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MOLECULAR SYSTEMATICS OF SOLANUM SERIES *CIRCAEIFOLIA* (*SOLANUM* SECTION *PETOTA*) BASED ON AFLP AND RAPD MARKERS

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Abstract

The three or four taxa of *Solanum* series *Circaeifolia*, a small group of wild tuber-bearing *Solanum* endemic to Bolivia, could not be clearly separated from each other in an earlier morphological study. Therefore, RAPD and AFLP data sets were generated to investigate the status of and the relationships among these taxa. Especially the AFLP data enable the distinction of four groups. These results are compared to the earlier morphological analysis and the consequences for the classification of the series are discussed.

Introduction

Solanum ser. *Circaeifolia* Hawkes is a small, well-defined group of wild tuber-bearing Solanums in sect. *Petota* Dumort. consisting of 3 or 4 taxa, endemic to Bolivia. The plants are relatively small (height usually up to 30 cm) with slender stems, a terminal leaflet somewhat larger than the laterals, white, stellate flowers, and small, narrowly conical fruits. Hawkes and Hjerting (1989) accepted three species in this series, with one species consisting of two subspecies: *S. capsicibaccatum* Cárdenas, *S. circaeifolium* Bitter subsp. *circaeifolium*, *S. circaeifolium* subsp. *quimense* Hawkes and Hjert. and *S. soestii* Hawkes and Hjert. Ochoa (1990) on the other hand recognised only two species, one of these consisting of two varieties: *S. circaeifolium* var. *circaeifolium*, *S. circaeifolium* var. *capsicibaccatum* (Cárdenas) Ochoa and *S. soestii*.

In a paper on a numerical analysis of the taxa of ser. *Circaeifolia* based on morphology we have discussed the classification of the taxa in this series (Van den Berg and Groendijk-Wilders 1999). This morphology-based phenetic analysis did not result in a clear separation of the presumed taxa. Both the dendrogram and the PCA showed much overlap among the accessions of *S. capsicibaccatum* and *S. circaeifolium* subsp. *quimense* (see Fig. 2 and Fig. 3 in Van den Berg and Groendijk-Wilders 1999). None of the character states was species-specific. Lack of clear character states distinguishing species is a recurrent problem in the taxonomy of *Solanum* sect. *Petota*. Several studies in sect. *Petota* (Spooner et al. 1995; Van den Berg et al. 1996; Van den Berg et al. 1998) documented the need of a polythetic classification concept, i.e. grouping taxa that have the greatest number of shared features, no single feature of which is essential for group membership or is sufficient to make an organism a member of a group (Sokal and Sneath 1963; Stuessy 1990). The lack of clearly distinguishing morphological characters led us to reinvestigate the taxa of ser. *Circaeifolia* with molecular methods.

The PCR based RAPD and AFLP methods provide a quick and effective assessment of similarities and differences of the whole genome, and have been successfully applied in studies in sect. *Petota*. For example, a RAPD study of the three taxa in *Solanum* sect. *Etuberosum* (Juz.) A. Child, a group of non-tuber-bearing species closely related to *Solanum* sect. *Petota* showed that RAPDs are suitable for both intra- and interspecific studies in this section (Spooner et al. 1996). A RAPD study on the identification of potato cultivars (Demeke et al. 1993) was able to distinguish 36 commercial cultivars using only 2 primers. Kardolus et al. (1998) demonstrated that the level of variation found with AFLPs enables the grouping of individual plants in their accessions, and of accessions in species.

The objectives of this study are to use the RAPD and AFLP variation detected within and among the taxa of *Solanum* ser. *Circaeifolia* to investigate the relationship of these taxa and compare the results with the earlier morphological analysis (Van den Berg and Groendijk-Wilders 1999).

Material and Methods

Plant material

The same accessions used in the prior morphological analysis (Van den Berg and Groendijk-Wilders 1999) were investigated, supplemented by other accessions which became available through the CGN (Centre for Genetic Resources, the Netherlands). Although some new material of *S. capsicibaccatum* and *S. circaeifolium* was collected subsequently (Spooner et al. 1994) none of these collections passed quarantine in time for analysis. Table 1a summarises the 13 accessions of ser. *Circaeifolia* and two other taxa (*S. acaule* Bitter and *S. brevicaule* Bitter) which were examined in the AFLP analysis. In 1999 material of *S. soestii*, which was grown by the CGN in Wageningen, was analysed by Keygene n.v. and added to the dataset. In the AFLP analysis one to five genotypes per accession were studied, and the genotype was the Operational Taxonomic Unit (OTU).

