as one species, *S. incanum*.

The modern recognition of distinct groups of taxa within series *Incaniformia* began with Jaeger (1986), and was later modified by Lester and Hasan (1991) and Samuels (1996). The groupings described by Lester and Hasan (1991; Table 1) have been widely commented on in recent literature, and have been followed by most subsequent authors, e.g. Sakata and Lester (1994) and Samuels (1996). However, other authors, e.g. Karihaloo and Gottlieb (1995), Karihaloo and Rai (1995), and Karihaloo et al. (1995), have criticised the distinction of the four groups (morphoforms) of weedy and cultivated forms of *S. melongena* (groups E–H) as being artificial. Recent work by Samuels (1996) gave further support for the recognition of distinct groups within the species aggregate, and in particular within groups E–H, which led him to recommend recognition at the species level of *S. campylacanthum* Hochst. ex A. Rich. (group A, with group B relegated to *S. campylacanthum* subsp. *panduriforme)*, *S. incanum* L. (group C), *S. lichtensteinii* Willd. (group D), *S. insanum* L. (group E), *S. cumingii* Dunal (group F), and *S. melongena* L. (encompassing groups G and H). A recent paper by Sakata and Lester (1997) based on chloroplast DNA diversity gave further support to some of Samuels' claims, in particular the recognition of *S. lichtensteinii* as a distinct species. However, very little work has been done, so far, to address the problem of the status of the cultivated eggplant, and its relationship with near relatives, by utilizing nuclear DNA diversity. Only one study, to-date, has focused directly on nuclear genomic diversity by undertaking RAPD analysis (Karihaloo et al. 1995). Previous studies to address the problem have focused mainly on morphology (e.g. Deb 1989; Karihaloo and Rai 1995), crossability (Hasan and Lester 1990a), anatomy (Hasan and Lester 1990b), isozymes (e.g. Lester and Hasan 1991; Isshiki et al. 1994; Karihaloo and Gottlieb 1995) and chloroplast DNA diversity (e.g. Sakata et al. 1991; Sakata and Lester 1994, 1997).

The objectives of the present study were: (1) to detect AFLP variation among the cultivated eggplant, *S. melongena*, and related species of the genus *Solanum*; (2) to determine species relationships among the eggplant taxa; and (3) to evaluate the usefulness of AFLPs as systematic characters, with regards to the family Solanaceae. For comparative purposes, the same accessions have been used here as in other companion studies utilising isozymes, ITS (the internal transcribed spacer of nuclear ribosomal DNA) sequences and morphological analyses (Mace et al., unpublished).

Materials and methods

Plant material

A total of 36 accessions of the cultivated eggplant, *S. melongena*, and related species from series *Incaniformia*, together with six accessions from series *Macrocarpa* and *Aculeastrum*, were used in this study. Table 2 lists the sources of the accessions obtained from the genebanks in Birmingham (BIRM), Montfavet (INRA) and Nijmegen (NIJM). The plant material was grown at the Botanical Garden of the University of Nijmegen, The Netherlands, and at INRA, Station d'Amélioration des Plantes Maraichères, Montfavet, France.

DNA isolation

DNA was extracted from 0.4 g of 49 freeze-dried leaf samples of these 36 accessions using the QIAGEN Genomic DNA Purification from Plant Leaves protocol (QIAGEN GmbH, Max-Volmer-Strasse 4, 40724 Hilden, Germany). The DNA was then purified by the QIAGEN Genomic-tip Protocol, using the midi prep volumes.

AFLP analysis

The AFLP protocol was performed essentially as described by Zabeau and Vos (1993) and Vos et al. (1995), with some minor modifications. All primer and adaptor sequences were designed by Keygene (Table 3). DNA (0.5 µg) was digested at 37°C for 1.5 h using 5 U of *Eco*RI (Boehringer Mannheim) or *Hind*III (Boehringer Mannheim), 5 U of *Mse*I (Biolabs), 5 µl of 10 × restriction-ligation buffer (100 mM Tris HCl, 100 mM MgAc, 500 mM KAc) in a final volume of 50 µl. Adaptor ligation was achieved by adding 5 pmol of the *Eco*RI-adaptor or the *Hind*III-adaptor, 50 pmol of the *Mse*I-adaptor, 10 mM of ATP, 1 U of T4-DNA ligase (Biolabs), 1 µl of 10 × restriction-ligation buffer and sterile H_2O to the double-digested DNA sample (60 µl final volume) and incubating for 3.5 h at 37°C. Then, 5 µl of DNA (digested and ligated) was mixed with 75 ng of the *Eco*RI/*Hind*III primer +1 selective nucleotide, 75 ng of the *Mse*I primer +1 selective nucleotide, 2 mM dNTPs (Pharmacia), 5 µl of 10 × PCR buffer (Pharmacia) and 1 U of *Taq* DNA polymerase (Pharmacia), in a final volume of 50 µl. The reaction mixtures were overlaid with one drop of mineral oil (Sigma Chemical Co.) prior to a PCR being run on a MWG thermal cycler, using the following temperature profile: 20 cycles of denaturation at 94°C for 30 s, followed by annealing at 56°C for 60 s, ending with extension at 72°C for 60 s. Only *Eco*RI/*Hind*III primers (i.e. the rare cutters) were labelled. Sufficient primer was prepared for 50 selective amplifications by mixing 4 µl of

