A biosystematic analysis of *Solanum acaule*

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A biosystematic analysis of *Solanum acaule*

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Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit Wageningen,
dr. C.M. Karssen,
in het openbaar te verdedigen
op woensdag 11 februari 1998
des namiddags te vier uur in de Aula.

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Kardolus, J.P.

A biosystematic analysis of *Solanum acaule* /
J.P. Kardolus. - [S.l.: s.n.]. Fig., Tab.
Thesis Wageningen, - with ref. - with summary in Dutch.
ISBN 90-5485-829-X

Subject headings:
Solanum / AFLP / evolution / potato / taxonomy

Druk:
Ponsen & Looijen BV, Wageningen

STELLINGEN

1 *Solanum acaule* en *Solanum demissum* zijn niet onafhankelijk van elkaar ontstaan, zoals door aardappeltaxonomen tot nu toe werd verondersteld, maar zijn juist genetisch nauw verwant.

(Ochoa C.M. (1990) The potatoes of South America: Bolivia: p.32; Ugent D. (1981) Phytologia 48: 85-95; dit proefschrift)

2 De DNA merker-techniek AFLP is een efficiënt en flexibel gereedschap, en zal steeds vaker gekozen worden voor studies in de genetica, moleculaire biologie en taxonomie.

(Breyne P. *et al.* (1997) Applications of AFLP in plant breeding, molecular biology and genetics. Belg. Journ. Bot. 129: 107-117; dit proefschrift)

3 Voor het onderzoek naar hybride-soortsvorming zijn nog geen geschikte fylogenie reconstructie-programma's beschikbaar.

(dit proefschrift)

4 De genetische variabiliteit van de zelfbevruchter *Solanum acaule* blijft het best bewaard door, indien nodig, met name die genenbank-collecties aan te houden die verzameld zijn aan de randen van het verspreidingsgebied.

(dit proefschrift)

5 Een beter inzicht in de biosystematische relaties van wilde soorten zal veredelaars helpen bij de planning van hun veredelingsprogramma's, waarbij verwant materiaal gekozen of juist vermeden kan worden.

(Spooner D.M & Van den Berg R.G. (1992) Gen. Res. Crop Evol. 39: 23-37)

6 De grote ideeën liggen vaak besloten in combinaties van kennisvelden.

(Paul Crutzen, winnaar Nobelprijs, Volkskrant 9 december 1995)

7 Taxonomisch moeilijke groepen zijn biosystematisch het meest interessant.

8 'Human population with its egosystem is threatening virtually all ecosystems'.

(Nevling L.I. (1996), Ann. Missouri Bot. Gard. 83: 574-580)

9 De referentieprocedure van wetenschappelijke artikelen zou objectiever verlopen wanneer de auteurs van een manuscript anoniem blijven voor degene die het betreffende artikel refereert, in plaats van andersom.

10 Je kunt geen moeilijke vragen beantwoorden als je er niet over nagedacht hebt, omdat ze nog niet eerder gesteld zijn.

(Frank de Grave, televisie-programma NOVA, 1 juli 1996)

11 De isozym-techniek geeft relatief weinig datapunten in vergelijking met de AFLP-techniek, maar levert daarentegen wel grote hoeveelheden afwas op.

12 Een plantenveredelaar die werkt bij een vakgroep Plantentaxonomie, voelt zich regelmatig als een 'Englishman in New York'.

(naar Sting (1987), op de CD '...Nothing Like The Sun')

Stellingen behorende bij het proefschrift 'A biosystematic analysis of *Solanum acaule*'

Jouke P. Kardolus
Wageningen, 11 februari 1998

ABSTRACT

This thesis describes a biosystematic analysis of the allotetraploid species *Solanum acaule*. *Solanum acaule* is one of the circa 200 tuber-bearing *Solanum* species (*Solanum* sect. *Petota*, Solanaceae) and often used in plant breeding programs for its disease resistances and frost tolerance. This species has the widest distribution of all wild potato species, from Ecuador into Argentina, and occurs at altitudes up to 4500 meter in the Andes. Together with the hexaploid *S. albicans* it forms the polyploid taxonomic series *Acaulia*. This study focuses on the hybrid speciation of series *Acaulia*. *Solanum acaule* shows morphological similarities with species of series *Megistacroloba* and *Tuberosa*, which have been hypothesized as possible ancestors. A phylogenetic study is conducted to unravel evolutionary relationships of *Solanum acaule* and its putative progenitors. Several morphological aspects of series *Acaulia* and related species are examined, and combined with a study of molecular markers. The thesis is completed with the proposal of a new subspecies of *Solanum acaule*.

In a multivariate morphological analysis the boundaries of series *Acaulia* are determined and variation within the series is reconsidered. Inflorescence architectural traits, such as the dimensions of pedicels and corollas, are discussed in relation to the taxonomy of *Solanum* sect. *Petota* species, their habitat and breeding behavior. An unrecorded inflorescence type, which can be characterized as a monochasium with a strongly reduced peduncle and one or two 'extra' flowers in the axil of the subtending leaf, is described in series *Acaulia*. The taxonomy of *Solanum* sect. *Petota* often depends on quantitative characters. These are often variable, causing difficulties in species classification. However, series *Acaulia* is characterized by a qualitative character, i.e. a modified or even completely absent pedicel articulation. Normally, unfertilized flowers separate at the floral abscission zone which is located at this articulation. In an anatomical study this zone is proven to be absent in non-articulated pedicels of *Solanum acaule*. In *S. albicans* this zone is modified. These special features of pedicel articulation in series *Acaulia* are discussed in relation to the 'jointless'-mutations in tomato.

The AFLP™ molecular marker-technique is for the first time applied in *Solanum* taxonomy. Highly informative DNA fingerprints are produced with the AFLP-technique. Classifications based on AFLPs are generally in agreement with current taxonomic opinions, but also new evolutionary insights are revealed. The results of the present AFLP study casts doubt on the status of certain taxonomic series, and the classification of particular species into these series. Based on the neighbor-joining analysis of the AFLP data it is concluded that the hexaploid *Solanum demissum* (from Mexico) is likely to be closely related to both the South American series *Acaulia* and the Central American polyploid series *Longipedicellata*, and feasibly a hybrid descendant of these series. Series *Acaulia* itself is a monophyletic group in the cladistic analyses, and it is most advanced in the cladograms. In addition to the AFLP study, an isozyme analysis has been conducted on series *Acaulia* and its closest relatives. Based on both AFLP and isozyme data, it is concluded that series *Acaulia* is indeed most closely related to series *Tuberosa* and *Megistacroloba*. However, it is not a simple derivative of a hybridization event of species of these two series, but more probably one of its ancestors could have belonged to the series *Megistacroloba / Tuberosa* affiliation. The second ancestor that provided the unique characters to series *Acaulia* is still unknown, and possibly extinct.

KEYWORDS: *Solanum acaule*, section *Petota*, series *Acaulia*, Solanaceae; AFLP, allopolyploidy, Andes, biosystematics, DNA fingerprinting, floral abscission, hybridization, inflorescence, morphology, numerical taxonomy, phylogeny, potato, speciation, taxonomy

CONTENTS

GENERAL INTRODUCTION

THE POTATO

Potato is the fourth starch crop in the world, after wheat, rice and maize, with an annual world production of approx. 300 × 10^6 metric tons (FAO 1995). The cultivated potato is taxonomically a single species, *Solanum tuberosum* L. belonging to the nightshade family, the Solanaceae. Other members of this economic important family are genera such as *Capsicum* L. (chili peppers), *Petunia* Juss., *Physalis* L. and *Nicotiana* L. (tobacco). The large genus *Solanum*, that comprises an estimated number of 1100 (D'Arcy 1991) to 2000 species (Hawkes 1972), includes crops like the egg-plant (*S. melongena* L.), the pepino (*S. muricatum* Ait.), the tomato (*S. lycopersicum* L.), and various ornamentals. The tomato is often placed in a separate genus, as *Lycopersicon esculentum* Mill., but recent studies have confirmed its classification in *Solanum* (Child 1990, Spooner *et al.* 1993).

WILD RELATIVES

Solanum tuberosum is one of the circa 200 tuber-bearing *Solanum* species (*Solanum* section *Petota* Dumort.). Next to *S. tuberosum*, six other species are cultivated. These are mostly grown in the Andes in South America, the center of origin of the potato. The other tuber-bearing species are wild, most of them have a weedy character. In addressing the question of the origin of the cultivated potato, several wild species have been hypothesized to be directly related to cultivated potatoes. Members of the 'brevicaule-complex', a group of morphologically very similar wild species (Grun 1990, Van den Berg *et al.* 1996, 1998), have been named as closest relatives of the cultivated potato (Ochoa 1990, Van den Berg *et al.* 1996). Hawkes (1990) has explicitly identified *S. leptophyes* Bitter as the probable progenitor of the diploid cultigen *S. stenotomum* Juz. & Bukasov, which in its turn is ancestral to *S. tuberosum* ssp. *andigena*.

ECONOMIC RELEVANCE

A number of wild species are of interest for potato breeding because of their disease resistances and other agronomically interesting traits. However, the genetic resources of *Solanum* species have not been exploited exhaustively yet (Ross 1986, Janssen 1997). *Solanum acaule* Bitter, the main subject of this thesis, is one of the most often used wild species in plant breeding programs. *Solanum acaule* is known for its wide range of resistances (Bamberg *et al.* 1994), e.g. against Potato Virus X and Potato Leaf Roll Virus (Cockerham 1970, Kameraz *et al.* 1978), bacterial Ring Rot (Ishimura *et al.* 1994), and for its frost tolerance (Estrada 1980). *S. juzepczukii* Bukasov, a triploid cultigen grown in the Andes, has derived its frost tolerance most likely from *S. acaule* (Hawkes 1962, Schmiediche *et al.* 1982).

TAXONOMY OF *SOLANUM* SECTION *PETOTA*

In the recent treatment of Hawkes (1990) the tuber-bearing *Solanum* species are classified in nineteen series. Furthermore, Hawkes has included in section *Petota* two non-tuber-bearing series, series *Etuberosa* Juz. and series *Juglandifolia* (Rydb.) Hawkes. Recently these were raised to a higher level and classified as sections (Spooner *et al.* 1993). The series classification of Hawkes can be helpful to those who are interested in potatoes, but less acquainted with the taxonomy of this group, providing a basic insight in the species of section *Petota* and their phylogenetic relations. One would expect species within a series to be more affiliated to each other than to species that are classified in different series. Unfortunately, various examples have been presented lately (Spooner and Van den Berg 1992b, Spooner *et al.* 1995), and will be presented in this thesis (Chapters 4 and 5), that certain species belonging to different series are more related to each other than to the species of their own series. The classification into series is therefore not always a good predictor of mutual relationship.

SPECIES CONCEPTS

Also, the circumscription of species is problematic in this group. Many wild potato species are extremely variable in their characters and the main problem in taxonomic interpretations in sect. *Petota* is the lack of documentation of morphological variability within taxa (Spooner and van den Berg 1992a). Phenotypic plasticity necessitates a broad species concept encompassing the range of variability encountered. In contrast, many taxa in this group have been narrowly defined, ignoring the variability that causes overlap in the character-state ranges of presumably distinct entities.

Taxonomists working in this group have applied a typological species concept, where a distinction is made between representatives of so-called 'pure' species and deviating material that is interpreted as the result of hybridization mixing the species-characteristic features. A better alternative seems to be to acknowledge the variability within species that reflects ongoing speciation. This would imply the use of a polythetic species concept. In this concept the grouping depends on the greatest number of shared features, no single feature of which is essential to group membership or is sufficient to make an organism a member of the group (Sokal and Sneath 1963, Stuessy 1990). The use of this concept would also lead to the recognition of a lower total number of taxa than has been upheld up to now (Hawkes 1990).

POLYPLOID SPECIES

Hawkes (1990: Appendix II) has listed 38 taxa, on a total of 108 'better-known' tuber-bearing *Solanum* species, with a somatic ploidy level higher than diploid. In part, these are polyploid cytotypes of diploid (2n = 2x = 24 chromosomes) species, such as the tetraploid (4x) cytotype of *S. gourlayi* Hawkes. Some are triploid (3x) and pentaploid (5x) taxa, probably of recent hybrid origin, that do not reproduce sexually. In total 23 of the polyploids are well-established tetraploid or hexaploid species, without diploid representatives. So

about a quarter of the tuber-bearing *Solanum* species are polyploid biological species. For many other plant groups a similar or even higher percentage of polyploids is estimated (Averett 1980).

Hybridization is an important process in plant speciation and will only produce successfully new species if the hybrid reproduction is stabilized (Grant 1971). One method of stabilization is vegetative propagation. This may be accomplished by apomictic seed production or by specialized vegetative plant parts such as stolons and tubers. Triploid and pentaploid potatoes persist in this way. Mostly these are either cultivated taxa, such as the 3x species *S. juzepczukii* and *S. chaucha* Juz. & Bukasov, and the 5x *S. curtilobum* Juz. & Bukasov, or hybrids of wild and cultivated material (e.g. the pentaploid *S.* × *edinense* Berth., see Hawkes 1990: p. 197) that are probably of recent origin.

Another method of stabilization in hybrid reproduction is amphiploidy. Amphiploidy is a mode of speciation which involves the combination of the chromosome sets of the parental species. An advantage of amphiploidy is the combining of both homozygosity and heterozygosity. The amphiploid is homozygous as far as meiosis is concerned, and is therefore true-breeding, but it retains the gene systems of both parental species. Chromosomal sterility barriers in the hybrid, because of pairing problems of the homeologous chromosomes of both parents, are overcome by the doubling of the chromosome number. In a hypothetical allotetraploid species AABB a homologous chromosome pair exists of each chromosome type in each genome (Grant 1971).

Traditionally, the doubling of chromosomes has often been seen as a process taking place after the hybridization event. Harlan and De Wet (1975) have strongly opposed this idea of 'chromosome doubling' in the zygote, and explained that most polyploids arise by the functioning of unreduced gametes (2n-gametes). Harlan and De Wet (1975) have stated that genuine allopolyploids may be generated by the interspecific hybridization of autopolyploids. Another possibility would be the hybridization of a normal gamete of an autopolyploid with a 2n-gamete of a diploid. Den Nijs and Peloquin (1977) have indicated for *Solanum* sect. *Petota* the probability of polyploid evolution from the functioning of 2n-gametes.

ORIGIN OF *SOLANUM ACAULE*

The origin of the cultivated potato *S. tuberosum*, which is interpreted as an autotetraploid taxon (see Grant 1971), has attracted many studies. This thesis focuses on the origin of an allopolyploid potato species, *Solanum acaule*. The tetraploid *S. acaule* is classified in series *Acaulia* Juz., together with the hexaploid species *S. albicans* (Ochoa) Ochoa. They are both inbreeding species and distributed from Peru to Argentina, and also material from Ecuador has been cited recently (Spooner *et al.* 1992, Ochoa 1993). This Ecuadorian material is included in the present study. Series *Acaulia* occurs in the Andean region of South America at high altitudes (3000 to 4500 m). *S. acaule* has the widest distribution of all wild tuber-bearing species (Ochoa 1990).

Solanum acaule has often been declared to be an amphiploid or allotetraploid based on the bivalent pairing of chromosomes in the meiosis (Swaminathan and Howard 1953, Swaminathan 1954) and fixed heterozygosity, as was confirmed by isozyme studies (Cortés and Camadro 1989). Hybridization between two distinct species has been proposed, but

which species are involved is unknown (Hawkes 1990: p. 56). *Solanum acaule* shows morphological similarities with species classified in both series *Megistacroloba* Cárdenas & Hawkes and series *Tuberosa* (Rydb.) Hawkes, and its ancestors could belong to these groups as has been suggested in various papers (Ugent 1966, 1981, Kameraz *et al.* 1978, Chavez 1984, Hawkes 1990, Ochoa 1990). However, no phylogenetic study has been conducted to unravel such relations.

SCOPE OF THIS THESIS

The main objective of this study is to reveal the biosystematic relations of *Solanum acaule* with other tuber-bearing *Solanum* species. Before focusing on the evolutionary relations within this group, a multivariate analysis of mostly quantitative characters is presented in Chapter 1. In this study *S. acaule*, its closest relative *S. albicans* and species of other series are examined to determine the boundaries of series *Acaulia* and to reconsider the classification of the variation within series *Acaulia*.

Section *Petota* species often need to be keyed out on quantitative characters, like leaf dissection and pubescence density. As has been pointed out earlier in this introduction, the species are often quite variable. This frequently causes the problem of assigning particular plant material to the right species. However, some qualitative morphological characters are present in the group and these are worth a more detailed study. One of these is the 'absence of pedicel articulation' in series *Acaulia*. In Chapter 3 this character is examined anatomically. In Chapter 3 also the position of the articulation on the pedicel, which is used as a taxonomic character, is reconsidered. Similarly, in Chapter 2 the architecture of inflorescences in section *Petota* is studied. The results are discussed with regard to the breeding behavior and habitat of the studied taxa.

The feasibility of the molecular marker technique AFLP™ for biosystematic studies, with special emphasis on the potential of this technique for the taxonomic investigation of *Solanum* species, is studied in Chapter 4. In Chapter 5 the AFLP-technique is combined with an isozyme analysis. A large set of *Solanum* species has been investigated to provide a more detailed insight in the biosystematic relations of this group, with a focus on the evolution of the polyploid series *Acaulia*. Both in Chapter 4 and 5 phenetic and cladistic analysis methods are used and a comparison of both methods is given.

In Chapter 6 a new taxon is presented, that is classified as a new subspecies of *Solanum acaule*. This new subspecies is based on the combined morphological and molecular findings of the previous chapters of this thesis.

The thesis is completed with a synopsis of the main results, also presenting some general concluding remarks.

CHAPTER 1

Morphological variation within series *Acaulia*

ABSTRACT

Fifty-five genebank accessions of the species *Solanum acaule* and *S. albicans* have been morphologically examined. They were compared with 11 accessions of species from other series within *Solanum* sect. *Petota*, to verify series boundaries of *Acaulia*. Multivariate phenetic analyses indicate that series *Acaulia* is a well-defined group, except for a striking similarity between *S. albicans* and *S. demissum* (series *Demissa*) from Mexico. Within series *Acaulia* the tetraploid species *S. acaule* and the hexaploid *S. albicans* can be well separated. Plant material of *S. albicans* from Ecuador is morphologically discrete from material of this species from Peru and can probably be recognized as a new subspecies. Taxa within *S. acaule*, viz. subspecies *acaule*, *punae* and *aemulans*, are distinct entities, although some accessions of sspp. *acaule* and *aemulans* show overlap in certain characteristics. Results indicate that nine (out of 55) accessions of series *Acaulia* in this study had been misidentified in recent genebank inventories.

INTRODUCTION

Series *Acaulia* Juz. is a relatively well studied group. Especially the allotetraploid species *Solanum acaule* Bitter is well known to plant breeders for its virus and nematode resistance and frost-tolerance (Ross 1986, Ochoa 1990) and has therefore often been studied. Investigations have been made on crossability with other tuber-bearing *Solanum* species (Hermsen 1966, Hermsen and Ramanna 1969, Hawkes and Hjerting 1969, Okada 1973, Estrada 1984, Ochoa 1990), meiotic behavior (Swaminathan 1954, Hermsen 1966, Camadro *et al.* 1992), electrophoretic patterns (Camadro *et al.* 1992) and RFLP patterns (Hosaka and Spooner 1992).

Series *Acaulia* is geographically distributed from Ecuador to province La Rioja in Argentina (Ochoa 1990, Spooner *et al.* 1992). *Solanum acaule* is reported not to be directly related to any other species of wild potato, although there are some similarities with series *Megistacroloba* (Hawkes and Hjerting 1969, 1989). Morphologically, *S. acaule* bears a strong resemblance to *S. demissum* Lindley of series *Demissa* from Mexico, which is seen as its "ecological vicariad" (Hawkes and Hjerting 1969). Ochoa (1990) also does not explain the parallels between *S. demissum* and series *Acaulia* by a common evolutionary origin. He postulates that species in series *Megistacroloba* and *Tuberosa* may have played a role in the origin of *S. acaule*. However, recent RFLP data on nuclear DNA (Debener *et al.* 1990) indicate a correspondence of *S. acaule* with *S. demissum*. *Solanum megistacrolobum* Bitter appears to be less related to *S. acaule* with these data.

Within series *Acaulia* conflicting views on taxonomy still exist. Hawkes (1990) accepts two species, *S. acaule* and *S. albicans* (Ochoa) Ochoa. He divides *S. acaule* into three geographic subspecies: ssp. *punae* (Juz.) Hawkes & Hjerting (central Peru), ssp. *acaule*

(south Peru to province La Rioja, Argentina) and ssp. *aemulans* (Bitter & Wittm.) Hawkes & Hjerting (province La Rioja, Argentina). According to Okada and Clausen (1982), plants of ssp. *aemulans* from province Jujuy, Argentina, can be postulated as fertile hybrid derivatives of *S. acaule* × *S. megistacrolobum*. Consequently, Hawkes (1990) only recognizes material of province La Rioja, Argentina (type locality of ssp. *aemulans*) as ssp. *aemulans* and places previously identified ssp. *aemulans* material from province Jujuy now in ssp. *acaule*.

Ochoa (1990) also reports two species within the series: *S. acaule* and *S. albicans*. Within *S. acaule*, he recognizes two varieties: var. *acaule* and var. *aemulans*. He has placed ssp. *punae* in the synonymy of *S. acaule* var. *acaule*. Correll (1962) shared this view. Hawkes and Hjerting (1969), notwithstanding, state that sspp. *punae* and *aemulans*, both in greenhouse and field, kept their distinctive features, and are not habitat modifications of *S. acaule*.

Based on RFLP data, Hosaka and Spooner (1992) conclude that ssp. *punae* and ssp. *acaule* are indistinguishable. However, their RFLP data indicate that certain accessions of ssp. *punae* are clearly distinct from ssp. *acaule*, whereas some others fall within the variation of ssp. *acaule*. Misidentification of this material is suggested (Hosaka and Spooner 1992). They find regional differences between ssp. *aemulans* material of province Jujuy and of province La Rioja in Argentina. They also conclude that *S. albicans* should be treated as species, instead of a subspecies of *S. acaule*, as it was originally described by Ochoa.

These opinions indicate that as yet no consensus has been reached on the taxonomy of *S. acaule*. Morphological studies can contribute to elucidation of the taxonomical situation. This paper presents the results of a study on living plant material of series *Acaulia*. Morphological features are studied to define taxa within series *Acaulia*. The taxonomic treatment of Hawkes (1990) is followed in this text, although material of ssp. *aemulans* from province Jujuy, Argentina, is still considered to belong to this subspecies. The triploid hybrids *S.* × *indunii* Okada & Clausen and *S.* × *virsooi* Okada & Clausen have been placed in series *Acaulia* (Hawkes 1990). These hybrids of *S. acaule* with species from other series are not included in this study.

In the literature the following characters are used to distinguish between taxa within series *Acaulia* (Correll 1962, Hawkes and Hjerting 1969, Hawkes 1990):

1. Peduncle not forked; pedicel articulation generally not present, but if so, very high up, 2-3 mm below calyx; style not exserted; flowers violet-blue to purplish ..... *S. acaule*
- **2.** Plant very flat-rosetted, bearing long spreading hairs on stem, petiole and leaf rachis ..... *S. acaule* ssp. *punae*
- **2.** Plant with less flat rosettes, bearing short, crisped and sub-appressed hairs.
  - **3.** Pedicel articulation well-marked; terminal leaflet much larger than the 0-4 paired laterals which diminish rapidly towards leaf base; upper lateral leaflets broadly decurrent..... *S. acaule* ssp. *aemulans*
  - **3.** Pedicel articulation not well-marked, generally absent; terminal leaflet only slightly larger than the (2-)4-5(-7)paired laterals which do not diminish rapidly in size towards the leaf base; upper lateral leaflets not decurrent..... *S. acaule* ssp. *acaule*

1. Peduncle forked; pedicel articulation about 5-7 mm below calyx; style exserted 1.5-2.0 mm above anther column; flowers white ..... *S. albicans*

Plant material of series *Megistacroloba*, *Demissa*, *Tuberosa*, *Cuneoalata* and *Yungasensia* was examined to establish the degree of distinction from series *Acaulia*. Characters were analyzed with multivariate methods. These methods are suitable for resolving taxonomical problems within the group of tuber-bearing *Solanum* species (sect. *Petota*) (Van den Berg and Spooner 1992, Spooner and Van den Berg 1992b, Giannattasio and Spooner 1994).

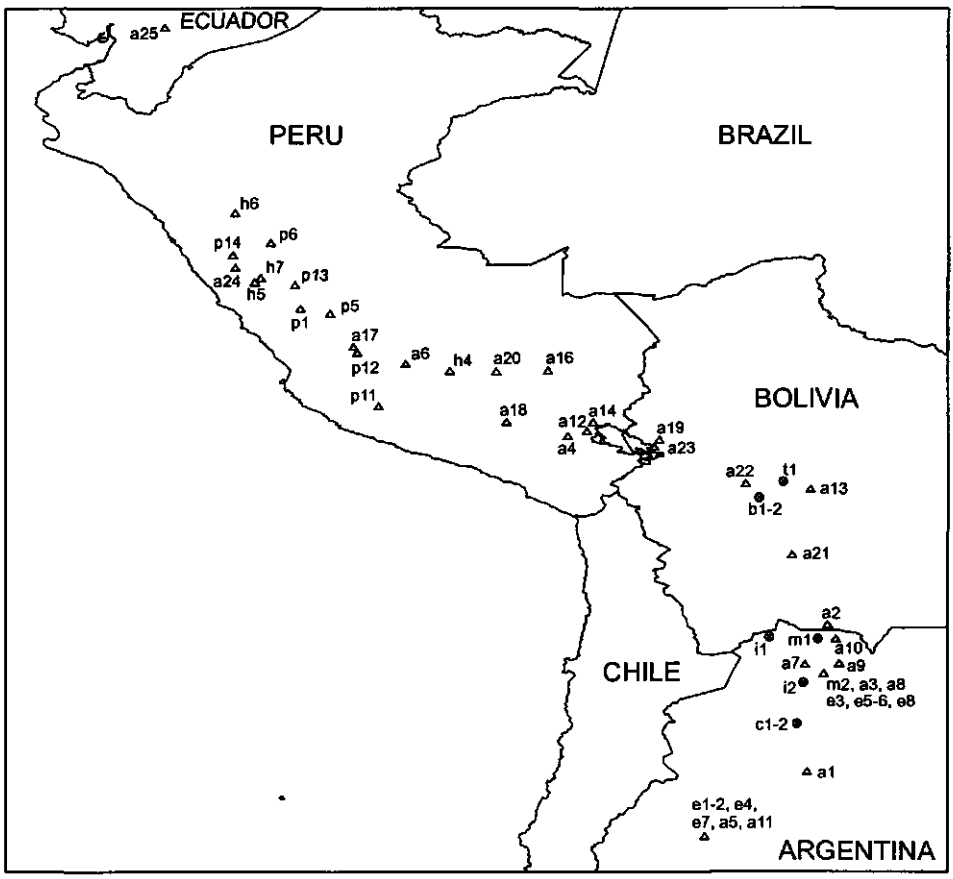


Figure 1. Geographic distribution of accessions.

MATERIALS AND METHODS

Plant material. In this study 55 genebank accessions of taxa within series *Acaulia* were examined (Table 1): *S. acaule* ssp. *acaule* (25 accessions), ssp. *punae* (15), ssp. *aemulans* (8) and *S. albicans* (7), and compared to a number of species from other series: *S. chacoense* Bitter (2) of series *Yungasensia*, *S. brevicaule* Bitter (2) of series *Tuberosa*, *S. demissum* (2) of series *Demissa*, *S. infundibuliforme* Philippi (2) of series *Cuneoalata*, *S. megistacrolobum* (2) and *S. toralapanum* Cárdenas & Hawkes (1) of series *Megistacroloba*. The geographic distribution of the accessions is, as far as known, mapped in figure 1, except for accessions of *S. demissum*, which were collected in Mexico.

All accessions were obtained from the Potato Introduction Station, National Research Program-6, Sturgeon Bay, Wisconsin, USA (Bamberg and Martin 1993). Seeds were planted in Sturgeon Bay in a greenhouse in early May, seedlings were transferred to peat pots in late May, and six individuals per accession were transplanted together in rows in a field plot in early June 1993. Identifications of these accessions have been provided in past years by J.G. Hawkes, J.P. Hjerting, C.M. Ochoa and K.A. Okada during visits to the Potato Introduction Station to inspect living representatives in field plots (Spooner and Bamberg 1991). The identification of the subspecies of *S. acaule* sometimes differed between taxonomists. Taxonomists did not consistently classify the same accession in different years and even in one year (Table 2). One accession, p14, was obviously a mixture of *S. acaule* ssp. *punae* and *S. albicans*, and was a priori split into two accessions: p14p (ssp. *punae*) and p14h (*S. albicans*).