A separate RAPD dataset of all the taxa of ser. *Circaeifolia* available in the U.S.A. (Potato Introduction Station at Sturgeon Bay) was generated. For the RAPD analysis DNA of five plants was mixed and in this case the OTU consisted of the pool of five genotypes. Only two accessions were shared between the AFLP and RAPD study. Besides the species of ser. *Circaeifolia* other species of different series in sect. *Petota* and one species of sect. *Etuberosum* were analysed in the RAPD analysis (Table 1b).

All plant material was grown in a greenhouse except one accession of *S. soestii*, which has been analysed from in vitro material because of the lack of seeds in the genebanks in The Netherlands and U.S.A.

Methods

RAPD procedure

The RAPD amplification and scoring procedures followed Miller and Spooner (1999), except the use of some different RAPD 10-mer primers. In this experiment 27 10-mer primers (Operon Technologies, Alameda, California) were reliably scored: A2, A4, A12,

A15, A16, A18, B14, D20, E3, E18, M2, Q1, R7, S3, S7, T1, AA2, AA10, AA14, AA17, AA20, AC5, AE5, AL18, AN15, AO1, AR16.

AFLP procedure

The AFLP-procedures followed Kardolus et al. (1998) with some modifications. The dilution of the ligation mixture was 10-fold, and after pre-amplification the first PCR product was diluted 50-fold. The gel system used was a 0.35 mm sequence system (GIBCO BRL, USA). The autoradiograms were exposed 2-10 days to visualise the AFLP fragments. The following three primer combinations were used: E+AAC/M+CAC, E+AAC/M+CAG and E+ACA/M+CAC.

Data analysis

The presence or absence of RAPD and AFLP bands was scored on the gels and the autoradiograms, respectively, and transferred into a 1 (present) and 0 (absent) matrix over all OTUs. Band intensity differences were not scored.

Phenetic analyses were performed with the program NTSYS-PC, version 2.01 (Rohlf 1999). Using the procedure SIMQUAL, similarities between OTUs were calculated with three different similarity coefficients: Simple Matching, Jaccard, and Dice coefficient. The OTUs were clustered with the procedure SAHN using three different clustering methods: UPGMA, WPGMA, and Single Linkage. Phenograms were generated with the TREE program of NTSYS and the goodness of the fit was estimated by calculating the cophenetic correlation coefficient. The data matrices were also phenetically analysed with TREECON 1.2 for Windows (Van de Peer and De Wachter 1994), using Nei and Li's distance coefficient and the neighbour-joining (NJ) and UPGMA algorithms, with 1000 bootstrap repeats to test the reliability of the branches in the tree. For the AFLP analysis trees were calculated for each primer combination separately as well as for the combined primer combinations. Cladistic analyses were performed using the program Hennig86 (Farris 1988).

Results and Discussion

General

The RAPD data consisted of 100 bands. In the AFLP analysis 408 fragments were generated using 3 primer combinations. Several studies where RAPD and AFLP data were compared (Powell et al. 1996; Milbourne et al. 1997) showed a higher marker index (a measure of the efficiency of the used marker) for AFLPs, mainly due to the higher number of loci investigated. Generally the AFLP bands were much easier to score than the RAPD bands. In a study on the reproducibility of RAPD, AFLP and SSR markers (Jones et al. 1997) the AFLP profiles showed extremely high reproducibility compared to that of the other markers. In our study, therefore, AFLP results might be more reliable than those of RAPDs. A comparison in the present study is, however, complicated by the fact that the investigated material is different for the two molecular approaches used (see Table 1a and 1b).

TABLE 1a. *Solanum* material examined in the AFLP study.

Code	Species	Source[1]	Collector[2]	Origin	Latitude	Longitude	[3]#GT AFLP
acl	*S. acaule* subsp. *acaule*	C17924	HOF 1870	Argentina	22°15'S	65°40'W	1
brc	*S. brevicaule*	C18030	EBS 2346A[4]	Bolivia	-	-	1
cap1	*S. capsicibaccatum*	C18254	HHA 6510	Bolivia	18°36' S	64°07' W	4
cap2	*S. capsicibaccatum*	C18268	HHA 6702	Bolivia	Coch.	Quillacollo	5
cap3	*S. capsicibaccatum*	C18291	EBS 2320[4]	-	-	-	2
cap4	*S. capsicibaccatum*	C18297	GND s.n.	Bolivia	-	-	3
cap5	*S. capsicibaccatum*	B32676	HHA 6469 x 6486	Bolivia	Coch.	-	3
cap6	*S. capsicibaccatum*	C18265	HHA 6561 x 6486	Bolivia	Coch.	-	4
crc7	*S. circaeifolium* subsp. *circaeifolium*	B27058	VSOA 62	Bolivia	15°40' S	68°37' W	4/5
crc8	*S. circaeifolium* subsp. *circaeifolium*	C18133	VSOA 73	Bolivia	15°47' S	68°40' W	5
qum9	*S. circaeifolium* subsp. *quimense*	C18127	VSOA 24	Bolivia	17°03' S	67°17' W	4
qum10	*S. circaeifolium* subsp. *quimense*	C18128	VSOA 30	Bolivia	16°50' S	67°16' W	5
qum11	*S. circaeifolium* subsp. *quimense*	C18158	VSA 182 + 183	Bolivia	17°03' S	67°18' W	5
sst 1[5]	*S. soestii*	B63075	SGV 6722	Bolivia	16°56' S	67°11' W	2
sst 2[5]	*S. soestii*	B63077	SGV 6724	Bolivia	16°56' S	67°11 W	1

