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Highly polymorphic AFLP markers as a complementary tool to ITS sequences in assessing genetic diversity and phylogenetic relationships of sweetpotato (*Ipomoea batatas* (L.) Lam.) and its wild relatives

Junchao Huang, Harold Corke and Mei Sun*
*Department of Botany and Zoology, The University of Hong Kong, Pokfulam Road, Hong Kong; *Author for correspondence (e-mail: meihk@yahoo.com; phone: 011 852 22990847; fax: 011 852 28188136)*

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Abstract

Comparative analyses of genetic diversity and phylogenetic relationships of sweetpotato (*Ipomoea batatas* (L.) Lam.) and its wild relatives in *Ipomoea* series *Batatas* were conducted using amplified fragment length polymorphism (AFLP) and sequence data from the internal transcribed spacer (ITS) region of the ribosomal DNA. Low ITS divergence among thirteen species of ser. *Batatas* resulted in poorly resolved relationships. More variable AFLP characters were found to be more efficient in characterizing genetic diversity and phylogenetic relationships at both intra- and interspecific levels within ser. *Batatas*. Highly informative AFLP fingerprints of 36 accessions representing 10 species of ser. *Batatas* were generated using only six primer combinations. Of the species examined, *I. trifida* was found to be the most closely related to *I. batatas*, while *I. ramosissima* and *I. umbraticola* were the most distantly related to *I. batatas*. The highly polymorphic AFLP markers are a valuable tool in assessing genetic diversity and phylogenetic relationships of sweetpotato and its wild relatives.

Introduction

Sweetpotato is the most important food crop in the genus *Ipomoea*, which is the largest genus in the family Convolvulaceae consisting of about 650 species. Currently, thousands of sweetpotato genotypes are cultivated around the world. Scientific interest in utilization of sweetpotato germplasm, and in identifying the evolutionary relationship of sweetpotato with wild species in the genus has increased in recent years. Until recently, the species allied with sweetpotato were poorly known. The systematics of the sweetpotato allies was not fully examined until 1978 (Austin 1978). Since then sweetpotato allies included in the ser. *Batatas* have been further revised (Austin 1991; Austin and Huaman 1996; Austin and Wilkin 1993) to contain 13 species and a named hybrid species, i.e. *I. batatas*, *I. cordatotriloba*, *I. cynanchifolia*, *I. grandifolia*, *I. lacunosa*, *I.* × *leucantha* (or *I. leucantha*), *I. littoralis*, *I. tabascana*, *I. tenuissima*, *I. tiliacea*, *I. trifida*, *I. triloba*, *I. ramosissima*, and *I. umbraticola*. All these species but *I. littoralis* were confined originally to the Americas where the group probably originated (Austin 1978, 1988). Cytological studies have shown that there are three ploidy levels within the series. The cultivated sweetpotato is a hexaploid with 2n = 90, *I. tiliacea* and *I. tabascana* are tetraploids with 2n = 60, and the remaining species are diploids 2n = 30.

Within the ser. *Batatas*, the phylogenetic relationships between sweetpotato and its wild relatives are difficult to reveal on the basis of morphological, fertility and cytological differences. Austin (1988) presented some phenetic analyses of the taxa based on morphological characters, but obtained different results between single link and UPGMA analysis. Recently, several molecular studies have been carried out on the origin of sweetpotato and its relationships with other species in the series using restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), and chloroplast (cp) DNA

(Huang and Sun 2000; Jarret et al. 1992; Jarret and Austin 1994). Although each data set alone has provided valuable information on the origin of sweetpotato, these molecular approaches have not fully resolved the relationships between sweetpotato and its allies.

In the present study, we used two complementary approaches, ITS sequencing and amplified fragment length polymorphism (AFLP), to investigate intra- and interspecific relationships in *I.* ser. *Batatas*. The two internal transcribed spacers (ITS1 and ITS2) of nuclear ribosomal RNA genes normally exhibit high rates of variability, and have proven to be useful in inferring phylogenetic relationships among plant taxa at various taxonomic levels (for review see Baldwin et al. (1995)). AFLP was described by Zabeau and Vos (1993) and Vos et al. (1995). This assay has the potential to generate a large number of polymorphic loci and has been used to analyze genetic diversity in many plant species. AFLP has also proven to be a

Table 1. Species and accessions of *Ipomoea* series *Batatas* studied.