Table 2 Accessions of *Solanum* species used for AFLP analysis

Taxon	Code[a]	Donor[b]	Acc. No.[b]	Origin[c]
S. aculeastrum Dunal	acl	INRA	MM 1169	Uganda
S. cerasiferum Dunal	cer	INRA	MM 866	Chad
S. dasyphyllum Thonn.	dsp	INRA	MM 1137	Benin
S. macrocarpon L.	mcr	INRA	MM 132	Réunion Island
S. macrocarpon L.	mcr	INRA	MM 150	Ivory Coast
S. macrocarpon L.	mcr	INRA	MM 1129	Togo
S. marginatum L. f.	mrg	NIJM	88–020	Stuttgart Bot. Gdn.
S. marginatum L. f.	mrg	BIRM	S. 0256	Mexico
S. sessilistellatum Bitter	ses	INRA	MM 1269	Kenya
S. incanum L. group A	A	BIRM	S. 0050	Tanzania
S. incanum L. group A	A	BIRM	S. 2027	Kenya
S. incanum L. group A	A	BIRM	S. 2461	Kenya
S. campylacanthum Hochst. ex A. Rich.	A	INRA	MM 210	Ethiopia
S. incanum ?	A	NIJM	92–118	Uganda
S. incanum L. group B	B	BIRM	S. 1382	Zambia
S. incanum L. group B	B	INRA	MM 668	Zimbabwe
S. panduriforme E. Mey. ex Dunal	B	NIJM	80–190	Pretoria Bot. Gdn.
S. incanum L. group C	C	BIRM	S. 0931	Israel
S. incanum L. group C	C	BIRM	S. 1512	Israel
S. incanum L. group C	C	BIRM	S. 1750	Iran
S. incanum L. group C	C	BIRM	S. 1793	Ethiopia
S. incanum L. group C	C	BIRM	S. 2459	Saudi Arabia
S. incanum L. group D	D	BIRM	S. 1692	S. Africa
S. incanum L. group D	D	BIRM	RNL 337	Zimbabwe
S. lichtensteinii Willd.	D	INRA	MM 674	S. Africa
S. melongena L. group E	E	BIRM	S. 1554	India
S. melongena L. group E	E	BIRM	S. 1992	India
S. melongena L. group E	E	INRA	MM 498	Japan
S. melongena var. *insanum* L.	E	NIJM	93–232	Auroville-India
S. melongena ?	F	BIRM	S. 1355	Indonesia
S. melongena L. group F	F	INRA	MM 686	Indonesia
S. melongena L. group G	G	BIRM	S. 2389	Malaysia
S. incanum	G	NIJM	92–202	Macau Bot. Inst.
S. incanum	G	NIJM	92–105	Havana Bot. Gdn.
S. melongena L. group H cv. Black Beauty	H	BIRM	S. 2458	Fothergills Seeds
S. melongena L. group ?	H	NIJM	88–026	Lyon Bot. Gdn.

[a] The Codes A–H are our identifications into groups A to H as defined in Table 1, and as used in Figures 2 and 3

[b] Accessions were obtained from genebanks in Birmingham (BIRM), Montfavet (INRA) and Nijmegen (NIJM). NIJM accession numbers are abbreviated by replacing '4750', the code for Solanaceae, by '-'

[c] Generally the country of original collection is shown, but for some accessions only the suppliers name or location is known.