Chromosome Numbers. Accessions a24 and a25 were checked for their chromosome number, because these two accessions are morphologically very similar to *S. albicans* in the field plot. A cell-spreading technique according to Pijnacker and Ferwerda (1984) was used.

Character Measurement. Thirty quantitative characters and four qualitative characters (stem anthocyanin intensity, presence/absence of pedicel articulation and two corolla colour characters) (Table 3) were determined in late August on flowering and fruit-bearing plants. The first three surviving plants per row were measured for all characters. Measurements of leaves were made on the largest leaf per plant. Means of the three plants are used as representative of each accession, the OTU (Operational Taxonomic Unit). Character LPA, length from calyx base to pedicel articulation, is only used in the univariate analyses, due to the lack of data when the articulation is absent.

Character analysis. In a first multivariate numerical analysis, cluster analysis was performed on all OTUs. In a second analysis, cluster analysis and principal component analysis (PCA) was performed on a subset of OTUs belonging to series *Acaulia* and to *S. demissum*. Multiple discriminant analysis was used to examine infraspecific variation within *S. acaule*. Character means and ranges were calculated of the groups, which were determined with multivariate analyses. The groups represent the presumed taxa in series *Acaulia*. A one-way analysis was performed to determine which characters differed significantly between taxa. NTSYS-PC, version 1.80 (Rohlf 1993), was used for computation of cluster analyses and PCA. SPSS-PC, version 5.0.1 was used for basic statistics, multiple discriminant analysis and one-way analysis (Norušis 1990a,b).

Cluster analysis. In the first analysis all OTUs are included, in the second only those of series *Acaulia* and of *S. demissum*. In NTSYS-PC similarity matrices for cluster analysis were generated with range-normalized data (STAND in SIMINT). As coefficients for similarity the euclidean distances (EUCL) are computed. Clustering was performed using the unweighted pair-group method (UPGMA) in SAHN. Dendrograms were plotted in TREE (figure 2).

Principal Component analysis. OTUs of series *Acaulia* and of *S. demissum* were analyzed. Product-moment correlation (CORR) among variables was generated in SIMINT with standardized data (STAND). The first three PCA-axes were extracted in EIGEN, the explained variation of the axes was also calculated in EIGEN. Projections of the OTUs on the PCA-axes (PROJ) were plotted in MXPLOT. A minimum-length spanning tree was calculated in MST from the euclidean distance matrix of the cluster analysis. The minimum spanning tree is a network that is superimposed over the PCA plots to help detect local distortions, i.e. pairs of points which appear close together in a plot but actually are far apart if other dimensions are taken into account (Rohlf 1975).

Table 1. Accessions of *Solanum* species used in this study

Taxon [1]	[2]Code	Collector	[3] PI	[4]Country	State	Latitude	Longitude	Altitude
S. acaule ssp. *acaule*	**a1**	Brücher 33	208856	Argentina	Tucumán	26°52' S	65°41' W	-
	a2	Hjerting 994	210029	Bolivia	Tarija	21°55' S	65°14' W	4000 m
	a3	Sleumer 4114	217450	Argentina	Jujuy	23°33' S	65°16' W	3950 m
	a4	Correll P180	246504	Peru	Puno	15°41' S	70°31' W	3900 m
	a5	HHRO 3407	320280	Argentina	La Rioja	28°58' S	67°42' W	3250 m
	a6	Ochoa S-39	365307	Peru	Ayacucho	13°16' S	73°51' W	4160 m
	a7	Hoffman 1644	472645	Argentina	Jujuy	23°13' S	65°42' W	4300 m
	a8	Okada 4360	472700	Argentina	Jujuy	23°35' S	65°12' W	4000 m
	a9	Okada 4465	472715	Argentina	Salta	23°10' S	65°00' W	4400 m
	a10	*Okada 6309*	472777	*Argentina*	Salta	22°22' S	65°04' W	*3700 m*
	a11	Okada 6081	472799	Argentina	La Rioja	28°58' S	67°42' W	3050 m
	a12	HHCH 4116	473313	Peru	Puno	15°30' S	70°08' W	3825 m
	a13	HHCH 4226	473316	Bolivia	Cochabamba	17°26' S	65°35' W	3550 m
	a14	HHCH 5071	473327	Peru	Puno	15°18' S	69°58' W	3950 m
	a15	Ochoa 10112	473439	Peru	Ayacucho	-	-	-
	a16	Ochoa 11605	473444	Peru	Cuzco	13°30' S	70°55' W	-
	a17	Hjerting 5610	473483	Peru	Huancavelica	12°42' S	74°54' W	4000 m
	a18	Hjerting 5904	473484	Peru	Arequipa	15°12' S	71°46' W	4100 m
	a19	EBS 1824	473512	Bolivia	Oruro	15°47' S	68°40' W	4500 m
	a20	EBS 1825	473513	Peru	Cuzco	13°31' S	71°59' W	3800 m
	a21	Astley 86	498066	Bolivia	Potosí	19°36' S	65°57' W	-
	a22	HHA 6571	498078	Bolivia	Cochabamba	17°15' S	66°54' W	3730 m
	a23	Ochoa 11818	498184	Bolivia	La Paz	16°01' S	68°46' W	3800 m
	a24	Ochoa 12067	498194	Peru	Ancash	10°05' S	77.18' W	4080 m
	a25	SCLp 5070	561642	Ecuador	Chimborazo	2°09' S	78°43' W	3750 m
S. acaule ssp. *aemulans*	**e1**	Hjerting 1898	275133	Argentina	La Rioja	28°58' S	67°42' W	3150 m
	e2	HHRO 3396	320279	Argentina	La Rioja	28°58' S	67°42' W	3250 m
	e3	Okada 4412	435071	Argentina	Jujuy	23°35' S	65°10' W	3700 m
	e4	Okada 6087	472775	Argentina	La Rioja	28°58' S	67°42' W	3050 m
	e5	Okada 4361	472793	Argentina	Jujuy	23°35' S	65°12' W	4000 m
	e6	Okada 4413	472796	Argentina	Jujuy	23°35' S	65°10' W	3700 m
	e7	Okada 6085A	472802	Argentina	La Rioja	28°58' S	67°42' W	3050 m
	e8	Okada 7622	500018	Argentina	Jujuy	23°35' S	65°13' W	3200 m
S. acaule ssp. *punae*	**p1**	Hjerting 1341	210031	Peru	Junín	11°27' S	75°58' W	3850 m
	p2	CCC 592	225620	Peru		-	-	-
	p3	IICA 3324	230469	Peru		-	-	-
	p4	Correll P298	246571	Peru	Lima	-	-	4050 m
	p5	Correll P751	266386	Peru	Junín	11°36' S	75°22' W	4100 m
	p6	*Ochoa S-83*	365312	*Peru*	*Huánuco*	*9°18' S*	*76°35' W*	*3700 m*
	p7	Ochoa 5106	473430	Peru		-	-	-
	p8	Ochoa 9012	473434	Peru	Apurímac	-	-	-
	p9	Ochoa 9829	473436	Peru	Apurímac	-	-	-
	p10	Ochoa 10113	473440	Peru	Ayacucho	-	-	-
	p11	Ochoa 11603	473442	Peru	Ayacucho	14°40' S	74°23' W	-
	p12	Hjerting 5488	473482	Peru	Huancavelica	12°54' S	74°49' W	4200 m
	p13	Ochoa 11296	498179	Peru	Pasco	10°40' S	76°05' W	-
	p14p	[5]Ochoa 12069	498196	Peru	Ancash	9°40' S	77°21' W	4400 m
	p14h	[5] as **p14p**						
	p15	Ochoa 12092	498200	Peru	Ancash	-	-	3950 m

Table 1. Continued

Taxon [1]	[2]Code	Collector	[3] PI	[4] Country	State	Latitude	Longitude	Altitude
S. albicans	**h1**	Correll P863	266381	Peru	Cajamarca	-	-	3300 m
	h2	Hawkes 2427	310986	Peru		-	-	-
	h3	Hawkes 2429	310987	Peru		-	-	-
	h4	Ochoa S-17	365305	Peru	Apurímac	13°30' S	72°56' W	3400 m
	h5	Ochoa S-21	365306	Peru	Lima	10°34' S	76°54' W	3650 m
	h6	Ochoa 2713	365376	Peru	La Libertad	8°17' S	77°18' W	-
	h7	Ochoa 13014	498203	Peru	Ancash	10°26' S	76°47' W	4100 m
S. brevicaule	**b1**	HOHL 196	545969	Bolivia	Cochabamba	17°37' S	66°43' W	3700 m
	b2	HOHL 256	545971	Bolivia	Cochabamba	17°38' S	66°43' W	3840 m
S. chacoense	**c1**	Okada 4907	472816	Argentina	Salta	25°11' S	65°47' W	2500 m
	c2	Okada 7497	558042	Argentina	Salta	25°11' S	65°48' W	2700 m
S. demissum	**d1**	TRHRG 123	498012	Mexico	Durango	24°15' N	104°23' W	2800 m
	d2	TRHRG 289	545764	Mexico	Veracruz	19°34' N	97°15' W	2400 m
S. infundibuliforme	**i1**	Okada 5970	472914	Argentina	Jujuy	22°14' S	66°27' W	3700 m
	i2	Okada 6000	472916	Argentina	Jujuy	23°43' S	65°40' W	3840 m
S. megistacrolobum	**m1**	Hoffman 1618	473111	Argentina	Jujuy	22°24' S	65°22' W	4000 m
	m2	Okada 4436	473135	Argentina	Jujuy	23°34' S	65°16' W	3900 m
S. toralapanum	**t1**	HHA 6616	498145	Bolivia	Cochabamba	17°14' S	66°03' W	3760 m

[1] Taxon identification according to genebank inventory (Bamberg and Martin 1993); [2] Coding number in this analysis
[3] Plant Introduction number (Bamberg and Martin 1993); [4] Country, State, Latitude, Longitude, Altitude: original locality data of germplasm (Bamberg and Martin 1993); [5] Accession is split because three plants were *S. albicans* (p14h) and three plants were *S. acaule* ssp. *punae* (p14p) in the field plot

Table 2. Conflicting identifications of accessions of *Solanum acaule* and its subspecies and of *S. albicans*, based on data from field books of the potato genebank at Sturgeon Bay.

Taxon	Code	[1] Field-identifications
S. acaule ssp. *acaule*	**a5**	mix of acl+aem (Hawkes'84 / Okada'85 / Hawkes'86); aem (Okada'86)
	a8	intermediate acl/aem (Okada'77); acl+aem (Okada'85); acl (Ochoa'87)
	a24	pne/alb? (Hawkes'86); alb (Hawkes'87); acl (Ochoa'87)
S. acaule ssp. *aemulans*	**e2**	acl × aem (Hawkes'87); acl (Ochoa'87)
	e4	intermediate acl/aem type (Okada'77); mix acl/aem (Okada'81); acl
	e6	appears aem, or mix aem+acl (Okada'77); acl (Hawkes'87 / Ochoa'87)
	e8	aem, but not true aem (Okada'85)
S. acaule ssp. *punae*	**p1**	pne (Hawkes'87); acl (Ochoa'87)
	p3	pne (authority unknown); acl (Hawkes'87 / Ochoa'87)
	p6	pne (Ochoa+Hawkes'69); acl (Ochoa'77)
	p7	acl (Ochoa'77 / Ochoa'78); pne (Hawkes'78); acl (Hawkes'87 / Ochoa'87)
	p8	acl (Hawkes+Ochoa'78); pne (Hawkes'78); acl (Hawkes'87 / Ochoa'87)
	p9	acl (Ochoa'77 / Hawkes+Ochoa'78); pne (Hawkes'78); acl (Hawkes'87 / Ochoa'87)
	p10	acl (Ochoa'77 / Hawkes+Ochoa'78); pne (Hawkes'78); acl (Hawkes'87 / Ochoa'87)
	p11	acl (Ochoa'77 / Hawkes+Ochoa'78); pne (Hawkes'78); acl (Hawkes'87 / Ochoa'87)
	p12	acl (Ochoa'77); pne (Hawkes'86 / '87); acl (Ochoa'87)
	p13	pne (Hawkes'86 / '87); acl (Ochoa'87)
	p14h/p	pne (Hawkes'86); alb (Hawkes'87); acl (Ochoa'87)
	p15	good pne (Hawkes'86); pne (Hawkes'87); acl (Ochoa'87)
S. albicans	**h4**	acl, strong articulation (Ochoa+Hawkes'69); outstanding pne (Hawkes'87); alb
	h5	articulated acl, light lilac flowers (Ochoa'69/'77); outstanding pne (Hawkes'87); alb

[1]Standard abbreviations are used for the taxa names (Hawkes 1990). Taxonomists and year of identifications are given.

Table 3. Characters used in this study

Character	Abbreviation
Plant height (mm)	PH
Stem wing, width (mm)	SWW
Stem anthocyanin, intensity[1] (0-4)	SA
Pubescence length, lower side leaf (mm)	PULL
Number of pairs of lateral leaflets	JN
Number of interjected leaflets on rachis	ILR
Leaf, length (mm)	LL
Ratio: leaf, length / width	LF
Rachis length between first[2] and second lateral leaflet (mm)	RL
Terminal leaflet lamina, length (mm)	TLDL
Ratio: terminal leaflet lamina, length / width	TLDF
Terminal leaflet petiole, length (mm)	TLPEL
Largest lateral leaflet, length (mm)	LLL
Ratio: largest lateral leaflet, length / width	LLF
Ratio: first[2] lateral leaflet length / second lateral leaflet length	LR
Petiolule length of first[2] lateral leaflet, acroscopic side (mm)	PEL
Decurrency of first[2] lateral leaflet: length from main vein towards end of mesophyll on rachis (mm)	DF
Inflorescence, length (mm)	IL
Peduncle, length (mm)	PDL
Number of pedicels per inflorescence	PEDN
Pedicel, length (mm)	PEDL
Articulation of pedicel[3] (0 - 1)	PA
Length from pedicel articulation to base of calyx (mm)	LPA
Calyx, length (mm)	CL
Ratio: calyx lobe length / calyx length	CLOF
Corolla, diameter (mm)	COD
Corolla lobe[4], length (mm)	COLL
Ratio: Corolla lobe[4], length / width	COLF
Corolla colour[5], adaxial side (0-4)	COC
Difference: (Corolla colour[5], adaxial side) - (Corolla colour[5], abaxial side)	COCD
Anther, length (mm)	AL
Style, length (mm)	STL
Fruit, diameter on widest point (mm)	FD
Ratio: diameter of fruit on widest point / length of fruit	FF

[1] Anthocyanin colour of stem: 0 - 4 = none to very much anthocyanin

[2] First lateral leaflet = first lateral leaflet from apex of leaf

[3] Articulation of pedicel: 0 = absent; 0.5 = uncertain; 1 = present

[4] Corolla lobe, length = "4" in figure 3 in Spooner and Van den Berg (1992b); width = "3" in same figure

[5] Corolla colour coding, comparable RHS colour-chart codes between brackets (Royal Horticultural Society 1986): 0 = white to very pale blue (155B/91D); 1 = pale blue/purple (91A,B,C); 2 = medium blue/purple (92B,C); 3 = rather dark blue/purple (92A); 4 = deep dark blue/purple (90A/86A)

Multiple Discriminant analysis. OTUs belonging to *S. acaule* were analyzed with a multiple discriminant analysis: a1-a23, e1-e8, p1-p13, p14p and p15. Three groups are defined on basis of both cluster analyses and PCA. Group 1 is ssp. *acaule*, group 2 is ssp. *punae* and group 3 is ssp. *aemulans*. OTUs which are not clearly classified in the first two analyses, are labeled "ungrouped". Pedicel articulation (PA) was excluded from analysis because values are constant in each group (0 in group 1 and 2, 1 in group 3). Variables were stepwise selected for inclusion in the multiple discriminant analysis based on minimizing Wilks' lambda. Projection of OTUs on the two Canonical Discriminant functions were plotted. Probabilities were calculated for "ungrouped" OTUs to fall in a group.

Univariate analysis. Duncan's multiple range test (DMRT, $P \leq 0.01$) was used in a one-way analysis to test for significant character differences between taxa in series *Acaulia* (Table 6). For this test accessions of taxa within the series were grouped according to the results of cluster, principal component and multiple discriminant analysis. Harmonic group means were used because of different group sizes between these entities.

Mean, minimum and maximum values were calculated for all characters of found entities within series *Acaulia* (Table 5).

RESULTS

Character analysis. Cluster analysis of all OTUs (Fig. 2) reveals that series *Acaulia* is clearly distinguishable from all other investigated taxa, with the exception of *S. demissum* of series *Demissa*. OTUs of this species cluster together with accessions of *S. albicans*. The phenetic tree indicates that among the species studied, series *Acaulia* has some morphological parallels with both species of series *Megistacroloba* and with *S. brevicaule* and *S. infundibuliforme*. Accessions of *S. chacoense* show the largest distance from all other accessions.

Within the cluster of series *Acaulia* a dichotomy can be seen (Fig. 2). One branch consists of OTUs of *S. acaule* sspp. *acaule* and *punae*. Three subgroups can be defined: a ssp. *acaule* cluster with some accessions of ssp. *punae* (p3, p4, p7, p8, p9, p10), a *punae* cluster with one *acaule* accession (a23), and a separate *acaule* group consisting a1, a8, a9 and a10.

The other branch of series *Acaulia* divides into two subgroups: a *S. albicans* group (h1-h7, p14h) with the two *S. demissum* accessions, and an *aemulans* group. Within the first group two accessions labeled as *acaule* (a24, a25), cluster together with *S. albicans*. Chromosome counts made clear that these two are hexaploid. OTU a24 falls readily within the variation of the other accessions of *S. albicans* and is obviously misidentified in the inventory. OTU a25, collected in Ecuador, is separated from the other *S. albicans* OTUs. The *S. acaule* ssp. *aemulans* accessions form a cluster. Two accessions of ssp. *acaule* (a5, a11) are placed within the "aemulans" group, indicating misidentification.

In the second cluster analysis with only OTUs of series *Acaulia* and of *S. demissum* (not shown) no major changes in tree topology are found. Again a dichotomy is clear in the phenetic tree. In one branch there is an "acaule" cluster and a "punae" cluster. OTU a23 now clusters with the other ssp. *acaule* accessions and p8 joins the *punae* group. In figure 2 this ambiguous situation is marked with a ⧖ sign. The other branch again consists of a hexaploid cluster (*S. albicans* and *S. demissum*) and an *aemulans* group. In this analysis,

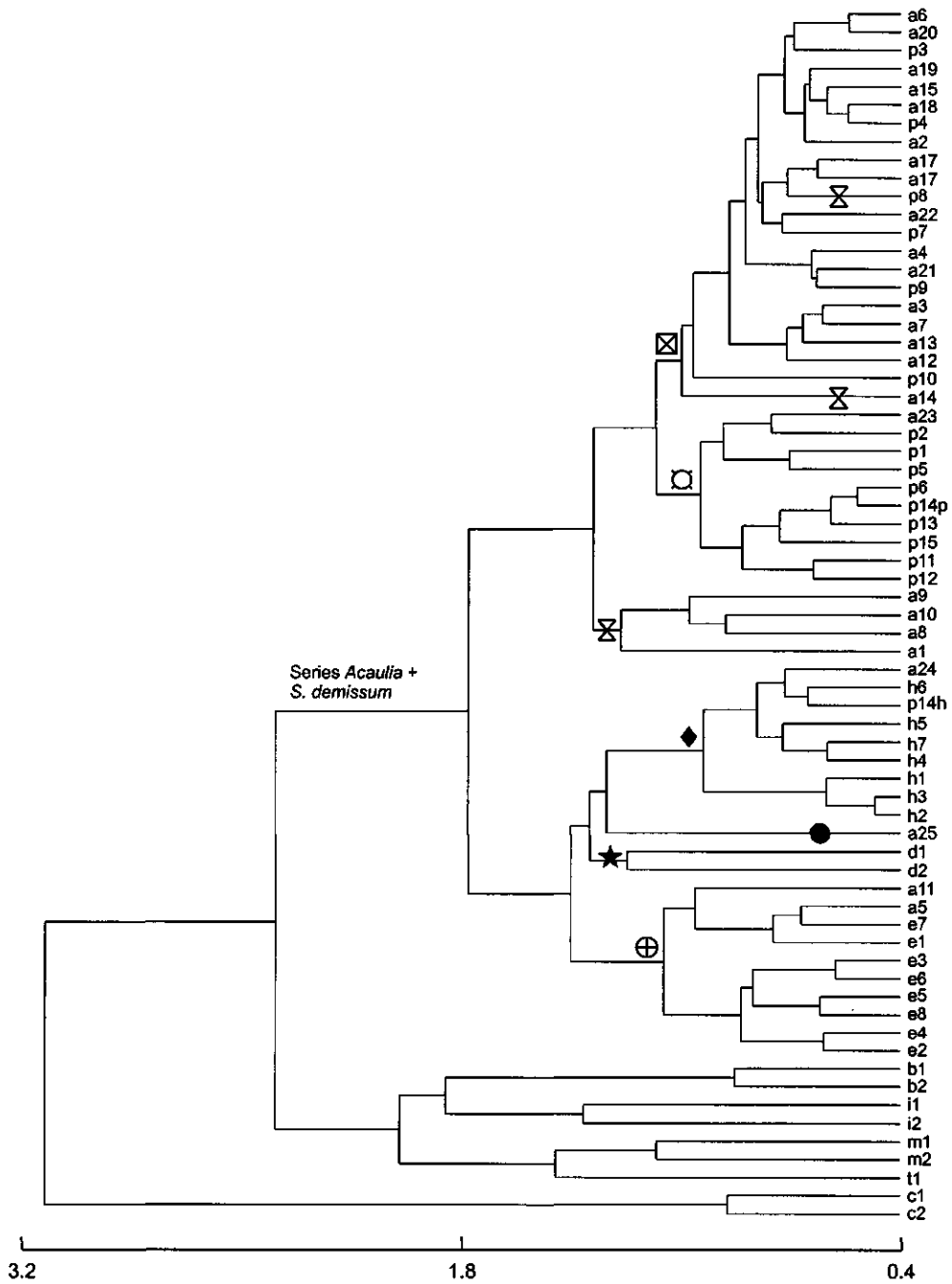


Figure 2. Phenetic tree (UPGMA) composed of 66 *Solanum* OTUs. Clusters are labeled as follows: *S. acaule* ssp. *acaule* ⊠ ; *S. acaule* ssp. *punae* ☊ ; *S. acaule* ssp. *aemulans* ⊕ ; *S. albicans* ♦ ; OTU **a25** ● ; *S. demissum* ★ ; ambiguous accessions ⧖.

OTUs a1, a8, a9 and a10 cluster with ssp. *aemulans* OTUs and are therefore also marked with a ⧖ sign in figure 2.

Principal Component analysis (PCA). The plot of the accessions on the first three principal components is shown in figure 3a,b. These axes explain 56.0 % of total observed variation. On the first component a segregation is demonstrated between a *S. acaule* sspp. *acaule* and *punae* group and a *S. acaule* ssp. *aemulans* and *S. albicans* group. Table 4 shows that the main characters explaining this separation are two leaf characters (TLDL and RL), anther length (AL), pedicel articulation (PA) and corolla diameter (COD).

The second component (Fig. 3a) reveals a split between *S. albicans* and *S. acaule* ssp. *aemulans*. Accessions of *S. albicans* have more leaflets per leaf (JN and ILR), bigger leafs (LLL), longer hairs (PULL) and more flowers per inflorescence (PEDN) (Table 4).

Along the third component (Fig. 3b) a ssp. *punae* group is separated by a higher length/width ratio of corolla lobe (COLF), a shorter peduncle (PDL), longer hairs (PULL), a larger difference between adaxial and abaxial corolla colour (COCD) and fruits with a higher width/length ratio (FF). Also the accessions a1, a8, a9 and a10 are separated from ssp. *aemulans* OTUs.

The projection of accessions e1, e7, a5 and a11 on the first three principal components show that these have the most distinct morphology of the ssp. *aemulans* material. The two other accessions of province La Rioja, e2 and e4, are grouped together with other *aemulans* OTUs from province Jujuy (e3, e5, e6, e8). The *S. demissum* OTUs d1 and d2 group together with *S. albicans* accessions on the first two axes. OTU d2 has an isolated position in the third direction, as does, even more extremely, OTU a25.

The Minimum Spanning Tree, superimposed on the principal component plots (Fig. 3), also revealed an indecisive grouping of OTUs a1, a8, a9 and a10. OTU a1 is attached to *S. albicans* OTU h4. OTUs a9 and a10 are connected with *S. acaule* ssp. *acaule* accessions. OTU a8 was placed intermediately between *S. acaule* ssp. *acaule* OTU a2 and ssp. *aemulans* OTU e6.

Multiple Discriminant analysis. Two discriminant functions were calculated for the three subspecies of *S. acaule*, i.e. ssp. *acaule*, ssp. *punae* and ssp. *aemulans*. Putative groups were constructed based on the results of cluster and principal component analysis. Group *acaule* consists of OTUs a2-4, a6-7, a12-22, p3-4, p7,9,10; group *punae* of OTUs p1-2, 5-6, p11-13, p14p, p15; group *aemulans* of e1-8, a5, a11. Ungrouped are p8, a1, a8, a9, a10 and a23. From the cluster analysis and PCA it can be concluded that accessions a1, a8, a9 and a10 can be classified in either *acaule* or *aemulans*. A23 and p8 are ambiguous between *acaule* and *punae*.

The articulation of pedicel (PA) is not a suitable character for discriminant analysis, because it lacks variation between putative groups (see Material and Methods). This character has the following scores for the possible *aemulans* accessions: OTU a1 articulated, a8 two plants not articulated, one uncertain, and a9-10 not articulated.

Figure 4 shows that all accessions a priori assigned to one of the three subspecies actually do group in this subspecies. Probabilities are calculated for ungrouped OTUs to belong to one of the subspecies. A23 falls readily within the variation of ssp. *acaule* (P=1.00). Accession a10 is also grouped with this group (P=1.00), but has higher positive scores on the first canonical function. OTU p8 takes an intermediate position between

acaule and *punae*, but has the highest probability to belong to ssp. *acaule* (P=1.00). OTUs a1, a8 and a9 all have a probability of 1.00 to belong to ssp. *aemulans*, although OTU a1 takes an intermediate position between sspp. *aemulans* and *acaule* in figure 4.

Univariate analysis. With cluster analysis, PCA and multiple discriminant analysis five groups within series *Acaulia* can be demonstrated: *S. acaule* ssp. *acaule* (accessions a2-4, a6-7, a10, a12-23, p3-4, p7-10); ssp. *punae* (accessions p1-2, 5-6, p11-13, p14p, p15); ssp. *aemulans* (accessions e1-8, a1, a5, a8, a9, a11); *S. albicans* (h1-h7, p14h, a24); *S. albicans* accession from Ecuador (a25).

Mean, minimum and maximum values for all characters of these groups are given in table 5. Table 6 shows characters significantly different between defined groups with DMRT ($P \leq 0.01$). All groups are significantly different in at least four and up to 18 characters.

Tables 5 and 6 show that in comparison with ssp. *acaule*, ssp. *punae* generally has a less developed stem (PH), longer hairs (PULL), smaller inflorescences (IL) and a more circular top leaflet (TLDF). Subspecies *aemulans* contrast with ssp. *acaule* in a bigger top leaflet (TLDL), fewer lateral leaflets (JN), a decline in length of lateral leaflets (LR), almost always a pedicel articulation (except accessions a8 and a9), bigger anthers (AL) and bigger (FD) and more globular fruits (FF). The *S. albicans* accessions from Peru differ from the one accession from Ecuador (a25) in the following characters without any overlap: a shorter stem, fewer leaflets and interstitials, clear pedicel articulation, shorter leaves, a higher ratio of corolla lobe length/width, and smaller anthers and fruits. A comparison of leaf shapes of taxa within series *Acaulia* is shown in figure 5.