Code	Species	Accession	Origin
1	*I. cynanchifolia* Meisn.	DPw 2554	Brazil
2	*I. leucantha* Jacquin	DLP 3354	Argentina
3		DLP 3004	Columbia
4		DLP 431	Ecuador
5		DLP 2931	Mexico
6		DLP 521	Peru
7	*I. ramosissima* (Poir.) Choisy	DLP 2760	Bolivia
8		DLP 3010	Columbia
9		DLP 1173	Ecuador
10		DLP 4679	Cyprus
11		DLP 2814	Peru
12	*I. triloba* L.	DLP 3003	Columbia
13		DLP 2982	Dominica
14		DLP 2943	Mexico
15		DLP 2429	Peru
16		DLP 4161	Paraguay
17	*I. umbraticola* House	DLP 2941	Mexico
18		DLP 4604	Nicaragua
19	*I. tiliacea* (Willd.) Choisy	DLP 2917	Mexico
20		DLP 4638	Nicaragua
21	*I. cordatotriloba* Dennst.	DLP 4148	Argentina
22		DLP 2762	Bolivia
23		DLP 3001	Columbia
24		DLP 3617	Paraguay
25	*I. grandifolia* (Dam.) O'Donell	DLP 4039	Argentina
26		DPw 2611	Brazil
27		DLP 4169	Paraguay
28		Vilaro 5	Uruguay
29	*I. trifida* (H.B.K.) G. Don	DLP 1084	Columbia
30		DLP 3685	Guatemala
31		DLP 2961	Mexico
32		DLP 4607	Nicaragua
33		DLP 714	Venezuela
34	*I. batatas* (L.) Lam.	Kyudei No.63	Japan
35		Kinang Kong	Philippines
36		CN 1108-13	Taiwan
37	*I. lacunosa* L.	Grif 6172 01 SD	United States
38	*I. tabascana* McDonald & Austin	PI 518479 01 SD	Mexico
39	*I. tenuissima* Choisy	PI 553012 01 SD	United States
40	*I. setosa* Ker Gawl.	CIP	Peru
41	*I. alba* L.	DLP 42	Peru
42	*I. aristolochiaefolia* G. Don	DLP 1254	Ecuador
43	*I. cairica* (L.) Sweet	DLP 496	Peru
44	*I. dumetorum* Willdenow ex Roemer & Schultes	DLP 3296	Peru

competent tool in bacterial and fungi taxonomic studies (Huys et al. 1996; Mueller et al. 1996) and in plant phylogenetic studies (Kardolus et al. 1998). To further improve the efficiency of AFLP, we used fluorescence-labeled primers for automatic DNA sequencer detection (Huang and Sun 1999). This modified AFLP has proven to be highly efficient in generating a large number of informative marker loci with only a few pairs of primers (Xu and Sun 2001). The objective of this study was to compare the suitability and efficiency of ITS sequences and AFLPs in detecting genetic diversity and phylogenetic relationships of sweetpotato and its wild relatives.

Materials and methods

Plant material and DNA extraction

All species in *Ipomoea* ser. *Batatas* except for *I. littoralis*, which was not available at the time of this study, were included for ITS sequence analysis. Five other species representing five sections of *Ipomoea* were also sampled for use as outgroups in this study (Table 1). Four species, *I. aristolochiaefolia*, *I. alba*, *I. cairica,* and *I. dumetorum*, are from *I.* subgenus *Quamoclit*. Each species represents one section of the subgenus. The other species *I. setosa* represents section *Eriospermum* in the subg. *Eriospermum*, where all species of ser. *Batatas* are also placed. These plant materials were obtained as seeds or in vitro plantlets from the International Potato Center (Lima, Peru). In most cases, one accession of each species was used to represent the species. However, two accessions, No. 8 of *I. ramosissima* (accession DLP 3010 from Columbia) and No. 20 of *I. tiliacea* (accession DLP 4638 from Nicaragua), which are distinctively different from other accessions of the same species (Huang and Sun 2000), were added to the ITS analysis. Thirty-six accessions representing ten species of *I.* ser. *Batatas* were used for AFLP analysis. Seeds were germinated to provide fresh young leaves for total DNA extraction. Five individuals of each accession were bulked to represent the accession for AFLP assays, and one individual of each species was used for ITS amplification. A modified DNA extraction protocol of Stewart and Via (1993) was used in this study (Huang and Sun 2000). DNA quantification was done by a DyNA fluorometer (Pharmacia).