δ-[^{33}P]ATP (100 µCi/µl), 2.6 µl of T4 kinase buffer (250 mM Tris HCl, 100 mM MgCl, 50 mM DTT, 5 mM Spermidine, pH 7.5), 5 µl of *Eco*RI/*Hind*III primer +3 selective nucleotides (50 ng/µl stock), 5 U of T4 kinase (Pharmacia) and 11.6 µl of sterile H_2O (final volume 24 µl). Samples were incubated at 37°C for 30 min then heated to 70°C for 10 min. The final selective PCR-amplification was performed with a [^{33}P]-labelled *Eco*RI/*Hind*III primer +3 nucleotides and an unlabelled *Mse*I primer +3 nucleotides. Five microliters of pre-amplified DNA was added to 0.5 µl of labelled *Eco*RI/*Hind*III primer +3 nucleotides, 30 ng of unlabelled *Mse*I primer +3 nucleotides, 2 mM of dNTP, 2 µl of 10 × PCR buffer (Pharmacia), 0.5 U of *Taq* DNA polymerase and 9.8 µl of sterile H_2O. The final PCR reaction was then performed using the following temperature profile: 1 cycle of denaturation at 94°C for 30 s, followed by annealing at 65°C for 30 s, ending with extension at 72°C for 60 s, followed by 12 cycles with the conditions listed above, except with a 0.7°C lower annealing temperature each cycle, and finally 23 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C. The PCR product was mixed with 20 µl of formamide dye (98% de-ionised formamide, 10 mM EDTA pH 8, bromophenol blue 0.05%, xylene cyanol 0.05%, 1 ml sterile H_2O) and denatured by incubation for 5 min at 95°C, and then immediately placed on ice prior to loading. The 5% polyacrylamide gels were prepared by mixing 75 ml of a 5% gel mix (125 ml of acrylamide-bisacrylamide stock solution 40:2), 450 g of urea and 50 ml of 10 × TBE (1 M Tris-HCl, 1 M boric acid, 20 mM EDTA; pH 8.3), de-gassing and adding 50 µl of TEMED (N,N,N',N'-Tetramethylethylenediamine p.A.; Merck no.1.10732.0100) and 750 µl of 10% ammonium persulphate. The gels were poured and left overnight before use and then pre-run at 58 W for 30 min. Shark-tooth combs with 96 wells were used, and samples of 2 µl were loaded per track. Gels were run for 2 h at 58 W constant power, fixed in 10% acetic acid for 30 min, then dried in an oven at 80°C for 1.5–2 h. Gels were exposed to X-ray film, at room temperature, for 2–4 days.

Data analysis

For each accession, a binary matrix reflecting specific AFLP-band presence (1) or absence (0) was generated. Only heavy bands were scored, faint bands were discarded. Estimates of similarity were based on three different measures: (1) Nei and Li's (1979) definition of similarity: $Sij=2a/(2a+b+c)$, where S*ij* is the similarity between two individuals, *i* and *j*, *a* is the number of bands present in both *i* and *j*, *b* is the number of bands present in *i* and absent in *j*, and *c* is the number of bands present in *j* and absent in *i*; this is also known as the Dice coefficient (1945), (2) Jaccard's coefficient (Jaccard 1908): $Sij=a/a+b+c$, and (3) the simple matching (SM) coefficient (Sokal and Michener 1958): $Sij=a+d/a+b+c+d$, where *d* is the number of bands absent from both *i* and *j*. The matrices of similarity were then analysed using various clustering methods, UPGMA (unweighted pairgroup method; Sokal and Michener 1958), WPGMA (weighted pairgroup method; Sneath and Sokal 1973), complete linkage (Lance and Williams 1967) and single linkage (Lance and Williams 1967) using the software NTSYS-pc, version 1.80 (Rohlf 1993). The dendrograms were created with the TREE program of NTSYS, and the goodness of

Table 3 Adaptor, + 1 primer and + 3 primer sequences (5´ - 3´) used for AFLP analysis. A total of 16 EcoRI/MseI + 3 primer combinations and 16 HindIII/MseI + 3 primer combinations were initially tested, of which 3 EcoRI/MseI and 5 HindIII/MseI were used in this study, as listed

Enzyme	Type	Sequence (5´ - 3´)
*Eco*RI	Adaptor (+) [a]	CTCGTAGACTGCGTACC
	Adaptor (-)	CTGACGCATGGTTAA
*Hind*III	Adaptor (+) [a]	CTCGTAGACTGCGTACC
	Adaptor (-)	CATCTGACGCATGGTCGA
*Mse*I	Adaptor (+) [a]	GACGATGAGTCCTGAG
	Adaptor (-)	TACTCAGGACTCAT
*Eco*RI	Primer + 1	AGACTGCGTACCAATTCA
	Primer + 3	GACTGCGTACCAATTCACT
		GACTGCGTACCAATTCAAC
		GACTGCGTACCAATTCACA
*Hind*III	Primer + 1	AGACTGCGTACCAGCTTA
	Primer + 3	GACTGCGTACCAGCTTACC
		GACTGCGTACCAGCTTAAT
*Mse*I	Primer + 1	GACGATGAGTCCTGAGTAAA
		GACGATGAGTCCTGAGTAAC
	Primer + 3	GATGAGTCCTGAGTAAACC
		GATGAGTCCTGAGTAAAGC
		GATGAGTCCTGAGTAAACA
		GATGAGTCCTGAGTAAAAG
		GATGAGTCCTGAGTAACAG
		GATGAGTCCTGAGTAACCA