DISCUSSION

Taxonomic status of series *Acaulia*. This study underlines the morphological distinctiveness of series *Acaulia*, when compared with representatives of a number of other series from sect. *Petota*. *Solanum demissum* forms an exception, because it resembles *S. albicans* in leaf and inflorescence structure, corolla shape and minuteness of anther and style. Although the traits in this study are almost all quantitative with overlapping character states, which are questionable for phylogenetic interpretations (Pimentel and Riggins 1987), a direct evolutionary link between *S. demissum* and series *Acaulia* could very well be suggested. Both *S. albicans* and *S. demissum* have a hexaploid genome. A study on nuclear RFLPs of 14 wild species and three cultivated *Solanum* species (Debener *et al.* 1990) indicates a phylogenetic relation between *S. acaule* and *S. demissum*. A study on species of series *Demissa* also reveals morphological parallels between *S. albicans* and *S. demissum* (Spooner *et al.* 1995).

However, these results contrast with those of chloroplast DNA (Hosaka *et al.* 1984). In that study closer relations between *S. acaule* and *S. multidissectum* Hawkes, *S. stenotomum* ssp. *goniocalyx* (Juz. & Bukasov) Hawkes and *S. phureja* Juz. & Bukasov (series *Tuberosa*) are found. Further research is needed to come to a better understanding of the phylogeny of sect. *Petota*. Special focus on affinities between *S. demissum* and series *Acaulia* can be of great interest.

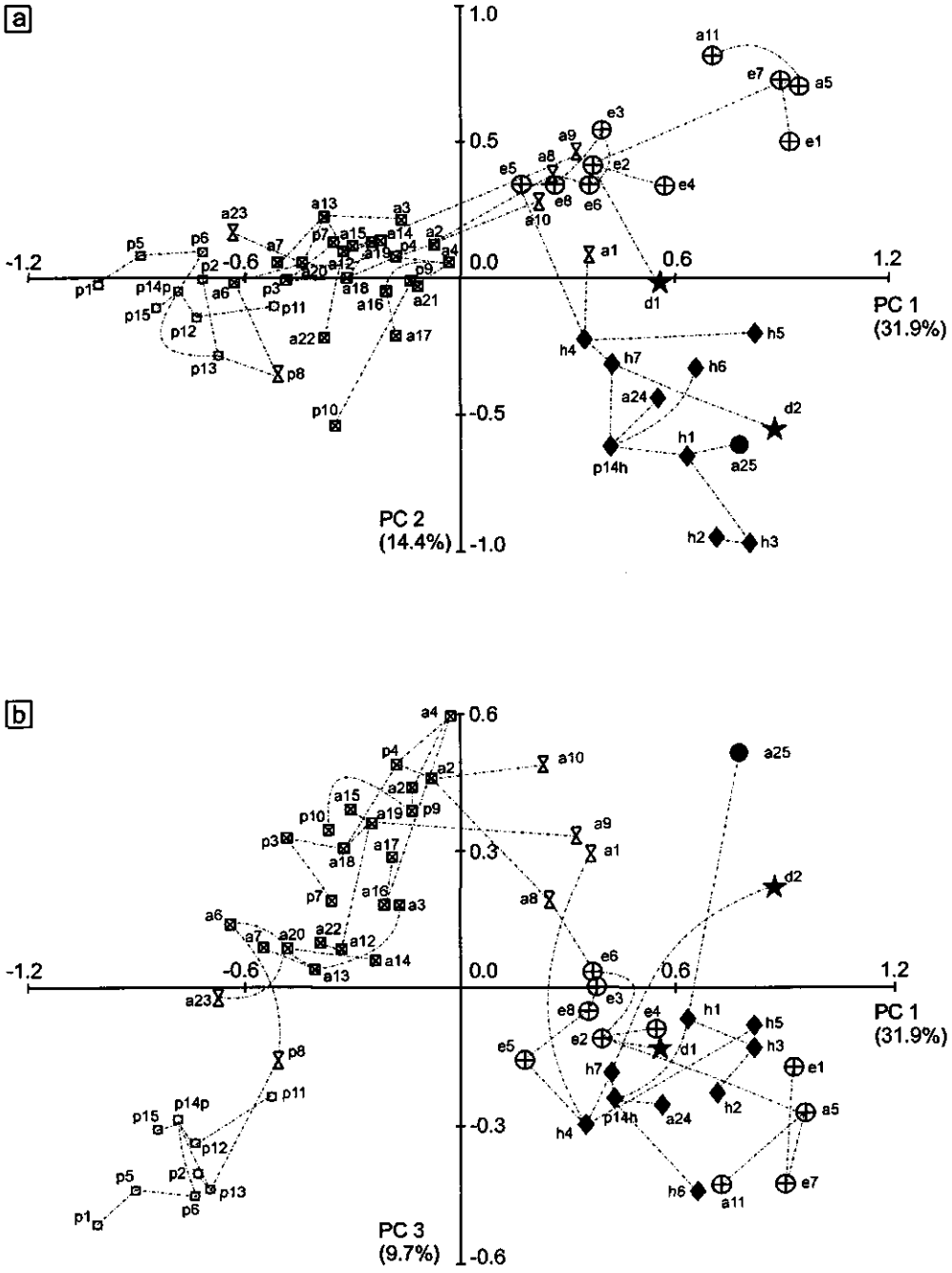


Figure 3. Projections of OTUs of series *Acaulia* and *Solanum demissum* onto the first two principal components (a) and onto the first and third component (b). Labels are explained in Figure 2.

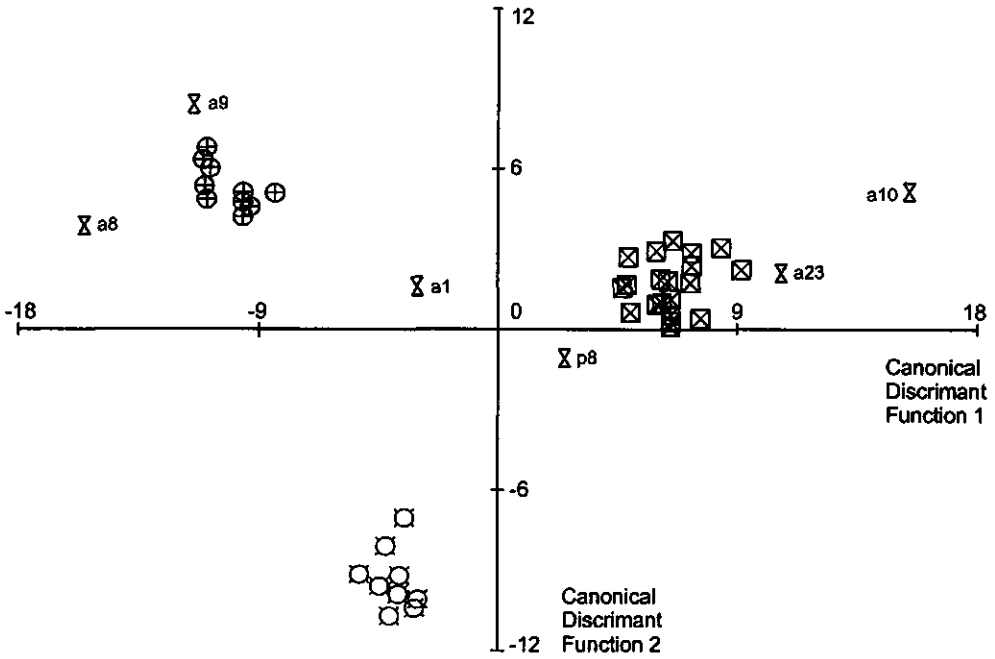


Figure 4. Projections of OTUs of *Solanum acaule* subspecies on Canonical Discriminant Functions. Labels as in Figure 2.

Table 4. Characters with highest factor loadings on first three principal components

Principal Component	1		2		3	
Factor loadings:	TLDL	= 0.91	ILR	= -0.80	COLF	= -0.54
	RL	= 0.88	PULL	= -0.74	PDL	= 0.51
	AL	= 0.88	PEDN	= -0.60	PULL	= -0.47
	PA	= 0.85	LLL	= -0.55	COCD	= -0.47
	COD	= 0.76	JN	= -0.51	FF	= -0.47

Species within the series. The current division of series *Acaulia* into two species, i.e. *S. acaule* and *S. albicans* (Ochoa 1983, Hawkes 1990), is supported by both this morphological study and RFLP data (Hosaka and Spooner 1992). *Solanum albicans* is sympatric in Peru with *S. acaule*, which is distributed from northern Peru to province La Rioja, Argentina (Fig. 1), but keeps it own characteristics by its ploidy-level.

Solanum albicans can be easily discriminated from *S. acaule* sspp. *acaule* and *punae* by the presence of an articulation. In ssp. *aemulans*, however, an articulation is present, but in this subspecies it is a distinct swollen ring on the pedicel, whereas in *S. albicans* it can be seen as a green pigment band on the pedicel.

Table 5. Mean, minimum (Min) and maximum (Max) for all characters of accessions of *Solanum acaule* subspecies, *S. albicans* and of one accession of *S. albicans* from Ecuador

	S. acaule ssp. *acaule* a2, a3, a4, a6, a7, a10, a12-a23, p3, p4, p7-p10			*S. acaule* ssp. *punae* p1, p2, p5, p6, p11-p13, p14p, p15			*S. acaule* ssp. *aemulans* e1-e8, a1, a5, a8, a9, a11			*S. albicans* Ecuador h1-h7, p14h, a24			*S. albicans* a25
	Min	**Mean**	Max	Min	**Mean**	Max	Min	**Mean**	Max	Min	**Mean**	Max	**Mean**
PH	223	**311**	430	100	**133**	186	155	**300**	446	90	**152**	250	**416**
SWW	0.2	**0.46**	0.7	0.1	**0.33**	0.6	0.3	**0.40**	0.6	0.43	**0.78**	1.2	**0.80**
SA	0.0	**1.17**	3.0	0.0	**2.00**	4.0	0.5	**1.8**	3.2	0.00	**0.71**	2.0	**1.0**
PULL	0.3	**0.83**	1.6	1.5	**2.09**	2.9	0.4	**0.57**	1.2	1.50	**2.2**	3.0	**2.1**
JN	5.0	**5.92**	7.0	4.7	**5.59**	6.0	3.3	**4.4**	5.3	4.67	**5.4**	6.0	**6.7**
ILR	2.3	**5.7**	13.0	1.7	**3.7**	5.7	0.0	**2.4**	4.5	4.3	**8.8**	13.0	**16.3**
LL	99	**162**	210	118	**132**	142	126	**172**	216	156	**185**	221	**224**
LF	3.0	**4.0**	5.2	3.2	**3.8**	4.4	3.0	**3.8**	4.7	2.4	**3.2**	3.7	**3.6**
RL	10.7	**16.6**	21.7	12.0	**13.6**	15.7	15.3	**21.0**	25.7	21.3	**24.9**	29.3	**28.7**
TLDL	19.0	**23.9**	30.7	16.0	**18.5**	20.3	27.25	**38.5**	52.3	28.0	**36.6**	46.3	**37.0**
TLDF	1.0	**1.2**	1.4	0.9	**1.0**	1.1	1.1	**1.3**	1.5	1.2	**1.3**	1.4	**1.2**
TLPEL	2.8	**5.8**	8.7	3.7	**4.6**	6.3	4.7	**7.5**	11.3	7.7	**10.6**	12.5	**13.7**
LLL	18.7	**23.5**	30.3	18.0	**20.1**	22.7	18.8	**25.8**	33.3	26.0	**35.5**	49.3	**36.2**
LLF	1.4	**1.5**	1.7	1.4	**1.5**	1.6	1.3	**1.6**	1.9	1.6	**1.7**	1.8	**1.5**
LR	0.8	**0.91**	1.0	0.8	**0.87**	0.9	0.9	**1.1**	1.4	0.9	**1.0**	1.1	**1.0**
PEL	0.0	**0.05**	0.5	0.0	**0.04**	0.3	0.0	**0.08**	0.7	0.0	**0.27**	1.3	**1.0**
DF	0.0	**0.50**	2.0	0.0	**1.6**	3.3	0.0	**3.9**	12.3	0.0	**1.4**	4.0	**1.2**
IL	21.7	**35.9**	48.0	19.0	**22.8**	27.0	37.7	**45.7**	58.3	31.5	**39.7**	47.0	**41.0**
PDL	1.1	**8.5**	16.0	0.8	**2.5**	3.9	4.0	**9.5**	15.0	3.9	**6.7**	10.0	**7.7**
PEDN	2.0	**3.9**	6.3	2.0	**3.1**	4.0	1.7	**3.1**	6.7	4.7	**5.5**	6.7	**5.0**
PEDL	14.6	**22.3**	34.0	11.8	**16.3**	21.2	24.7	**31.6**	44.3	21.0	**25.5**	28.0	**23.3**
PA	0	**0**	0	0	**0**	0	0	**0.87**	1.0	1.0	**1.0**	1.0	**0.50**
LPA	-	**-**	-	-	**-**	-	0.9	**1.8**	3.4	2.3	**4.0**	5.23	**2.0**
CL	3.9	**4.9**	6.1	4.0	**4.4**	4.6	4.6	**5.0**	5.7	4.6	**5.1**	5.5	**6.3**
CLOF	0.5	**0.58**	0.7	0.5	**0.56**	0.6	0.6	**0.60**	0.6	0.6	**0.62**	0.7	**0.60**
COD	13.6	**15.6**	19.5	12.2	**13.7**	17.3	15.7	**17.8**	20.7	14.7	**16.7**	20.0	**20.7**
COLL	2.3	**2.9**	3.8	2.2	**2.7**	3.6	2.8	**3.3**	4.1	3.3	**3.7**	4.2	**3.5**
COLF	0.3	**0.34**	0.4	0.3	**0.38**	0.5	0.3	**0.36**	0.5	0.4	**0.46**	0.5	**0.30**
COC	0.0	**1.6**	3.0	1.0	**1.9**	3.0	0.0	**1.1**	3.0	0.0	**0.68**	1.8	**2.0**
COCD	0.0	**0.38**	0.9	0.3	**0.80**	1.5	0.0	**0.7**	2.0	0.0	**0.56**	1.0	**0.67**
AL	2.3	**2.5**	2.8	2.0	**2.3**	2.6	2.6	**3.1**	3.7	2.8	**3.1**	3.5	**3.6**
STL	5.0	**5.8**	7.3	4.7	**5.1**	5.7	4.9	**6.5**	7.8	5.0	**5.8**	6.6	**6.2**
FD	15.2	**17.7**	23.6	14.3	**16.3**	18.0	18.1	**21.9**	25.8	17.1	**18.9**	21.3	**23.3**
FF	0.8	**0.93**	1.1	0.9	**1.0**	1.1	1.0	**1.11**	1.2	1.0	**1.10**	1.2	**1.0**

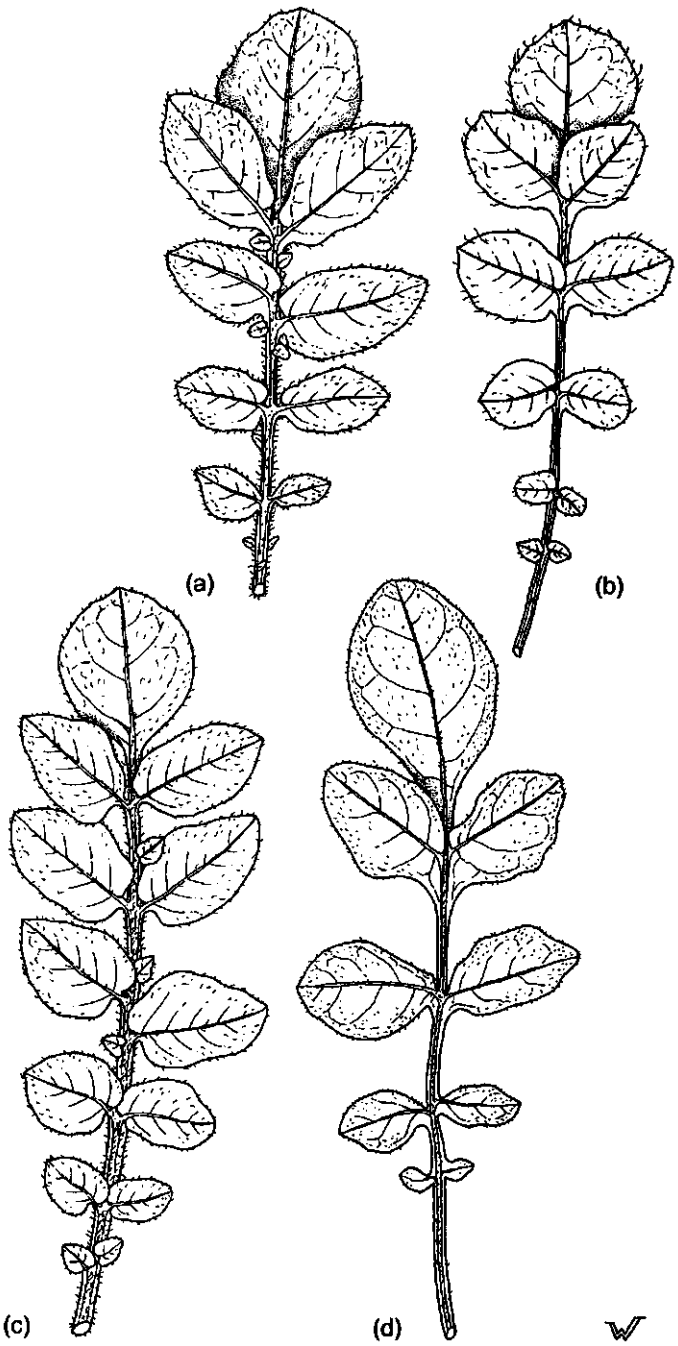


Figure 5. Comparison of leaf shapes (× 0.66). (a) *S. albicans* PI 310986 [= h2]; (b) *S. acaule* ssp. *punae*, [1,2]BGRC 7958 [same germplasm collection as p5]; (c) *S. acaule* ssp. *acaule*, drawn from [3]*Ochoa and Salas 11831*, (d) *S. acaule* ssp. *aemulans*, [1,2]BGRC 17182 [= a11].

[1] Plants grown in greenhouse Wageningen; [2] Braunschweig Genetic Resources Center; [3] herbarium specimen.

Table 6. Characters significantly different with DMRT (P≤0.01), for accessions of *Solanum acaule* subspecies, *S. albicans* and for accession a25 from Ecuador

	S. acaule ssp. *acaule* a2, a3, a4, a6, a7, a10, a12-a23,p3, p4, p7-p10	*S. acaule* ssp. *punae* p1, p2, p5, p6, p11, p12, p13, p14p, p15	*S. acaule* ssp. *aemulans* e1-e8, a1, a5, a8, a9, a11	*S. albicans* h1-h7, p14h, a24
S. acaule ssp. *punae* p1, p2, p5, p6, p11-p13, p14p, p15	PH, PULL, IL, TLDF			
S. acaule ssp. *aemulans* e1-e8, a1, a5, a8, a9, a11	JN, TLDL, LR, PA, AL, FD, FF	PH, PULL, JN, RL, TLDL, TLDF, LR, IL, PDL, PA, COD, AL, STL, FD		
S. albicans h1-h7, p14h, a24	PH, SWW, PULL, RL, TLDL, TLPEL, LLL, PA, COLL, COLF, AL, FF	SWW, ILR, LL, RL, TLDL, TLDF, TLPEL, LLL, LR, IL, PA, COLL, AL	PH, SWW, PULL, JN, ILR, LLL	
S. albicans, accession a25 from Ecuador	SWW, PULL, ILR, LL, RL, TLDL, TLPEL, LLL, PEL, PA, CL, COD, AL, FD	PH, SWW, JN, ILR, LL, RL, TLDL, TLPEL, LLL, PEL, IL, PEDN, PA, CL, COD, COLL, AL, FD	SWW, PULL, JN, ILR, LL, RL, TLPEL, LLL, LR, PEL, PEDN, PA, CL, COD, AL	PH, JN, ILR, PEL, PA, CL, COLF, AL, FD

Infraspecific variation within *S. albicans*. Accessions of *S. albicans* from Peru are quite homogeneous, reflected in cluster analysis and principal component analysis in the present study. The flower colour varies between white and medium purple. Correll (1962, p. 344) already mentioned that a white corolla colour is not a distinctive character for this taxon. The emphasis on the white colour has been a probable cause of some misidentifications in the past: *S. albicans* accessions with a medium purple corolla, h4 and h5, both have been classified as an "articulated *S. acaule*" and an "outstanding ssp. *punae*" (Table 2).

A recent collection of *S. albicans* from Ecuador, a25, is demonstrated in this study to have a hexaploid ploidy-level and does not belong to *S. acaule* (Spooner *et al.* 1992) but to *S. albicans*. In the field plot it differs from *S. albicans* material from Peru in the following characters: longer stems and composed leaves, more pairs of lateral and interstitial leaflets, longer petiolules, a less clear pedicel articulation, less pronounced corolla lobes (COLF), and bigger anthers and fruits. Because only one accession has been collected in Ecuador, it is at present not possible to ascertain the taxonomic status of this material. But, since the material differs from *S. albicans* as much as it does, the status of (geographical) subspecies would be appropriate, in conformity with the infraspecific classification of *S. acaule*. ***NB.*** *See Chapter 6, this thesis, for the latest insights on the taxonomic status of this material.*

Infraspecific variation within *S. acaule*. Multivariate analyses in this study point out that within *S. acaule* three groups can be demonstrated, i.e. ssp. *acaule*, ssp. *punae* and ssp. *aemulans*. This classification is in concordance with recent taxonomic views (Hawkes 1990), although the reduction of ssp. *punae* as a synonym of ssp. *acaule* has been effected (Ochoa 1990) or suggested (Hosaka and Spooner 1992).

Nonetheless, six of 45 *S. acaule* accessions in this study are intermediate in their characters between ssp. *acaule* and ssp. *punae*, or between ssp. *acaule* and ssp. *aemulans*. Probabilities have been calculated with multiple discriminant analysis for this material to fall into one of the subspecies. All six accessions have a probability of 1.00 to be classified in a subspecies. Accessions a1 and p8, however, retain rather intermediate positions between sspp. *acaule* and *aemulans*, and sspp. *acaule* and *punae*, respectively. And accessions a8 and a9 do not fall in the variation of ssp. *aemulans* and a10 not in the variation of ssp. *acaule*.

In a RFLP analysis of genomic DNA of *S. acaule* (Hosaka and Spooner 1992), focused on infraspecific variation, it is concluded that ssp. *punae* and ssp. *acaule* are indistinguishable. Of 15 ssp. *punae* accessions in the present study, seven were also studied by RFLP techniques (Hosaka and Spooner 1992). Accessions p1, p5 and p6 had a RFLP pattern that separated them clearly from ssp. *acaule* in a principal component analysis. This is in agreement with the data of the present study. Accessions p4, p8, p9 and p10 had RFLP-bands comparable with those of ssp. *acaule*, which is also in agreement with the present morphological study. The classification of this material as ssp. *punae* (Bamberg and Martin 1993), must be due to misidentifications. Accessions p3 and p7, which were not studied with RFLPs, also should be considered as ssp. *acaule*.

Subspecies *acaule* is a rather homogeneous entity, in spite of its wide geographical distribution from central Peru into northern Argentina. It is demonstrated in this study that in comparison with ssp. *punae*, ssp. *acaule* has a more robust habit and shorter hairs, and living plant material which is grown under identical circumstances is readily distinguishable.

Compared to ssp. *aemulans*, ssp. *acaule* has no pedicel articulation, a different leaf structure and smaller fruits. Especially between these subspecies intermediate forms have been found in this study. Accession a8 has some plants with an articulation and some without, and has an intermediate morphology. Accession a9 has no articulation, but has to be classified with *aemulans*. These intermediates might be caused by hybridization. In fact the intermediate forms do occur in the area of overlap between the subspecies (Fig. 1). Recent hypotheses (Okada and Clausen 1982, Hawkes 1990) only classify material of La Rioja as ssp. *aemulans*. This is opposed by the results of this study, because material of province Jujuy (e3, e5, e6, e7) does not differ in its morphological characters from some accessions from La Rioja (e2, e4).

Special attention must be paid to accession a1. It was collected in province Tucumán in Argentina and is in this study classified in ssp. *aemulans*. With RFLP analysis (Hosaka and Spooner 1992), material of this region takes a distinct position in the principal component analysis, together with some accessions out of provinces Salta, Jujuy and La Rioja, Argentina. These results indicate that a rich genetic variability occurs in northern Argentina, which is not easy to classify.

CHAPTER 2

The inflorescence architecture of *Solanum acaule* and related taxa of *Solanum* section *Petota*

ABSTRACT

In this morphological study a special and yet unrecorded inflorescence type is described in the polyploid series *Acaulia* (*Solanum* sect. *Petota*) and compared to those of other tuber-bearing *Solanum* species. This inflorescence can be characterized as a monochasium with a strongly reduced peduncle and one or two 'extra' flowers in the axil of the subtending leaf. Species showing this inflorescence architecture have a rosette-habit, which is correlated with the high altitudes in the Andes where they occur naturally. 'Extra' flowers and other inflorescence architectural traits such as the dimensions of pedicels, peduncles, and corollas are discussed in relation to the taxonomy of *Solanum* sect. *Petota* species, their habitat, and breeding behavior.

INTRODUCTION

Inflorescences and branching patterns within Solanaceae have been described by Danert (1967) and Child (1979). The general branching pattern within this family is acrotonic, i.e. the shoots terminate with a flower or inflorescence and a secondary sprout emerges from the axils of the top leaf or lower leaves of the primary shoot. After this new continuing sprout has produced a number of foliar organs, it ends again in an inflorescence. This reiterative process continues throughout the growing period. Each section of foliose leaves and flowers together is termed anthoclade (Weberling 1989). The morphological interpretation of structures in Solanaceae is troublesome because many parts of the plant can be metatopically displaced due to processes of recaulescence and/or concaulescence (Danert 1957, Troll 1957, Weberling 1989). For example, the partial inflorescence grows concaulescent with the terminal flower that ends the anthoclade (Danert 1957). These shifts make the interpretation of structures difficult as can be illustrated by the remarks of Correll (1962) on *Solanum* inflorescences. He incorrectly named the inflorescences pseudo-terminal (p. 29) and stated that technically they are always lateral. Actually, the inflorescences are terminal (Hayward 1938, Danert 1957) and 'pushed' aside laterally by the shoot growing from the axil of a lower leaf.

The three- to plurifoliate anthoclades of *Solanum* section *Petota* Dumort., comprising all tuber-bearing *Solanum* species, end in a thyrsoid inflorescence. The mono- to pleiochasial inflorescences (Child 1979, Child and Lester 1991) are composed of scorpioid, ebracteate, partial inflorescences (Hawkes and Hjerting 1989, Weberling 1989). The general inflorescence stalk or peduncle is mostly dichasial branched, but species such as *Solanum acaule* and species of series *Megistacroloba* normally have an unforked peduncle (Hawkes and Hjerting 1969, 1989).

Kardolus J.P. and N. Groendijk-Wilders (1998) Acta Botanica Neerlandica 47 (In press).

Studying the morphology of *S. acaule* and related species (Kardolus 1998), it became obvious that the inflorescence architecture of taxa of the polyploid series *Acaulia* (which includes two species, namely *S. acaule* and *S. albicans*) is not conform this general architecture. Often 'extra' flowers are found at the base of the inflorescence in these species. This observation contradicts the conclusion (Danert 1957) that inflorescences of *S. acaule* have the same morphology as those of *S. tuberosum* L., namely a dichasium. The main objective of our study is to compare the inflorescence structure of species of series *Acaulia* with that of related species of series *Demissa*, *Megistacroloba* and *Tuberosa*, and determine the taxonomic value of this trait.

Besides the general architecture of inflorescences, other inflorescence traits have been used in potato taxonomy. For example, the lengths of the unforked and forked parts of the peduncle can differ among species in section *Petota*. In most species the unforked part is much longer than the branches. However, in *S. acaule* and *S. megistacrolobum* ssp. *megistacrolobum* the peduncle is very short (Hawkes and Hjerting 1989). The compact habit of these taxa seems to be associated with their extreme environment. Their distribution is in the high-mountain areas of the Andes at elevations up to 4400 meter, where both species occasionally have been found at the same locality (Hawkes and Hjerting 1989: p.192, Ochoa 1990). However, *S. acaule* and *S. megistacrolobum* have different pollination mechanisms. *Solanum acaule* and *S. albicans* are self-fertile and inbreeding (Hawkes and Hjerting 1969). Sometimes their pollination is even cleistogamous (Ugent 1981). The polyploid species *S. demissum* from Mexico, that has been demonstrated to be closely related to *S. acaule* and *S. albicans* (Kardolus *et al.* 1998), shows the same breeding pattern. The diploid species of series *Megistacroloba* and *Tuberosa* are outbreeders and need pollinating insects. In this study, we investigate inflorescences to determine a possible correlation between the morphometric proportions of the inflorescences of *S. acaule* and related species and their habitat and breeding behavior.