Amplification and sequencing of ITS regions

ITS sequences were amplified using primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′). These primers were constructed from conserved regions of the 3′ end of the rDNA gene coding for the 18S rRNA and from the 5′ end of the 26S rDNA (White et al. 1990). PCR amplifications were done using a total volume of 50 μL, containing 3 mM $MgCl_2$, 0.1 μM of each primer ITS5 and ITS4, 0.2 mM dNTP mixture, 2.5 μL DMSO, 20 ng genomic DNA and 1.5 units of recombinant Taq DNA polymerase (Life Technologies). PCR was performed in a MJ Research thermal minicycler as follows: (1) 5 min initial denaturation step at 94 °C; (2) 1 min at 94 °C; (3) 1 min at 57 °C annealing temperature; and (4) 1 min at 72 °C; followed by a 5 min at 72 °C extension step after 35 cycles. The PCR products were purified with a High Pure™ PCR Product Purification Kit (Cat.1732676, Boehringer Mannheim) and then used for direct sequencing on a Pharmacia ALFexpress automated sequencer, following the procedure described in the manual of Autocycle™ 200 sequencing kit. Both DNA strands were sequenced using primers ITS2 (5′-GCTACGTTCTTCATCGATGC-3′), ITS3 (5′-GCATCGATGAAGAACGTA-3′) in addition to primers ITS4 and ITS5. All 36 ITS1 and ITS2 sequences obtained from this study are available in GenBank (accession numbers: AF256614–256649).

AFLP analysis

AFLP analysis was performed as described by Vos et al. (1995) and Huang and Sun (1999). Total genomic DNA (250 ng) was digested using two restriction enzymes, *Eco*RI and *Mse*I (Gibco). Pre-amplifications were performed in a volume of 20 μL of 1 × PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 6 pmol primer *Eco*RI + A and *Mse*I + C, and 1 unit Taq polymerase and 2 μL diluted fragments, using 20 cycles of 94 °C for 30 s, 56 °C for 60 s, and 72 °C for 60 s. One μl of the diluted (1 : 5) pre-amplification products was used as template for the selective amplification using three *Eco*RI primers (E-AAG, E-ACT, E-AGC) and six *Mse*I primers (M-CAA, M-CAC, M-CAT, M-CTA, M-CTC, M-CTG). Only *Eco*RI primers were labelled with fluorescein Cy-5 (Pharmacia) at the 5′ end for the selective amplification. The selective amplification was carried out using the following cycling parameters: 1 cycle of 30 s at 94 °C,

30 s at 65 °C, and 60 s at 72 °C, followed by 12 cycles in which the annealing temperature decreases 0.7 °C per cycle, and followed by 23 cycles of 1 min at 94 °C, 30 s at 56 °C, and 1 min at 72 °C.

Following PCR amplification, reaction products were mixed with an equal volume of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue and xylene cyanol). The resulting mixtures were denatured for 3 min at 90 °C, chilled on ice and run on a 4.25% polyacrylamide gel with an ALFexpress™ automated sequencer (Pharmacia) using the fragment option. The resulting gel image was analyzed with the software package FRAGMENT ANALYSIS 1.2 (Pharmacia).

Data analysis

The DNA sequences were aligned visually after initial alignment with the Clustal × 1.7 program. The alignments among the species studied are fairly unequivocal due to many highly conserved regions. The boundaries of the ITS regions were identified by comparison with data for *Daucus carota* (Yokota et al. 1989). Phylogenetic analyses were performed with PAUP 4.0 (Swofford, D.L., 1999). A heuristic search was employed with 100 random additions of sequences and the TBR branch-swapping option. Bootstrap values were calculated from 500 replicates.

All AFLP fragments (bands) were treated as dominant markers and scored as binary characters: either present (1) or absent (0). Bands ranging from 100 bp to 500 bp were used in the data matrix. Fragments of the same size in different accessions were considered to be the same allele. Phylogenetic analyses were performed as described above for ITS sequences.

Results

ITS sequence divergence and phylogenetic relationships

Among the twenty taxa studied, the length of ITS1 ranged from 190 bp to 221 bp, and ITS2 ranged from 217 to 225 bp. However, the lengths of ITS1 (except for *I. umbraticola*) and ITS2 were nearly constant within the ser. *Batatas* varying from 190 to 191 bp for ITS1 and from 217 to 218 bp for ITS2. The GC content of all taxa investigated ranged from 53 to 60% in ITS1 and from 63 to 71% in ITS2. The GC content in ITS2 is 10% higher than that in ITS1.The GC content of the ITS1 and ITS2 regions combined ranged from 56% for *I. dumetorum* to 65% for most of the ingroup species. The average GC content was 63% over all taxa.