[a] Plus and minus adaptor strands for each restriction site were synthesized separately, mixed and allowed to anneal to form complete adaptors

fit of the clustering to the data was calculated using the COPH and MXCOMP programs (Rohlf 1993). In addition, a principal coordinate analysis (PCO) was carried out in NTSYS-pc using the DCENTER and EIGEN procedures. Cut-off points were assigned to group the accessions into clusters on all dendrograms produced by selecting an appropriate similarity measure. The cut-off points, and consequently the number of clusters, varied between the taxa and depended on the number of accessions and the level of diversity within each taxon. Therefore, the cut-off point had to be flexible in order to take account of the variations between taxa.

Results

The AFLP primer combinations *Hind*III+ACC and *Mse*I+ACC, *Hind*III+ACC and *Mse*I+AGC, *Hind*III+ACC and *Mse*I+ACA, *Hind*III+ACC and *Mse*I+AAG, *Hind*III+AAT and *Mse*I+ACA, *Eco*RI+ACT and *Mse*I+CAG, *Eco*RI+AAC and *Mse*I+CAG, and *Eco*RI+ACA and *Mse*I+CCA were used to analyse 36 *Solanum* accessions. They yielded 45, 39, 44, 22, 35, 30, 34 and 44 polymorphic AFLPs respectively. An example of the level of polymorphism detectable with the *Hind*III+ACC and *Mse*I+ACC primer combination for the *Solanum* taxa is presented in Fig. 1. The sizes of the AFLP fragments were determined by comparing sequencing ladders of control template DNA to AFLP patterns. AFLP fragment sizes ranged from approximately 50 to 700 base pairs (bp). Polymorphic fragments were distributed across the entire size range with the major proportion being between 150 and 300 bp.

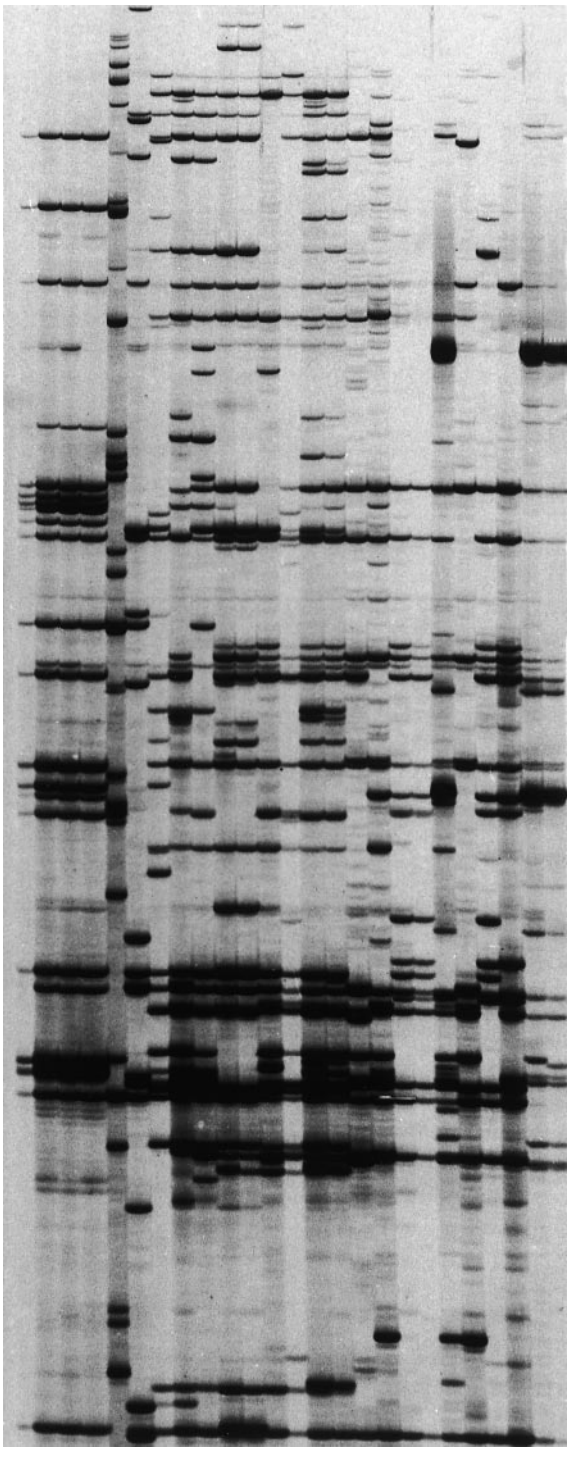

Fig. 1 An example of the AFLP polymorphism amongst *Solanum* samples, detected with the *Hind*III+ACC and *Mse*I+ACC primer combinations