MATERIALS AND METHODS

Morphological observations. In total 44 genebank accessions of 17 (sub)species belonging to four different taxonomic series in section *Petota* were examined from 1993 until 1996 (Table 1). The accessions were received as seeds from the potato genebank collections at Braunschweig - Germany, presently relocated in Wageningen at the Centre for Genetic Resources - The Netherlands (BGRC numbers), and at Sturgeon Bay - USA (PI numbers). At least 5 seedlings of each accession were grown in pots in a standard greenhouse. Plants were studied two times a week during their flowering period to follow the development of inflorescences. Variation in inflorescence structure, within and between species, was described. Simple drawings were made of the different inflorescence types. Photographs of all inflorescence types were taken in the greenhouse and detailed botanical drawings were made of the most important types. The inflorescences were also observed for other properties such as the presence of leaves in the inflorescence. Morphological comparisons with plants collected in the wild were made by studying herbarium material of *S. acaule* and *S. albicans* (Table 2).

Table 1. List of plant material studied

Taxonomic series	Species	Genebank number
Acaulia Juz.	*Solanum acaule* Bitter ssp. *acaule*	BGRC 16835, 17090, 17111, 17128,17141, 17181, 24579, 27206, 27244, 27361, 27362,
	S. acaule ssp. *aemulans* (Bitter et Wittm.) Hawkes et Hjert.	BGRC 17180, 17182
	S. acaule ssp. *punae* (Juz.) Hawkes et Hjert.	BGRC 7958; PI 24657
	S. albicans (Ochoa) Ochoa	BGRC 18287; PI 365376, 498203, 561642
Megistacroloba Cárdenas et Hawkes	*S. boliviense* Dunal	BGRC 27248
	S. megistacrolobum Bitter ssp. *megistacrolobum*	BGRC 8113, 8117, 17642, 27262
	S. megistacrolobum ssp. *toralapanum* (Cárdenas et Hawkes) Giannattasio et Spooner	BGRC 28020
	S. raphanifolium Cárdenas et Hawkes	BGRC 7207, 8185
	S. sanctae-rosae Hawkes	BGRC 15454, 17568
Demissa Bukasov	*S. demissum* Lindl.	BGRC 9990, 10022, 10030
Tuberosa (Rydb.) Hawkes	*S. brevicaule* Bitter	BGRC 18291, 28038
	S. bukasovii Juz.	BGRC 7993, 15424; PI 210044

Table 2. List of flowering herbarium specimens of *Solanum acaule* and *S. albicans* (marked with an asterisk) studied

Herbarium	Specimen
CIP	HHCH 4236, 4243, 4271, 4324, 4416; Ochoa 2065* (holotype), 2495*, 11961, 11989, 12083*, 13238*, 16023*, 16173*; Ochoa & Salas 11823,11824, 11826, 11830, 11831, 11833, 11895, 14947, 14949-14953, 15468, 15476, 15519, 15569, 15570, 16024*, 16028*; SOA 53, 54; SCLp 5070*
G	Hieronymus & Niederlein 474
K	Balls 5987, 6026, 6201; Cabrera-Schwabe 11; Correll P298, B636; HHR 3813; Iltis & Ugent 1435; Sandeman 3933; Saunders 819, 1384; Sharpe 120; Stafford 431; Vargas 2005
LIL	Budin 7439; Castillon 468, 3195; Krapovickas 8745; Lillo 1231, 2957, 4212, 5521, 7403, 11476, 18154; O'Donell 4596; Olea 221, 253; Schreiter 352, 353, 4841, 6203, 6965, 7097, 7103; Shepard 231; Sleumer 228, 1838, 2689, 2750, 3252, 3462, 3589, 4113; Sparre 5989, 6102
S	Correll B601; Fiebrig 3429 (lectotype ssp. *acaule*); Hammarlund 128; Kurtz 11396; Regnell 979
US	Balls 6404; Cabrera 8264; Cárdenas 606; Cook & Gilbert 180, 181, 182a; Ochoa & Salas 11895; Shepard 231; Venturi 6672, 6993, 9526

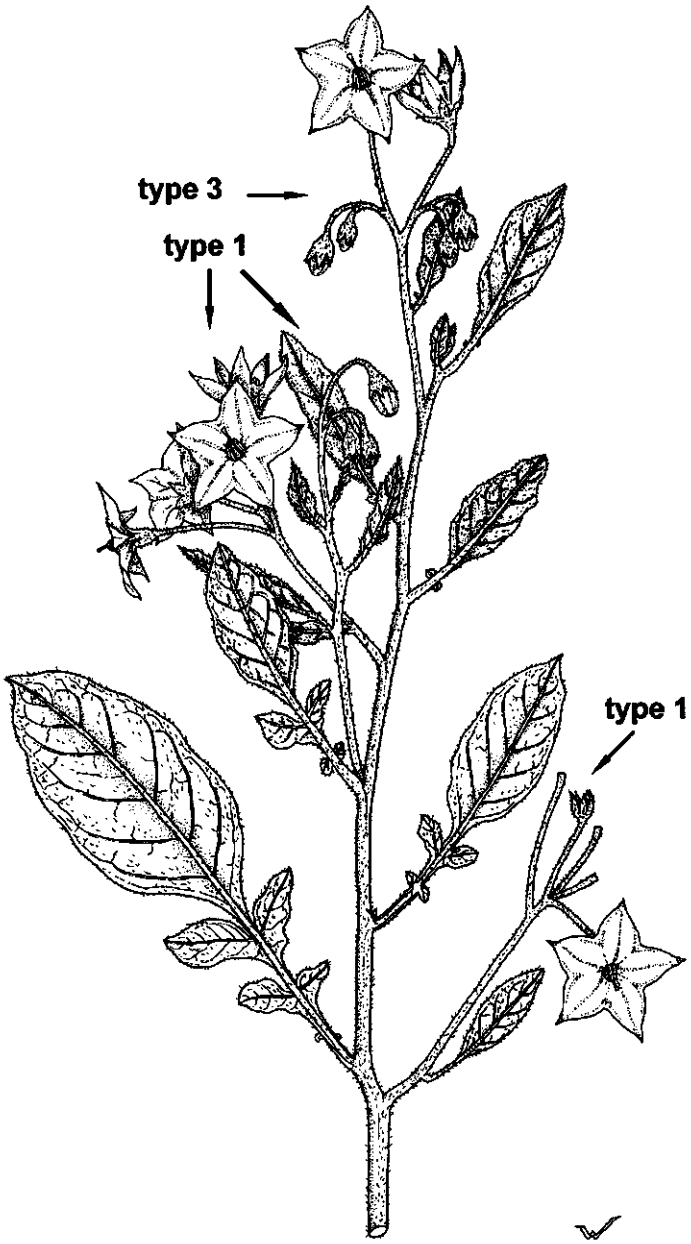


Figure 1. Botanical drawing (× 0.66). *Solanum megistacrolobum* (BGRC 17642) with the lower three inflorescences as type 1, and an upper inflorescence with a forked peduncle (type 3).

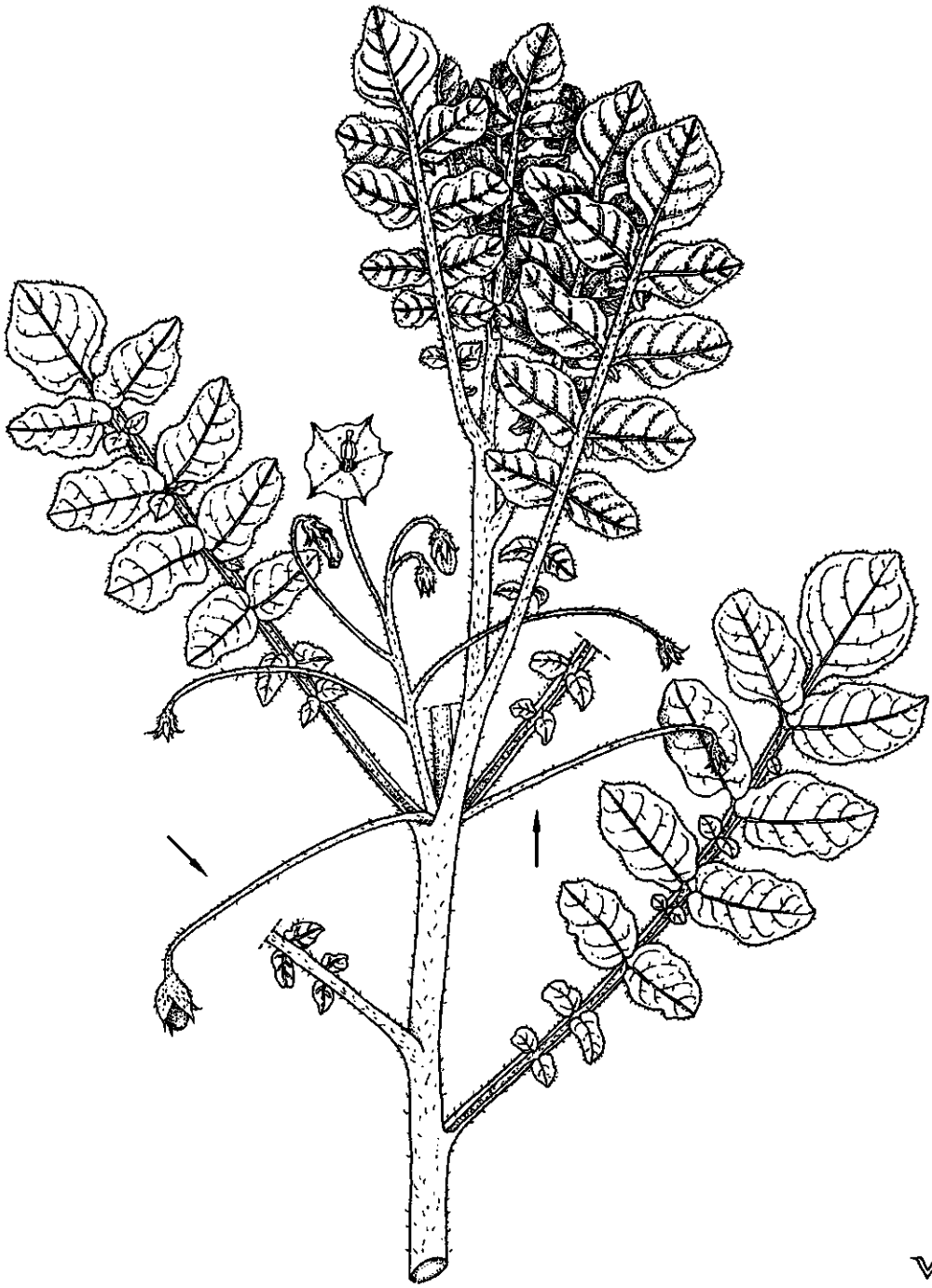


Figure 2. Botanical drawing (× 1). A type 2A-inflorescence in *S. acaule* ssp. *acaule* (BGRC 16835): a monochasium with 2 'extra' flowers in the axil of the leaf. Arrows point at the 'extra' flowers.

Figure 3. Botanical drawing (× 0.66). *Solanum brevicaule* (BGRC 28038): inflorescence with a forked peduncle bearing two monochasia (type 3).

Morphometric data. Data were measured in the trial field in Sturgeon Bay in 1993. Plant material of this experiment has been described in detail by Kardolus (1998). The identifications of the accessions belonging to series *Acaulia* are displayed in Table 5 of the same paper. Three plants per accession of the following taxa were studied: *S. acaule* ssp. *acaule* (25 accessions) / ssp. *punae* (9 accessions) / ssp. *aemulans* (13 accessions), *S. albicans* (9 accessions), *S. brevicaule* (2 accessions), *S. demissum* (2 accessions), and *S. megistacrolobum* ssp. *megistacrolobum* (2 accessions) / ssp. *toralapanum* (1 accession). Five characters of one inflorescence per plant were analyzed: 1. The length of the peduncle from the subtending leaf (or when there is no subtending leaf, from the stem) to the first fork or pedicel; 2. The total number of flowers per inflorescence; 3. The length of the pedicel, that is the length of the ultimate flower stalk up to the base of the calyx; 4. The diameter of the corolla; 5. The exsertion of the style from the anther column. Data of these five characters were analyzed statistically using the JMP® software package, version 3.1.4 (SAS Institute Inc., Cary, NC, USA). Tukey-Kramer honestly-significant-difference-test (Tukey 1953, Kramer 1956) was applied to check for significant differences among the character means of all pairs of the eight taxa listed above.

RESULTS

Morphological observations. The architecture of the inflorescences of the taxa examined was variable. Nevertheless, a morphological pattern could be distinguished. We assigned the inflorescences to three general 'types', viz. (1) the monochasium, (2) the monochasium with an 'extra' flower at the base of the monochasium and (3) a dichasial branched inflorescence. The architecture of the inflorescence is not a constant species-specific diagnostic trait. Often more types of inflorescences were present within a species (Table 3) and even in one plant (Fig. 1). The frequency of occurrence of the observed inflorescence types differed among species. Furthermore, the second type of inflorescence occurred only in a part of the studied taxa (Table 3).

Monochasiums, i.e. inflorescences with an unforked peduncle, were observed in all taxa. We named this **'Type 1'** (Figs. 1, 4a-c, e). Type 1 was most frequent in series *Megistacroloba* and *S. demissum* (Table 3). In series *Acaulia* type 1-inflorescences were characterized by a relatively short peduncle (see *morphometric data*).

In series *Acaulia* a second type was often observed (Fig. 4d-e). We entitled this type of inflorescence **'Type 2'**. It differs in an exceptional way from the 'normal' monochasium. The peduncle is absent or strongly reduced in length and one 'extra' flower is placed in the axil of the leaf blade. This flower is placed separately from the monochasium. The 'extra' flowers were the first developed flowers in the inflorescences and often also the largest flowers in an inflorescence. The pedicels of the 'extra' flowers curved downwards soon after anthesis (Figs. 2, 4h). About half of the studied inflorescences of *S. acaule* were type 2, and a quarter of the *S. albicans* inflorescences. Also inflorescences with two 'extra', single flowers were observed and called **'Type 2A'** (Figs. 2, 4g-h). These were mainly recorded in *S. acaule* ssp. *acaule* (Table 3).

In *S. demissum*, *S. megistacrolobum*, and *S. boliviense* type 2-inflorescences were also present (Table 3), but regularly with a short peduncle. In most of their inflorescences the structural position of the 'extra' flower differed from that in series *Acaulia*: the 'extra' flower was not positioned separately in the axil of the subtending leaf, but placed at the base of the monochasium and definitely forming part of it (Fig. 4f). However, in some

S. megistacrolobum inflorescences the 'extra' flower was positioned in the axil of a leaf as in series *Acaulia*. Type 2-inflorescences were never observed in series *Tuberosa*. Also *S. sanctae-rosae* and *S. raphanifolium* (series *Megistacroloba*) did not exhibit type 2-inflorescences.

Inflorescences with a forked peduncle bearing two monochasia, named **'Type 3'**, were present in all taxa (Table 3; Figs. 1, 3, 4i-j). One might speak of a real dichasium when a terminal flower is present within the inflorescence (**'Type 3A'**). In *S. brevicaule* and *S. bukasovii* more than 90% of the inflorescences were type 3 or 3A (Table 3).

Usually one leaf grows concaulescent with the inflorescence (Fig. 4b). This leaf resembles the leaves in the vegetative part of the anthoclade, but is often smaller. Also inflorescences without this leaf on the peduncle were observed. On most peduncles of type 3-inflorescences only one leaf was present (Fig. 1, upper inflorescence, and Fig. 3). Leaves within the inflorescences were not often observed. In *S. megistacrolobum* and *S. demissum* we did find occasionally small leaves (Fig. 4f). They were randomly distributed within the inflorescence, but never above the articulation of the pedicel. Regularly placed bracts were not observed in the material of this study.

The plants grown in the greenhouse were much longer and more elongated than the herbarium specimens of material collected in South America. This facilitated the observations on the structure of inflorescences, especially of the plants belonging to series *Acaulia* and *S. megistacrolobum* (see Figs. 1, 2). In herbarium material of *S. acaule* and *S. albicans* (Table 2) it was possible to observe 'extra' flowers in certain specimens (*S. acaule* specimens *Hawkes, Hjerting, Cribb & Huamán 4243, Ochoa & Salas 11824, 11830* and *11895, Venturi 6993*).

Table 3. Percentage of inflorescence types observed

Species	Total number of inflorescences observed	Type 1	Type 2	Type 2A	Type 3	Type 3A
Solanum acaule ssp. *acaule*	117	9%	44%	29%	12%	6%
ssp. *aemulans*	36	19%	45%	-	33%	3%
ssp. *punae*	28	14%	61%	-	25%	-
S. albicans	32	3%	25%	3%	50%	19%
S. boliviense	104	76%	19%	-	5%	-
S. megistacrolobum ssp. *megistacrolobum*	51	67%	25%	2%	6%	-
ssp. *toralapanum*	47	68%	13%	6%	13%	-
S. raphanifolium	37	73%	-	-	24%	3%
S. sanctae-rosae	49	98%	-	-	2%	-
S. demissum	56	38%	29%	6%	18%	9%
S. brevicaule	56	9%	-	-	91%	-
S. bukasovii	38	8%	-	-	76%	16%

Morphometric data. In the trial field at Sturgeon Bay (USA) the plants showed a more compact habit such as found under natural conditions. The reduced length of inflorescences in series *Acaulia* (*S. acaule* and *S. albicans*) and *S. demissum* and *S. megistacrolobum* ssp. *megistacrolobum* was expressed in the relative shortness of their peduncle (see Fig. 5a). The plants of these taxa had peduncles with an average length of less than one centimeter. *Solanum brevicaule* and *S. megistacrolobum* ssp. *toralapanum* had a significantly longer peduncle compared to these four species (Tukey-Kramer HSD-test, $\alpha = 0.05$).

S. brevicaule had the highest number of flowers (mean = 11) per inflorescence compared to all other taxa (Fig. 5b). This high number of flowers in *S. brevicaule* is obviously correlated with the high percentage of forked peduncles bearing two monochasia (Table 3). Monochasia, also those with 'extra' flower(s) (Type 2/2A), generally consist of few flowers. For example, the inflorescences of *S. acaule* (all three subspecies) which are mostly of type 2/2A and type 1 (Table 3), had only three to four flowers (Fig. 5b).

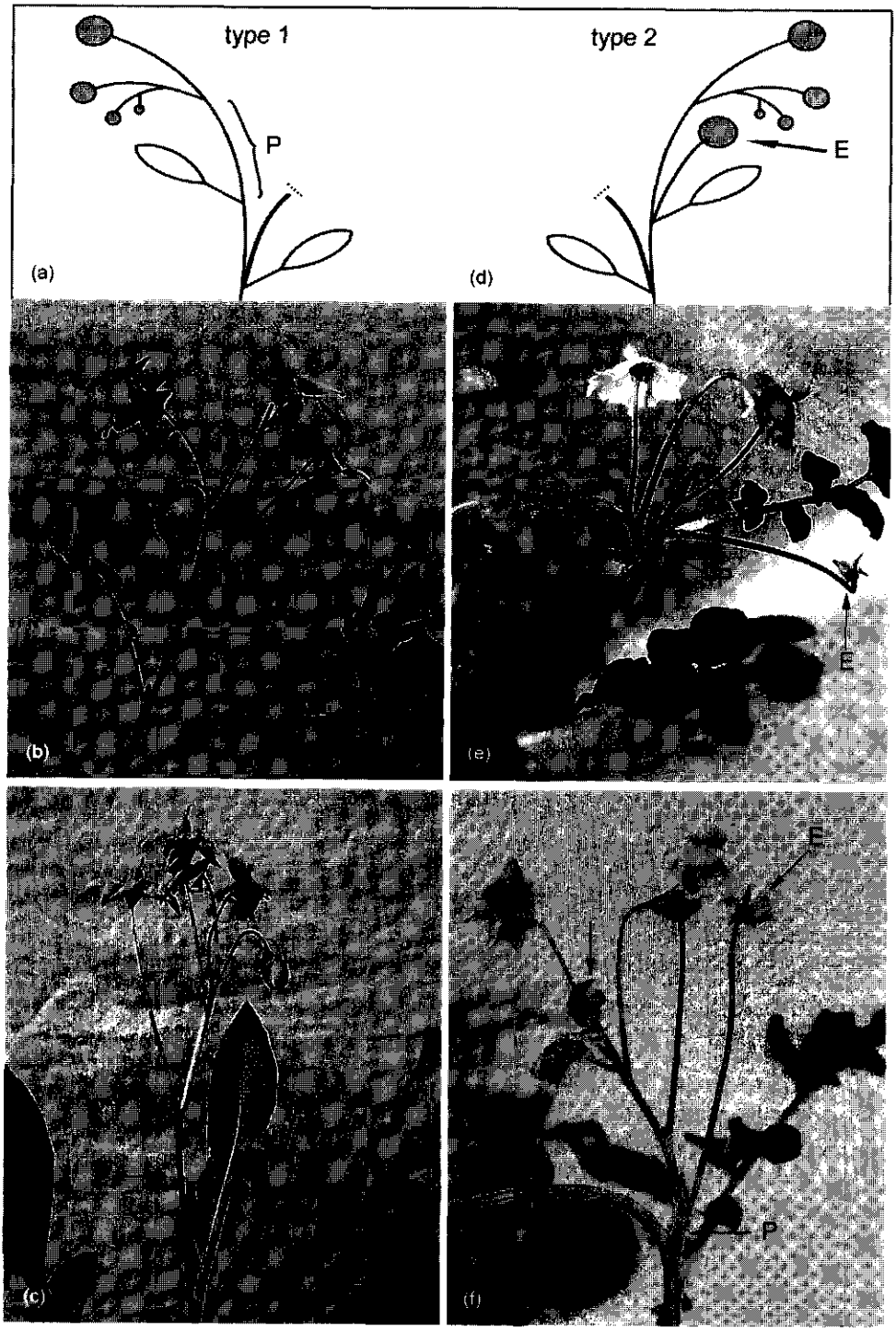
All the taxa, except *S. megistacrolobum* ssp. *megistacrolobum*, had a pedicel length between 15 and 30 mm (Fig. 5c). No correlation could be found between peduncle length and pedicel length of the observed taxa. *Solanum megistacrolobum* ssp. *megistacrolobum* has conspicuously long pedicels with an average length of 40 mm. These pedicels position the flowers beyond the leaves of this compact plant. The studied accession of *S. megistacrolobum* ssp. *toralapanum* displayed relatively long peduncles with medium-length pedicels. In series *Acaulia* the flowers are hidden in the foliage, which is most obvious in the herbarium specimens of plants collected in South America.

The flowers of the inbreeding species *S. acaule* and *S. albicans* were relatively small (Fig. 5d). The average corolla diameter of these taxa ranged between 13.5 and 17.5 mm. The outbreeders *S. brevicaule* and *S. megistacrolobum* had statistically significant (HSD-test, $\alpha = 0.05$) larger corollas (22 - 23 mm). The corolla dimensions of *S. demissum* were of intermediate size.

The styles of all three inbreeding species (*S. acaule*, *S. albicans*, and *S. demissum*) were generally not much exserted from the anther column. The average style exsertion ranged in this group of five taxa from 1.4 mm (*S. acaule* ssp. *punae*) to 2.2 mm (*S. acaule* ssp. *aemulans*). The exsertion in the two outbreeding species was larger: in *S. megistacrolobum* ssp. *toralapanum* the average exsertion was 2.5 mm and in *S. brevicaule* even 3.4 mm, which is significantly different from all inbreeding taxa.

DISCUSSION

Inflorescence architecture. The 'Type 2' inflorescence has never been mentioned explicitly in taxonomic studies of *Solanum*. We studied plants grown in a greenhouse. Their habit was quite elongated. To ascertain that this unnatural environment did not cause aberrations in the inflorescence architecture, such as the formation of 'extra' flowers, a comparison was made with herbarium material. In herbarium specimens of *S. acaule* we also observed 'extra' flowers. Apparently, the architecture of the plants did not change fundamentally by the growth under greenhouse conditions. Until now, the structure of this type of inflorescence has apparently been overlooked due to the compact habit of species having inflorescences with 'extra' flowers.


type 1
P
(a)
(b)
(c)
type 2
E
(d)
(e)
E
E
P
(f)

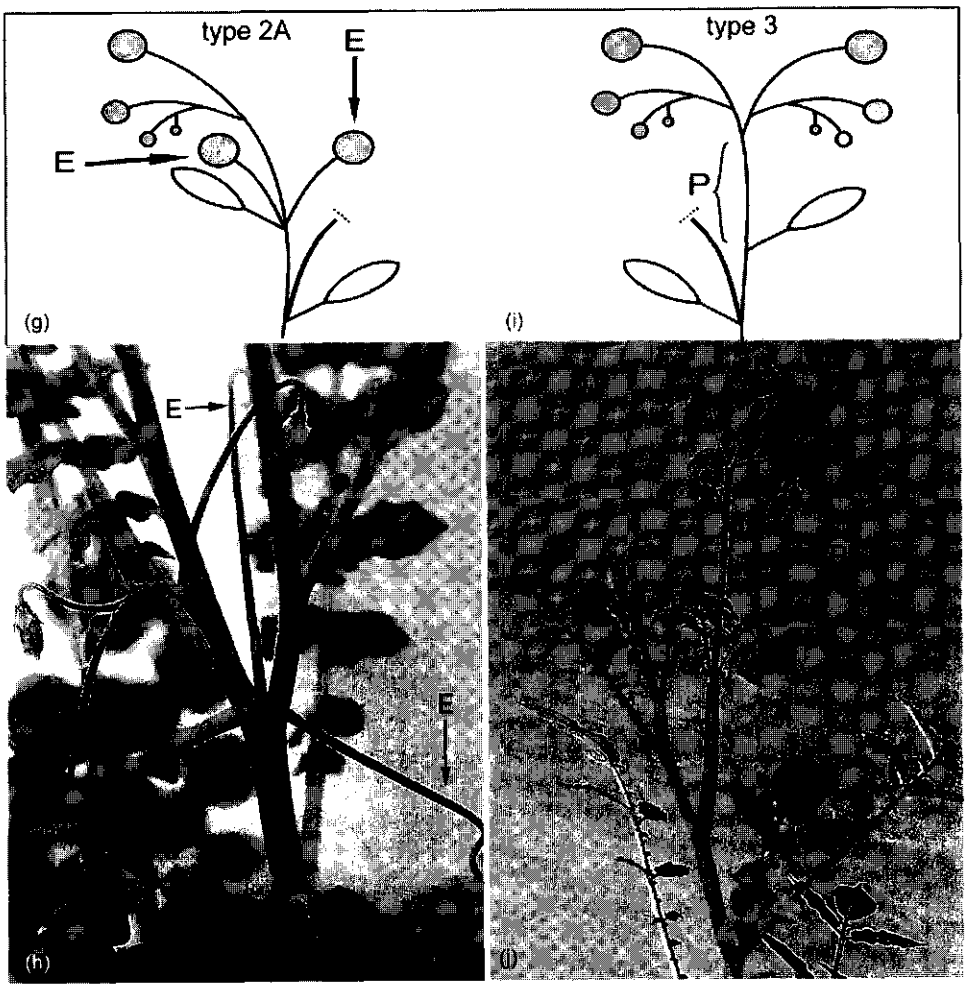


Figure 4. Overview of the inflorescence types as described in this study. P = peduncle; E = 'extra' flower. (a) Schematic drawing of type 1, the monochasium. (b) A photograph of *S. sanctae-rosae* (BGRC 24674) showing a type 1-inflorescence. (c) Type 1-inflorescence in *S. megistacrolobum* ssp. *toralapanum* (BGRC 27115). (d) Type 2, a monochasium with an 'extra' flower in the axil of the subtending leaf. (e) Type 1 (left) and type 2 (right) inflorescences in *S. acaule* (BGRC 16835). (f) Type 2-inflorescence with a short peduncle in *S. demissum* (BGRC 9990). Note the randomly placed leaves in the inflorescence (arrow). (g & h) Schematic drawing and photograph (*S. acaule*, BGRC 16835) of a type 2A-inflorescence, a monochasium with two 'extra' flowers. (i) Type 3: the forked peduncle with two monochasia. This is the most common type of inflorescence in *Solanum* sect. *Petota*. (j) *S. brevicaule* (BGRC 28038) showing this type of inflorescence.

