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Genetic diversity and relationships of sweetpotato and its wild relatives in *Ipomoea* series *Batatas* (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA

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Abstract Genetic diversity and relationships of 40 accessions of *Ipomoea*, representing ten species of series *Batatas*, were examined using ISSR markers and restriction-site variation in four non-coding regions of chloroplast DNA. A total of 2071 ISSR fragments were generated with 15 primers in these accessions and, on average, 52 bands per accession were amplified. Most of the primers contained dinucleotide repeats. The ISSR fragments were highly polymorphic (62.2%) among the 40 accessions studied. Restriction analysis of chloroplast (cp) DNA revealed 47 informative restriction-site and length mutations. Phylogenetic analyses of ISSR and cpDNA datasets generally revealed similar relationships at the interspecific level, but the high polymorphism of ISSRs resulted in a better separation of intraspecific accessions. However, the combined ISSR and cpDNA dataset appeared to be appropriate in resolving both intra- and interspecific relationships. Of the species examined, *I. trifida* was found to be the most closely related to cultivated sweetpotato, the hexaploid *I. batatas*, while *I. ramosissima* and *I. umbraticola* were the most distantly related to *I. batatas* within the series. *Ipomoea triloba*, hitherto considered to be one of the ancestors of sweetpotato, was only distantly related to sweetpotato based on ISSR similarity index.

Key words Chloroplast DNA · Genetic diversity · *Ipomoea batatas* · ISSR · RFLP · Sweetpotato

Introduction

Sweetpotato [*I. batatas* (L.) Lam.] is an important food crop grown in more than one hundred countries, with an annual production worldwide exceeding 1.3×10^8 million tons (Food and Agriculture Organization 1990). For further improvement of sweetpotato, other *Ipomoea* spp. may play an important role in providing new genes, such as those for resistance to various diseases and insects. Many wild *Ipomoea* species possess agriculturally desirable traits. For example, resistances to sweetpotato weevil (*Cylas* spp.), scab [*Elsinoe batatas* (Saw.), Viegas and Jenkins], and black rot (*Ceratocystis fimbriata* Ell. et Halst.) have been found in *I. trifida* and *I. littoralis* (Iwanaga 1988). A better knowledge of genetic diversity and relationships between sweetpotato and its wild relatives would aid in the development of breeding programs that efficiently utilize wild *Ipomoea* germplasm.

Ipomoea series *Batatas* contains sweetpotato and 13–14 other taxa (Austin 1978, 1988, 1991, 1997; Austin et al. 1993; MacDonald and Austin 1990). The phylogenetic relationships of the species in the series have previously been studied based on morphological characters (Austin 1988). However, phylogenetic reconstruction based on morphological characters alone is difficult due to morphological similarity, hybridization, and phenotypic plasticity of the species. Recent investigations of phylogenetic relationships of sweetpotato and other species in ser. *Batatas* have used restriction fragment length polymorphisms (RFLPs) of genomic DNA (Jarret et al. 1992) and random amplified polymorphic DNAs (RAPDs) (Jarret and Austin 1994). Conventional RFLP analysis is time-consuming and relatively expensive and requires considerable technical expertise. Although RAPDs have been widely used in the study of germplasm genetic diversity, problems of reliability and reproducibility have been noted (e.g., Ellsworth et al. 1993). So far, only limited success has been achieved in resolving the relationships of sweetpotato with the wild species in the series, and some inconsistencies still exist.

In this study, we used 40 accessions of *Ipomoea*, representing ten species of the series *Batatas*, to examine genetic diversity and relationships of the cultivated sweetpotato and its wild relatives using two polymerase chain reaction (PCR)-based marker systems: inter-simple

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sequence repeat (ISSR) and chloroplast (cp) DNA PCR-RFLP. ISSR is a new source of genetic markers that overcomes many of the technical limitations of RFLP and RAPD analyses (Tsumura et al. 1996), and ISSR markers have higher reproducibility than RAPDs (Meyer et al. 1993; Fang and Roose 1997). ISSR markers involve the PCR amplification of DNA using single primers composed of microsatellite sequences. These primers target microsatellites that are abundant throughout the eukaryotic genome (Tautz and Renz 1984; Kijas et al. 1995) and evolve rapidly (Levinson and Gutman 1987). ISSR analysis has been used to assess genetic diversity in dent corn and popcorn (Kantety et al. 1995) as well as to identify cultivars of chrysanthemum (Wolff et al. 1995), oilseed rape (Charters et al. 1996) and citrus (Fang and Roose 1997). However, ISSR markers have rarely been used to study relationships at the interspecific level, likely due to concerns over the non-homology of some co-migrating bands typical of fast-evolving DNA markers, as shown for RAPDs (Rieseberg 1996). Comparative studies using ISSR and a more conservative marker system, such as RFLPs of cpDNA, are needed to test the suitability of ISSRs as markers for studying relationships at both intra- and interspecific levels. CpDNA has been frequently utilized for phylogenetic analysis in plants because many cpDNA regions appear to evolve at rates appropriate for resolving relationships at the interspecific level and higher (Palmer et al. 1988; Liston 1992; Petersen and Doebley 1993). Almost all these regions are non-coding and interspersed in the chloroplast genome, and universal primers can be designed in the flanking coding regions. Sixteen pairs of cpDNA primers, which are homologous to the most conserved coding regions of cpDNA but amplify the more variable non-coding regions, have been designed and tested on a broad taxonomic range (Demesure et al. 1995; Dumolin-Lapegue et al. 1997). Similarly, universal primers have also been designed for amplifying non-coding regions of plant mitochondrial (mt) DNA (Demesure et al. 1995; Dumolin-Lapegue et al. 1997).

Materials and methods

Plant materials

Seeds of 35 accessions representing nine wild species of *Ipomoea* ser. *Batatas* and in vitro plantlets of five accessions of cultivated *I. batatas* were obtained from the International Potato Center (Lima, Peru) for this study (Table 1). Pooling of DNA from five individuals within accessions has been found in lupin germplasm to be the most appropriate strategy for assessing large quantities of plant material using ISSR-PCR (Gilbert et al. 1999). In our study, about ten seeds of each accession were germinated. A pre-screening was conducted to compare the ISSR banding patterns among individuals within accessions. We found that the bulk leaf sample of five individuals was adequate for the DNA extraction to represent each accession of the nine wild species. Similar to the findings by Gilbert et al. (1999), band profiles generated from DNA pools containing five individuals were fully representative of all constituent individuals used in the mix. All plant material in each tissue culture tube was used for DNA extraction to represent each of the five accessions of sweetpotato.