The dendrograms constructed using the three different similarity matrices (Dice, Jaccard's and SM) and various different clustering methods (UPGMA, WPGMA, complete linkage and single linkage) were examined and the co-phenetic correlation values produced by each coefficient compared (Table 4). The UPGMA technique gave

Table 4 Comparison of cophenetic correlation values obtained from the three similarity coefficients and four clustering methods employed for analysing the present AFLP data

	DICE	Jaccard's	SM
UPGMA	0.955	0.965	0.918
WPGMA	0.944	0.956	0.907
Complete linkage	0.933	0.947	0.914
Single linkage	0.928	0.942	0.879

consistently higher co-phenetic correlation scores, where $r > 0.9$ indicates a very good fit; $r = 0.9–0.8$ indicates a good fit; and $r < 0.8$ indicates a poor fit. Jaccard's coefficient of similarity gave consistently higher co-phenetic correlation values than either the Dice or SM coefficients, although they are all very high and Dice had very similar scores to Jaccard's coefficient. This was also found in a recent study by Milbourne et al. (1997). By comparing all the dendrograms produced, and selecting a cut off point of 50% similarity, four main clusters appear, which are almost identical in all analyses. When NTSYS analysis was performed with each primer combination individually, the major clusters found were similar to those with all the primer combinations together (data not shown). However, the internal structure of the clusters did vary somewhat, indicating that each primer combination provided slightly different but complementary information. This was also demonstrated by Tohme et al. (1996). Milbourne et al. (1997) also demonstrated the ability of AFLPs to distinguish individuals based on an individual assay basis.

Figure 2 shows the dendrogram produced by Jaccard's coefficient and the UPGMA clustering method, with four main clusters identified. The 49 samples of 36 accessions are labelled according to the abbreviations listed in Table 2. Cluster 1 consists of four accessions of two taxa; three accessions of *S. macrocarpon* (MM132, MM150 and MM1129) and one accession of *S. dasyphyllum* (MM1137), all of which are placed in series *Macrocarpa* of section *Melongena*. Cluster 2 consists of *S. incanum* group A accessions (S.2461, S.0050, two individuals of MM210, four individuals of S.2027, and 92-118) and *S. incanum* group B accessions (S.1382, 80-190 and two individuals of MM668). Cluster 3 consists of four accessions of *S. incanum* group C (S.2459, S.1793, S.1750 and S.0931) and three accessions of *S. incanum* group D (RNL337, S.1692 and two individuals of MM674). Cluster 4 is a large cluster containing accessions of *S. melongena* group E (MM498, 93-232, S.1554 and S.1992), group F (MM686 and S.1355), group G (two individuals of S.2389 and four each of 92-202 and 92-105) and group H (S.2458 and 88-026), and also an accession of *S. incanum* group C from Israel, S.1512.

Five accessions are not included in the above four clusters at the 50% level in Fig. 2; *S. aculeastrum* (MM1169), *S. sessilistellatum* (MM1269), *S. cerasiferum* (MM866) and *S. marginatum* (S.0256 and 88-020).