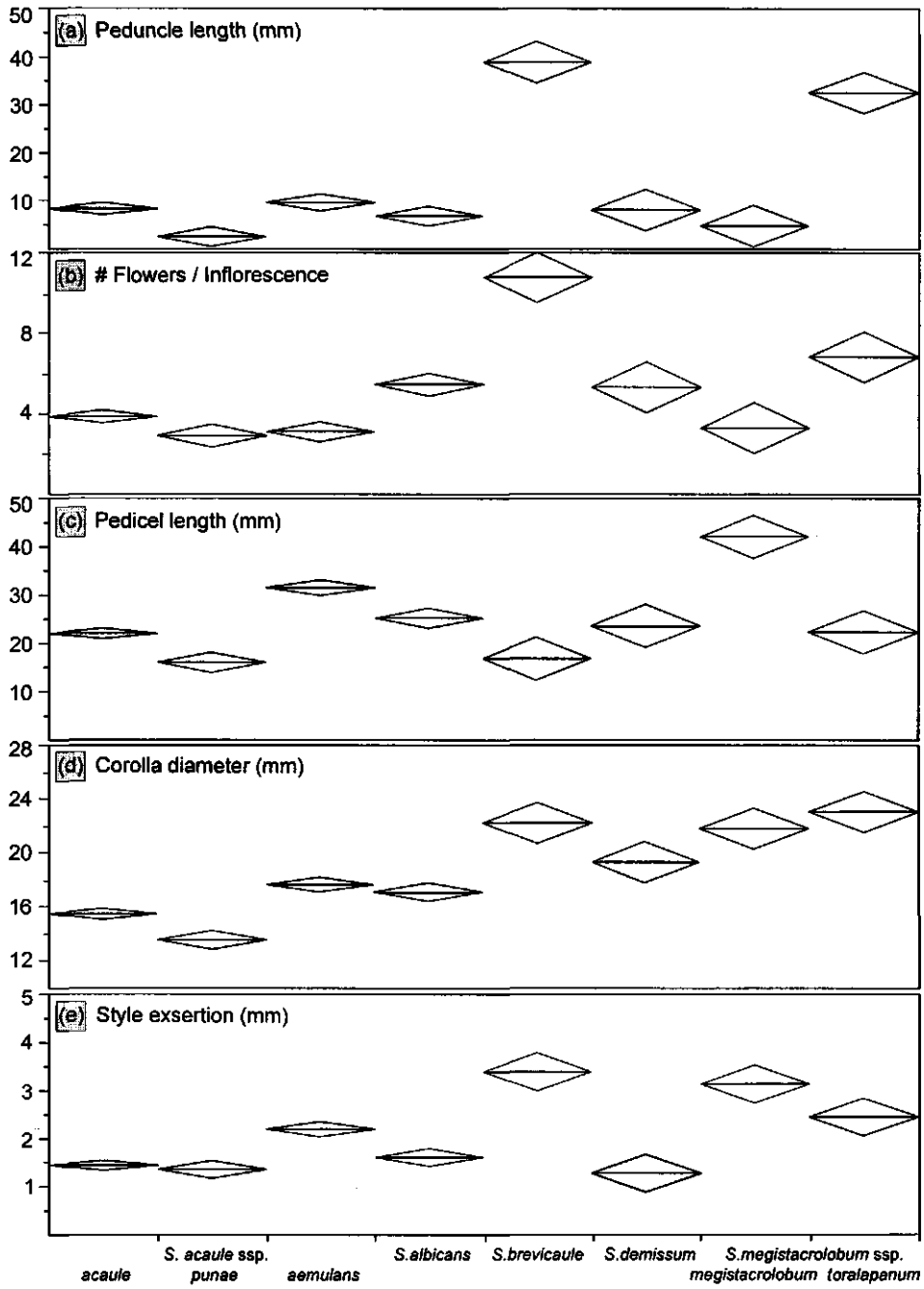


Figure 5. Morphometric data on eight taxa, measured in a field-plot at Sturgeon Bay. Displayed are the means (horizontal lines) and 95% confidence interval (diamond) of: (a) the peduncle length from the subtending leaf to the first fork or pedicel, (b) total number of flowers per inflorescence, (c) pedicel length, (d) corolla diameter and (e) style exsertion.

In the taxonomic diagnoses of tuber-bearing *Solanum* species, inflorescence structural characters such as the 'extra' flower(s) in type 2-inflorescences of *S. acaule*, *S. albicans*, *S. megistacrolobum*, *S. boliviense*, and *S. demissum*, should be used with care. The inflorescence architecture is often quite variable, even within individual plants. However, most other *Solanum* sect. *Petota* species do not exhibit these 'extra' flowers in the inflorescence. During this and earlier studies (Van den Berg *et al.* 1996, 1998) we have never observed 'extra' flowers in series *Tuberosa*.

Regarding the interpretation of the 'extra' flower(s) in type 2-inflorescences of series *Acaulia*, *S. megistacrolobum*, *S. boliviense*, and *S. demissum*, two different structural situations have been described in the results. In the first situation, the 'extra' flower is placed directly in the axil of the subtending leaf and is not associated with the accompanying monochasium. This configuration was observed in most inflorescences of series *Acaulia* plants and infrequently in *S. megistacrolobum*. On the other hand, in *S. megistacrolobum*, *S. boliviense and S. demissum* the 'extra' flower is generally placed at the very base of, and in the same plane as the monochasium, and is undoubtedly part of it (Fig. 4f). Below the 'extra' flower usually a (short) peduncle can be recognized. This could be the result of the incomplete concaulescence of the monochasium with the terminal flower, or the descent of the terminal flower. Accordingly, the type 2-inflorescences of these three taxa are then transformed type 1-monochasia. The terminal flower is separately placed from the monochasium and appears as an 'extra' flower.

The first situation, in which the 'extra' flower is not connected to the monochasium, needs some further discussion. Only Danert (1957) has described the inflorescence of *S. acaule* in more detail. In his view the inflorescence of *S. acaule* is a normal dichasium, as in *S. tuberosum*. He showed a figure of an inflorescence of *S. acaule* in flower bud stage. In this figure one flower bud is unmistakably larger than the other buds. He stated that this was the terminal flower of the dichasium. In our view this bud is the 'extra' flower as described above. A hypothesis to explain the occurrence of the 'extra' flower could be that, as described above, this flower is indeed the terminal flower of the anthoclade, and that the accompanying monochasium did not grow concaulescent with this flower. However, this explanation does not take into account that thirty percent of the subspecies *acaule* inflorescences have two 'extra' flowers (Table 3: type 2A; Fig. 4g-h).

Other considerations could be put forward on the presence of 'extra' flowers. In his treatment on the structure of inflorescences, Weberling (1989) described the formation of accessory buds in inflorescences. The 'extra' flowers could have been developed from these buds. Weberling (1989) has given an example of *Ipomopsis rubra* (L.) Wherry (Polemoniaceae). In this species accessory buds grow out to flowering accessory shoots after anthesis of the primary flowers. In *S. acaule* the 'extra' flowers are the first flowers and develop before the rest of the flowers of the monochasium. So the explanation on 'extra' flowers as accessory buds may be questioned. Finally, it could be proposed that one of the two 'extra' flowers is the terminal flower of a single-flowered partial inflorescence. The other 'extra' flower could then again be assigned as the terminal flower of the monochasium, as described above. It may be concluded that no satisfying hypothesis on the explanation of 'extra' flowers can be given, but the presence of these flowers in series *Acaulia* is of importance for potato taxonomy.

The highest leaf in the main axis has been considered by Danert (1967) as the bract of the first partial inflorescence. Danert designated this subtending leaf on the peduncle as a diagnostic character for sect. *Petota*. The diagnostic value of this trait may be questioned because we regularly observed inflorescences without such a leaf in section *Petota*. Danert (1967) also remarked that in compound inflorescences with two, three or more forks an equal number of leaves on the peduncle could be observed. Danert previously (1957) asserted that the upper partial inflorescence in a dichasium is placed without a subtending leaf ("tragblattlos") and that the leaf on the peduncle belongs to the terminal flower of the inflorescence. Usually we also observed only one leaf on the peduncle.

Rather frequently we found small normal to bract-like leaves in the inflorescences of various taxa, e.g. in *S. demissum* (Fig. 4f). The placement of these leaves is random and hence it is questionable to define these leaves as bracts subtending the individual flowers. However, the term "ebracteate" of the inflorescences of *Solanum* sect. *Petota* described by Hawkes and Hjerting (1989) is too strict. Danert (1957) has displayed a scheme of a potato inflorescence with bracts at the base of every pedicel. In recent studies on tuber-bearing *Solanum* species of various taxonomic series (Spooner and Van den Berg 1992b, Spooner *et al.* 1995, Van den Berg *et al.* 1996, 1998, Kardolus 1998, Van den Berg and Groendijk-Wilders 1998) these bracts were never observed.

The inflorescence architecture in relation to habitat and breeding behavior. The wild potato species are most abundantly distributed in the high mountains of South and Central America (Correll 1962). Taxa such as the species belonging to Series *Acaulia* have a rosette-habit and inflorescences with short peduncles (Figs. 4e, 5a). Therefore they can prosper at elevations up to even 4400 meters (Bamberg *et al.* 1996).

The breeding behavior of the polyploid species in series *Acaulia* and *S. demissum* differs from the diploid and obligate outcrossing wild potato species. *Solanum acaule*, *S. albicans*, and *S. demissum* are self-pollinators and the flowers can be cleistogamically pollinated (Hawkes and Hjerting 1969, Ugent 1981). This mechanism is facilitated by the short, hardly exserted style (Fig. 5e). Inbreeding potato species do not have to attract insects. Consequently they can suffice with less pronounced inflorescences with relatively few and small flowers (Fig. 5b, d). *Solanum megistacrolobum*, however, is a diploid outbreeder and needs to exhibit its flowers to pollinators. Notwithstanding the compact habit of *S. megistacrolobum*, the flowers are well presented because of the length of the pedicels (Fig. 5c) and relatively large corollas (Fig. 5d). In series *Acaulia* the flowers are positioned among the foliage. Moreover, the peduncles and pedicels bend downwards with maturing fruits (Hawkes and Hjerting 1969, 1989, Ochoa 1990), especially the pedicels of the 'extra' flowers. Due to this geotrophic tendency the fruits of these high-Andes species are embedded in the soil and in this way ensure seed production in areas of early frost.

CHAPTER 3

The floral abscission zone in series *Acaulia* and related taxa of *Solanum* section *Petota*

ABSTRACT

Species of the genus *Solanum* usually possess pedicels with a floral abscission zone, which is designated the 'articulation' or 'joint'. A distinct group of tuber-bearing wild potatoes, series *Acaulia*, is characterized by an indistinct or completely absent articulation. The anatomy of pedicels without articulation is compared with that of articulated pedicels. No abscission zone is observed in pedicels without an articulation, a situation found in the tetraploid *Solanum acaule* ssp. *acaule* and *Solanum acaule* ssp. *punae*. The hexaploid *Solanum albicans* of series *Acaulia* has an anatomically incompletely differentiated abscission zone. *Solanum acaule* ssp. *aemulans* has articulated pedicels and a floral abscission zone. The absence of a floral abscission zone is presumably a recessive trait. The special features of pedicel articulation in series *Acaulia* are discussed in relation to the 'jointless'-mutations in tomato. The position of the articulation on the pedicel is concluded to be less significant for taxonomy than generally considered.

INTRODUCTION

The floral abscission zone is an important character in taxonomic studies of potato, *Solanum tuberosum* L., and related wild species. This zone is visible as a constriction of the pedicel and has been designated as the 'articulation'. Dorsey (1919) and Cutter (1978) have described abscission at the articulation in several stages of flower and berry development: the flowers may already fall in the bud-stage or at full anthesis, and also shedding of immature fruits often occurs. Mature potato berries might break off at the articulation for dispersal (Bitter 1912).

In *Solanum* L. sect. *Petota* Dumort., in which the cultivated potato is classified, both the presence or absence of the articulation and the position of this zone on the pedicel has been used to distinguish taxa (Hawkes 1990). Both aspects are of interest in the polyploid series *Acaulia* Juz., which is classified in sect. *Petota*. Hawkes and Hjerting (1969: p. 106) have remarked that "no abscission layer is developed in series *Acaulia*, where the articulation is generally not very well marked and may often be completely absent". If present, the pedicel position in series *Acaulia* is "high up underneath the calyx" (Hawkes 1990). Furthermore, Hawkes has characterized the articulation of the tetraploid (2n=48) *S. acaule* Bitter ssp. *acaule* as "not well-marked or generally completely absent" compared to *S. acaule* ssp. *aemulans* (Bitter & Wittm.) Hawkes & Hjert. where it is "well-marked". The second species classified in series *Acaulia*, the hexaploid *S. albicans* (Ochoa) Ochoa, exhibits a peculiar articulation of the pedicel. Its articulation can be seen as a pale green band at anthesis (Kardolus 1998), but the fruits do not separate at this articulation; and the articulation is positioned further from the calyx than in *S. acaule* (Hawkes 1990). It is clear

from the above that a distinction between the articulation and the abscission zone should be made and that even a pedicel lacking an abscission zone can still be articulated. Until now no anatomical studies have been presented to determine whether in series *Acaulia* the abscission zone is truly absent.

In order to define the abscission zone and the abscission layer unequivocally, we will use the definition of Wittenbach and Bukovac (1972): "The abscission zone is a zone generally at the base of a leaf, or fruit, or flower, or other plant part, that contains the abscission layer and the protective layer, both of which (may) play a role in the separation of the plant part from the plant. The abscission layer is the layer of cells in the abscission zone that after disjunction or breakdown causes the separation of the plant part from the plant". Wittenbach and Bukovac (1972) have described two abscission zones in sweet cherry (*Prunus avium* L.): one zone between pedicel and peduncle, and one between the fruit and the receptacle. Biaiñ de Elizalde (1980) has also interpreted two abscission zones within the inflorescence of tomato, *Solanum lycopersicum* L. (syn. *Lycopersicon esculentum* Mill.). Using the same terminology as Wittenbach and Bukovac (1972), she has described a proximal abscission zone ("between pedicel and peduncle", see next paragraph) where flowers and immature fruits abscise, whereas the ripe fruits abscise at the distal abscission zone ("between fruit and pedicel"). Kendall (1918) conducted an extensive study on abscission processes in the Solanaceae, but did not include the falling of mature berries from the receptacle. Generally, the articulation of the pedicel is considered to be the only abscission zone in the inflorescence of *Solanum* section *Petota*.

The term pedicel has mostly been defined in the Solanaceae as the flower stalk extending from the base of the calyx to the place of attachment to the peduncle (Hawkes 1990). One might argue, however, that the part of the flower stalk above the articulation has specific morphological features and needs to be distinguished from the part below the articulation. Hawkes and Hjerting (1969: 106) have reported that "If the calyx pubescence differs markedly from that of the stem and peduncle, this same difference is also developed on the upper part of the pedicel, whilst the lower part, below the articulation, shows the pubescence type of peduncles and stems", which seems to support such a distinction. In the concept of Biaiñ de Elizalde (1980), describing the proximal floral abscission zone (the 'articulation') being placed "between peduncle and pedicel", the pedicel is delimited to the part above the abscission zone. This opinion is not followed in other taxonomic studies on Solanaceae. Velenovsky (1910) has given a third interpretation of the flower stalks of the Solanaceae, among other plant groups. He interpreted the flower stalk below the calyx unto the abscission zone as an extension of the receptacle and named it the "pericladium". In his view the pedicel is the proximal part of the flower stalk below the abscission zone to the peduncle. Danert (1957) has opposed this opinion, arguing that in anatomical sections the part of the flower stalk below and above the articulation is identical. For practical reasons we have used the most widely adapted definition of pedicel, that is "the entire ultimate flower stalk bearing a flower" (see also Rickett 1954 for further discussions).

When the position of the articulation is used in taxonomic studies, two points need special attention: besides the question how to describe the position of the joint unequivocally, it is important to know whether this characteristic is taxonomically as useful as often suggested (Correll 1962, Hawkes and Hjerting 1969, 1989, Hawkes 1990, Ochoa 1990). Most species classified in series *Tuberosa* (Rydb.) Hawkes have the articulation in the middle of the pedicel as in *S. tuberosum*. The articulation can also be more eccentrically

placed towards the top of the pedicel (e.g. *S. acaule* ssp. *aemulans*) or at its base (e.g. *S. brevidens* Phil.). However, in certain species such as *S. bukasovii* and *S. fendleri* the position has been described as 'well above the middle' (Correll 1962) and 'very high' (Hawkes 1990), without a precise definition of these circumscriptions. We want to re-evaluate the usefulness of the position of the articulation on the pedicel, and examined a representative sample of taxa.

We observed the floral abscission zone in *Solanum* sect. *Petota* to determine if this zone is the anatomical equivalent to the morphological term 'articulation of the pedicel'. In examining plant material of series *Acaulia* and related taxa we investigated whether the floral abscission zone in series *Acaulia* is actually absent. Besides the anatomical evaluation of the floral abscission zone, we studied morphometric data to evaluate the suitability of 'the position of the articulation' as a taxonomic trait.

Table 1. List of material used in the anatomical experiment

Species	Accession[1]	Origin	Articulation[2]	Observation[3]
series *Acaulia* Juz.				
Solanum acaule Bitter ssp. *acaule*	B 17148	Argentina	±	2
S. acaule ssp. *acaule*	B 17181	Argentina	±	2
S. acaule ssp. *acaule*	B 27206	Bolivia	-	2
S. acaule ssp. *acaule*	B 24555	Argentina	-	2
S. acaule ssp. *punae* (Juz.) Hawkes et Hjert.	B 07958	Peru	-	2
S. acaule ssp. *aemulans* (Bitter et Wittm.) Hawkes et Hjert.	B 17180	Argentina	±	2
S. albicans (Ochoa) Ochoa	B 18287	Peru	±	2
S. albicans	[4] PI 561642	Ecuador	± / -	2
series *Megistacroloba* Cárdenas et Hawkes				
S. megistacrolobum Bitter	B 17642	Argentina	+	2
S. boliviense Dunal	B 27248	Bolivia	+	2
series *Longipedicellata* Bukasov				
S. fendleri A.Gray	B 23568	USA	+	2
series *Tuberosa* (Rydb.) Hawkes				
S. brevicaule Bitter	B 28038	Bolivia	+	2
S. coelestipetalum Vargas	B 07993	Peru	+	2
S. bukasovii Rybin	B 15424	Peru	+	1
S. juzepczukii Bukasov	[5] CIP 702445	?	±	1
artificial hybrids				
ssp. *acaule* × *S. fendleri*	B 24555 × 23568	-	±	2
ssp. *acaule* × ssp. *aemulans*	B 24555 × 17180	-	±	2
ssp. *aemulans* × ssp. *acaule*	B 17180 × 24555	-	±	2

[1] **B:** BGRC-number of the Centre for Genentic Recources, Wageningen, The Netherlands; **PI:** Plant Introduction-number NRSP-6, Sturgeon Bay, WI - USA; **CIP:** International Potato Center - Peru

[2] – : pedicel articulation absent; + : pedicel articulation present; ± : pedicel articulation present but less pronounced

[3] **1:** only macromorphological observations; **2:** macromorphological and anatomical observations

[4] This hexaploid (2n=72) accession is morphologically somewhat aberrant from typical *S. albicans*. Molecular studies are in progress to determine its taxonomic status.

[5] received as in-vitro clone

MATERIALS AND METHODS

Anatomical experiment. Plants, received as seeds (Table 1) from the Centre for Genetic Resources (Wageningen, The Netherlands) and the Potato Introduction Station NRSP-6 (Sturgeon Bay, USA), were grown in pots in a greenhouse at the Department of Plant Taxonomy, Wageningen Agricultural University. Also an in-vitro clone was studied from CIP, Lima, Peru (see Table 1). Vouchers of each accession are deposited in WAG.

Per genebank accession three pedicels were taken at anthesis and three at the ripe-berry stage. Some flowers were pollinated manually with pollen from the same genebank accession to stimulate fruit set. The pedicels were stored in ethanol 70%. To study the development of the pedicel articulation of *Solanum albicans* during fruit set, additional pedicels were taken at days 14, 28 and 42 after anthesis. After dehydration in ascending concentrations of ethanol (70%, 90%, 96%, 100%), the pedicels were passed through a series of ascending concentrations of Technovit® 7100 (Heraeus Kulzer GmbH, Wehrheim, Germany), and embedded in the same. From this material longitudinal sections were cut with a Leitz microtome at a thickness of 2-4 μm. Sections were mounted on microscope slides and stained for 10 minutes with 0.5 % (1N HCl) toluidine blue (BDH laboratory supplies, Poole, England). Subsequently, preparations were rinsed thoroughly with water, air dried, mounted in DPX (BDH laboratory supplies, Poole, England) and covered with cover slips. Photographs were taken with a Zeiss Axiophot photo-microscope.

Morphometrical experiment. In 1993 and 1995, plants were grown in field plots at the agricultural research stations at Sturgeon Bay and Hancock, Wisconsin, USA. In this morphometrical experiment the following eleven taxa were examined: *S. acaule* ssp. *acaule*, ssp. *punae* and ssp. *aemulans*, *S. albicans*, *S. megistacrolobum* (material described in Kardolus 1998), *S. demissum* Lindl. (material described in Spooner *et al.* 1995), *S. brevicaule*, *S. bukasovii*, *S. fendleri*, *S. oplocense* Hawkes and *S. verrucosum* Schltdl. (see Van den Berg *et al.* 1998). Per taxon between 5 and 24 genebank accessions were studied, and of *S. megistacrolobum* only two.

At complete anthesis two characters were measured on one of the first opening flowers of an inflorescence: 1) the total pedicel length in mm (PedLength), and 2) the distance from the base of the pedicel to the articulation point in mm (ArtLow). Two characters were derived from these two measurements: 3) ArtUp, the length (in mm) from the articulation to the base of the calyx (PedLength - ArtLow); 4) ArtRatio, the relative position of the articulation on the pedicel (ArtLow / PedLength). These inflorescence characters were examined on three genotypes per accession. Data were analyzed with the JMP® software package, version 3.1.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Anatomical observations. Most of the examined accessions had articulated pedicels (see Table 1). Only two accessions of *S. acaule* ssp. *acaule* (BGRC 24555, 27206) and the ssp. *punae* accession BGRC 7958 had pedicels without an articulation. In the articulated plants the articulation was marked as a band around the pedicel. In general, this band was clearly visible as an constriction above a more or less developed thickening (Fig. 1a, 2a). The thickening became more obvious as the fruits matured, and a difference in pigmentation below and above the articulation was often observed, well demonstrated in *S. bukasovii*

(Fig. 1b). The part of the flower stalk below the articulation was coloured strongly with anthocyanin, and a sharp boundary could be noticed at the articulation.

Also the F1-seedlings from crossings between material without an articulation (*S. acaule* ssp. *acaule* BGRC 24555) and articulated material (*S. acaule* ssp. *aemulans* and *S. fendleri*) had pedicels with an articulation, but the constriction was less pronounced than in the articulated parents (not shown). We attempted to self the F1-hybrids of ssp. *acaule* × *S. fendleri* to produce an F2, and tried back-crossings with both parents, but no berries were formed. The F1-hybrids of *S. acaule* ssp. *acaule* × ssp. *aemulans* and reciprocal were also unexpectedly self-sterile. In *S. acaule* ssp. *acaule* accessions BGRC 17148 and 17181, in *S. acaule* ssp. *aemulans* and in *S. juzepczukii*, the articulation was somewhat less pronounced (like in the F1-genotypes) than in 'normal' articulated material as e.g. *S. brevicaule*. Especially in BGRC 17148 the articulation was quite inconspicuous at anthesis, and during the ripening of the berries the articulation swelled only weakly.

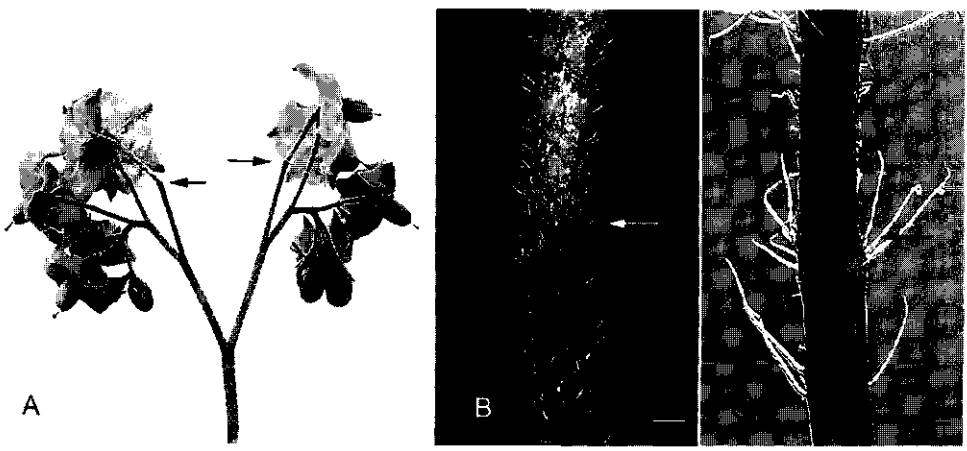


Figure 1. (a) Inflorescence of *S. brevicaule* showing the articulation (arrows) of the pedicels. (b) *S. bukasovii* BGRC 15424: below the articulation (arrow) the pedicel is darkly pigmented with anthocyanin, above the articulation no anthocyanin pigmentation is visible. (c) Pedicel of *S. albicans* BGRC 18287, arrow points to the articulation which is visible as a pale green band and faint constriction. Flowers photographed at full anthesis. Bar in b, c = 0.5 mm.

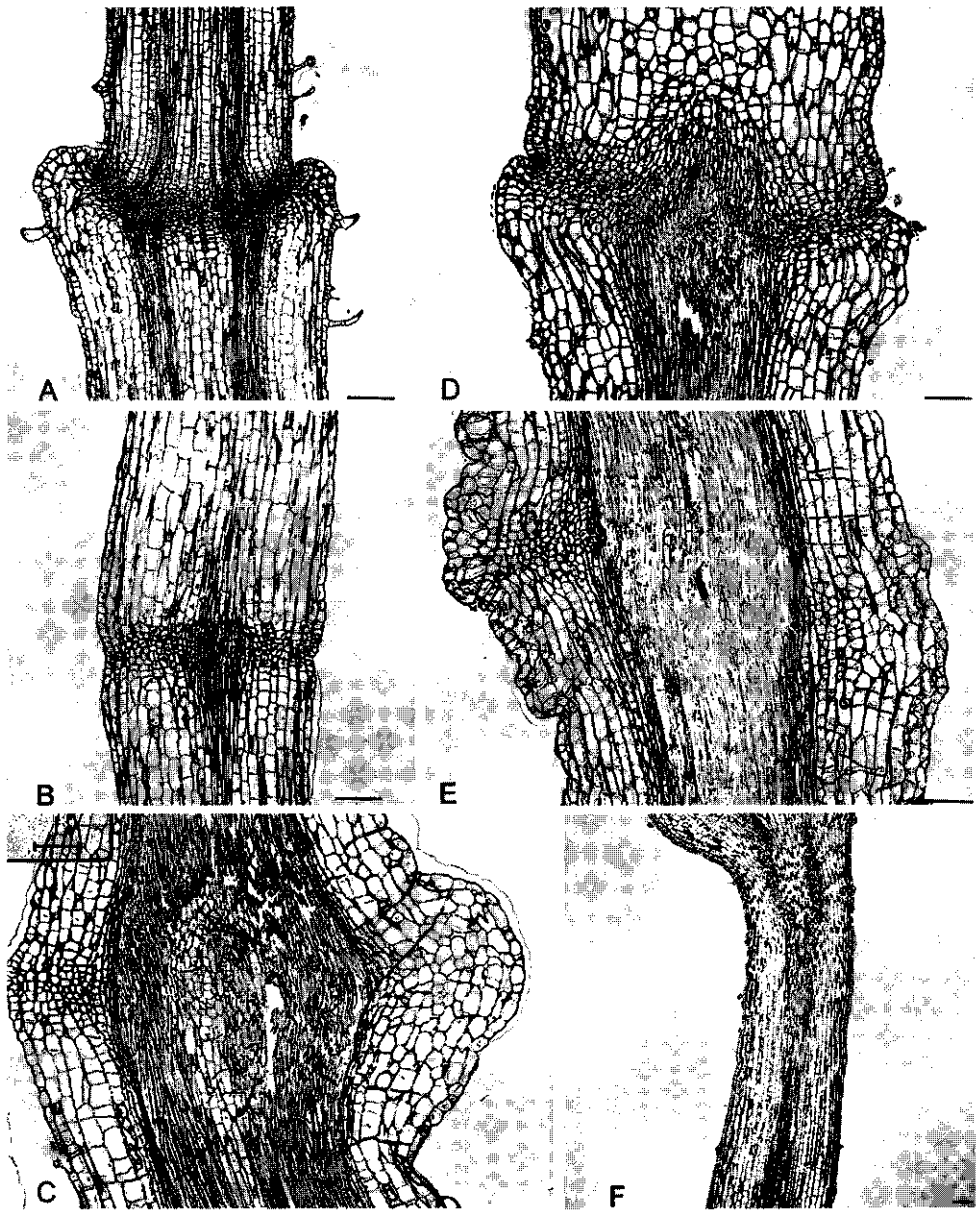


Figure 2. Longitudinal sections of pedicels, stained with toluidine-blue. Flowers or berries are positioned to the upperside. Bar = 155 μm. (a) *S. brevicaule* BGRC 28038: floral abscission zone at anthesis showing small undifferentiated cells. (b) Pedicel at anthesis of *S. albicans* BGRC 18287. The constriction is inconspicuous compared to Fig. 2a, but the small cells are present. (c) Ripe berry stage, also of accession BGRC 18287. The small cells in the articulation zone are partly (right hand side) transformed to larger parenchymatic cells. (d) Pedicel at ripe berry stage of articulated *S. acaule* ssp. *acaule* BGRC 17148 showing small cells at the articulation. (e) Pedicel at day 14 after anthesis of *S. albicans* PI 561642. The articulation in this accession is not pronounced. (f) *S. acaule* ssp. *punae* (BGRC 7958) at anthesis, pedicel without a layer with small, undifferentiated cells.