The aligned data set had 455 nucleotides with 268 constant characters and 187 variable characters among all taxa studied. For parsimony analysis, 85 polymorphic sites were found to be informative (36 in ITS1 and 49 in ITS2). The alignment resulted in 69 gaps, which were treated as missing data. Within the ser. *Batatas*, only 33 variant sites were found among the 13 species, of which 18 occurred in *I. umbraticola* and 10 were parsimony-informative (five in ITS1 and five in ITS2). Only four indels occurred among the ingroup taxa, including a 25-bp insertion present in *I. umbraticola*. Although there are alternative alignments for positions 71–99 of ITS1 among these taxa, the alternative alignments of these positions did not affect tree topologies. Within the positions 71–99 of ITS1, sequence divergences are much higher than those within other positions. All ingroup taxa except *I. umbraticola* and accession No. 8 of *I. ramosissima* had a deletion of 28 base pairs compared to the outgroup species *I. aristolochiaefolia*. The other four outgroup species had a 8 to 24-bp insertion. Interestingly, accession No. 8 of *I. ramosissima*, which has been shown here to be distant from other accessions of *I. ramosissima* based on AFLPs, or ISSRs and cpDNA PCR-RFLPs (Huang and Sun 2000), had a 26-bp insertion at the position.

DNA divergence values (uncorrected p-distances, Kumar S., Tamura K. and Nei, M. (1993)) are shown in Table 2. Interspecific sequence diversity among the taxa ranged from 0% (between *I. cordatotriloba* and *I. grandifolia*) to 25.9% (between *I. aristolochiaefolia* and *I. dumetorum*). The lengths of the ITS regions, high GC contents (56–65%), and DNA divergence values among the 20 taxa of *Ipomoea* were within those reported for other angiosperm species (Baldwin et al. 1995; Nickrent et al. 1994). The highly conserved ITS1 sequence motif of flowering plants, GGCRY-(4 to 7N)-GYGYCAAGGAA (Liu and Schardl 1994) was also found in *Ipomoea* ITS1 between positions 119 and 139. Liu and Schardl (1994) suggested that this sequence plays a role in the processing of rRNA gene transcripts. Sequences at or near the 3′ end of ITS1 and ITS2 are generally more variable.

Phylogenetic analysis of the sequence data matrix

Table 2. Divergence values (uncorrected p-distances) of ITS sequences between 20 taxa of *Ipomoea* studied.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 *I. aristolochiaefolia*																			
2 *I. alba*	0.13932																		
3 *I. cairica*	0.14797	0.13527																	
4 *I. lacunose*	0.17053	0.14885	0.1344																
5 *I. tabascana*	0.16799	0.14617	0.1318	0.0049															
6 *I. tenuissima*	0.17309	0.14897	0.1346	0.0098	0.0098														
7 *I. leucantha*	0.17817	0.1569	0.1423	0.01225	0.01225	0.01716													
8 *I. grandifolia*	0.17562	0.15423	0.1397	0.0098	0.0098	0.01471	0.00245												
9 *I. ramosissima* 7a	0.17562	0.1542	0.1396	0.00735	0.00735	0.01225	0.0098	0.00735											
10 *I. cordatotriloba*	0.17401	0.15323	0.1376	0.0098	0.0098	0.01225	0.00247	0	0.00737										
11 *I. cynachifolia*	0.17221	0.15394	0.1417	0.0147	0.01467	0.00981	0.01229	0.01474	0.01718	0.01229									
12 *I. triloba*	0.17468	0.1513	0.1391	0.00981	0.00978	0.00981	0.01233	0.00985	0.01229	0.00988	0.00978								
13 *I. tiliacea 19*	0.17218	0.15134	0.1366	0.01226	0.01222	0.01226	0.01477	0.01229	0.01474	0.01234	0.01222	0.00244							
14 *I. tiliacea* 20	0.16466	0.14866	0.129	0.00981	0.00978	0.01474	0.01232	0.00985	0.01229	0.00988	0.01467	0.00978	0.00733						
15 *I. trifida*	0.17053	0.14884	0.1343	0.00735	0.00735	0.01225	0.01471	0.01225	0.0098	0.01227	0.01711	0.01222	0.01467	0.00733					
16 *I. batatas*	0.17053	0.14885	0.1344	0.00245	0.00245	0.00735	0.0098	0.00735	0.0049	0.00735	0.01222	0.00733	0.00978	0.00733	0.0049				
17 *I. umbraticola*	0.17726	0.15681	0.1538	0.04892	0.04402	0.05137	0.05627	0.05382	0.05137	0.05168	0.05627	0.05382	0.05631	0.04896	0.04647	0.04647			
18 *I. ramosissima* 8	0.18707	0.1724	0.1657	0.05598	0.0512	0.05587	0.05826	0.05587	0.05348	0.05341	0.06071	0.06082	0.06331	0.05607	0.05362	0.05359	0.0626		
19 *I. setosa*	0.16869	0.15785	0.1671	0.09863	0.09615	0.10606	0.10854	0.10606	0.10359	0.10623	0.11092	0.10598	0.1085	0.1011	0.09369	0.09863	0.10534	0.11049	
20 *I. dumetorum*	0.25928	0.25445	0.2319	0.24042	0.24045	0.24018	0.24327	0.24569	0.24306	0.2455	0.24409	0.24439	0.24199	0.237	0.23541	0.24044	0.25596	0.25027	0.23782