[1] B- Braunschweig Genetic Resources Collection, Germany; C- Center for Genetic Resources, The Netherlands
[2] Collector: EBS- Edwin Bauer Sortiment; GND- Gandarillas; HHA- Hawkes, Hjerting and Aviles; HOF- Hoffman; SGV- Spooner, Garcia and Van den Berg; VSA- Van Soest and Alarcon; VSOA- Van Soest, Okada and Alarcon
[3] Number of genotypes studied with AFLP
[4] Donornumber, no collectionnumber available
[5] Analysed by Keygene n.v.

In the AFLP analysis the cophenetic correlation coefficients of the phenograms constructed using three different similarity coefficients (SM, Jaccard and Dice) and various clustering methods (UPGMA, WPGMA and Single Linkage) were compared. The UPGMA method with Jaccard's coefficient gave consistently the highest cophenetic correlation coefficients, with values between 0.974 and 0.987, which means a very good fit. This goodness of fit indicates that the phenograms faithfully reflect the distances in the similarity matrices. The UPGMA/Jaccard's coefficient combination was also found to produce high cophenetic correlation coefficients in recent AFLP work on the wild relatives of *Solanum melongena* L. (Mace et al. 1999a) and on the genera *Datura* L. and *Brugmansia* Pers. (Mace et al. 1999b). The cophenetic correlation values of the other similarity coefficients and clustering methods were also very high, however, with a lowest value of 0.956.

Notwithstanding the inherent problems of phylogenetic analyses of these kinds of molecular datasets, cladograms were produced using the Hennig86 program in order to compare the tree topologies resulting from phenetic and cladistic analyses. In most

cases the topology of the cladograms was similar to those of the phenograms, as was also found in an AFLP study on other representatives of sect. *Petota* (Kardolus 1998).

TABLE 1b. Solanum material in the RAPD study.

Code	Species	Series[1]	Source[2]	Collector[3]	Origin	Latitude	Longitude
brd180	*S. brevidens*	ETU	PI558180	SCL 4539	Argen-tina	40°51'S	71°33'W
buk155	*S. bukasovii*	TBR	PI414155	HHCH 5225	Peru	13°39'S	73°23'W
blb196	*S. bulbocastanum*	BUL	PI275196	HAW 1593	Mexico	17°30'N	96°27'W
cap036	*S. capsicibaccatum*	CIR	PI210036	HJT 1638	Bolivia	17°25' S	66°13' W
cap120	*S. capsicibaccatum*	CIR	PI498120	HHA 6510	Bolivia	18°36'S	64°07'W
cap458	*S. capsicibaccatum*	CIR	PI473458	OCH 11915	Bolivia	17°02'S	67°15'W
cap560	*S. capsicibaccatum*	CIR	PI205560	GND s.n.	Bolivia	Coch.	-
cph213	*S. cardiophyllum* subsp. *ehrenbergii*	PIN	PI275213	HAW 1428	Mexico	20°36'N	100°23'W
crc461	*S. circaeifolium* subsp. *circaeifolium*	CIR	PI473461	OCH 11909	Bolivia	15°47' S	68°40' W
qum116	*S. circaeifolium* subsp. *quimense*	CIR	PI498116	HHA 6532	Bolivia	17°46' S	64°41' W
qum117	*S. circaeifolium* subsp. *quimense*	CIR	PI498117	HHA 6578	Bolivia	17°07' S	66°56' W
qum118	*S. circaeifolium* subsp. *quimense*	CIR	PI498118	HHA 6581	Bolivia	17°07' S	66°56' W
qum119	*S. circaeifolium* subsp. *quimense*	CIR	PI498119	HHA 6588	Bolivia	17°12'S	66°53'W
qum973	*S. circaeifolium* subsp. *quimense*	CIR	PI545973	HOHL 217	Bolivia	17°02' S	67°15' W
qum974	*S. circaeifolium* subsp. *quimense*	CIR	PI545974	HOHL 226	Bolivia	17°04'S	67°16'W
hyp477	*S. hypacrarthrum*	PIU	PI473477	OCH 11607	Bolivia	11°25'S	76°38'W
ktz360	*S. kurtzianum*	TBR	PI498360	HOF 1758 x 1759	Argen-tina	32°51'S	68°49'W
mlt055	*S. multidissectum*	TBR	PI210055	HJT 1583	Peru	14°10'S	71°06'W
pnt766	*S. pinnatisectum*	PIN	PI347766	TRN 205A	Mexico	21°07'N	101°40'W
sst6	*S. soestii*	CIR		SGV 6722	Bolivia	16°56' S	67°11' W
sst46	*S. soestii*	CIR		SGV 6719	Bolivia	16°57' S	67°11 W
sst56	*S. soestii*	CIR		SGV 6724	Bolivia	16°56' S	67°11 W