Table 1 Species and accessions of *Ipomoea* ser. *Batatas* studied. Accession codes of 1–40 were assigned for the present study

Code	Species	Accession	Origin
1	*I. cynanchifolia*	DPw 2554	Brazil
2	*I. leucantha*	DLP 3354	Argentina
3		DLP 3004	Colombia
4		DLP 431	Ecuador
5		DLP 2931	Mexico
6		DLP 521	Peru
7	*I. ramosissima*	DLP 2760	Bolivia
8		DLP 3010	Colombia
9		DLP 1173	Ecuador
10		DLP 4679	Nicosia
11		DLP 2814	Peru
12	*I. triloba*	DLP 3003	Colombia
13		DLP 2982	Dominica
14		DLP 2943	Mexico
15		DLP 2429	Peru
16		DLP 4161	Paraguay
17		DLP 751	Venezuela
18	*I. umbraticola*	DLP 2941	Mexico
19		DLP 4604	Nicaragua
20	*I. tiliacea*	DLP 2917	Mexico
21		DLP 4638	Nicaragua
22	*I. cordatotriloba*	DLP 4148	Argentina
23		DLP 2762	Bolivia
24		DLP 3001	Colombia
25		DLP 2936	Mexico
26		DLP 3617	Paraguay
27	*I. grandifolia*	DLP 4039	Argentina
28		DPw 2611	Brazil
29		DLP 4169	Paraguay
30		Vilaro 5	Uruguay
31	*I. trifida*	DLP 1084	Colombia
32		DLP 3685	Guatemala
33		DLP 2961	Mexico
34		DLP 4607	Nicaragua
35		DLP 714	Venezuela
36	*I. batatas*	Kyudei No. 63	Japan
37		Kinang Kong	Philippines
38		CN 1108-13	Taiwan
39		Torreblanca	Peru
40		Vsp	Philippines

DNA isolation

DNA was extracted from the bulk fresh young leaves of five individuals or from the plantlets in each tissue culture tube for each of the 40 accessions for the ISSR and cpDNA PCR-RFLP assay. The DNA extraction procedure was a modification of the protocol of Stewart and Via (1993). Because of the high amounts of polysaccharides and proteins present in *Ipomoea* species, we repeated the steps from adding CTAB to chloroform extraction after RNAse digestion. Our modified DNA extraction procedure yielded high-quality DNA with absorbance ratios (A_{260}/A_{280}) ranging from 1.82 to 1.90. This is because CTAB removed the polysaccharides in the two separate steps, and the two-step chloroform extraction removed the proteins (including added RNAse). Sample DNA was quantified by means of fluorometric analysis.

PCR-amplification of ISSRs

One hundred SSR primers from the Biotechnology Laboratory, University of British Columbia (UBC Primer set no. 9) were tested for PCR amplification. We initially examined the optimal conditions for detecting ISSRs for the five accessions of sweetpotato. The effects of the concentration of the primer, DNA template, and Mg^{2+} and the influence of annealing temperature on the reproduc-

Table 2 Nucleotide sequences of the 15 SSR primers and universal primers for cpDNA and mtDNA used in this study, and the annealing temperature used for PCR reaction

Primer	Sequence (5´–3´)[a]	Annealing temperature (°C)
ISSR primer[b]		
807	AGAGAGAGAGAGAGAGT	50.5
811	GAGAGAGAGAGAGAGAC	53
817	CACACACACACACACAA	51.5
819	GTGTGTGTGTGTGTGTA	52.5
825	ACACACACACACACACT	52.5
835	AGAGAGAGAGAGAGAGYC	53.5
841	GAGAGAGAGAGAGAGAYC	51.5
850	GTGTGTGTGTGTGTGTYC	55
857	ACACACACACACACACYG	55
861	ACCACCACCACCACCACC	51.5
864	ATGATGATGATGATGATG	52
878	GGATGGATGGATGGAT	50.5
888	BDBCACACACACACACA	52
890	VHVGTGTGTGTGTGTGT	50
891	HVHTGTGTGTGTGTGTG	51.5
Universal primer pair for cpDNA		
1 *trnC-*	CCAGTTCAAATCTGGGTGTC	58
-trnD	GGGATTGTAGTTCAATTGGT	
2 *trnM-*	TGCTTTCATACGGCGGGAGT	58
-rbcL	GCTTTAGTCTCTGTTTGTGG	
3 *trnD-*	ACCAATTGAACTACAATCCC	53
-trnT	CTACCACTGAGTTAAAAGGG	
4 *trnS-*	GAGAGAGAGGGATTCGAACC	53
-trnfM	CATAACCTTGAGGTCACGGG	
Universal primer pair for mtDNA		
1 *nadl 2-*	GCATTACGATCTGCAGCTCA	55
-nadl 3r	GGAGCTCGATTAGTTTCTGC	
2 *nad4 1-*	CAGTGGGTTGGTCTGGTATG	55
-nad4 2r	TCATATGGGCTACTGAGGAG	
3 *nad4 2c-*	CTCCTCAGTAGCCCATATGA	55
-nad4 3r	AACCAGTCCATGACTTAACA	
4 *nad5 1-*	TTTTTTCGGACGTTTTCTAG	55
-nad5 2r	TTTGGCCAAGTATCCTACAA	
5 *nad7 2-*	GCTTTACCTTATTCTGATCG	55
-nad7 3r	TGTTCTTGGGCCATCATAGA	
6 *nad7 3-*	TCTATGATGGCCCAAGAACA	55
-nad7 4r	ACACCAAATTCTCCTTTAGG	

[a] Y: pyrimidine; B: C, G, or T; D: A, G, or T; H: A, C, or T; V: A, C, or G
[b] Primer number follows that in UBC Set 9 (no. 801–900)

ibility of banding patterns were examined. Although all these parameters affected the banding patterns, only primer concentration and annealing temperature had a large influence. In standard reaction conditions, the banding patterns of SSR-primed PCR were highly reproducible, likely due to the high stringency of annealing.