[a] number represents accession code for the species with more than one accession studied.

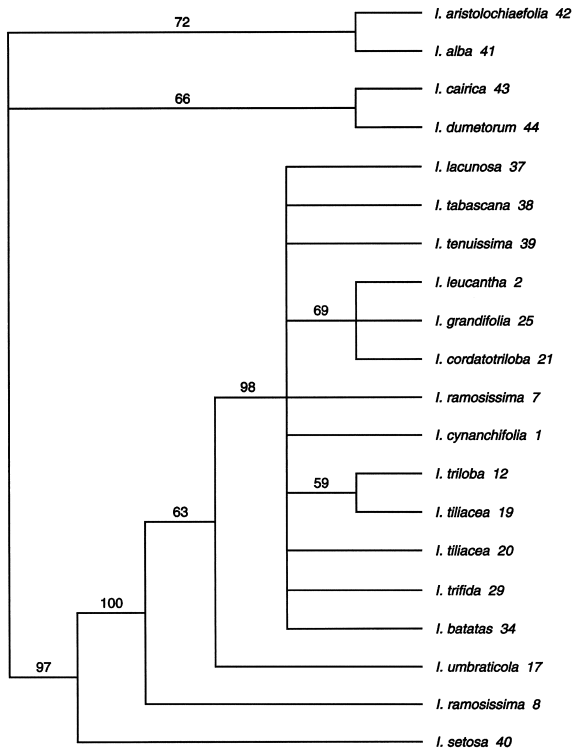

Figure 1. Consensus tree of 87 most parsimonious trees constructed based on ITS sequences. Numbers above branches are bootstrap values. Numbers following each species name represent the accession code as given in Table 1.

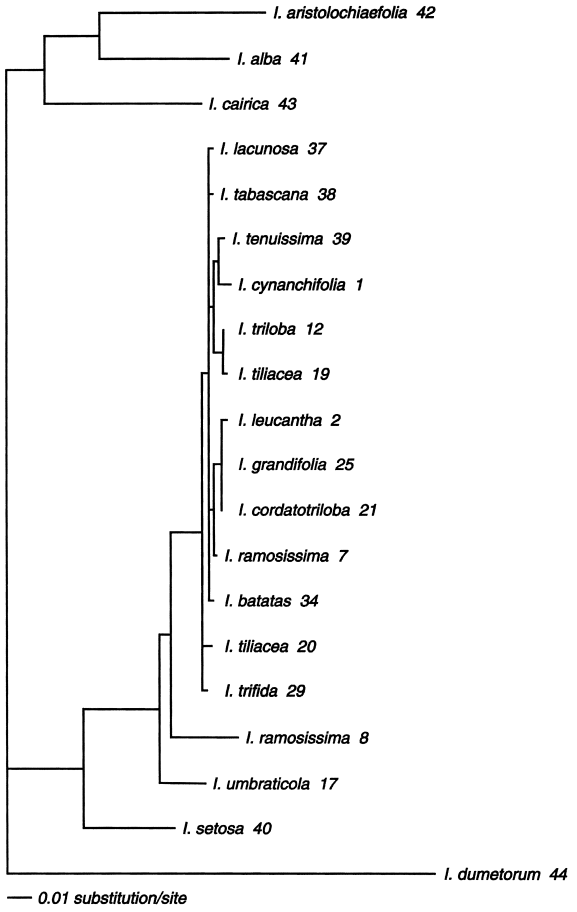

Figure 2. Maximum likelihood tree constructed based on ITS sequences. Numbers above branches are bootstrap values. Numbers following each species name represent the accession code as given in Table 1.