[1] Series: BUL- Bulbocastana; CIR- Circaeifolia; ETU- Etuberosa; PIN- Pinnatisecta; PIU- Piurana; TBR- Tuberosa.
[2] PI- Plant Introduction Station, USA (Bamberg et al. 1996).
[3] Collector: GND- Gandarillas; HAW- Hawkes; HHA- Hawkes, Hjerting and Aviles; HHCH- Hawkes, Hjerting, Cribb and Huaman; HJT- Hjerting; HOF- Hoffman; HOHL- Hawkes, Okada, Hermsen and Landeo; OCH- Ochoa; SCL- Spooner and Clausen ; SGV- Spooner, Garcia and Van den Berg; TRN- Tarn.

RAPDs

In all analyses the representatives of ser. *Circaeifolia* clustered in a group separate from the species from other unrelated series. The bootstrap value for the ser. *Circaeifolia* branch is very high (91% in the NJ and 96 % in the UPGMA tree, respectively; Fig. 1), but those of the connections between the outgroup species are rather low with exception of the clustering of *S. bukasovii* Juz. with *S. multidissectum* Hawkes (bootstrap value 97%) and the position of *S. bulbocastanum* Dunal as an extreme outlier (supported by 100% bootstrap). So, although the series appears to be a well-defined group, its relationships to the other investigated species is not clear.

Within ser. *Circaeifolia* several clusters are formed but, except for a cluster of all three accessions of *S. soestii*, the clusters do not contain all the representatives of a taxon, but either subsets of a taxon or a mix of different taxa. Notably, in the *S. soestii* branch the two representatives of the accession 'sst6' are less similar to each other than one of them is to the other two accessions ('sst46', 'sst56') of this taxon.

No complete separation is present between *S. circaeifolium* subsp. *quimense* and *S. capsicibaccatum*, while the position of *S. circaeifolium* subsp. *circaeifolium* is difficult to interpret since only one accession was included. These results only weakly support the taxonomic status of the 4 proposed taxa within the series, except for the distinction of *S. soestii*, and largely agree with the morphological analysis of Van den Berg and Groendijk-Wilders (1999) where especially *S. circaeifolium* subsp. *quimense* and *S. capsicibaccatum* proved to be difficult to distinguish from each other.

AFLPs

In the AFLP analysis the primer combination E+AAC/M+CAC generated 139 scoreable bands, the primer combination E+ACA/M+CAC 164 scoreable bands and the combination E+AAC/M+CAG 105 scoreable bands. The phenograms resulting from the NTSYS analyses of separate primer combinations showed the same major groups as those formed with two or all three primer combinations combined.

Furthermore, accessions cluster according to the taxa to which they were assigned with very high bootstrap values for those clusters (mostly between 90 and 100%), and individual plants mostly cluster in their accession, be it with lower bootstrap values. Thus, the AFLP data show a high degree of hierarchical structure, indicating the possibility of a taxonomic interpretation of the results.

The phenogram generated from the primer combination E+ACA/M+CAC with the UPGMA method and Jaccard's coefficient (Fig. 2) showed the highest cophenetic correlation coefficient: 0.979. The phenogram based on the combination of all three primercombinations (Fig. 3) could include only one of the *S. soestii* accessions and lacks one of the *S. circaeifolium* subsp. *circaeifolium* accessions.