The PCR amplification conditions adopted were similar to those of Nagaoka and Ogihara (1997) except for the annealing temperature. We found that appropriate annealing temperatures could reduce the background or smear of the PCR products. When the annealing temperature for each primer was adjusted by an increase or decrease of 1–3°C from a standard 52°C (Table 2), good results were obtained. Formamide also reduced background and smear through influencing primer-template annealing or melting temperature. Of the 100 primers 15 that gave strong, reproducible bands were finally selected for use in this study (see Table 2 for primer sequences). Amplifications were carried out in 1.5 m*M* $MgCl_2$, 2% formamide, 200 n*M* primer, 1 U of *Taq* polymerase, and 10 ng of genomic DNA per 20-µl reaction. PCR amplification was performed using the following cycle profile: 1 cycle at 94°C for 5 min followed by 45 cycles at 94°C for 45 s, 50–55°C (depending on primers used) for 45 s, 72°C for 1.5 min; and a final 7-min extension at 72°C. The amplified products were electrophoresed on 1.5% agarose gels and detected by staining with ethidium bromide.

PCR-amplification of cpDNA and mtDNA non-coding regions

Four sets of chloroplast primers and six sets of mitochondrial primers were used to amplify non-coding regions of cpDNA and mtDNA (Demesure et al. 1995). PCR reactions were performed in a PCT-100™ thermocycler (MJ Research) with a 5-min denaturation at 94°C; 35 cycles of 1 min at 94°C; a 1 min annealing at 58°C (for the *trnC-trnD* and *trnM-rbcL* regions), 53°C (for the *trnD-trnT* and *trnS-trnfM* regions), or 55°C (for mtDNA primers); and a 2-min extension at 72°C. The success of each PCR reaction was verified by electrophoresing 2 µl of the reaction products with 1% agarose gel in 0.5×TRIS-borate (TBE) buffer and staining the gel with ethidium bromide. The sizes of the amplified fragments were determined by comparison with known fragment sizes of DNA marker. The PCR-amplified DNA fragments were digested with the restriction endonucleases: *Alu*I, *Cfo*I, *Dde*I, *Hae*III, *Hinf*I, *Mbo*I, *Msp*I, *Rsa*I, *Taq*I for the four cpDNA regions and one mtDNA region, and *Eco*RI, *Kpn*I, *Sty*I for the two regions, *trnC-trnD* and *trnM-rbcL*. The restriction digests were visualized on 2–3% agarose gels stained with ethidium bromide.

Data analysis

For ISSR analysis, the banding patterns were recorded using a gel documentation system (Bio-Rad Gel Doc 1000). The fragment size scored ranged from 300 to 1500 bp. Weak bands were excluded from final data analysis. Bands with the same molecular weight and mobility were treated as identical fragments. In the data matrices, the presence of a band was coded as 1, and its absence was coded as 0. Amplification failure of a sample or missing data was coded as 9. The data matrices were analyzed by the SIMQUAL program of NTSYS-pc, version 1.8 (Rohlf 1994), and genetic similarities between accessions were estimated using the Nei and Li's (1979) band sharing coefficient (or similarity index), estimated as $S=2N_{XY}/(N_X+N_Y)$, where N_X and N_Y are the numbers of DNA fragments observed in accession X and Y, respectively, and N_{XY} is the number of fragments shared by both accessions. Phylogenetic analysis was performed using PAUP* 4.0 (beta version 4.0b2; Swofford 1999), using heuristic searches with 100 random additions of characters and TBR branch-swapping options. Bootstrap values were obtained based on 100 replications in each analysis. Phylogenetic trees were produced from the ISSR data matrices using the unweighted pair group method with arithmetic average (UPGMA) or parsimony method.

For cpDNA PCR-RFLP data analysis, the presence or absence of a restriction site was recorded. In addition, the restriction fragments of cpDNA were recorded in the same fashion as ISSR bands, i.e., the presence of a fragment was coded as 1, and its absence was coded as 0, in order to avoid errors in ascertaining the presence or absence of a restriction site or in distinguishing length polymorphism from restriction site polymorphism. To facilitate comparison between the two marker systems, we analyzed the cpDNA-RFLP data matrices using the same computer programs as described above for ISSR data analysis.

Results

ISSR diversity

Of the 15 SSR primers used, 11 were designed to anneal to dinucleotide repeats, 3 were designed to anneal to trinucleotide repeats, and 1 was designed to anneal to tetranucleotide repeats. Zietkiewicz et al. (1994) noted that

Table 3 Intraspecific ISSR polymorphism and the average similarity index (Nei and Li's coefficient) between intraspecific accessions of *Ipomoea* ser. *Batatas* species

Species[a]	Number of accessions	Number of ISSR fragments		Percentage polymorphism	Similarity index (%)
		Total	Polymorphic		
I. batatas	5	246	137	55.7	65.8
I. cordatotriloba	5	270	167	61.9	61.5
I. cynanchifolia	1	44	–	–	–
I. grandifolia	4	238	156	65.5	61.5
I. leucantha	5	276	189	68.5	59.8
I. ramosissima	5	268	204	76.1	59.0
I. tiliacea	2	96	53	55.2	43.0
I. trifida	5	237	185	78.1	52.5
I. triloba	6	321	226	70.4	66.0
I. umbraticola	2	107	30	28.0	72.2

[a] Only 1 accession of *Ipomoea cynanchifolia* was available for this study; the between-accession polymorphism and similarity index could not be computed

Table 4 Interspecific similarity index (Nei and Li's coefficient) between *Ipomoea* ser. *Batatas* species based on ISSR data

Species	*I. leucantha*	*I. ramosissima*	*I. triloba*	*I. umbraticola*	*I. tiliacea*	*I. cordatotriloba*	*I. grandifolia*	*I. trifida*	*I. batatas*
I. cynanchifolia	53.5	25.7	53.7	37.8	46.0	43.1	37.6	26.6	25.1
I. leucantha		33.4	53.7	34.9	45.3	49.3	47.8	33.6	33.4
I. ramosissima			36.6	27.0	30.9	31.5	33.8	30.8	32.1
I. triloba				35.8	48.6	49.2	44.1	28.4	27.6
I. umbraticola					33.2	37.3	35.6	30.3	31.4
I. tiliacea						42.9	41.6	35.0	33.7
I. cordatotriloba							54.3	39.1	35.0
I. grandifolia								38.3	33.7
I. trifida									46.2
I. batatas									-