The longitudinal sections through the pedicel of almost all material studied (except for *S. albicans* and non-articulated *S. acaule* accessions) showed the articulation as 7-10 rows of relatively small cells (Fig. 2a) with dense cytoplasmic contents and large nuclei. The cell contents of these cells were darker stained by toluidine blue than those of adjacent cells. The vascular bundles continued uninterrupted through the layer. During the development of the berries, the entire pedicel thickened, primarily due to the expansion of the pith (compare Figs. 2b and C). In ripe-berry stage the abscission layer was often still visible because the cells remained small and undifferentiated (Fig. 2d). A clear abscission layer was observable in the pedicel sections of the F1-offspring of the crossings between non-articulated and articulated material, and the articulated *S. acaule* ssp. *acaule* and ssp. *aemulans* accessions. The layer remained intact during ripening of their fruits (not shown).

S. albicans exhibited a special type of floral abscission zone. In both *S. albicans* accessions the articulation at anthesis was expressed as a pale green band on the pedicel and only a faint constriction (Fig. 1c). When fruit-set was prevented by emasculation, the flowers and the part of the pedicel above the articulation did not fall off but merely shriveled, whereas 'normal' articulated species such as *S. brevicaule* do shed their flowers after emasculation. In the *S. albicans* accession PI 561642 the articulation was often hard to distinguish or even not visible externally, this variation being observed within one inflorescence.

The sections of the pedicel at anthesis of *S. albicans* BGRC 18287 showed a distinct zone with small cells, but only slightly constricted (Fig. 2b). In material collected 14 days after anthesis, the cells in this layer were enlarged to parenchyma-like cells and the pedicel was swollen at the articulation zone. However, this swelling was irregular and often unequally enlarged around the pedicel. At the mature fruit stage it appeared as a somewhat lump-like outgrowth without a clear constriction (Fig. 2c). In the *S. albicans* accession PI 561642 from Ecuador, the layer was less developed (Fig. 2e) than in accession BGRC 18287. In both accessions the zone became less pronounced and finally disappeared during ripening of the berries.

Only the pedicels of two *S. acaule* ssp. *acaule* accessions (BGRC 24555 and 27206) and of *S. acaule* ssp. *punae* (Juz.) Hawkes & Hjerting (BGRC 07958) did not show any visible articulation; no difference in pigmentation or thickening was seen in their pedicels, neither at a very early stage nor at a later stage. In the sections of the non-articulated accessions of *S. acaule* ssp. *acaule* and ssp. *punae* (Fig. 2f) there was no trace of an abscission zone. To verify a possible shift of this zone towards an extreme proximal or distal position, the ends of the pedicel towards the calyx and the peduncle were also checked but no such zone was observed.

Morphometrical investigation. In the observations on the dimensions of pedicels a strong correlation ($r = 0.95$, Fig. 3) was found between the total pedicel length (PedLength) and the length of the pedicel below the articulation (ArtLow). The length above the articulation (ArtUp) and PedLength were uncorrelated ($r = -0.08$). The taxa examined in this study, except for *S. megistacrolobum*, had a mean PedLength of 11 to 30 mm (Fig. 4). *S. megistacrolobum* had relatively long pedicels (mean length 42 mm). ArtUp varied from 4 to 8 mm, apart from *S. acaule* ssp. *aemulans* where the articulation was always positioned within 3 mm below the calyx. ArtRatio (ArtLow / PedLength), reflecting the position of the articulation on the pedicel, was highest in *S. megistacrolobum* and *S. acaule* ssp.

aemulans; *S. albicans* and *S. demissum* had their articulation also in the upper quarter of the pedicel.

A comparison of the distribution of ArtRatio in *S. acaule* ssp. *aemulans* and *S. fendleri* is given in Fig. 5. In *S. fendleri* the relative position of the articulation differed enormously among pedicels, its ArtRatio varying from below the middle of the pedicel (0.4) to very high up (0.9). The variation of this trait was considerably lower in ssp. *aemulans*. In general, ArtRatio was much more variable in the five species with the articulation positioned around the 'middle' of the pedicel; the standard deviation of ArtRatio in *S. brevicaule*, *S. bukasovii*, *S. fendleri*, *S. oplocense* and *S. verrucosum* is at least twice as high as in taxa with the articulation at a relatively high position (Fig. 4).

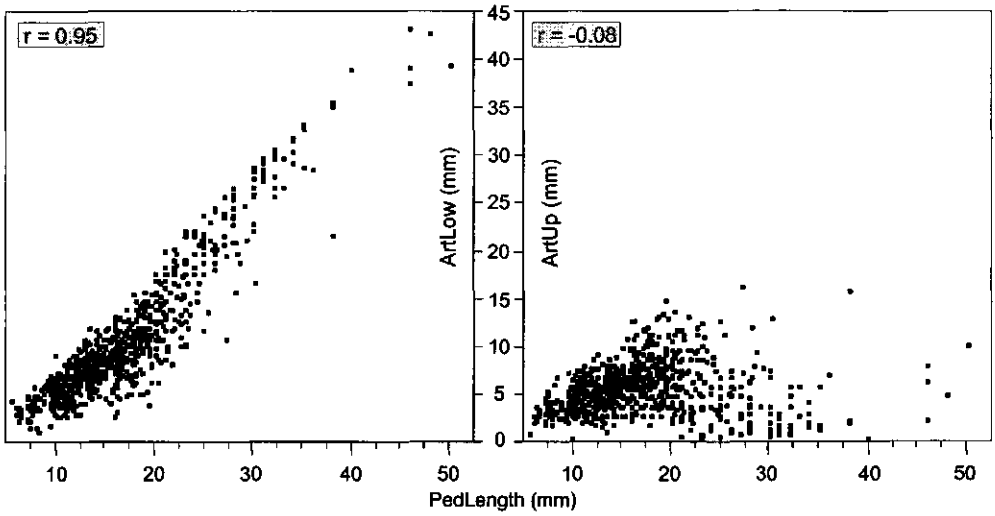


Figure 3. Scatter-plots of pedicel length below the articulation (ArtLow) and the pedicel length from the articulation up to the calyx (ArtUp) against the total length of the pedicel (PedLength). In this diagram measurements on all plant material of the morphometrical experiment have been used, except that from the *S. acaule* ssp. *acaule* and ssp. *punae* accessions. In total data on 364 pedicels are depicted. r = correlation between characters.

DISCUSSION

Pedicel articulation in series *Acaulia* and related species. To describe the articulation of the pedicel in the inflorescence unequivocally, it is necessary to apply a clear definition of the term 'pedicel'. Our study confirmed Danert's observations (1957) that below and above the articulation no anatomical differences are present in the pedicel and that the vascular bundles continue uninterruptedly through the abscission zone. Nevertheless, differences in morphology above and below the articulation have been observed in both pubescence (Hawkes and Hjerting 1969) and pigmentation, e.g. in *S. bukasovii* (Fig.1b). However, it would be impossible to describe pedicels lacking a floral abscission zone, as found in series

Acaulia, when the definition of 'pedicel' is restricted to the part above the articulation, as was used by Biaiñ de Elizalde (1980). In potato taxonomy the term pedicel is mostly seen (Correll 1962, Hawkes 1990) as the flower stalk bearing a single flower; Hawkes (1990: 228) has rather roughly defined the pedicel as "the flower stalks which grow from the upper branches of the peduncle". For taxonomic purposes this is a pragmatic and effective definition, as was also concluded after Rickett's (1954) extensive discussion.

Species of *Solanum* section *Petota* normally display a clear pedicel articulation. In the anatomical study of articulation, we found an abscission zone as defined by Wittenbach and Bukovac (1972) in most of the material studied. D'Arcy's (1972: 256) hypothesis on the articulation, i.e. "it may indicate the site of an ancestral bracteole", is not supported by the observed configuration of continuing vascular bundles. Exceptions to the general pattern of articulated pedicels were observed in series *Acaulia*. An abscission zone was totally absent in the two *S. acaule* ssp. *acaule* accessions and in the *S. acaule* ssp. *punae* accession. In a related study using more material of series *Acaulia* (Kardolus 1998), most accessions of *S. acaule* ssp. *acaule* and all of ssp. *punae* exhibit pedicels without an articulation. Bitter (1912) assumed that the articulation of *S. acaule* could have made a shift to the extreme tip of the pedicel just below the calyx. We exclude this hypothesis because no abscission zone was found in anatomical sections of the non-articulated accessions, whereas such a zone was present in the two articulated accessions of *S. acaule* ssp. *acaule* and the ssp. *aemulans* accession. In this material it was positioned a few millimeters below the calyx.

The dispersal of *S. acaule* ssp. *aemulans*, a subspecies always having a clear articulation, is confined to Argentina (Hawkes and Hjerting 1969). In germplasm of *S. acaule* ssp. *acaule* from Argentina the pedicel articulation is variably present (e.g. in BGRC 17148, 17181) or absent (e.g. BGRC 24555). In a study on 47 *S. acaule* accessions the *S. acaule* specimens from Peru and Bolivia did not have an abscission zone (Kardolus 1998). The occurrence of a floral abscission zone in *S. acaule* is apparently linked to the southern part of its area of distribution. Hawkes and Hjerting (1969) considered the absence of pedicel articulation as a derived character. They suggested consequently that ssp. *aemulans* is the most primitive taxon of the whole series *Acaulia* because of the presence of the most distinct pedicel articulation, followed by ssp. *acaule*, ssp. *punae*, and *S. albicans*. However, Okada and Clausen (1982) have postulated another hypothesis for the presence of the articulation in *S. acaule* ssp. *aemulans*. They suggested that this character could have been introgressed in *S. acaule* from an articulated species, e.g. *S. megistacrolobum*. On the other hand, when the non-appearance of a floral abscission zone is caused by some gene mutation (see **A comparison with 'jointless' in tomato**), we hypothesize that the occurrence of articulated pedicels in ssp. *aemulans* could just as well be the result of a reversal of this mutation.

The floral abscission zone is always located at the articulation but the term 'pedicel articulation' is not equivalent to the term 'floral abscission zone'. In the hexaploid species *S. albicans* the articulation is generally observed as a pale green band only at anthesis. In accession PI 561642 it is sometimes externally indistinguishable. In anatomical sections a layer with small cells is present in *S. albicans* at anthesis, but in later stages no clear layer can be identified. Emasculated or poorly fertilized flowers shrivel up completely on the plant, as do flowers of non-articulated *S. acaule* accessions. It may be concluded that *S. albicans* has articulated pedicels, but the articulation is just an incompletely differentiated and non-functional floral abscission layer.

A comparison with 'jointless' in tomato. The F1 off-spring of crosses between non-articulated *S. acaule* ssp. *acaule* and plants with a floral abscission zone, had a distinct abscission layer but it was not as well developed as in 'normal' articulated species. In *S. juzepczukii* the articulation was present, but likewise less pronounced. This can be explained by the hypothesis (Kardolus *et al.* 1998) that the triploid cultigen *S. juzepczukii* originated from a crossing between *S. acaule* and some cultivated diploid potato. The absence of an abscission zone is apparently a recessive character, with probably some modifying genes influencing the expression of this trait. Unfortunately, it was not possible to produce an F2 and determine the genetic basis of this trait more thoroughly.

In tomato, *S. lycopersicum*, and its wild relatives two different 'jointless' mutants, *j1* and *j2*, are known (Butler 1936, Rick 1956, Philouze 1978, Wing, Zhang and Tanksley 1994). *Jointless j1* (also known as *j*) is defined as a single recessive mutation that completely suppresses the formation of the pedicel abscission zone. It was mapped on chromosome 11 of *S. lycopersicum*. Jointless mutation *j2* is another single recessive mutation non-allelic to *j1*. Rick (1956) described that *j2* was not completely recessive because the fruits of an F1 hybrid *J2/j2* were firmer attached to the plant than in *J2* homozygous genotypes ('normal' jointed phenotype). No F2 plants of a cross between *S. esculentum* (*jointless j2/j2*) and *S. pennellii* Correll (*J2/J2*) could be unambiguously scored as 'jointless' (Wing *et al.* 1994). This effect was also noted by Vulkova-Achkova (1982), who described a new phenotype, "secondary jointlessness", in a segregating population of jointed *J2/J2* (*S. lycopersicum*) × jointless *j2/j2* (*S. pimpinellifolium* B. Juss). The first inflorescences of the F2-plants showed no joints, whereas subsequent ones showed "a swelling at the place of the joint, but without a separating layer". Suggested was that "secondary jointlessness" is a result of the interaction between the *j2* allele of *S. pimpinellifolium* and modifying genes of *S. lycopersicum*. One may postulate that a similar situation of 'jointlessness' and 'secondary jointlessness' exists in series *Acaulia*. The non-articulated *S. acaule* ssp. *punae* and *acaule* accessions exhibit the phenotype 'jointless'. The hexaploid *S. albicans* shows a swelling at the articulation when the fruits mature, but a clear abscission zone in the pedicel is absent. This phenotype could be called 'secondary jointlessness' analogous to Vulkova-Achkova's terminology (1982). Gene synteny studies could confirm the genetic equivalence of jointed genes in *Solanum* sect. *Petota* to those in tomato.

Evaluating the use of the articulation position for taxonomic purposes. Not only the presence or absence of an articulation is used for the identification of *Solanum* sect. *Petota* species, but also the position of this articulation on the pedicel. In keys to series and species (Hawkes and Hjerting 1969, 1989, Hawkes 1990, Ochoa 1990) the height of the articulation has mostly been defined rather imprecisely by means of terms such as 'basal articulation' and 'articulation at the middle or very high up'. Correll (1962) has consequently given the total pedicel length together with a description of the articulation position which makes it possible to determine a more absolute position of the articulation. Usually, only for material articulated at the very base (non-tuber-bearing species of *Solanum* sect. *Etuberosum* (Buk. & Kameraz) Child, previously classified in sect. *Petota* as series *Etuberosa* Juz. [Hawkes 1990]) or just below the calyx (series *Acaulia, Megistacroloba*) a value for the length from articulation to the base of the pedicel or to the calyx is given (Correll 1962, Hawkes and Hjerting 1969, Hawkes 1990, Ochoa 1990).

We examined both the absolute length from calyx to articulation (ArtUp) and the relative character ArtRatio (Fig. 4). When comparing these two characters it is remarkable that the absolute length from calyx to articulation is similar in *S. verrucosum* and *S. megistacrolobum*, but the ratio differs enormously between both taxa. In *S. megistacrolobum* the relative position is much higher because of its high ArtLow. On the other hand, the ratio in *S. albicans* and *S. megistacrolobum* is similar, but the mean length above the articulation (ArtUp) is obviously shorter in *S. albicans*. In conclusion, in order to give an unequivocal description of the position of the articulation it is necessary to combine a relative trait as ArtRatio with an absolute pedicel length value.

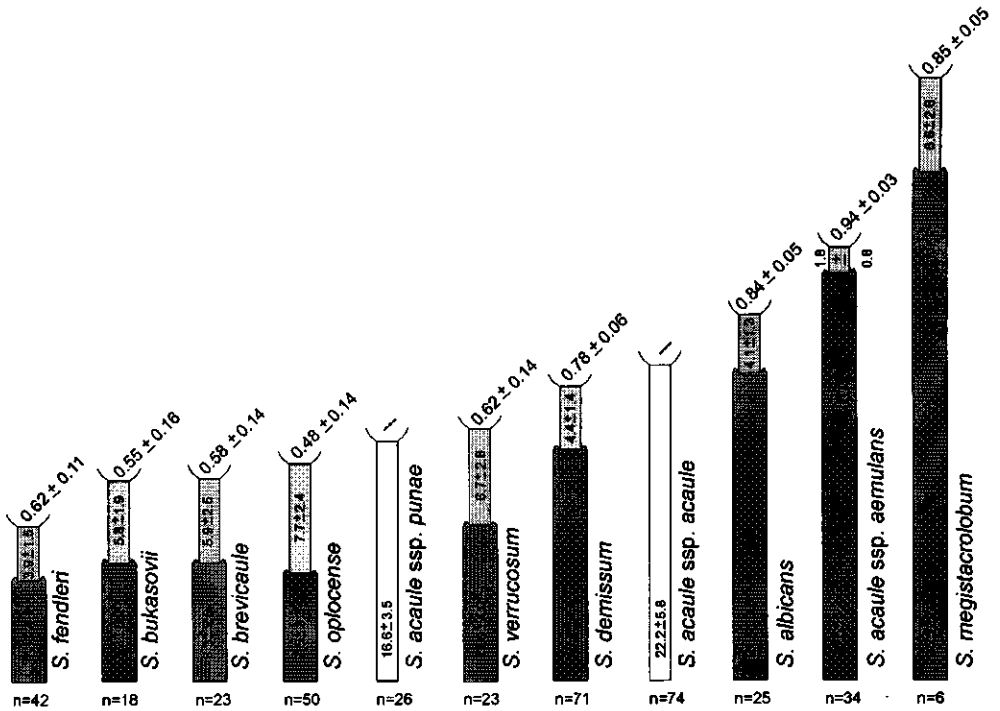


Figure 4. Dimensions of pedicels of the various taxa in the morphometrical experiment of this study. The darker part represents the pedicel part below the articulation down to the uppermost furcation of the peduncle (ArtLow, in mm). The light gray part reflects the part of the pedicel above the articulation up to the calyx (ArtUp, in mm). The schematic pedicels of the *S. acaule* ssp. *acaule* / ssp. *punae* accessions are not coloured because no articulation was present in this material. Character means and standard deviations are placed in the respective plant parts. In the schematic flower ArtRatio with its standard deviation is given. n = number of plants.

The position of the articulation can be used as a diagnostic character. Solanum *acaule* ssp. *aemulans* and *S. albicans* were distinguishable from each other in the height of the articulation, as has also been described by Hawkes (1990: p. 183). ArtUp in ssp. *aemulans* proved to be statistically significantly shorter than in *S. albicans* (Fig. 4). Most species show a large variation in the height of the articulation, however. The taxa in this study with a mean ArtRatio of about 0.6 have a normal distribution of ArtRatio; the pedicels of *S. fendleri* (Fig. 5) are articulated below the middle to rather high up. The mean ArtRatio of *S. bukasovii* is 0.55, which contradicts the descriptions in Correll (1962) and Hawkes (1990), who describe the articulation as being 'high'.

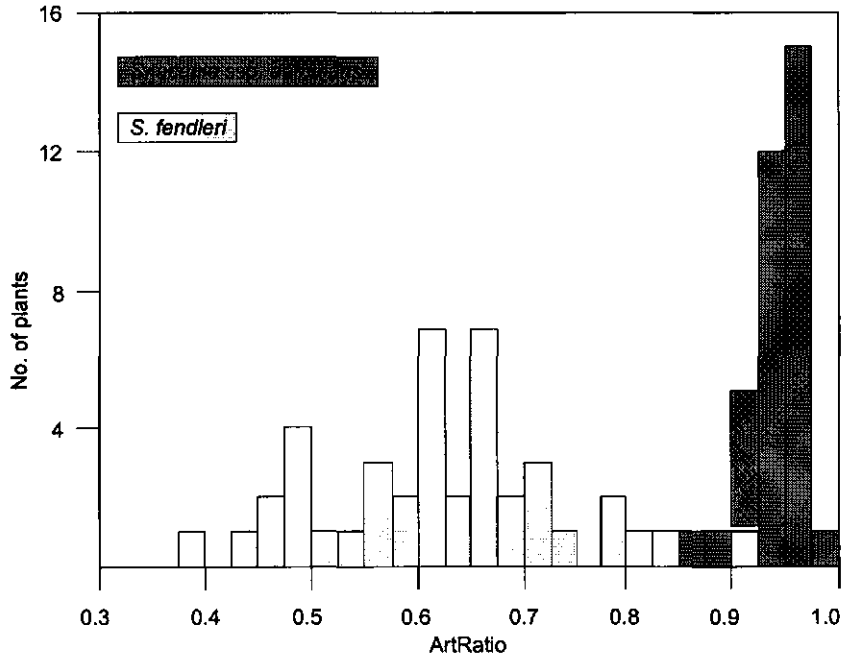


Figure 5. Distribution of ArtRatio in *S. fendleri* (42 plants) and *S. acaule* ssp. *aemulans* (34 plants).

The total pedicel length (PedLength) of the taxa of this study, which all have an articulation clearly above the base of the furcation of the pedicel, was strongly correlated (Fig. 3) with the length of the pedicel below the articulation (ArtLow). However, the length of the pedicel above the articulation (ArtUp) was not correlated with the total pedicel length. Therefore is ArtUp the most appropriate absolute value to be used in combination with the relative trait ArtRatio to describe the position of the articulation. ArtUp can even be used solely, as demonstrated by the difference between *S. acaule* ssp. *aemulans* and *S. albicans*. Most *Solanum* sect. *Petota* taxa are articulated around the middle of the pedicel, also in species such as *S. bukasovii* with a so-called 'high' articulation, and the variability of the trait is then high. Concluding, only for taxa with a relatively high or low articulation the position can be used in diagnostic keys (series *Acaulia*, *Megistacroloba* and sect. *Etuberosum*).

CHAPTER 4

The potential of AFLPs in biosystematics: a first application in *Solanum* taxonomy

ABSTRACT

Using the AFLP technique highly informative DNA fingerprints were generated from 19 taxa of *Solanum* section *Petota* (potatoes) and three taxa of *Solanum* section *Lycopersicum* (tomatoes). Both phenetic and cladistic analyses were conducted from the individual genotypic level to the species level. An AFLP fingerprint, using a combination of suitable AFLP primers, generated 12 to 71 scorable fragments per genotype which was sufficient for taxonomic interpretation. The classifications based on the molecular markers were generally in agreement with current taxonomic opinions. Unexpectedly, *S. microdontum* was associated with series *Megistacroloba* rather than with series *Tuberosa*, and *S. demissum* (series *Demissa*) and species of series *Acaulia* appeared closely affiliated. AFLP is an efficient and reliable technique to generate biosystematic data and therefore a promising tool for evolutionary studies.

INTRODUCTION

Molecular techniques have become of great significance for biosystematic studies (Soltis *et al.* 1992). Both the analysis of chloroplast and nuclear DNA have proven their potential for systematic botany (Chase *et al.* 1993, Olmstead and Palmer 1994). The use of several DNA marker systems for taxonomic purposes has been discussed by Bachmann (1992) and Whitkus *et al.* (1994). These authors conclude that RFLPs are commonly detected within and among species, but the laboratory procedures are relatively labor intensive. The detection of RAPD markers involves less complex and costly procedures, and a genomic library is not necessary. However, questions regarding the reliability of the RAPD technique have been raised (Williams and St.Clair 1993, Smith *et al.* 1994).

A novel multilocus DNA fingerprinting technique, named AFLP, has recently become available (Vos *et al.* 1995). The AFLP technique is based on the selective PCR amplification of DNA restriction fragments under stringent PCR conditions. The technique involves digestion of genomic DNA with a combination of two restriction endonucleases. Double-stranded oligonucleotide adapters are ligated to the resulting fragments to generate template DNA for amplification. The sequence of the adapters and the adjacent restriction site serve as primer binding site for subsequent PCR amplification of the restriction fragments. Selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. The DNA fragments, amplified with primers of which one is radioactively labeled, are separated on denaturing polyacrylamide gels, and visualized by autoradiography.

Until now AFLP has predominantly been applied in genetic mapping studies (Ballvora *et al.* 1995, Becker *et al.* 1995, Meksem *et al.* 1995, Van Eck *et al.* 1995). The technique is semi-

Kardolus J.P., Van Eck H.J. and R.G. van den Berg (1998) Plant Systematics and Evolution (In press).

quantitative because the intensity of AFLP bands can be used to determine the zygosity. Therefore AFLP can be used as codominant marker (Van Eck *et al.* 1995). They have also demonstrated that AFLP markers map genome-wide, hitting several loci on all twelve linkage groups of potato with every primer combination tested. Recently, AFLP has proven to be a competent tool in bacterial taxonomic studies (Huys *et al.* 1996, Janssen *et al.* 1996) and it has also been used for the identification of fungal isolates (Mueller *et al.* 1996). Folkertsma *et al.* (1996) have applied AFLP for the characterization of populations of two nematode species, *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone (Loof & Bakker). They concluded an extensive differentiation at the molecular level of these two morphologically nearly identical species. Folkertsma *et al.* (1996) also demonstrated that the difference in level of infraspecific variation of both species was concordant with RAPD data.

In order to assess the potential and the efficiency of the AFLP technique for plant systematic purposes, we examined genetic and biosystematic aspects of AFLP data. In this study a number of taxa of *Solanum* section *Petota* Dumortier, the wild relatives of the potato, were analyzed. Section *Petota* is divided in the subsections *Estolonifera* Hawkes and *Potatoe* G. Don. Subsection *Potatoe* comprises more than 200 tuber-bearing species classified in 19 series (Hawkes, 1990). Many taxa in this group are difficult to key out on the basis of their morphology. Often they can only be classified by means of multivariate analysis of quantitative characters and on the basis of geographic origin (Giannattasio and Spooner 1994, Van den Berg *et al.* 1996, Van den Berg and Groendijk-Wilders 1998). The scarcity of qualitative morphological markers makes a reliable phylogenetic analysis almost impossible (see also Bachmann 1995).

Studies at the molecular level, using nuclear and chloroplast RFLP patterns, and RAPDs, have contributed to new insights in phylogenetic relationships and taxonomic classifications within section *Petota* (Hosaka *et al.* 1984, Debener *et al.* 1990, Spooner *et al.* 1991, 1996). However, the interpretation of variation patterns in section *Petota* remains very difficult because sexual hybridization and polyploidization have presumably played an important role in this group. Consequently, speciation and species boundaries are unclear in many cases. AFLPs could be a powerful tool in solving these problems.

In order to assess the prospects of AFLP for systematic research we applied the AFLP technique to material of different taxonomic levels, that is from genotypes within individual accessions to species belonging to series of *Solanum* section *Petota* and section *Lycopersicum* (Mill.) Wettst. The choice of potato species in this study reflects the range of variability in section *Petota*, including on the one hand the morphologically distinct *Solanum pinnatisectum* Dunal (series *Pinnatisecta* (Rydb.) Hawkes) and, on the other hand, several species of series *Tuberosa* (Rydb.) Hawkes that are more difficult to distinguish from each other (Van den Berg *et al.* 1996). Our results show to what extent these differences in similarity show up in the AFLP fingerprints. The present study also included polyploid taxa to examine the influence of ploidy level on the number of AFLP fragments and on the results of the biosystematic analysis.