S. acaule Bitter and *S. brevicaule* Bitter were used as species outside the ser. *Circaeifolia*. The cluster of these taxa display a bootstrap value of 100% and they are joined to the representatives of ser. *Circaeifolia* at a low level of similarity (0.17 using Jaccard's coefficient) indicating the relatively distant relationship of ser. *Circaeifolia* to these representatives of the rest of sect. *Petota*. The same result was found by Kardolus (1998), who studied 171 accessions of *Solanum* species, mostly of sect. *Petota*, with AFLP markers and found that the two studied OTUs of *S. circaeifolium* did not group in the 'potato cluster' which encompass the wild tuber-bearing species that are allied to the cultivated potato. All the species that fell outside this 'potato cluster' have an endosperm balance number (EBN) of one (Hawkes 1990).

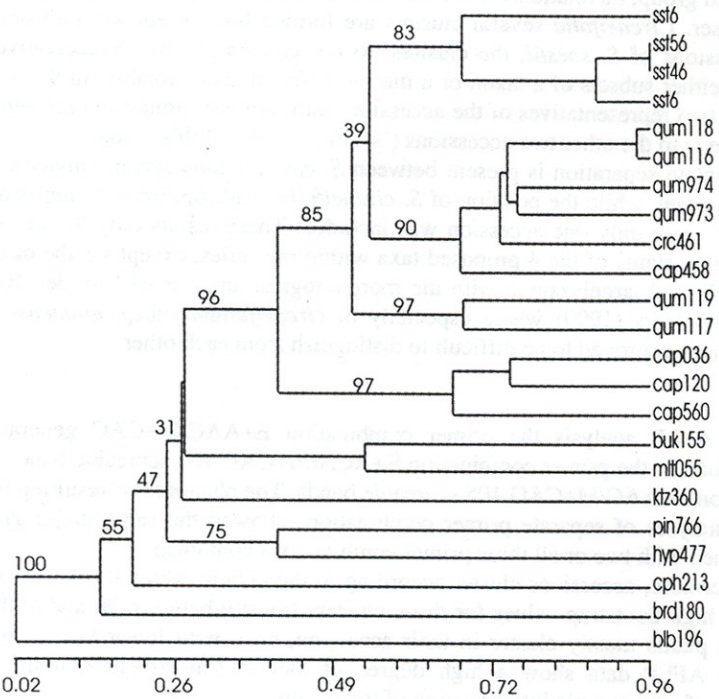

FIGURE 1. RAPD results, r=0.979

The accessions of ser. *Circaeifolia* in this study are grouped in four well distinguished branches, each branch comprising all the representatives of only one taxon, and showing a very high bootstrap value (98-100%, see Fig. 2). Using Jaccard's coefficient as a measure of similarity, individual plants cluster in their accessions at levels around 0.8, while the different accessions of a taxon cluster at levels around 0.6. The members of ser. *Circaeifolia* cluster at levels between 0.3 and 0.4, while the outgroups connect at levels around 0.2. In an AFLP analysis of the tribe Datureae (Mace et al. 1999b) the *Brugmansia* species formed a distinct cluster linked with the herbaceous *Datura* species at a level of 0.2. The similarity level of the species within both the *Datura* and the *Brugmansia* cluster tends to be as high as 0.8, while the outgroup, the genus *Atropa* L., is joined to the Datureae at a level of around 0.12. The *Circaeifolia* AFLP data show a comparable pattern and the level of similarity based on Jaccard's coefficient gives reliable information on group membership.

Mace et al. (1999a) suggested using a cut off point of 50 % similarity and discuss the four main clusters that are then apparent in the *Solanum melongena* complex. Applying this 50% cut off point to our *Circaeifolia* data the four clusters mentioned above, each representing a taxon, are apparent.