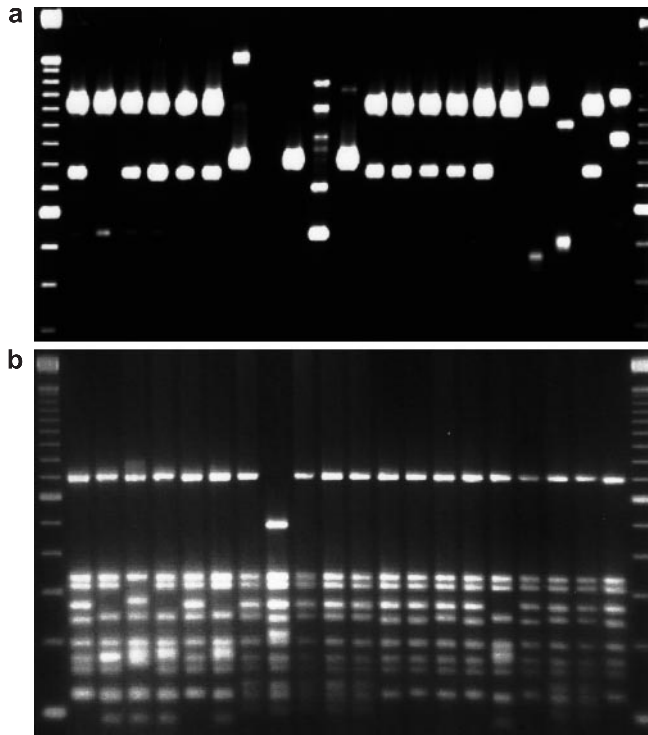

Fig. 1. a Photograph of ethidium bromide stained gel of ISSR fragments using primer UBC-825. DNA samples from left to right: *lane 1* DNA marker (100 bp), *lanes* 2–22 corresponding to the first 21 accessions of *Ipomoea* series *Batatas* as listed in Table 1, *lane 23* DNA marker. **b** *Taq*I restriction fragments of the *trnC–trnD* region of cpDNA. *Lane 1* DNA marker (100 bp), *lanes* 2–21 correspond to the first 20 accessions of *Ipomoea* series *Batatas* as listed in Table 1, *lane 22* DNA marker

primers anchored at the 5' end displayed broader specificity than those anchored at the 3' end. Fang and Roose (1997) found that in *Citrus*, 5'-anchored primers generally produced clearer patterns but fewer and larger fragments than 3'-anchored primers. However, 11 out of 15 primers used in this study were anchored at the 3' end, and they produced as clear patterns as those primers anchored at the 5' end.

ISSR analysis using the 15 primers generated a total of 2071 DNA bands in the 40 accessions of *Ipomoea* surveyed. On average, 52 bands per accession and 207 bands per species were amplified with 15 primers, ranging from 2–5 bands per accession per primer. Each of the 15 primers used detected numerous polymorphisms among accessions (Fig. 1a). At the intraspecific level, ISSR polymorphism was measured as the proportion of polymorphic loci to the total number of loci scored in all accessions of the same species (Table 3). Of the ten species studied, *I. umbraticola* had the lowest level of ISSR polymorphism (28.0%), while *I. trifida* had the highest level of ISSR polymorphism (78.1%). In all species with 2 or more accessions surveyed, intraspecific accessions exhibited much higher levels of genetic similarity than interspecific accessions (Tables 3, 4). With some exceptions, most of the intraspecific accessions sampled for each species were clustered together (Fig. 2). Among the nine species, *I. trifida* was found to be the most closely related to sweetpotato, especially those accessions of *I. trifida* from Guatemala (*32*), Mexico (*33*) and Nicaragua (*34*), and *I. ramosissima* was the most distantly related to *I. batatas*. However, based on pairwise interspecific ISSR similarity index, *I. cynanchifolia* and *I. triloba*