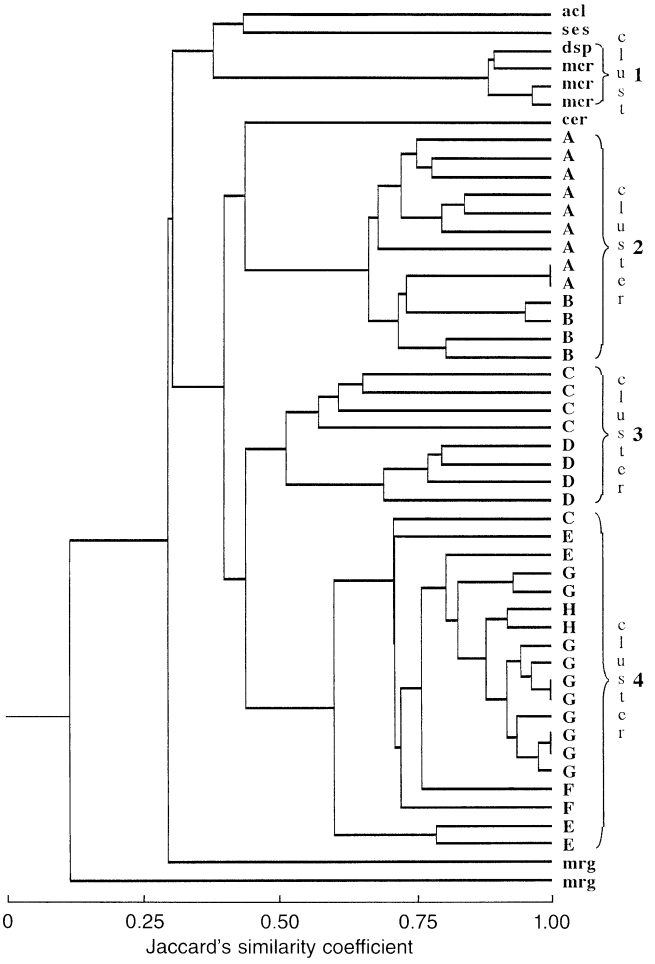

Fig. 2 A dendrogram constructed from the AFLP data, using Jaccard's coefficient of similarity and UPGMA clustering. The samples are labelled with the codes listed in Table 2

The clusterings of these accessions vary slightly between the dendrograms constructed with different similarity coefficients and clustering methods, in particular the placement of *S. cerasiferum* (MM866). In Fig. 2, it groups at a relatively low level of similarity (45% similarity) with cluster 2; however, in the dendrograms constructed with the simple matching coefficient, *S. cerasiferum* is grouped with *S. aculeastrum* (MM1169) and *S. sessilistellatum* (MM1269). This implies that the inclusion of bands absent from both accessions reduces the level of similarity between the *S. cerasiferum* and *S. incanum* accessions, and increases the affinity of *S. cerasiferum* to *S. aculeastrum* and *S. sessilistellatum*. However, as mutual absence does not necessarily imply similarity, the grouping of *S. cerasiferum* in Fig. 2 is probably a more true reflection of the species relationships.

Figure 3 shows a 3-dimensional PCO plot of the eggplant AFLP data; 23.4% of the total variation is represented on the *x* axis, the next 15.1% is represented on the *y* axis, and the next 13.0% on the *z* axis. The accessions plotted are labelled according to their informal grouping

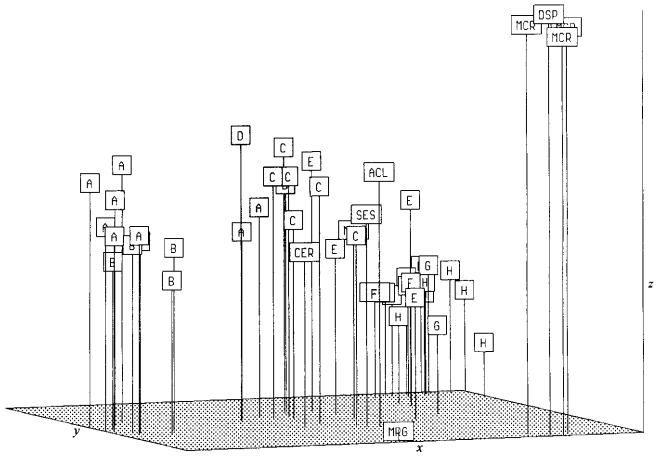

Fig. 3 A 3-D plot of the Principal Coordinates Analysis of the AFLP data. The samples are labelled with the codes listed in Table 2

within series *Incaniformia*; A, B, C, D, E, F, G and H, and species names are abbreviated as indicated in Table 2. It clearly distinguishes the cultivated *S. melongena* taxa (groups H, G and F) from the wild *S. incanum* taxa (groups A, B, C and D). The weedy taxon, *S. melongena* group E, is intermediate between the wild and cultivated taxa. Within the wild taxa, groups A and B are clearly distinct from groups C and D, with two exceptions. *S. cerasiferum*, MM866, is placed very near *S. incanum* group C and D accessions. Interestingly, one accession of *S. marginatum*, 88-020, which clustered with the other eggplant accessions only at a very low level in Fig. 2, is now placed very close to *S. incanum* groups C and D. *S. macrocarpon* and *S. dasyphyllum* are grouped together separately from all the other accessions, supporting their maintenance in a different series, *Macrocarpa*.

Discussion

The AFLP technique has been found to be a useful and robust tool for detecting genetic diversity and determining genetic relationships within and among a group of *Solanum* species. The repeatability of the AFLP banding patterns was very high, providing credibility to the conclusions derived from the analyses. As a new molecular technique with few guidelines on the possible ways in which unexpected results can arise, reproducibility is the only easy way of assessing the quality of the data (Karp et al. 1996). Likewise, the general consensus among the dendrograms produced by the different similarity coefficients and clustering methods proved the robustness of the data set and gave further support to the resulting groups of taxa.