MATERIAL AND METHODS

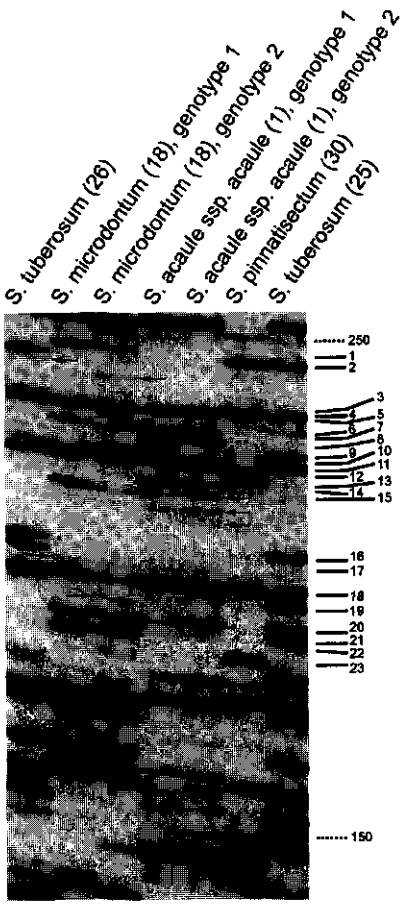
Plant material. Thirty accessions of 19 taxa of *Solanum* section *Petota* and three accessions of three taxa of *Solanum* section *Lycopersicum* were analyzed (Table 1). Accessions were received as seed lots or clones from genebanks and research institutes and their taxonomic identification was checked with our own observations. The taxonomy of section *Petota* follows Hawkes (1990) and that of section *Lycopersicum* follows Child (1990) and Spooner *et al.* (1993). In the biosystematic analyses one seedling or clone represented each accession as the Operational Taxonomic Unit (OTU) except for four OTUs that were represented by a DNA-mix of five individuals per accession (see Table 1). In the analysis of variation within accessions four genotypes per accession were studied and the AFLP data on these four genotypes were also incorporated in one of the biosystematic analyses (Fig. 3). Plant material was grown in a greenhouse. Voucher specimens are deposited at WAG.

AFLP protocol. Nuclear DNA was extracted from nitrogen-frozen young leaves and sprouts using the CTAB-method (Bernatzky and Tanksley 1986). The AFLP technique has been described by Vos *et al.* (1995). The whole procedure was performed according to Van Eck *et al.* (1995) with some small modifications: after sticky-end ligation of adapters to the *Eco*RI (adapter **E**) and *Mse*I (adapter **M**) restricted DNA, the selection of biotinylated ligation products using streptavidin coated magnetic beads was replaced by a 20-fold dilution of the ligation mixture. Selective restriction fragment amplification is achieved by adding one or more additional nucleotides onto the PCR primers, which will only then be successfully extended if matching the complementary sequence in the fragment flanking the restriction site. During pre-amplification, using a stringent touch-down temperature profile, the primers had one additional 3' nucleotide. In the subsequent PCR amplification a radioactively labeled (^{33}P) **E + 3** primer was used in combination with a **M + 3** primer. Three different primer combinations were tested: **E + AAC / M + CAG** (experiment A); **E + ACA / M + CAC** (experiment B); **E + ACA / M + CGT** (experiment C).

^{33}P-labeled amplification products were loaded, after 15 minutes pre-run, on a 5% denaturing polyacrylamide gel buffered with 1x TBE. A 0.4 mm sequence gel system (BioRad, Richmond, USA) was used. The anodal 1x TBE buffer was supplemented with 0.5 M sodium acetate to establish a salt gradient to slow down the migration of the smallest fragments. The gels were dried on Whatman 3MM paper. Subsequently, autoradiograms (X-ray film, Konica, Japan) were exposed for 1-7 days to the gels to visualize AFLP fragments.

Data analysis and experimental design. The presence or absence of AFLP fragments was scored on the autoradiograms and transferred into a 1 (present) and 0 (absent) matrix over all OTUs. Band intensity differences were not scored so the AFLPs were used as dominant markers. The phenetic analysis was performed with the program NTSYS-PC (Rohlf 1993) using the procedure SIMQUAL. Similarities between OTUs were calculated with the coefficient of Dice (1945). This coefficient omits consideration of negative matches (00) and gives more weight to matches (11) than to mismatches (01 and 10). Subsequently, OTUs were clustered with procedure SAHN using the UPGMA cluster criterion.

The cladistic analysis was performed by using the program PAUP 3.1.1 (Swofford 1993). The AFLP-data were analyzed using Wagner parsimony (Farris 1970). The most-parsimonious trees were sought using the heuristic procedure with BRANCH SWAPPING option TBR and STEPWISE ADDITION of OTUs. As a control, other heuristic settings such as branch swapping options SPR, NNI and No-Swapping, and Random Addition of OTUs (10 times), were tested and gave the same (or longer) most-parsimonious trees. Bootstrap values (Felsenstein 1985) on the resulting branches were obtained from 100 replicates.



fragment position	S. tuberosum (26)	S. microdontum (18), genotype 1	S. microdontum (18), genotype 2	S. acaule ssp. acaule (1), genotype 1	S. acaule ssp. acaule (1), genotype 2	S. pinnatisectum (30)	S. tuberosum (25)
1	0	1	1	0	0	0	0
2	1	0	0	0	0	1	1
3	1	0	0	0	0	0	0
4	0	0	0	0	0	1	0
5	1	1	1	1	1	0	1
6	0	1	0	0	0	0	1
7	0	0	1	1	1	0	0
8	0	0	0	1	1	0	0
9	1	0	0	0	0	0	1
10	0	1	1	0	0	0	0
11	1	1	1	1	1	0	1
12	0	0	0	0	0	1	0
13	0	0	0	0	0	1	0
14	0	0	0	1	1	0	0
15	0	1	0	0	0	0	0
16	1	0	0	0	0	0	1
17	1	0	0	0	0	0	0
18	1	1	1	1	1	1	1
19	0	0	0	0	0	0	1
20	0	1	1	1	1	0	1
21	0	0	0	0	0	0	1
22	0	1	1	0	0	0	0
23	0	0	0	0	0	1	0

Figure 1. Part of an AFLP fingerprint of seven genotypes generated with the primer combination **E+AAC / M+CAG.** The window shown in the left panel covers the region with AFLP bands having a mobility of 150-250 nucleotides, relative to a size ladder (not shown). The table to the right of this image is an example of the scoring matrix of the upper part of these seven lanes reflecting the presence (1) and absence (0) of fragments. Arrows point to some unclear fragments which were not scored in this study. OTUs (numbers between brackets) are described in Table 1.

Variation within accessions was determined (Fig. 2 and Table 2) by studying four seedlings of five accessions (OTUs 1, 17, 18, 20 and 21). The polymorphism rate within an accession was defined as the percentage of AFLP fragment positions showing polymorphy among the genotypes of that accession. For inter-accession analyses and species comparisons, OTUs were studied with the primer combinations as described in Table 1. In experiment A (primer combination **AAC / CAG**) the largest number of OTUs was studied, including *Solanum* section *Lycopersicum* genotypes (Fig. 3). The datasets of all experiments were combined and analyzed both phenetically (Fig. 4a) and cladistically (Fig 4b). In these combined analyses a subset of 25 OTUs of section *Petota* was examined. *S. pinnatisectum* (OTU 30) served as the outgroup in the cladistic analysis based on prior chloroplast DNA analyses (Hosaka *et al.* 1984, Spooner *et al.* 1991).

Table 1. Plant material examined in this study. The last three columns list the number of AFLP fragments scored per genotype, as obtained with Primer Combinations A (**E+AAC/M+CAG**), B (**E+ACA/M+CAC**) and C (**E+ACA/M+CGT**)

OTU no.	Species	Series [1]	Chr. no.	Source [3]	P.C. A	P.C. B	P.C. C
	section *Petota* Dumort.						
1	*Solanum acaule* Bitter ssp. *acaule*	Acaulia	48	BGRC 27206	52**	58**	36**
2	*S. acaule* ssp. *acaule*	ACA [2]	48	PI 246571	51	57	35
3	*S. acaule* ssp. *acaule*	ACA	48	BGRC 17111	-	60	31
4	*S. acaule ssp. punae* (Juz.) Hawkes & Hjert.	ACA	48	PI 473442	57	58	34
5	*S. acaule ssp. aemulans* (Bitter & Wittm.) Hawkes & Hjert.	ACA	48	PI 208856	53	56	-
6	*S. acaule* ssp. *aemulans*	ACA	48	PI 472715	-	59	32
7	*S. albicans* (Ochoa) Ochoa	ACA	72	PI 498194	62	58	34
8	*S. albicans*	ACA	72	PI 365305	63	60	39
9	*S. albicans*	ACA	72	PI 561642	59	71	34
10	*S. demissum* Lindl.	Demissa	72	BGRC 10030	60	59	35
11	*S. megistacrolobum* Bitter	Megistacroloba	24	BGRC 17642	47	52	20
12	*S. boliviense* Dunal	MEG	24	BGRC 24568	45	48	18
13	*S. raphanifolium* Cárdenas & Hawkes	MEG	24	BGRC 7207	41	34	18
14*	*S. canasense* Hawkes	Tuberosa (wild)	24	BGRC 7162	39	58	22
15	*S. multidissectum* Hawkes	TUBw	24	BGRC 8145	45	53	27
16	*S. multidissectum*	TUBw	24	BGRC 15459	51	61	24
17	*S. multidissectum*	TUBw	24	BGRC 15426	44**	56**	26**
18	*S. microdontum* Bitter	TUBw	24	BGRC 24649	46**	41**	19**
19*	*S. gourlayi* Hawkes	TUBw	24	BGRC 17338	53	54	25
20	*S. brevicaule* Bitter	TUBw	24	BGRC 24571	46**	46**	27
21	*S. brevicaule*	TUBw	24	BGRC 28023	41**	43**	25**
22	*S. brevicaule*	TUBw	24	BGRC 18291	45	47	19
23*	*S. infundibuliforme* Phil.	Cuneoalata	24	BGRC 17242	44	54	25
24*	*S. stenotomum* Juz. & Bukasov	TUBc (cultivated)	24	BGRC 7478	58	50	28
25	*S. tuberosum* L.	TUBc	48	F1 'Certa' × 'Gloria'	57	58	28
26	*S. tuberosum*	TUBc	48	[4] 77.2102.37	51	-	-
27	*S. tuberosum*	TUBc	48	[4] USW 5337.3	53	56	25
28	*S. ajanhuiri* Juz. & Bukasov	TUBc	24	CIP 702677 'Yari'	47	-	-
29	*S. juzepzcukii* Bukasov	TUBc	36	CIP 703262 'Morado Pingo Luki'	64	64	36
30	*S. pinnatisectum* Dunal	Pinnatisecta	24	BGRC 8175	35	12	13
	section *Lycopersicum* (Mill.) Wettst.						
31	*S. lycopersicum* L.	Lycopersicon	24	'Moneymaker'	28	-	-
32	*S. agrimoniifolium* (Dunal in DC.) J.F. Macbr. [5]	Eriopersicon (C.H. Muller) A.Child	24	[6] CGN 15790	33	-	-
33	*S. pennellii* Correll	Neolycopersicon (Correll) A.Child	24	TGRC LA0716	33	-	-

[1] Taxonomy of Solanum section Petota according to Hawkes (1990), of Solanum section Lycopersicum according to Child (1990) and Spooner *et al.* (1993)

[2] Series abbreviations according to Hawkes (1990)

[3] BGRC *Braunschweig Genetic Resources Collection*; PI *Plant Introduction* (Bamberg and Martin 1993); CIP *International Potato Center*, Peru; CGN *Center for Genetic Resources*, the Netherlands; TGRC *Rick Tomato Genetics Resource Center*, USA

[4] Described in Van Eck *et al.* (1995); [5] Synonym *Lycopersicon hirsutum* Miller

[6] G1.1560, see Lindhout *et al.* (1994)

- not examined; * OTU represented by a DNA-mix of five genotypes; ** Mean number of bands of four genotypes

RESULTS

Effects of ploidy level, mating system and primer combination. AFLP fingerprints consisted of 12 to 71 scorable amplification products per genotype (Table 1). Fragments sized from about 50 to 700 nucleotides. Figure 1 shows an image of part of the fingerprints of seven genotypes belonging to four species after PCR with primer combination **E + AAC / M + CAG** (experiment A). An example is given of the scoring matrix of this autoradiogram. From visual inspection of the AFLP-image in Fig. 1 it is already obvious that the fingerprints from genotypes that belong to the same species are alike. The fingerprint of *S. pinnatisectum* is hugely different from cultivated potato (*S. tuberosum*), hardly sharing corresponding bands.

In experiments A, B and C, respectively 200, 213 and 138 fragments (Table 2) were identified of which 93% were polymorphic. A fourth primer combination (**E + ATG / M + CTA**) gave such a dense fingerprint that reliable scoring was not possible. The ploidy level was clearly reflected in the number of AFLP fragments amplified per genotype (Table 1 and 2). Averaged over three primer combinations, the hexaploid genotypes showed 159 (± 5) fragments per genotype, the tetraploid material 142 (± 5) and the diploid 112 (± 19). Particularly the diploid *Solanum pinnatisectum* genotype and the material of section *Lycopersicum* showed fewer bands (Tables 1, 2).

Large contrasts in polymorphism rates could be detected (Fig. 2, Table 3) between genotypes from the inbreeding species *S. acaule* (OTU 1) and the outbreeders *S. multidissectum* (OTU 17), *S. microdontum* (OTU 18) and *S. brevicaule* (OTUs 20 and 21). In *S. acaule* only one fragment was polymorphic, on a total of more than 50 bands per primer combination, among the four genotypes in experiments A and B. With primer combination C no polymorphisms could be detected among the *S. acaule* genotypes. In contrast, accessions of the outbreeding species displayed polymorphism rates from 35% to 69% among the four genotypes sampled.

To compare the information obtained with individual primer combinations, UPGMA dendrograms were constructed using the data of those primer combinations. The topology of the resulting dendrograms remained generally the same with primer combinations A, B and C. This is evident in the dendrograms of accessions 1, 17, 18, 20 and 21 with four genotypes each (Fig. 2a, b). The genotype-clustering within accessions was not consistent based on the data of primer combinations A and B, but the four genotypes of an accession do cluster systematically together per accession. The resulting dendrograms of all accessions studied with primer combinations A (Fig. 3) and B, C (not shown) were quite similar. Regarding the six clusters recognized in Fig. 3 (see **Biosystematic analysis** below) rearrangements occurred only between clusters III and IV in the comparison of the three primer combinations. Part of the OTUs regrouped within their clusters with different primer combinations.

Biosystematic analysis. With primer combination **AAC / CAG** (experiment A) the largest number of accessions was studied. In the resulting UPGMA-dendrogram (Fig. 3) six clusters can be observed: (I) the section *Lycopersicum* taxa, (II) *S. pinnatisectum* (OTU 30), (III) the taxa of series *Megistacroloba* together with the series *Tuberosa* species *S. microdontum* (OTU 18) and *S. gourlayi* (OTU 19) at the basis of that cluster. Clusters (IV) and (V) mainly consisting of species of series *Tuberosa* with the apparently related *S. infundibuliforme* (series *Cuneoalata*), and cluster (VI) series *Acaulia* with the triploid *S. juzepczukii* (series *Tuberosa*) and the hexaploid *S. demissum* (series *Demissa*).

The dendrogram of the combined dataset of primer combinations A, B and C (Fig. 4a), based on 551 AFLP-bands and 25 OTUs of section *Petota*, showed a similar tree structure with minor rearrangements within the clusters of some OTUs. Particularly *S. juzepczukii* and *S. demissum* were placed within the series *Acaulia* cluster and OTU 19 (*S. gourlayi*) was attached to *S. infundibuliforme* and these together to the cluster with *S. brevicaule* OTUs.

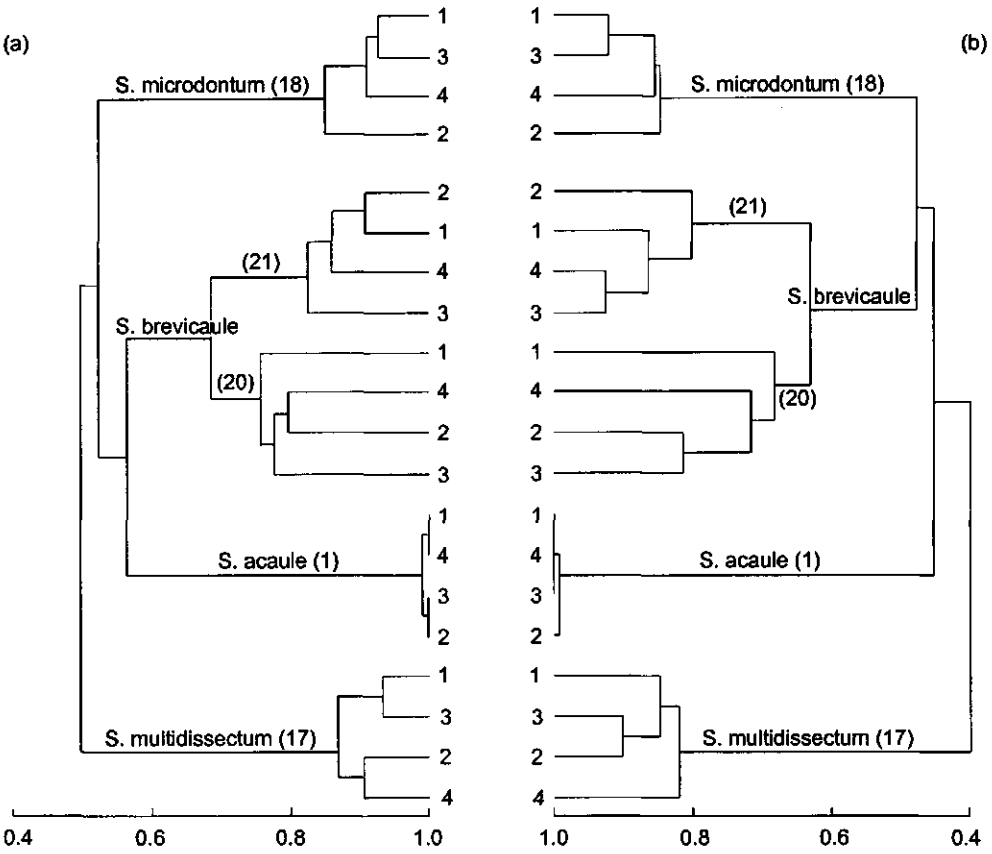


Figure 2. UPGMA dendrograms of five accessions with four genotypes (numbered 1 to 4) per accession. Cluster analysis results of primer combinations A (**AAC/CAG**) and B (**ACA/CAC**) are pictured in Fig. 2a and 2b, respectively. The similarity on the x-axis is based on the coefficient of Dice (1945). OTUs (numbers between brackets) are described in Table 1.

Table 2. Total number of AFLP fragments (between square brackets) with primer combinations (p.c.) A **(E+AAC/M+CAG)**, B **(E+ACA/M+CAC)** and C **(E+ACA/M+CGT)** in *Solanum* sect. *Petota* and sect. *Lycopersicum*. Mean number of fragments per genotype listed according to ploidy level and mating system (- not examined)

Plant material	Mating system	p.c. A [200] Mean number of fragments per genotype	p.c. B [213]	p.c. C [138]
Diploids (OTUs 11-13, 15-18, 20-22, 28)	outbreeding	46	53	27
Disomic tetraploids (OTUs 1-6)	inbreeding	54	58	33
Tetrasomic tetraploids (OTUs 25-27)	outbreeding	54	58	26
Hexaploids (OTUs 7-10)	inbreeding	62	62	36
Solanum pinnatisectum (OTU 30), diploid	outbreeding	36	13	12
S. lycopersicum (OTU 31), diploid	inbreeding	28	-	-
S. agrimoniifolium / S. pennellii (OTUs 32-33), diploid	outbreeding	33	-	-

Table 3. Total number of fragments scored in four genotypes of five accessions and the polymorphism rates in those five accessions (- not examined)

Plant material	Primer combination	Total number of fragments scored per accession	Percentage of fragments showing polymorphy
Solanum acaule (OTU 1)	A	52	2%
	B	58	2%
	C	36	0%
S. multidissectum (OTU 17)	A	52	35%
	B	72	46%
	C	33	48%
S. microdontum (OTU 18)	A	58	41%
	B	55	42%
	C	25	40%
S. brevicaule (OTU 20)	A	64	58%
	B	55	69%
	C	-	-
S. brevicaule (OTU 21)	A	51	43%
	B	59	42%
	C	31	45%

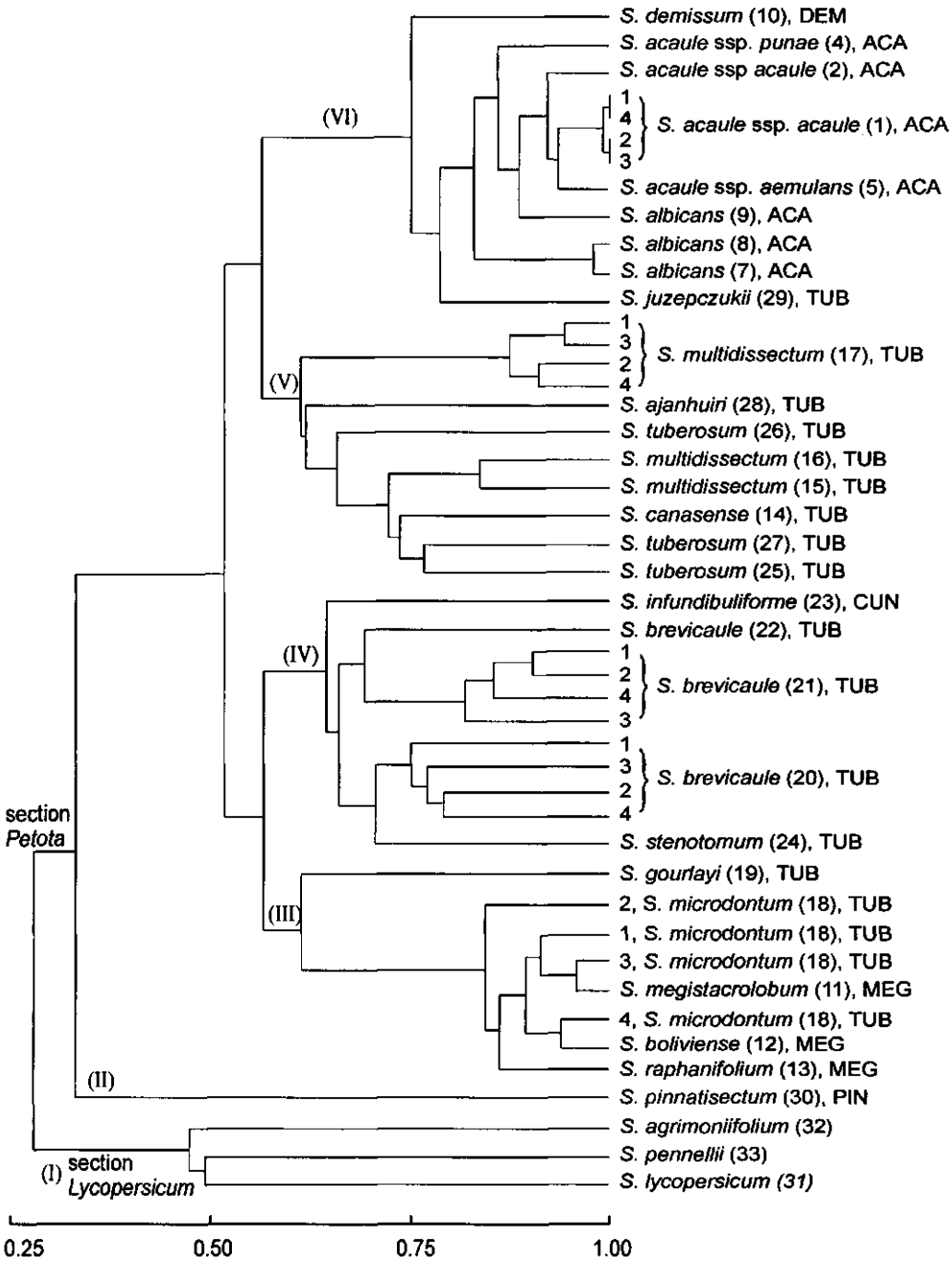


Figure 3. UPGMA dendrogram of 31 accessions, based on the data of primer combination A. The similarity on the x-axis is based on the coefficient of Dice (1945). Roman numbering refers to clusters as described in **Results**. OTUs (numbers between brackets) are described in Table 1. OTUs 1, 17, 18, 20 and 21 are represented by four individual genotypes.

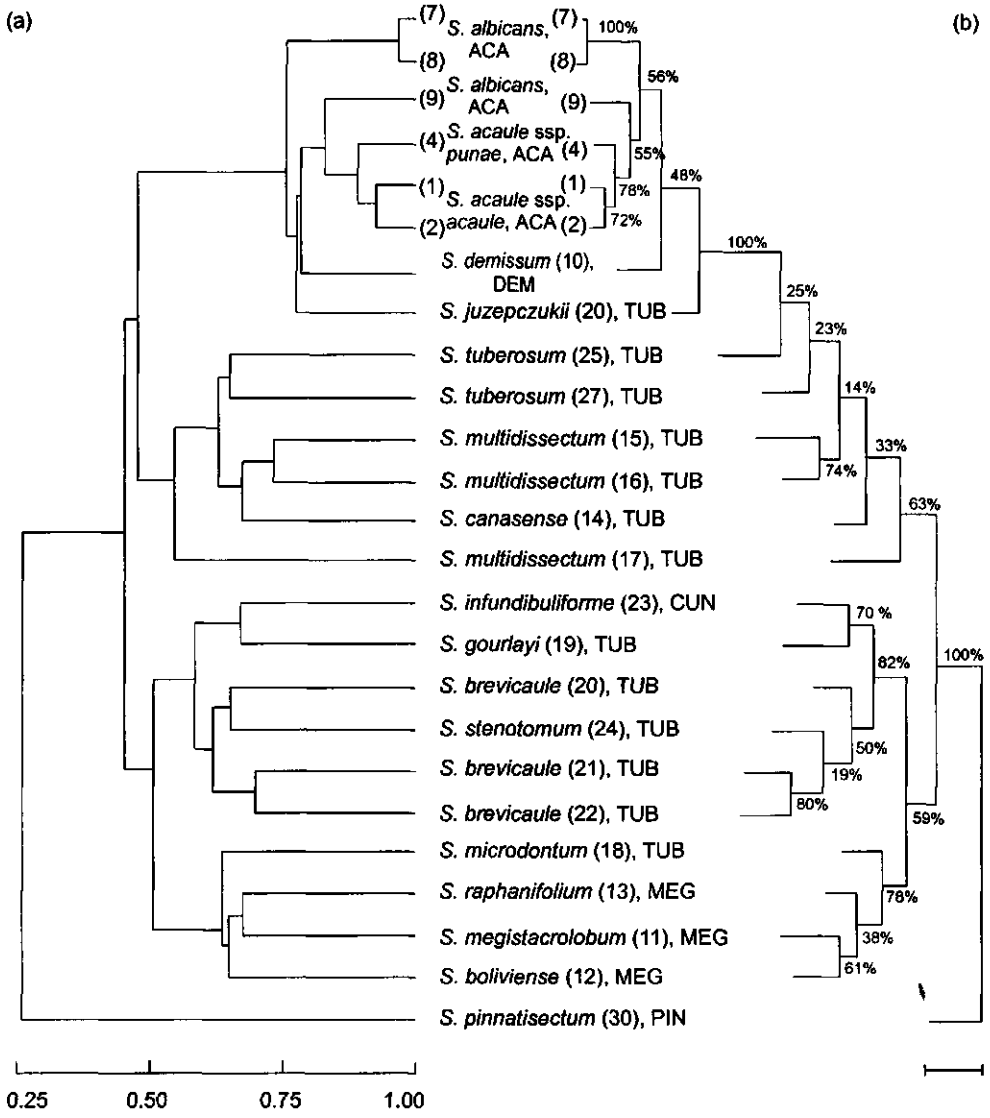


Figure 4. Comparison of a phenetic analysis and a cladistic analysis of the combined AFLP dataset on 25 OTUs and primer combinations A, B and C. The axis of the UPGMA dendrogram (Fig. 4a) is based on the coefficient of Dice (1945). Figure 4b represents one of the two most parsimonious Wagner trees of the cladistic analysis with bootstrap values provided for each branch. Branch lengths are based on the number of mutations supporting each branch (bar length = 40 mutations). OTUs (numbers between brackets) are described in Table 1.

The cladistic analysis of the same dataset resulted in two equally parsimonious 1206-step Wagner trees with a consistency index of 0.32 (without autapomorphies). The two cladograms differ in the placing of *S. microdontum* and *S. raphanifolium* at the base of the series *Megistacroloba* clade only. The topology of the displayed Wagner tree (Fig. 4b) is largely in concordance with the dendrogram (Fig. 4a). The *S. juzepczukii* and *S. demissum* OTUs are now positioned at the base of the *Acaulia* clade, which is characterized by 54 unique characters, nine of which are present in all OTUs of that clade. Furthermore, the cluster with the *S. tuberosum*, *S. multidissectum* and *S. canasense* OTUs present in the dendrogram, has been resolved in weakly supported branches with low bootstrap values in this most parsimonious tree. In the majority rule consensus tree (not shown) based on the bootstrap analysis of these data, this cluster had a bootstrap value of 58%.