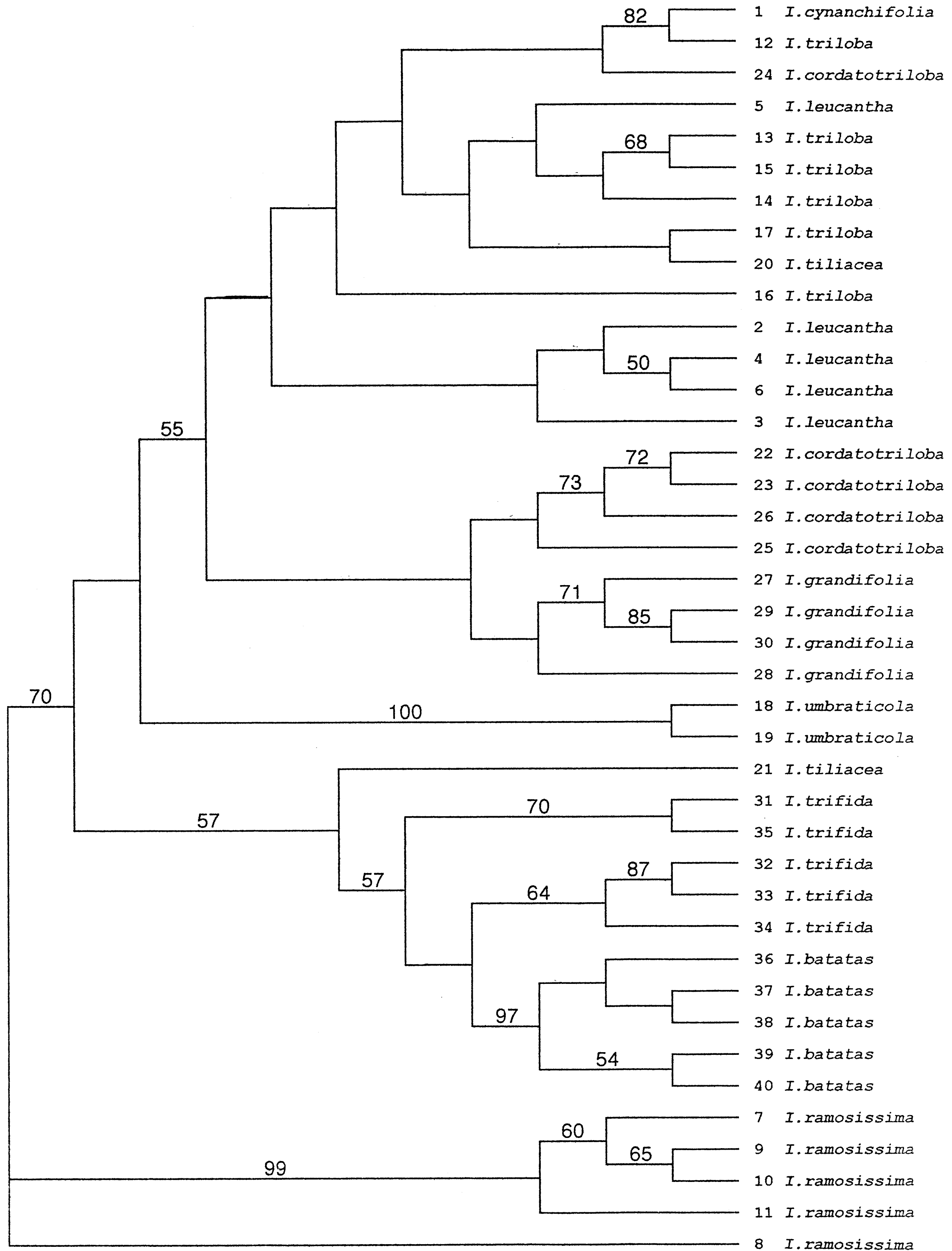

Fig. 2 UPGMA tree for 40 accessions representing ten species of *Ipomoea* ser. *Batatas* based on ISSR dataset. *Number* preceding each species name represents the accession code as in Table 1. Bootstrap frequencies are given *above branches* (bootstrap frequencies below 50% are not given)

Table 5 Summary of cpDNA amplification and restriction analysis of 40 accessions of *Ipomoea* ser. *Batatas*

Primer pair	Fragment amplified (bp)	Number of restriction sites (4-base enzymes)	Number of restriction sites (6-base enzymes)	Sequence length surveyed (bp)	Site and length polymorphism
trnC–trnD	2800 or 3000	37	14	232	32
trnM–rbcL	2600	30	17	222	0
trnD–trnT	1200	15	0	60	5
trnS–trnM	1300	17	0	68	10
Total	7900–8100	99	31	582	47

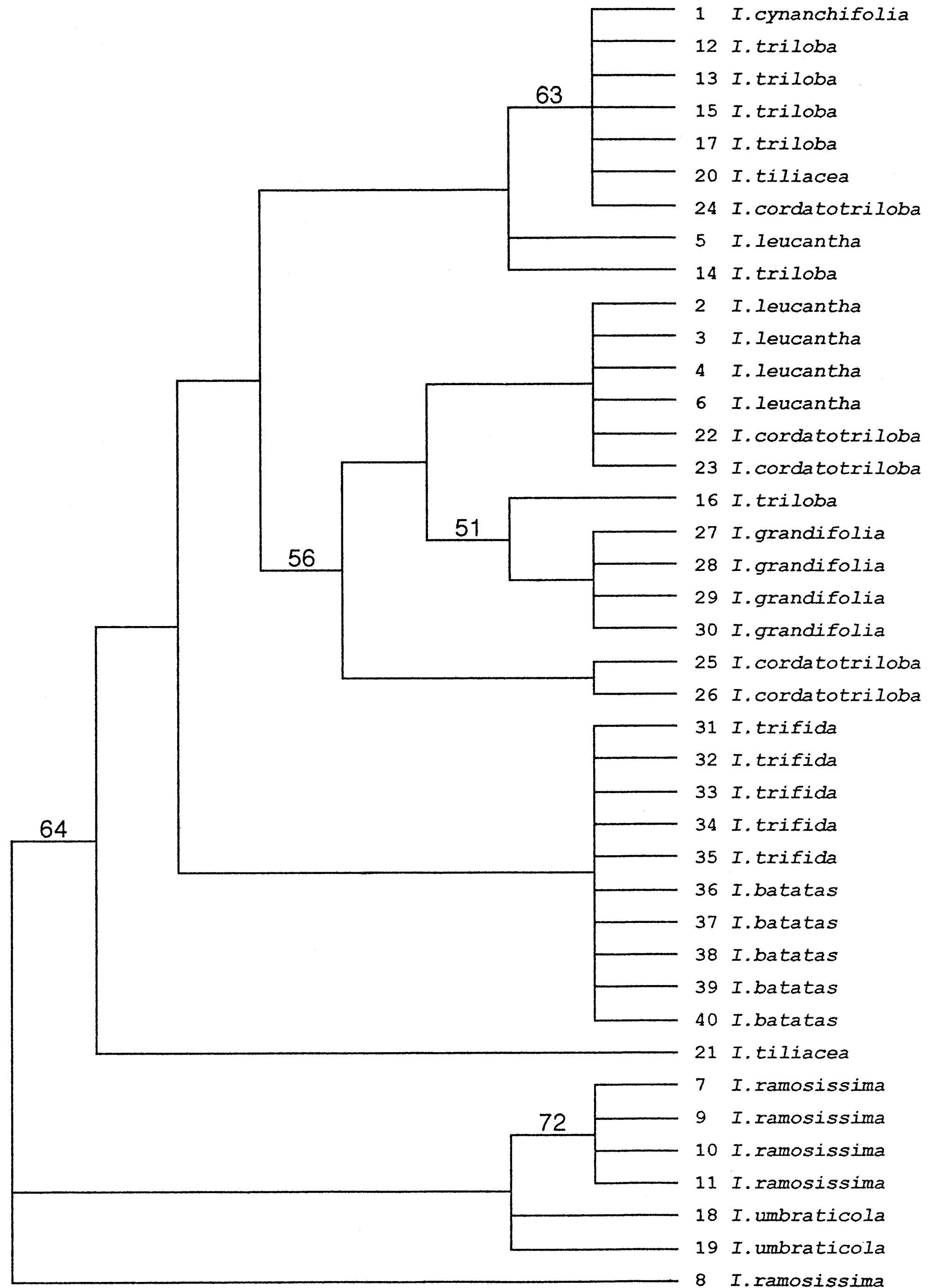

Fig. 3 Strict consensus tree of six most parsimonious trees based on cpDNA restriction site dataset. Bootstrap frequencies are given *above branches* (bootstrap frequencies below 50% are not given)