The detailed analysis of the AFLP data from the *Solanum* accessions supports the following conclusions. *S. campylacanthum* (*S. incanum* group A) and *S. panduriforme* (*S. incanum* group B) of subseries *Campylacantha* (Fig. 2 cluster 2, and Fig. 3) have been found to be very distinct from taxa of subseries *Euincana* (Fig. 2 cluster 3, groups C and D). However, although accessions of groups A and B are not intermingled, they are only just separated and are 70% similar, whereas some duplicate samples of a single accession (e.g. S. 2027) are only 80% similar. Thus, although the separation between groups A and B is definite, it is not great, and group B might be assigned subspecies rank under *S. campylacanthum* (group A), as *S. campylacanthum* subsp. *panduriforme* (Samuels 1996), or else it may be maintained as a distinct species, *S. panduriforme* (synonym *S. delagoense*) (Lester 1997). There is a high degree of variation among the accessions in subseries *Campylacantha* and particularly group A, which again is in line with the theory that *S. incanum* group A is a very ancient species: great variation among group A was recently reported by Sakata and Lester (1994), who concluded that this variation was evidence of both the great age of the taxon, and that Bitter's (1923) treatment of this taxon as many species should be taken more seriously.

Within subseries *Euincana* (Fig. 2 cluster 3) two separate clusters are formed. The accessions of group C form a distinct group, supporting the opinion that members of *S. incanum* group C belong to a separate species, *S. incanum* L. *sensu stricto*, as initially suggested by Lester and Hasan (1991) and later supported by Samuels (1996). Also, the even greater distinctness of group D supports Lester and Hasan's (1991) proposal that members of group D should not be treated merely as a variety of *S. incanum* (Bitter 1923) but should be reinstated as a separate species, under the name *S. lichtensteinii*, a view later supported by Samuels (1996) and Sakata and Lester (1997).

It is interesting that all accessions of group C are more divergent in their DNA than is group A from group B (but S.1512 of group C clusters anomalously with *S. melongena*), although morphologically they are more similar. This emphasises that DNA often changes at a different rate from the evolutionary divergence of morphological characters.

Some support is also given for the recognition of taxa of group E as a separate group within the *S. melongena* complex, contradicting Karihaloo and Gottlieb's (1995) findings that *S. insanum* (i.e. group E) was not distinct from the rest of *S. melongena*. The four accessions of *S. melongena* group E were not placed amongst those of groups G and H, but neither were they clustered together with one another. Such grouping indicates a high degree of variation in group E, and is in accord with the theory that it is a weedy relative of *S. melongena* (Lester and Hasan 1991). The dendrogram also indicates that *S. melongena* group F is distinct and deserves recognition as a separate group; however, as only two accessions were available for analysis, it is very difficult to draw any firm conclusions about the status of this taxon. Therefore Samuels' (1996) suggestion that *S. melongena* group F should be recognised as a separate species, *S. cumingii*, can not be evaluated

with confidence. The results also indicate that *S. melongena* groups G and H have a very close genetic relationship and, as such, should not be treated as separate species despite the great morphological diversity in the fruits of these domesticated plants. The results obtained here differ from those obtained by Karihaloo et al. (1995), who used the RAPD technique to study the variation among the cultivated and weedy taxa of *S. melongena*. The RAPD technique was not able to distinguish between the different groups of taxa, here classed as groups E, F, G and H, and they therefore concluded that it was not appropriate to distinguish them taxonomically, even as groups within *S. melongena*. The results from the present AFLP data clearly oppose this view, with the wild and weedy taxa (groups E and F), being distinct from the primitive and advanced cultivars (groups G and H).

S. cerasiferum, also included in series *Incaniformia*, was shown to be relatively closely related to the taxa in subseries *Campylacantha* and *Euincana*. However, the exact relationship of *S. cerasiferum* to the other species in series *Incaniformia* is not well understood. Jaeger (1986) considered that *S. cerasiferum* belonged to the *S. incanum* aggregate, but it was not studied by Lester and Hasan (1991). Samuels (1996) further delimited the position of *S. cerasiferum* in series *Incaniformia* by including *S. cerasiferum* in subseries *Euincana* following Bitter (1923). The results presented here are consistent with this suggestion, as *S. cerasiferum* is shown to be fairly closely related to members of subseries *Euincana*, particularly by the 3D PCO plot (Fig. 3). Hybrids of *S. cerasiferum* with *S. melongena* are as fertile as those of the *S. incanum* groups (Daunay et al. 1998).