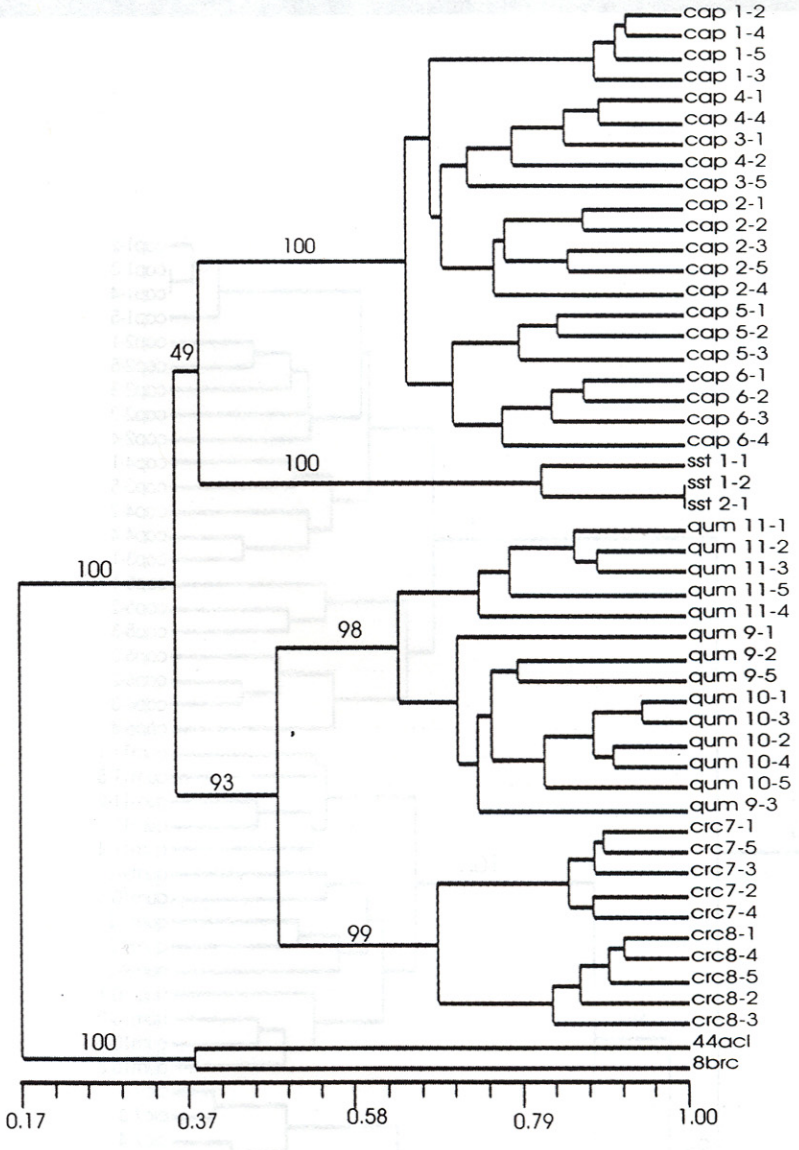

FIGURE 2. AFLP results primer combination E+ACA/M+CAC, r=0.979

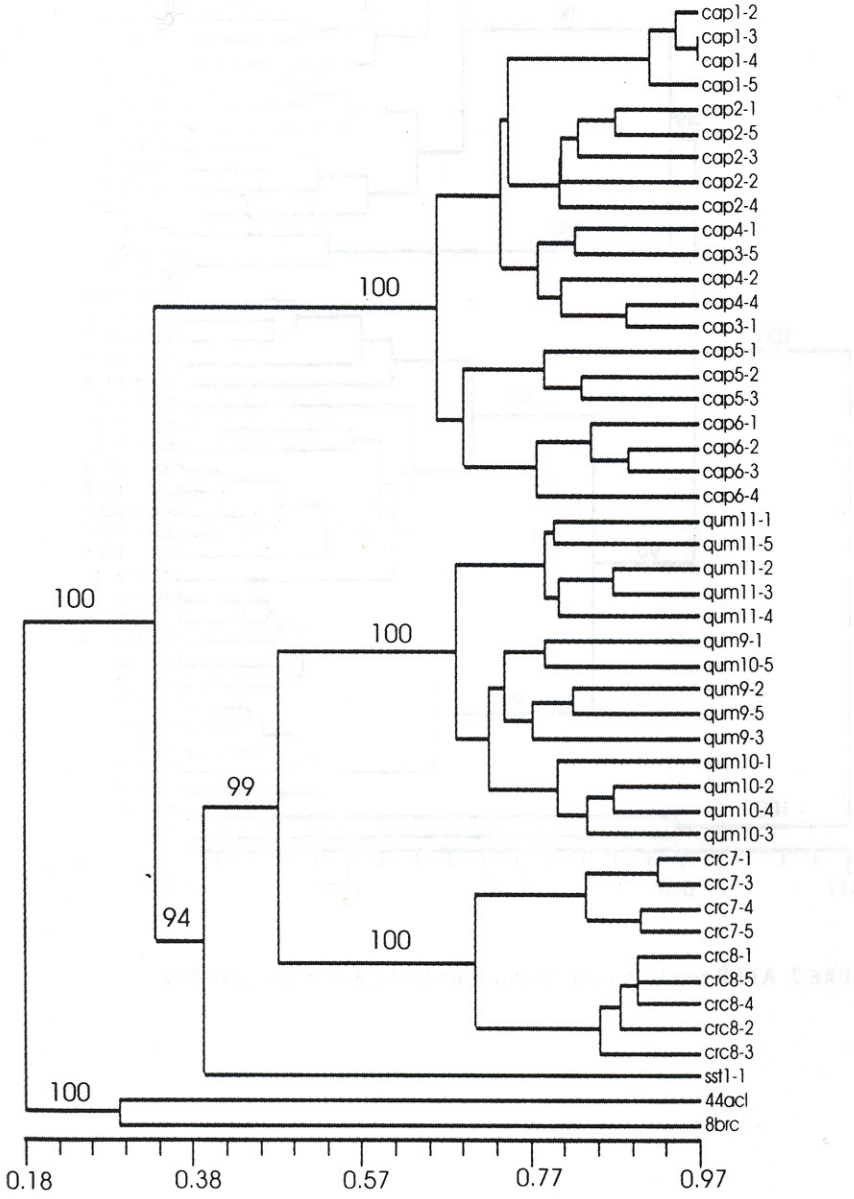

FIGURE 3. AFLP results three primer combinations, r=0.991

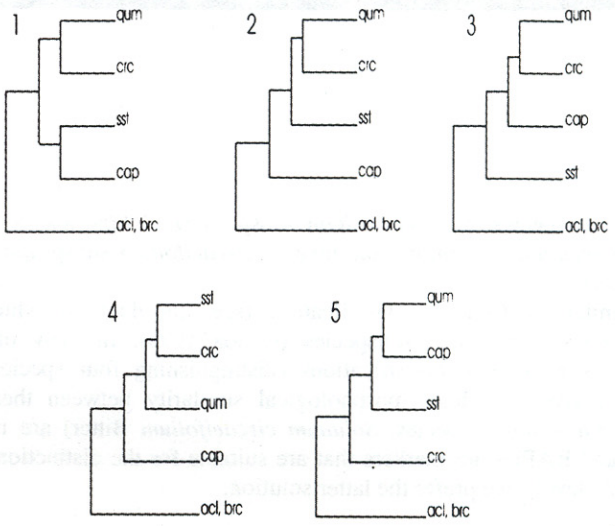

FIGURE 4. Tree topologies derived from the cluster analyses of the AFLP data using different algorithms and distance measures

From the phenograms resulting from the cluster analyses and the cladograms resulting from the phylogenetic analyses five different tree topologies can be derived (Fig. 4). Three of these (nrs. 1-3) are very similar, differing only in the relative positions of *S. capsicibaccatum* and *S. soestii*. Topology nr. 1 has the most support (it was found 25 times out of the 40 performed analyses), while topologies nr. 4 and nr. 5 occur only rarely (twice and once, respectively). The topology most often encountered (topology nr. 1) shows *S. circaeifolium* subsp. *quimense* and *S. circaeifolium* subsp. *circaeifolium* grouping together, with *S. capsicibaccatum* and *S. soestii* sharing another branch. This connection between *S. circaeifolium* subsp. *quimense* and *S. circaeifolium* subsp. *circaeifolium* is also present in topologies nr. 2 and nr. 3.

Thus Hawkes' classification of ser. *Circaeifolia* containing three species with *S. circaeifolium* subsp. *quimense* and *S. circaeifolium* subsp. *circaeifolium* as two subspecies within *S. circaeifolium* is supported by the majority of the results of the AFLP analysis. However, although the four entities are clearly distinguished, the way these four clusters are interconnected in the dendrograms is less stable when comparing the results of different primer combinations, different distance measures, and different cluster algorithms. The bootstrap support for the interconnections between clusters is generally weaker than those of the clusters themselves.