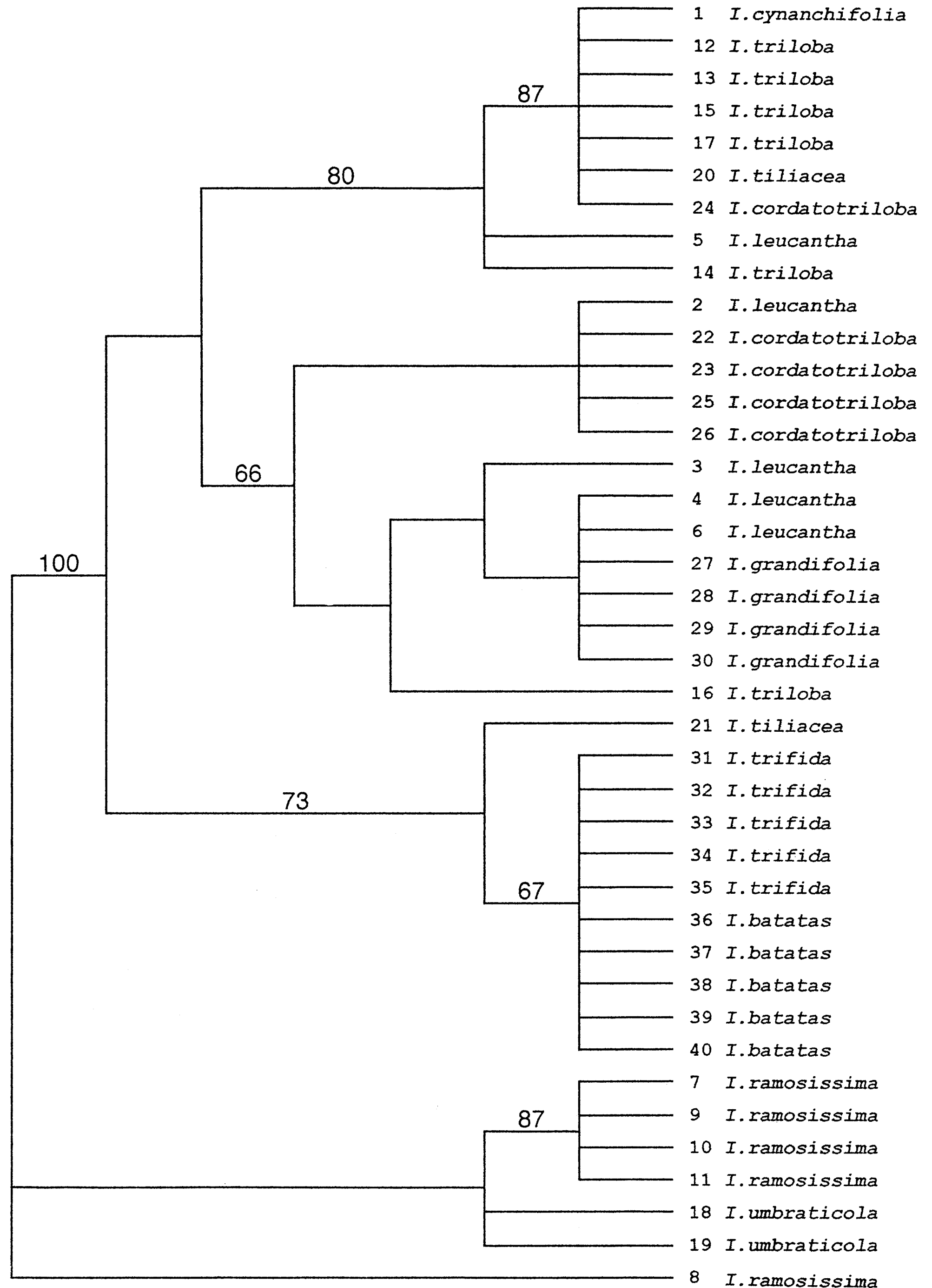

Fig. 4 Strict consensus tree of six most parsimonious trees based on cpDNA fragment dataset. Bootstrap frequencies are given *above branches* (bootstrap frequencies below 50% are not given)

were found to be more distantly related to sweetpotato than others among the nine wild species sampled in the ser. *Batatas* (Table 4).

Restriction analysis of PCR-amplified cpDNA regions

The *trnC-trnD* primer pair amplified a product that was approximately 2800 bp long in most species. A length polymorphism was detected common to species *I. ramosissima* and *I. umbraticola*, in which the amplified product is 200 bp longer than that from other species. The other three primer pairs amplified products with no detectable length polymorphism among the species (Table 5).

A total of 130 restriction sites were found in the four amplified cpDNA regions, of which 83 were common to all species and 47 were informative. A typical gel of cpDNA restriction fragments is shown in Fig. 1b. The results are summarized in Table 5. The restriction sites were partitioned as follows: 51 in the *trnC–trnD* region (32 were informative); 47 in the *trnM-rbcL* region (none