composed of 20 taxa resulted in 87 most parsimonious trees (299 steps; consistency index = 0.789; retention index = 0.672). A consensus of most parsimonious trees derived from this analysis is shown in Figure 1. The 50% bootstrap consensus tree obtained from neighbor-joining analysis (not shown here) had a topology quite similar to the consensus tree obtained from parsimonious analysis (figure not shown). The same tree topology for the ingroup taxa was obtained when the five outgroup species were excluded and *I. umbraticola* was selected as the outgroup for rooting purposes. However, maximum likelihood analysis resulted in a tree different in topology (Figure 2), but with the major relationships similar to those shown in Figure 1.

AFLP polymorphism and phylogeny

Six primer combinations (E-AAG/M-CTA, E-AAG/M-CAC, E-ACT/M-CAA, E-ACT/M-CTG, E-AGC/M-CAT, E-AGC/M-CTC) were used to analyze 36 accessions representing ten species of *I.* ser. *Batatas*. The application of fluorescence-labelled primers in the selective amplifications in combination with DNA sequence gel analysis resulted in a large number of clearly distinguishable AFLP fragments across the 36 accessions. A total of 1182 AFLP bands (loci) were identified, of which 891 were polymorphic across all accessions. The number of scored loci amplified by each pair of primers varied from 152 to 256, and averaged 197 per reaction. The number of bands obtained per accession ranged from 159 to 228 with all six primer combinations. Differences in ploidy level were not reflected in the number of AFLP fragments amplified with these primer combinations (data not shown).

Phylogenetic analyses of the combined data matrix generated from all six primer combinations showed that the trees constructed with parsimony, genetic

distances or neighbor-joining all had the same topologies. A single most parsimonious tree is shown in Figure 3. With few exceptions, the AFLP tree clustered all intraspecific accessions together according to their species designation. Phylogeny was also constructed based on genetic distance using the AFLP data sets derived from each primer combination. The resulting tree topologies (figures not shown) were generally similar to that constructed based on the combined data set.

Discussion

The phylogenetic relationships based on analysis of ITS sequences (Figure 1,2) agree with the current taxonomic treatment of ser. *Batatas* at the subgeneric level. *Ipomoea* ser. *Batatas* was once placed in *I.* subgenus *Quamoclit*. However, morphological, chloroplast DNA, and the morphological/palynological data (Austin and Wilkin 1993; McDonald and Austin 1990; McDonald and Mabry 1992) all indicate that *I.* ser. *Batatas* is better placed in *I.* subg. *Eriospermum* near ser. *Setosae*. Our results support this settlement. *Ipomoea setosa* and all species of ser. *Batatas* of the subgenus *Eriospermum* form a monophyletic clade with strong bootstrap support (97%). However, the four species of subgenus *Quamoclit* (*I. aristolochiaefolia*, *I. alba*, *I. dumetorum*, and *I. cairica*) were placed in different clades, suggesting that the species of subgenus *Quamoclit* are not monophyletic.

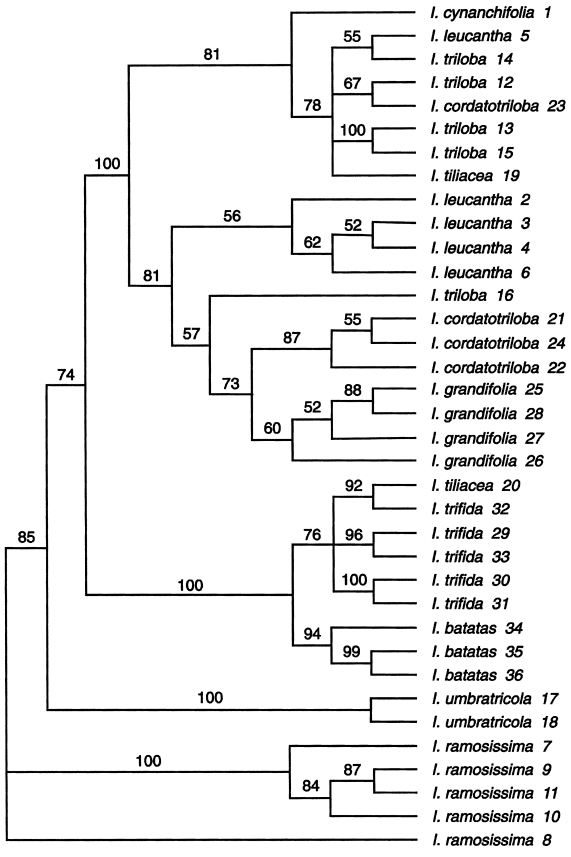

Figure 3. A single most parsimonious tree based on combined AFLP data set generated with all six primer combinations. Numbers above branches are bootstrap values. Numbers following each species name represent the accession code as given in Table 1.