The results of the previous morphological study (Van den Berg and Groendijk-Wilders 1999) indicated that of the four presumed taxa *S. circaeifolium* subsp. *circaeifolium* and *S. soestii* were clearly distinct but *S. circaeifolium* subsp. *quimense* and *S. capsicibaccatum* displayed much overlap. A classification based on these results would recognize only two species, *S. soestii* and *S. circaeifolium*, dividing the latter

species in two subspecies: *S. circaeifolium* subsp. *circaeifolium* and *S. circaeifolium* subsp. *capsicibaccatum* (combining the taxa *S. circaeifolium* subsp. *quimense* and *S. capsicibaccatum*).

This is similar to Ochoa's classification (see Introduction) which, however, employed varieties rather than subspecies (Ochoa 1990). In view of the present molecular results alternative classifications (distinguishing four species within ser. *Circaeifolia* or, given the high morphological similarity between these taxa, four subspecies within a single species, *Solanum circaeifolium* Bitter) are not ruled out. Since AFLPs and RAPDs are markers that are suitable for the distinction of groups at lower taxonomic levels, we prefer the latter solution.

References

Bamberg, J.B., Martin, M.W., Schartner, J.J. and Spooner, D.M. 1996. Inventory of tuber-bearing *Solanum* species. Catalog of potato germplasm. Potato Introduction Station, NRSP-6, Sturgeon Bay, WI, U.S.A.

Demeke, T., Kawchuk, L.M. and Lynch, D.R. 1993. Identification of potato cultivars and clonal variants by random amplified polymorphic DNA analysis. Am. Potato J. 70: 561-570.