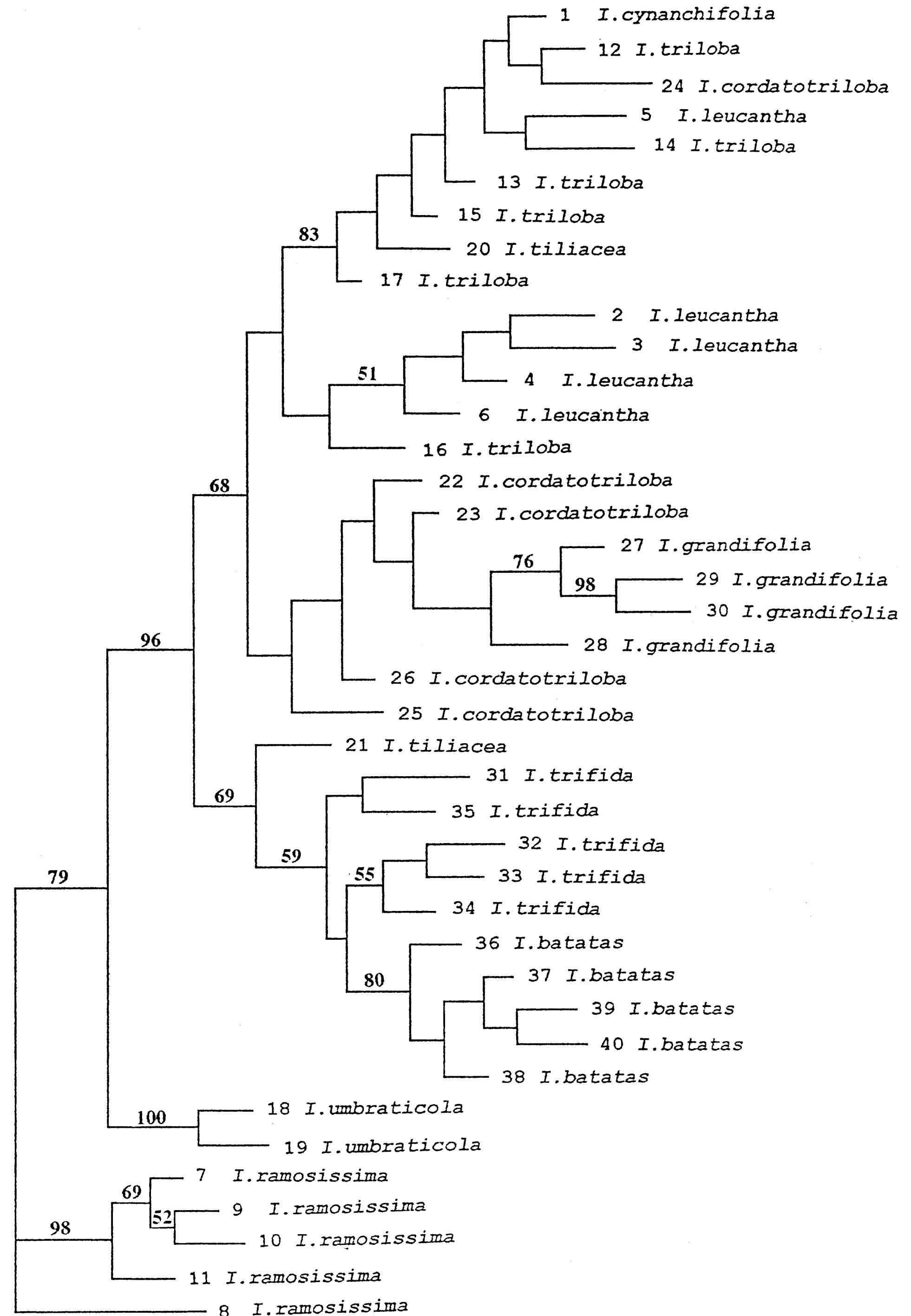

Fig. 5 Single most parsimonious tree based on the combined ISSR and cpDNA dataset. Bootstrap frequencies are given *above branches* (bootstrap frequencies below 50% are not given)

were informative); 15 in the *trnD–trnT* region (5 were informative); and 17 in the *trnS–trnM* region (10 were informative). This represents a maximum of 582 nucleotides surveyed if these restriction sites do not overlap. Comparing the cpDNA-based phylogenetic trees (Figs. 3, 4) with the ISSR-based trees (Fig. 2), similar relationships were found. The close relationship between sweetpotato and *I. trifida* was confirmed, and the same two species, *I. ramosissima* and *I. umbraticola*, were most distantly related to sweetpotato. One accession of *I. ramosissima* (*8*) from Colombia was found to be distantly related to its conspecific accessions as well as to other species. A major difference between the ISSR and cpDNA trees was the relationship between *I. ramosissima* and *I. umbraticola*, which formed a single clade based on a shared cpDNA fragment length polymorphism (Figs. 3, 4).

Parsimony analysis of the combined ISSR and cpDNA data matrix resulted in a single most parsimonious tree (Fig. 5). The clade in this tree comprising 5 of

Table 6 Summary of mtDNA amplification and restriction analysis of 40 accessions of *Ipomoea* ser. *Batatas*

Primer pair	Fragment amplified (bp)	Total no. of restriction sites	Site and length polymorphism
nad1 2–nad1 3r	1550	–	0
nad4 1–nad4 2r	2000	32	0
nad4 2c–nad4 3r	2700	–	0
nad5 1–nad5 2r	2200	2	0
nad7 2–nad7 3r	1500	–	0
nad7 3–nad7 4r	2700	–	0

the 6 accessions of *I. triloba* studied and 4 other species/accessions was better supported (*boot*=83%) than the corresponding clade in the trees based on either the ISSR or cpDNA dataset alone. The placement of the clade containing 4 *I. leucantha* accessions (*2*, *3*, *4*, *6*) in Fig. 5 was not well supported due to the differences between ISSR and cpDNA groupings (Figs. 2-4). However, one major advantage of the combined dataset was a better resolution of the relationships among *I. ramosissima, I. umbraticola* and the remaining eight species studied. *Ipomoea umbraticola* was placed as a sister clade to the group comprising eight other species but not to the *I. ramosissima* clades.

Six non-coding regions of mtDNA were also amplified with the universal primers (Table 6). PCR amplification of these regions revealed no length polymorphism among species. The sequence amplified with the *nad4 1- nad4 2* primer pair was digested using 8 of the four-base recognition restriction endonucleases, but neither site variation nor length polymorphism was revealed among the 40 accessions studied. The sequence amplified with the *nad5 1–nad5 2r* primer pair was cut by *Cfo*I, and again no polymorphism was detected. These results showed the conservative nature of mtDNA in these species. Thus, no further restriction analysis was considered necessary for all other amplified mtDNA sequences.

Discussion

In *Ipomoea* species, sufficient variation exists at the ISSR level to allow an analysis of intraspecific diversity. With some exceptions, most of the intraspecific groupings were congruent with current species assignments. Some accessions of *I. tiliacea*, *I. triloba*, *I. cordatotriloba,* and *I. leucantha* intermixed with the grouping of other species, suggesting that these species may represent a diverse group or have greater intraspecific genetic diversity. For instance, the 2 accessions of *I. tiliacea* studied were distantly placed in two groups, and 1 accession of *I. cordatortriloba* (*24*) was consistently clustered closer to *I. triloba* and *I. cynanchifolia* than to its conspecific accessions. The congruity between the ISSR and cpDNA groupings in the present study suggests that this type of discrepancy between molecular grouping and morphology-based species assignment is not likely to arise from a lack of sufficient variation within and between accessions. The anomalous clustering in the present case may be due to various ambiguous morphological distinctions used to classify species and accessions. As species of ser. *Batatas* are morphologically similar, and several species may be of hybrid origin, errors in morphologically based species identification could easily occur. This points to the value of molecular genetic characterization of sweetpotato germplasm resources.