The present study shows that ITS sequence diversity is informative for resolving phylogenetic relationships at the subgeneric level in *Ipomoea*. However, relationships within the ser. *Batatas* clade remain incompletely resolved. Phylogenetic analysis of ITS sequences supports the monophyly of these species, in that all taxa identified as members of sweetpotato alliance were found within a single clade (Figure 1,2). This group was strongly supported (98%) with both parsimony and neighbor-joining analyses. Within this group, *I. umbraticola* and *I. ramosissima* (accession no. 8) were placed into different clades at the base of the ser. *Batatas*, whereas the placement of accession no. 7 of *I. ramosissima* was within the clade containing *I. batatas*. This suggests that *I. ramosissima* is not a monophyletic species. Similarly, the ITS phylogeny fails to support the monophyly of *I. tiliacea*, with the two sampled accessions placed into different clades.

Within the clade containing *I. batatas*, however, very little resolution is obtained. As shown in the consensus tree (Figure 1), *I. leucantha*, *I. cordatotriloba* and *I. grandifolia* form a subclade, and *I. tiliacea* (accession no. 19) and *I. triloba* form another subclade, whereas all other taxa are placed sister to these subclades and to each other. Sequences of ITS have often been shown to provide good phylogenetic resolution at the species level, however, they are apparently not sufficiently divergent in resolving relationships within *I.* ser. *Batatas*. Lack of breeding barriers and frequent hybridisation among the members of ser. *Batatas* may result in homogeneity of ITS sequences. The low divergence of the ITS sequences among this group of species provides strong support for changing its status from section to series, as recently suggested by Austin and Huaman (1996).

The removal of the four more distant related

outgroup species did not alter the tree topology (figures not shown). In most analyses, *I. umbraticola* seems to be most distant to all other ingroup species. *I. umbraticola* was formerly placed in section *Eriospermum*, but was recently moved to ser. *Batatas* (formerly section *Batatas*), based mainly on morphological traits (Austin and Wilkin 1993). Classifications and phylogenetic hypotheses should be based on as many characters as possible. Other molecular markers, such as RFLP (Jarret et al. 1992) and ISSR and cpDNA PCR-RFLP (Huang and Sun 2000) also revealed that *I. umbraticola*, as well as *I. ramosissima* were distant to other species in ser. *Batatas*. However, the ITS sequence data fail to separate *I. ramosissima* (accession no. 7) from other species in ser. *Batatas* in the present study. Considering the presence of a large insertion in accession no. 8 of *I. ramosissima*, which is absent in all ingroups except *I. umbraticola*, this accession may be a misidentification and does not belong to *I. ramosissima*, as also suggested by other molecular evidence, such as ISSR (Huang and Sun 2000). The large deletion of ITS sequence at this position may be used for circumscription of ser. *Batatas*, which would exclude *I. umbraticola* from the series.

AFLP analysis has great potential as a powerful fingerprinting technique for DNA samples of any origin or complexity (Vos et al. 1995). Using fluorescence-labeled primers in combination with gel analysis with an automated DNA sequencer, a large number of amplified fragments can be distinctively resolved. As shown in the present study, this is an efficient and reliable marker system for studying germplasm diversity and phylogenetic relationships within ser. *Batatas*. An average of 152 informative bands were generated with each primer combination. High levels of AFLP diversity were detected, ranging from 54.3 – 98.0% in polymorphism across all accessions. The polymorphism obtained with each single primer combination is sufficient for characterizing relationships at both intra- and interspecific levels within ser. *Batatas*. The relationships revealed with each individual primer combination are similar to those detected with the combined data set based on several different primer combinations (Figure 3). The 36 accessions are grouped mainly according to their species designations, with the exception of accessions *I. leucantha 5*, *I. cordatotriloba 23*, *I. triloba 16*, *I tiliacea*, and *I. ramosissima 8*. The polyphyletic origins of *I tiliacea* accessions and genetic uniqueness of *I. ramosissima 8* are congruent with the ITS data. One major incongruence between the AFLP tree and ITS phylogeny is the basal placement of *I. ramosissima* species clade which includes accession *I. ramosissima* 7, whereas *I. umbratricola* is placed sister to the large clade containing the rest of eight taxa in the ser. *Batatas*, including *I. batatas*. Two major groups exist within this clade, both with strong bootstrap support (100%), indicating that *I. trifida* and accession *I. tiliacea 20* are the closest relatives to *I. batatas*. Other relationships revealed include the *I. cynanchifolia – I. triloba – I. tiliacea 19* grouping, and the *I. leucantha – I. cordatotriloba – I. grandifolia* grouping. Despite its low diversity, ITS phylogeny also reveals these relationships. In addition, similar tree topologies were previously obtained with ISSR and cpDNA-RFLP markers (Huang and Sun 2000).