The continued inclusion of *S. marginatum* in subseries *Euincana* is not supported. The dendrogram in Fig. 2 indicates quite clearly that the two accessions of this species are very distinct from the other species in section *Melongena*; however, the PCO plot in Fig. 3 places one accession of *S. marginatum* very near the other members of subseries *Euincana* namely, *S. incanum* groups C and D and *S. cerasiferum.* Sakata and Lester (1994) recently questioned the continued inclusion of *S. marginatum* in series *Incaniformia* based on the results from a cpDNA study, and also on the results of previous authors, e.g. Pearce and Lester (1979) studying crossability and Lester and Hasan (1991) using seed-coat anatomy, who found *S. marginatum* to be more distantly related to species of series *Incaniformia* than were some species in the morphologically distinct section *Oliganthes*. Due to taxa from section *Oliganthes* being unavailable for inclusion in this analysis, a firm conclusion cannot be reached regarding the relative relationship of *S. marginatum* to members of sections *Melongena* or *Oliganthes*. However, the results presented here do lend some weight to Sakata and Lester's theory, as both *S. marginatum* accessions included in this analysis are shown in Fig. 2 to be less closely related to members of series *Incaniformia* than are the accessions in series *Macrocarpa* and *Aculeastrum* of section *Melongena*.

Table 5 Proposed classification for *Solanum* series *Incaniformia*, based on AFLP and other data

Series *Incaniformia* Bitter	Lester and Hasan's Groups
Subseries *Campylacantha* Bitter	
S. campylacanthum Hochst. ex A. Rich.	*S. incanum* group A
S. panduriforme E. Meyer ex Dunal	*S. incanum* group B
Subseries *Euincana* Bitter	
S. incanum L. *s. str.*	*S. incanum* group C
S. lichtensteinii Willd.	*S. incanum* group D
S. cerasiferum Dunal	
Subseries *Melongena* Bitter	
S. melongena L. subsp. *insanum* L.	*S. melongena* group E
S. melongena L. subsp. *cumingii* Dunal	*S. melongena* group F
S. melongena L. subsp. *ovigerum* Salis.	*S. melongena* group G
S. melongena L. subsp. *melongena* L.	*S. melongena* group H

The exact placement of the species *S. aculeastrum*, *S. sessilistellatum*, *S. macrocarpon* and *S. dasyphyllum* within section *Melongena* is also uncertain. Both the dendrogram in Fig. 2 and the PCO plot in Fig. 3 support the distinction of *S. macrocarpon* and *S. dasyphyllum*, which belong to series *Macrocarpa* of section *Melongena*, from the species of series *Incaniformia*, and also provide evidence for their close relationship to one another, indicating that they are not distinct species. This close relationship between *S. macrocarpon* and *S. dasyphyllum* is supported by previous findings, e.g. Jaeger (1986) who considered *S. macrocarpon* to be a domesticated modification of the wild plants known as *S. dasyphyllum*. Jaeger assigned the wild form a subspecies status under *S. macrocarpon*, the earlier name. The relationship between *S. macrocarpon* and *S. sessilistellatum*, which is also placed in series *Macrocarpa*, is less clear, and the clustering in Fig. 2 suggests that the continued inclusion of *S. sessilistellatum* in series *Macrocarpa* may not be justified.

In comparison with another recent study of the systematic relationships of *S. melongena* and related species, using the same accessions, which looked at morphological and ITS-1 sequence variation (Mace et al., unpublished), the conclusions derived from both studies were found to be mostly congruent. In particular, the separate specific status of taxa of *S. campylacanthum* (*S. incanum* group A) was supported by both the ITS-1 and morphological results, as was the recognition also of *S. insanum* (*S. melongena* group E) as a separate species. The close genetic relationship between *S. melongena* groups G and H was also reflected in the morphological and ITS-1 analysis by Mace et al. (unpublished) indicating that these taxa should be regarded at the most as subspecies of *S. melongena*. To conclude, the classification for taxa of series *Incaniformia* shown in Table 5, and compared to Lester and Hasan's (1991) informal groups, is supported by the results presented here from the AFLP analyses.

This research represents one of the most comprehensive studies of DNA diversity for the eggplants and is among the first (cf. Kardolus et al. 1998) to report on the effectiveness of the AFLP technique for determining ge-

netic relationships in the Solanaceae. AFLP analysis has been shown to be quick, robust and effective, and requires only minimal preliminary work to detect a large number of genetic loci, which far exceeds that possible in the same amount of time and at the same cost by using other techniques.

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