At the interspecific level, four major groups could be distinguished (Figs. 2–5). *I. ramosissima* accessions formed two clades that were deeply separated from the other nine species. The next branch included two groups. The UPGMA trees separated *I. umbraticola* from the remaining eight species more frequently than that shown in Fig. 2, in which *I. umbraticola* was clustered with several other species with weak bootstrap support. The clade comprising eight other species was strongly supported in the parsimony analyses of cpDNA fragment data (Fig. 4) and the combined ISSR and cpDNA data (Fig. 5). Of these eight species, *I. trifida* appeared to be the closest relative of sweetpotato, as shown in all trees (Figs. 2–5). All 5 accessions of *I. trifida* studied were grouped together with all 5 accessions of *I. batatas*, and the 3 accessions of *I. trifida* from Guatemala, Mexico, and Nicaragua had the highest similarities to the sweetpotato. Other clusters revealed close relationships between *I. cynanchifolia* and *I. triloba,* and between *I. grandifolia* and *I. cordatotriloba*. In general, the resulting trees largely corroborate the current series classification, which is based on an integration of morphological and other characteristics (McDonald and Austin 1990; Austin 1991; Austin et al. 1993; Jarret et al. 1992).

The ISSR data in the present study support the traditional view that *I. trifida* is one of the most likely ancestors of the cultivated sweetpotato (Austin 1988). Jarret et al. (1992) examined systematic relationships in ser. *Batatas* using RFLPs. The RFLP data suggested that *I. trifida* was one of the diploid species most closely related to the cultivated hexaploid *I. batatas,* while species *I. tiliacea*, *I. ramosissima* and *I. umbraticola* were more distantly related to *I. batatas*. Jarret and Austin's (1994) RAPD analysis also identified *I. trifida* as one of the two wild species closest to both tetraploid and hexaploid *I. batatas*, while several other species studied, including *I. grandifolia*, *I. cordatotriloba*, and *I. triloba*, were grouped in a separate cluster.

Comparative studies of genetic relationships have shown that ISSR markers can provide the same level of accuracy as RFLP markers (e.g., Nagaoka and Ogihara 1997). Our results provide further support for the utility of ISSRs in resolving relationships at both intra- and interspecific levels compared to the results obtained with restriction analysis of nuclear DNA by Jarret et al. (1992).

The utility of cpDNA variation is also demonstrated in the present study as a means of checking the validity of the newly proposed ser. *Batatas* in the genus *Ipomoea*.

cpDNA restriction patterns were compared among the 40 accessions representing ten component species of the series. Of the four non-coding regions of cpDNA amplified, the *trnC–trnD* region provided the most informative restriction sites, in addition to a length polymorphism among the species. Two other regions, *trnD–trnT* and *trnS–trnM*, exhibited moderate levels of restriction site polymorphisms. However, no polymorphism was detected in the *trnM–rbcL* region among all 40 accessions studied. This indicates that rates of evolution in the noncoding regions in the chloroplast genome may vary greatly.

With a few exceptions, the 47 restriction site and length polymorphisms together revealed a pattern of interspecific relationships very similar to that revealed by ISSR data. No site or length polymorphisms were found between *I. batatas* and *I. trifida* based on cpDNA analysis. This provides further support that in the ser. *Batatas*, *I. trifida* is most likely one of the diploid progenitors in the origin of hexaploid *I. batatas*, the cultivated sweetpotato. Based on geographical distribution also, *I. trifida* is the most likely candidate involved in the origin of sweetpotato. The highest diversity of sweetpotato germplasm is in northwestern South America and parts of Central America, and these are the same geographical regions where *I. trifida* is also abundant (Austin 1988).

Ipomoea tiliacea, a tetraploid with storage roots, has also been considered to be a close relative of sweetpotato because of their morphological similarity (Austin 1988). In addition, *I. tiliacea* has the same genome type as sweetpotato and *I. trifida* (Nishiyama and Sakamoto 1975). In the present study, only 1 accession of *I. tiliacea* (21) from Nicaragua was placed closest to the sweetpotato – *I. trifida* group (Figs. 2, 4, 5).

One other species, *I. triloba*, has also been considered to be a close relative of sweetpotato (Austin 1988). Based on morphological characters, especially sepal shape and pubescence, Austin (1988) considered that *I. triloba* was most likely the second candidate for a genome donor in the origin of sweetpotato. However, this hypothesis has not been supported by other molecular investigations (e.g. Jarret et al. 1992; Jarret and Austin 1994). In the present study, we found that *I. triloba,* among the nine wild species sampled in the ser. *Batatas*, was actually one of the two species most distantly related with sweetpotato, based on the ISSR similarity index (27.6%; Table 4).

The groupings of *I. cordatotriloba*, *I. leucantha,* and *I. grandifolia* based on cpDNA data in this study are largely consistent with known relationships among the species. *Ipomoea leucantha* is a named hybrid in the ser. *Batatas*, derived from *I. cordatotriloba* x *I. lacunosa* (Austin 1978, 1988). In the present study, a few accessions of *I. leucantha* are placed close to *I. cordatotriloba*. *Ipomoea grandifolia* has the same hybrid status in the series, also with *I. cordatotriloba* as one of the progenitors (Austin 1988). The discrepancy at the species level is that some accessions of *I. leucantha* and *I. cordatotriloba* are dispersed into other clusters. This may be caused by the introgression of other cytoplasmic genomes. Phylogenetic analyses in many plant genera have suggested the introgression of cytoplasmic genomes between species, thereby causing incongruence among characters in nuclear and chloroplast genomes (Rieseberg and Soltis 1991).

The accession of *I. ramosissima* (*8*) from Colombia was distinctly separated from all other accessions of the species based on either ISSR or cpDNA data. This level of genetic distinctiveness can not be explained by the introgression of cytoplasmic genomes because both ISSR and cpDNA data give the same result. It is likely that the accession from Colombia represents a different species or a hybrid whose morphological traits are very similar to *I. ramosissima*.

Mitochondrial PCR-RFLPs showed no site or length polymorphism in the present study. This indicates that the slow evolutionary rate of the mitochondrial genome makes mtDNA PCR-RFLPs unsuitable for resolving phylogenetic relationships at the intrageneric level. In comparison with the high level of restriction site polymorphism detected in cpDNA for the same set of species, this study again provides evidence supporting that the nucleotide substitution rate of mtDNA in plants is much lower than that of cpDNA (Palmer 1992).

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