Farris, J.S. 1988. Hennig 86 reference, version 1.5. University of Stoney Brook, New York.

Hawkes, J.G. 1990. The potato: Evolution, biodiversity and genetic resources. Belhaven Press, London.

Hawkes, J.G. and Hjerting, J.P. 1989. The potatoes of Bolivia: their breeding value and evolutionary relationships. Oxford University Press, Oxford.

Jones, C.J., Edwards, K.J., Castaglione, S., Winfield, M.O., Sala, F., Van de Wiel, C., Bredemeijer, G., Vosman, B., Matthes, M., Daly, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Malcevschi, A., Marmiroli, N., Aert, R., Volckaert, G., Rueda, J., Linacero, R., Vazquez, A. and Karp, A. 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol. Breed. 3: 381-390.

Kardolus, J.P. 1998. A biosystematic analysis of *Solanum acaule*. Ph.D. thesis, Wageningen.

Kardolus, J.P., Van Eck, H.J. and Van den Berg, R.G. 1998. The potential of AFLPs in biosystematics: a first application in *Solanum* taxonomy (Solanacaeae). Plant Syst. Evol. 210: 87-103.

Mace, E.S., Lester, R.N. and Gebhardt, C.G. 1999a. AFLP analysis of genetic relationships among the cultivated eggplant, *Solanum melongena* L., and wild relatives (Solanaceae). Theor. Appl. Genet. 99: 626-633.

Mace, E.S., Gebhardt, C.G. and Lester, R.N. 1999b. AFLP analysis of genetic relationships in the tribe Datureae (Solanaceae). Theor. Appl. Genet. 34: 634-641.

Milbourne, D., Meyer, R., Bradshaw, J.E., Baird, E., Bonar, N., Provan, J., Powell, W. and Waugh, R. 1997. Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. Mol. Breed. 3: 127-136.

Miller, J.T. and Spooner, D.M. 1999. Collapse of species boundaries in the wild potato *Solanum brevicaule* complex (Solanaceae, *Solanum* sect. *Petota*): molecular data. Plant Syst. Evol. 214: 103-130.

Ochoa, C.M. 1990. The potatoes of South America: Bolivia. Cambridge University Press, Cambridge.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite markers for germplasm analysis). Mol. Breed. 2: 225-238.

Rohlf, F.J. 1999. NTSYS-PC. Numerical Taxonomy and multivariate analysis system. Version 2.01. Applied Biostatistics Inc., New York.

Sokal R.R. and Sneath, R.R. 1963. Principles of numerical taxonomy. Freeman, San Francisco.

Spooner, D.M., Tivang, J., Nienhuis, J., Miller, J.T., Douches, D.S. and Contreras-M., A. 1996. Comparison of four molecular markers in measuring relationships among the wild potato relatives *Solanum* section *Etuberosum* (subgenus *Potatoe*). Theor. Appl. Genet. 92: 532-540.

Spooner, D.M., Van den Berg, R.G. and Bamberg, J.B. 1995. Examination of species boundaries of *Solanum* series *Demissa* and potentially related species in series *Acaulia* and series *Tuberosa* (sect. *Petota*). Syst. Bot. 20: 295-314.

Spooner, D.M., Van den Berg, R.G., Garcia, W. and Ugarte, M.L. 1994. Bolivia potato germplasm collecting expeditions 1993, 1994: Taxonomy and new germplasm resources. Euphytica 79: 137-148.

Stuessy, T.F. 1990. Plant Taxonomy: the systematic evaluation of comparative data. Columbia University Press, New York.

Van de Peer, Y. and De Wachter, R. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Aplic. Biosci. 10: 569-570.

Van den Berg, R.G. and Groendijk-Wilders, N. 1999. Numerical analysis of the taxa of series *Circaeifolia* (*Solanum* section *Petota*). In: M. Nee, D.E. Symon, R.N. Lester and J.P. Jessop (eds.), Solanaceae IV: Advances in Biology and Utilization, pp. 213-226. The Royal Botanic Gardens, Kew.

Van den Berg, R.G., Groendijk-Wilders, N. and Kardolus, J.P. 1996. The wild ancestors of cultivated potato: the *brevicaule*-complex. Acta Bot. Neerl. 45: 157-171.

Van den Berg, R.G., Miller, J.T., Ugarte, M.L., Kardolus, J.P., Villand, J., Nienhuis, J. and Spooner, D.M. 1998. Collapse of morphological species in the wild potato *Solanum brevicaule* complex (Solanacaeae: sect. *Petota*). Am. J. Bot. 85: 92-109.