In the present study, only one accession of *I. tiliacea* was found to be closely related to *I. batatas*. Other molecular markers such as ISSRs and cpDNA-RFLPs also showed a close relationship between the same accession of *I. tiliacea* and *I. batatas* (Huang and Sun 2000). *I. tiliacea* has been considered as very closely allied with sweetpotato (Austin 1988). Similar to sweetpotato, it produces storage roots. Also, *I. tiliacea* has the same genome type B as sweetpotato and *I. trifida*. Based on morphological traits, especially on the sepal shape and pubescence, Austin (1988) considers *I. triloba* to be the best possibility of taxa that is the second candidate for a genome involved in the evolution of sweetpotato. In the present study, accession *I. tiliacea* 19 from Mexico is grouped with *I. triloba* species group, which indicates some relatedness between the two species. These species also have similar geographical origins. *I. tiliacea* and *I. triloba* are both from Caribbean, and *I. trifida* is Circum-Caribbean.

Other close relationships in the ser. *Batatas*, such as *I. leucantha*, *I. cordatofolia* and *I. grandifolia*, as revealed by the present AFLP and ITS data sets and other molecular evidence (Huang and Sun 2000), are congruent with Austin (1988) results. *I. leucantha* is a hybrid taxa between *I. cordatotriloba* and *I. lacunosa*; and *I. grandifolia* is likely also of hybrid origin, with *I. cordatotriloba* as one of the progenitors (Austin 1988). Thus, this group may actually represent a parental species together with two half-sister taxa.

Comparing our data with the results obtained from

other molecular studies, several species relationships in the genus *Ipomoea* can be confirmed. Austin (1988) postulated that *I. trifida* is one of the closest relatives of cultivated sweetpotato, and likely one of its ancestors. Jarret et al. (1992) examined systematic relationships in ser. *Batatas* using RFLPs, and their data show that *I. trifida* is one of the two species most closely related to the cultivated hexaploid *I. batatas* whereas *I. ramosissima* and *I. umbraticola* were more distantly related to *I. batatas*. The RAPD analysis by Jarret et al. (1992) also indicates that *I. trifida* is the closest relative to the hexaploid *I. batatas* among eight species from ser. *Batatas*. A more recent study based on ITS sequences (Miller et al. 1999) also places *I. setosa* and *I. umbraticola* in the same basal position relative to *I. batatas* and two other species in the ser. *Batatas*. All these relationships are supported by our studies based on ITS, AFLP (this study), and ISSR and cpDNA (Huang and Sun 2000).

In addition, comparative analysis of species relationships using various molecular techniques has demonstrated the phylogenetic utility of AFLP markers, especially for studying closely related species. In comparing the present AFLP analysis with other molecular approaches, AFLP with fluorescence-labelled primers has several advantages over other marker techniques for the analysis of genetic diversity and phylogeny. First, it is much faster to assay large numbers of DNA loci. For example, the 1182 characters used in the AFLP dataset were generated in about eight days. In contrast, only 189 characters were generated with 15 ISSR primers in 16 days (data not shown). Secondly, the repeatability of AFLPs is much higher than RAPDs or ISSRs, and thus more accurate markers can be generated for the studies of genetic diversity and phylogeny. A possible source of error in the AFLP data could be the non-homology of co-migrating fragments. However, the chance of co-migrating AFLP fragments representing different alleles of the same genetic locus between closely related species is small, due to the highly selective amplification of a small subset of the genome and the sharp resolution of polyacrylamide sequencing gels. AFLPs have been proven suitable for studying phylogenetic relationships up to the species level (Hill et al. 1996; Kardolus et al. 1998; Sharma et al. 1996). Our results also indicate that AFLP data are adequate in detecting phylogenetic relationships among the species in ser. *Batatas*, a valuable alternative in the case of low ITS resolution.

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