DISCUSSION

AFLPs and genetic variation studies. Our results indicate that AFLP is a highly informative and reproducible DNA marker suitable for biosystematic studies. The evaluation of the AFLP fingerprints of three different primer combinations resulted in a dataset of 551 polymorphisms. AFLP patterns generated by different primer combinations represented the genetic variation similarly as demonstrated by the dendrograms based on the subset of polymorphisms generated by one primer combination (Fig. 2 a, b). Polymorphisms obtained with only one primer combination already reflected the relationships between species well (Fig. 3).

In the analyses of genetic variation within and between species (Tables 1, 2 and 3) the number of fragments per genotype was positively correlated with the ploidy level. The mode of sexual reproduction resulted in a high level of homozygosity in the autogamous tetraploid species *S. acaule*, and a high level of heterozygosity in the allogamous diploid species (see Fig. 2). Consequently, the total number of fragments per accession of the diploid accessions is comparable with the number of amplified fragments per accession of *S. acaule*. Between the inbreeding disomic tetraploid (*S. acaule*) and the outbreeding tetrasomic tetraploid material (*S. tuberosum*) no clear difference in number of fragments per genotype was observed, indicating that the disomic chromosome pairing of *S. acaule* leads to fixed heterozygosity (Camadro *et al.* 1992).

The high level of genetic variation detected by AFLP within the outbreeding species (Table 3) in this study has introduced some conflict in the interspecific analysis. The heterozygosity of *S. microdontum* OTU 18 leads to the clustering of its individual genotypes between OTUs of series *Megistacroloba* (Fig. 3). The AFLP-pattern of one genotype sampled from such a variable population does not completely reflect the variation of that population and can cause some inconclusiveness in the analysis. However, the even more variable accessions of *S. multidissectum* (OTU 17) and of *S. brevicaule* (OTU 20 and 21) remained together per accession in Fig. 3. Dendrograms based on AFLP fingerprints of accessions represented by one genotype are therefore sufficiently informative for biosystematic interpretation.

A possible source of error in the data could be the non-homology of co-migrating fragments, as discussed for RAPD data by Spooner *et al.* (1996). On the basis of theoretical considerations the chance that two co-migrating AFLP fragments do not represent identical alleles of one genetic locus is small, because of the highly selective amplification of a small

subset of the whole genome and the sharp resolution of the polyacrylamide sequencing gels. Practically, co-migrating fragments have been sequenced by Rouppe van der Voort *et al.* (1997) and 19 out of 20 were identical. They have also demonstrated that co-migrating fragments in almost all cases could be localized on indistinguishable positions on genetic maps.

Biosystematic analysis of *Solanum* section *Petota*. In our study a subset of *Solanum* section *Petota* has been examined with AFLPs and compared with representatives of section *Lycopersicum*. We conclude that the section *Lycopersicum* material is clearly distinct (Fig. 3), and that within section *Petota* (Fig. 4) *S. pinnatisectum* and series *Acaulia* together with *S. juzepczukii* and *S. demissum*, constitute discrete groups. This classification based on AFLP polymorphisms is only partly in agreement with the taxonomy as proposed by Hawkes (1990), which is mainly based on morphological similarity. Some striking differences between our results of only a limited set of potato species (Figs. 3, 4) and Hawkes's (1990) classification can be observed. Firstly, *S. microdontum* (series *Tuberosa*) appeared closely related to OTUs of series *Megistacroloba*, rather than to the other representatives of series *Tuberosa*. Until now this association was never suggested. More representatives of both series should be analyzed in future studies to confirm the present findings concerning this connection. Furthermore, *S. demissum* (series *Demissa*) is placed on the same clade as material of series *Acaulia*. This relationship will be discussed under **Polyploid material**. A third observation that was not expected was the clustering of *S. infundibuliforme* (series *Cuneoalata*) together with species of series *Tuberosa* such as *S. brevicaule* and *S. stenotomum*. Our results suggest that the current taxonomy of section *Petota* (Hawkes 1990), particularly regarding the classification into series, should be reviewed as proposed by Spooner *et al.* (1995).

The concordance of our AFLP results (Figs. 3, 4) with previous molecular taxonomic studies on *Solanum* sect. *Petota* (Hosaka *et al.* 1984, Debener *et al.* 1990) suggests that AFLP markers have taxonomic relevance in elucidating genetic distances and phylogenetic relationships among the studied taxa. It may even be concluded that biosystematic analyses based on molecular markers such as AFLPs are more informative and more reliable than those based on morphological traits, because of the abundance of discrete, binary characters obtained, and the exclusion of environmental variance having a substantial influence on quantitative characters.

The OTUs of series *Tuberosa*, except *S. microdontum*, were separated into two clusters (Fig. 4a) that reflect their geographic origin. One cluster consists of OTUs from Bolivia and Argentina, comprising *S. brevicaule*, *S. gourlayi*, the cultivated *S. stenotomum* (and *S. infundibuliforme* of series *Cuneoalata*). The other cluster included *S. tuberosum* OTUs and wild species from Peru (*S. canasense* and *S. multidissectum*). In the RFLP study by Debener *et al.* (1990) *S. canasense* is also closely associated with the cultigens *S. tuberosum* ssp. *tuberosum* and ssp. *andigena* Hawkes, indicating that the cultivated potato might have a Peruvian origin.

Within series *Tuberosa* the grouping of different OTUs of one species is not always clear. For example, OTU 17 of *S. multidissectum* did not cluster directly with OTUs 15 and 16 (also of *S. multidissectum*), but was placed at the base of a larger cluster comprising also *S. tuberosum* and *S. canasense* OTUs (Figs. 3, 4). This scattered pattern of clustering of taxa is not due to a lack of resolution of AFLP polymorphism, because inconclusive clustering has also been observed in a morphological analysis of the 'brevicaule-complex', studying the same

material (Van den Berg *et al.* 1996). The AFLP data obtained in this study support the proposals by Correll (1962: 434) and Ugent (1966) to classify this 'brevicaule-complex' into fewer taxa. Additional morphological and molecular studies with larger sets of material have been conducted in 1995 in cooperation with the research group of David Spooner (University of Wisconsin, USA).

Polyploid material. *Solanum demissum* of series *Demissa* from Mexico appeared to be closely associated with the South American series *Acaulia* (Fig. 3 and 4). These polyploid taxa shared many apomorphies, that is AFLP fragments not present in the rest of the material studied. The OTUs of series *Acaulia* and *S. demissum* had also a set of other AFLP fragments in common with material of series *Tuberosa*, which therefore could have provided a common ancestral genome. Matsubayashi (1991) has referred to this relationship as the common AA-genome of section *Petota* present in both the polyploid series *Acaulia* and series *Demissa*. The synapomorphic AFLP-characters demonstrated in series *Acaulia* and *Demissa* should then be assigned to the A^aA^a-genome (Matsubayashi 1991) of series *Acaulia*. This phylogenetic relationship between series *Acaulia* and *S. demissum* based on AFLPs is in agreement with morphological studies (Spooner *et al.* 1995, Kardolus 1998) and a RFLP-study (Debener *et al.* 1990). However, it is in conflict with the genome description $AADDD^dD^d$ of *S. demissum* by Matsubayashi (1991). Furthermore, series *Longipedicellata* Bukasov and *S. verrucosum* Schlechtd., which were not analyzed here, have been mentioned in the origin of *S. demissum* (Marks 1955, 1965, Hawkes 1990). Follow-up studies will be necessary to elucidate this unexpected close relationship between these geographically disjunct taxa.

The triploid cultigen *S. juzepczukii* OTU 29 also shared the apomorphic characters of series *Acaulia* and *S. demissum*. *Solanum juzepczukii* has been assumed to be a hybrid between a diploid *Tuberosa* species and the tetraploid species *S. acaule* (Hawkes 1962, Schmiediche *et al.* 1982). Our results are consistent with this hypothesis, because *S. juzepczukii* showed AFLP-fragments that were present in both putative parental taxa.

Sexual species hybrids and branching trees. In biosystematic studies the interpretation of hybridization patterns remains indeterminate. Cladistic methods (and for that matter phenetic methods as well) only produce divergent branching patterns and can therefore never give the correct phylogeny for a group that includes taxa of hybrid origin. In a phylogenetic analysis with hybrids using morphological characters (McDade 1990), the hybrids appeared as a basal lineage to the branch that included its most derived parent. In our analysis the polyploids of presumed hybrid origin formed an advanced clade (Fig. 4b). An explanation of this incongruity could be the set of unique AFLP fragments demonstrated in the polyploids. Future analyses of groups including hybrid taxa should use computer algorithms, not readily available yet, that can deal with reticulate branching patterns to establish the most likely phylogeny.

Conclusion. The AFLP fingerprinting technique allows the visualization of DNA polymorphisms suitable for biosystematic purposes. The technique can be applied to study genetic variation at the population level up to a taxonomic level at which insufficient homologous DNA fragments are present in the OTUs under comparison. Our results indicate that in *Solanum* section *Petota* the AFLP technique is suitable up to the species level. Possibly AFLP might fail in biosystematic studies at the genus level due to a lack of homologous AFLPs and an increasing chance of co-migration of non-homologous fragments.

CHAPTER 5

A biosystematic analysis of the polyploid series *Acaulia* and related potato species

ABSTRACT

Relationships between the allopolyploid series *Acaulia* and related tuber-bearing *Solanum* species (section *Petota*) were inferred using the DNA fingerprinting-technique AFLP™ and a combined neighbor-joining and parsimony analysis. In addition, enzyme electrophoresis was applied to series *Acaulia* and its most closely related species. The higher degree of variation found with AFLPs, compared to chloroplast variation, and the relatively large set of 171 genebank accessions studied, provided a detailed insight in the phylogeny of section *Petota*. Previously revealed phylogenetic insights were supported by our data, and augmented with new views. Some evolutionary lineages were correlated with geographical distribution, such as a split of series *Tuberosa* species coming from Peru and those coming from Bolivia/Argentina. Other clades combined species that are now classified in different series. Series *Acaulia* itself was a monophyletic group, distinct in both morphology and molecular constitution. One progenitor of series *Acaulia* appears to belong to series *Megistacroloba* or *Tuberosa*, two closely related groups. The second ancestor is still unknown, and might be extinct. Furthermore, the neighbor-joining analysis indicated phyletic links of the hexaploid species *S. demissum* with series *Acaulia*, as well as of *S. demissum* with other polyploid species of Mexico/USA.

INTRODUCTION

The polyploid *Solanum* series *Acaulia* Juz. consists of two species, according to the latest treatments (Hawkes 1990, Kardolus 1998): the tetraploid (2n=48) species *S. acaule* Bitter and the hexaploid (2n=72) *S. albicans* (Ochoa) Ochoa. Series *Acaulia* is of significance for potato-breeding because of resistances against various diseases and tolerance for cold stress (Bamberg *et al.* 1994). Both species occur in the high Andes-region, with *S. acaule* having the widest distribution (Ochoa 1990) of all ca. 200 tuber-bearing *Solanum* species (*Solanum* sect. *Petota* Dumort.). *S. acaule* is distributed from Peru to northern Argentina, whereas *S. albicans* is confined to northern Peru and southern Ecuador. Series *Acaulia* forms a morphologically rather distinct entity among the wild tuber-bearing *Solanum* species with a rosette-habit and compact inflorescence (Kardolus 1998, Kardolus and Groendijk-Wilders 1998). A special feature for series *Acaulia* is the modified pedicel articulation. In *S. acaule* the floral abscission zone is generally absent and the pedicel is then unarticulated, and in *S. albicans* this zone is present but modified compared to other potato species (Chapter 3, this thesis).

The genome constitution of the species of series *Acaulia* suggests that the series evolved through hybridization events. Chromosomal studies of *Solanum acaule* have shown regular bivalent pairing (Swaminathan and Howard 1953, Swaminathan 1954) in meiosis.

Kardolus J.P. and R.G. van den Berg (Submitted).

Furthermore, isozyme studies have revealed fixed heterozygosity for the aspartate aminotransferase system (Cortés and Camadro 1989, Echeverría *et al.* 1989, Camadro *et al.* 1992). These findings lead to the conclusion that *S. acaule* has two non-homologous sets of chromosomes and can be called an amphidiploid. Although the evidence of the amphidiploid nature of *S. acaule* is strong, the parental species are unknown. The morphology of series *Acaulia* shows similarities with both *S. megistacrolobum* Bitter of series *Megistacroloba* Cárdenas & Hawkes (rosette-habit and inflorescence architecture) and species of series *Tuberosa* Hawkes (leaves and corolla), and these two series have frequently been hypothesized as being ancestral to series *Acaulia* (Ugent 1966, 1981, Kameraz *et al.* 1978, Chavez 1984, Hawkes 1990, Ochoa 1990). Next to the evolutionary relationships with these two series, Hawkes and Hjerting (1989) also mentioned series *Cuneoalata* Hawkes as a possibly related group.

The hexaploid species *S. albicans* has been hypothesized to be derived from a hybridization of *S. acaule* with some diploid sect. *Petota* species (Hawkes 1963, Hosaka and Spooner 1992). Hawkes (1990) has put forward *S. cajamarquense* as a candidate for providing the third genome in *S. albicans*. *S. cajamarquense* is a distinctive diploid species from Peru with a dense pubescence of long, thick, white hairs. A direct evolutionary connection between *S. albicans* and the hexaploid *S. demissum* Lindl. from Mexico has been demonstrated by nuclear DNA studies (Debener *et al.* 1990, Kardolus *et al.* 1998). These studies are concordant with described morphological and ecological similarities (Correll 1962, Hawkes and Hjerting 1969, Ochoa 1990, Spooner *et al.* 1995, Kardolus 1998).

Various molecular markers have been used for the study of botanical diversity. The major advantage over morphological methods is the immense number of discrete characters they reveal (Karp *et al.* 1996). Especially for section *Petota* species this is of significance because not many qualitative morphological characters can be scored in this group. Gaps in the character states of continuous morphological characters, that have been described to differ among taxa, are often ambiguous. Accordingly, the classification of many tuber-bearing *Solanum* species requires a polythetic species concept (Spooner *et al.* 1995, Van den Berg *et al.* 1998). In this concept the grouping depends on the greatest number of shared features, no single feature of which is essential to group membership or is sufficient to make an organism a member of the group (Sokal and Sneath 1963, Stuessy 1990).

Solanum acaule and related *Solanum* sect. *Petota* species have been studied by chloroplast DNA restriction site variation. *S. acaule* is then most closely related to the series *Tuberosa* species *S. multidissectum* Hawkes (Hosaka *et al.* 1984) or it forms its own clade, based on one unique mutation (Spooner and Castillo 1997). Spooner and Castillo (1997) have suggested that, because of the relatively low resolving power of chloroplast DNA in the terminal clades of section *Petota*, studies with less conservative molecular markers might provide more insight in the phylogeny of these closely related species. The AFLP™ DNA fingerprinting technique (Vos *et al.* 1995) has been described as a promising molecular marker technique for potato taxonomy (Kardolus *et al.* 1998) as well as for other plant groups (Hill *et al.* 1996, Sharma *et al.* 1996, Breyne *et al.* 1997). AFLP markers are randomly distributed over the genome of *Solanum tuberosum* L. (Van Eck *et al.* 1995), and provide a polymorphism level appropriate for the study of relationships between potato species (Kardolus *et al.* 1998).

Table 1. *Solanum* material examined in this study

Code	Species	Series[1]	Source[2]	Chr. no.	Origin	Latitude	Longitude	[3]# GT AFLP	[4]# GT isoz.
acl1	*S. acaule* Bitter ssp. *acaule*	ACA	PI230469	48	Peru	-	-	-	3
acl2	*S. acaule* ssp. *acaule*	ACA	PI473430	48	Peru	-	-	-	3
acl3	*S. acaule* ssp. *acaule*	ACA	PI473440	48	Peru	-	-	-	3
acl4	*S. acaule* ssp. *acaule*	ACA	PI246571	48	Peru	11°19' S	76°33' W	10	3
acl5	*S. acaule* ssp. *acaule*	ACA	PI473327	48	Peru	15°18' S	69°58' W	10	2
acl6	*S. acaule* ssp. *acaule*	ACA	B27139	48	Bolivia	16°53' S	68°49' W	-	4
acl7	*S. acaule* ssp. *acaule*	ACA	B28026	48	Bolivia	17°15' S	66°03' W	10	4
acl8	*S. acaule* ssp. *acaule*	ACA	PI498078	48	Bolivia	17°15' S	66°54' W	-	3
acl9	*S. acaule* ssp. *acaule*	ACA	B27147	48	Bolivia	18°05' S	67°30' W	-	3
acl10	*S. acaule* ssp. *acaule*	ACA	B27206	48	Bolivia	18°17' S	66°49' W	10	3
acl11	*S. acaule* ssp. *acaule*	ACA	B27244	48	Bolivia	18°50' S	65°53' W	-	3
acl12	*S. acaule* ssp. *acaule*	ACA	B27361	48	Bolivia	21°38' S	65°03' W	10	3
acl13	*S. acaule* ssp. *acaule*	ACA	B16835	48	Argentina	-	-	10	4
acl14	*S. acaule* ssp. *acaule*	ACA	B17087	48	Argentina	21°55' S	66°09' W	-	3
acl15	*S. acaule* ssp. *acaule*	ACA	B17090	48	Argentina	22°06' S	66°08' W	-	3
acl16	*S. acaule* ssp. *acaule*	ACA	B17076	48	Argentina	22°08' S	65°45' W	-	3
acl17	*S. acaule* ssp. *acaule*	ACA	B17156	48	Argentina	22°14' S	65°17' W	-	3
acl18	*S. acaule* ssp. *acaule*	ACA	B17111	48	Argentina	22°15' S	65°40' W	10	3
acl19	*S. acaule* ssp. *acaule*	ACA	B24555	48	Argentina	22°26' S	66°11' W	7	3
acl20	*S. acaule* ssp. *acaule*	ACA	B17139	48	Argentina	22°58' S	65°26' W	-	3
acl21	*S. acaule* ssp. *acaule*	ACA	B17059	48	Argentina	23°13' S	65°42' W	-	3
acl22	*S. acaule* ssp. *acaule*	ACA	B47624	48	Argentina	23°17' S	65°54' W	-	3
acl23	*S. acaule* ssp. *acaule*	ACA	B17148	48	Argentina	24°39' S	66°12' W	-	5
acl24	*S. acaule* ssp. *acaule*	ACA	B17142	48	Argentina	24°29' S	66°13' W	-	3
acl25	*S. acaule* ssp. *acaule*	ACA	B17128	48	Argentina	25°08' S	65°06' W	-	3
acl26	*S. acaule* ssp. *acaule*	ACA	B47627	48	Argentina	25°10' S	65°52' W	10	3
acl27	*S. acaule* ssp. *acaule*	ACA	B17181	48	Argentina	28°57' S	67°41' W	10	5
aem1	*S. acaule* ssp. *aemulans* Hawkes & Hjert.	ACA	[5]PI472715	48	Argentina	23°10' S	65°00' W	10	10
aem2	*S. acaule* ssp. *aemulans*	ACA	[5]PI208856	48	Argentina	26°52' S	65°41' W	10	10
aem3	*S. acaule* ssp. *aemulans*	ACA	B17180	48	Argentina	28°58' S	67°48' W	10	10
aem4	*S. acaule* ssp. *aemulans*	ACA	B17182	48	Argentina	28°58' S	67°42' W	10	19
aem5	*S. acaule* ssp. *aemulans*	ACA	PI320280	48	Argentina	28°58' S	67°42' W	10	10
pne1	*S. acaule* ssp. *punae* Hawkes & Hjert.	ACA	PI365312	48	Peru	9°16' S	79°35' W	10	3
pne2	*S. acaule* ssp. *punae*	ACA	B7958	48	Peru	11°36' S	75°35' W	10	3
pne3	*S. acaule* ssp. *punae*	ACA	PI473481	48	Peru	12°51' S	74°51' W	-	3
pne4	*S. acaule* ssp. *punae*	ACA	PI473442	48	Peru	14°40' S	74°23' W	10	3
alb1	*S. albicans* Ochoa	ACA	PI266381	72	Peru	-	-	-	5
alb2	*S. albicans*	ACA	[6]PI498197	72	Peru	7°28' S	76°37' W	1	-
alb3	*S. albicans*	ACA	PI365376	72	Peru	8°17' S	77°18' W	10	6
alb4	*S. albicans*	ACA	[6]PI498195	72	Peru	9°52' S	77°12' W	1	8
alb5	*S. albicans*	ACA	[6]PI498194	72	Peru	10°05' S	77°18' W	10	5
alb6	*S. albicans*	ACA	PI498203	72	Peru	10°26' S	76°47' W	-	5
alb7	*S. albicans*	ACA	PI365306	72	Peru	10°34' S	76°54' W	-	3
alb8	*S. albicans*	ACA	B18287	72	Peru	11°19' S	76°05' W	-	3
alb9	*S. albicans*	ACA	PI365305	72	Peru	13°30' S	72°56' W	6	5
alb10	?	ACA	[7]PI561642	72	Ecuador	2°09' S	78°43' W	10	7
blb	*S. bulbocastanum* Dunal	BUL	B8009	24	Mexico	19°29' N	98°54' W	3	-
cap	*S. capsicibaccatum* Cárdenas	CIR	B31182	24	-	-	-	-	2
crc	*S. cicaeifolium* Bitter ssp. *circaeifolium*	CIR	B27058	24	Bolivia	15°40' S	68°37' W	10	5
qum	*S. circaeifolium* ssp. *quimense* Hawkes & Hjert.	CIR	B27034	24	Bolivia	17°03' S	67°17' W	10	-

Table 1. Continued

Code	Species	Series[1]	Source[2]	Chr. no.	Origin	Latitude	Longitude	[3]# GT AFLP	[4]# GT isoz.
oxc	*S. oxycarpum* Schiede	CON	B53011	48	Mexico	19°34' N	97°14' W	10	-
pcj	*S. paucijugum* Bitter	CON	B18583	48	-	-	-	10	-
ifd1	*S. infundibuliforme* Phil.	CUN	B17212	24	Argentina	21°53' S	66°11' W	4	-
ifd2	*S. infundibuliforme*	CUN	B17242	24	Argentina	23°08' S	65°14' W	9	7
bcp	*S. brachycarpum* Corr.	DEM	B8100	72	Mexico	19°34' N	100°22' W	10	-
dms1	*S. demissum* Lindl.	DEM	B10030	72	Mexico	-	-	10	-
dms2	*S. demissum*	DEM	B10022	72	Mexico	-	-	10	6
dms3	*S. demissum*	DEM	B9990	72	Mexico	20°08' N	98°40' W	10	-
agr	*S. agrimoniifolium* J.F. Macbr. [8]	ERI [9]	CGN15790	24	-	-	-	8	-
brd1	*S. brevidens* Phil.	ETU	B28472	24	Chili	38°41' S	71°50' W	10	-
brd2	*S. brevidens*	ETU	B17441	24	Argentina	40°12' S	71°27' W	10	-
etb1	*S. etuberosum* Lindl.	ETU	B28476	24	Chili	-	-	9	-
etb2	*S. etuberosum*	ETU	B8082	24	Chili	-	-	10	-
sit	*S. sitiens* Johnston	JUG	B60459	24	Chili	-	-	1	-
lgl	*S. lignicaule* Vargas	LIG	B8106	24	Peru	13°26' S	71°51' W	12	-
hje	*S. hjertingii* Hawkes	LON	[10] B8088	48	Mexico	25°25' N	101°00' W	9	-
fen	*S. fendleri* A. Gray ssp. *fendleri*	LON	[11] B7230	48	Mexico	-	-	10	-
azn	*S. fendleri* ssp. *arizonicum* Hawkes	LON	B23568	48	USA	31°54' N	109°16' W	10	5
sto	*S. stoloniferum* Schlechtd. & Bouché	LON	B7229	48	Mexico	-	-	10	-
esc	*S. lycopersicum* L. [12]	LYC [13]	'Moneymaker'	24	-	-	-	8	-
mgl	*S. maglia* Schlechtd.	MAG	B23571	24	Chili	-	-	10	-
blv1	*S. boliviense* Dunal	MEG	B24568	24	Bolivia	18°59' S	65°22' W	10	11
blv2	*S. boliviense*	MEG	B27248	24	Bolivia	19°38' S	65°20' W	10	10
blv3	*S. boliviense*	MEG	B27342	24	Bolivia	20°44' S	64°51' W	10	-
mga1	*S. megistacrolobum* Bitter ssp. *megistacrolobum*	MEG	B27149	24	Bolivia	17°10' S	68°29' W	-	10
mga2	ssp. *megistacrolobum*	MEG	B8113	24	Bolivia	19°55' S	65°40' W	10	12
mga3	ssp. *megistacrolobum*	MEG	B17642	24	Argentina	21°55' S	66°08' W	10	9
tor1	*S. megistacrolobum* ssp. *toralapanum* Glannattasio & Spooner	MEG	B28020	24	Bolivia	17°14' S	66°03' W	10	10
tor2	*S. megistacrolobum* ssp. *toralapanum*	MEG	B27119	24	Bolivia	17°40' S	66°32' W	-	9
tor3	*S. megistacrolobum* ssp. *toralapanum*	MEG	B8125	24	Bolivia	19°09' S	64°55' W	10	-
rap1	*S. raphanifolium* Cárdenas & Hawkes	MEG	B15445	24	Peru	-	-	10	-
rap2	*S. raphanifolium*	MEG	B8185	24	Peru	13°31' S	71°59' W	10	11
rap3	*S. raphanifolium*	MEG	B7207	24	Peru	15°13' S	73°44' W	10	10
sct1	*S. sanctae-rosae* Hawkes	MEG	B15454	24	Argentina	26°33' S	66°30' W	10	10
sct2	*S. sanctae-rosae*	MEG	B17568	24	Argentina	26°46' S	65°45' W	7	8
sct3	*S. sanctae-rosae*	MEG	B17051	24	Argentina	27°34' S	67°12' W	10	10
bst1	*S. brachistotrichum* Rydb.	PIN	B7986	24	Mexico	-	-	10	-
bst2	*S. brachistotrichum*	PIN	B7987	24	Mexico	29°08' N	106°05' W	10	-
pnt1	*S. pinnatisectum* Dunal	PIN	B8168	24	Mexico	-	-	10	-
pnt2	*S. pinnatisectum*	PIN	B8175	24	Mexico	20°40' N	103°20' W	10	1
pcs1	*S. paucissectum* Ochoa	PIU	B8162	24	Peru	-	-	6	-
pcs2	*S. paucissectum*	PIU	B55216	24	Peru	5°20' S	79°26' W	10	-
les	*S. lesteri* Hawkes & Hjert.	POL	B55219	24	Mexico	16°17' N	96°33' W	10	-
ajh1	*S. ajanhuiri* Juz. & Bukasov	TUBc	CIP702677	24	-	-	-	10	1
ajh2	*S. ajanhuiri*	TUBc	BOL2914	24	-	-	-	-	1
cha1	*S. chaucha* Juz. & Bukasov	TUBc	CIP700145	36	-	-	-	1	1
cha2	*S. chaucha*	TUBc	CIP702013	36	-	-	-	-	1

Table 1. Continued

Code	Species	Series[1]	Source[2]	Chr. no.	Origin	Latitude	Longitude	[3]# GT AFLP	[4]# GT isoz.
cha3	*S. chaucha*	TUBc	CIP701328	36	-	-	-	-	1
cha4	*S. chaucha*	TUBc	CIP701568	36	-	-	-	1	1
cha5	*S. chaucha*	TUBc	CIP704217	36	-	-	-	1	-
juz1	*S. juzepczukii* Bukasov	TUBc	CIP703262	36	-	-	-	1	-
juz2	*S. juzepczukii*	TUBc	CIP702445	36	-	-	-	1	-
juz3	*S. juzepczukii*	TUBc	'Pingo Luki'	36	-	-	-	1	1
juz4	*S. juzepczukii*	TUBc	CIP702078	36	-	-	-	1	-
juz5	*S. juzepczukii*	TUBc	CIP703260	36	-	-	-	1	-
phu1	*S. phureja* Juz. & Bukasov	TUBc	B15482	24	-	-	-	10	-
phu2	*S. phureja*	TUBc	B50199	24	-	-	-	10	-
stn	*S. stenotomum* Juz. & Bukasov ssp. *stenotomum*	TUBc	B27165	24	Bolivia	-	-	10	13
gon	*S. stenotomum* ssp. *goniocalyx* Hawkes	TUBc	B7478	24	Peru	-	-	10	10
tbr1	*S. tuberosum* L. ssp. *tuberosum*	TUBc	'Certa'x'Gloria'	48	-	-	-	1	1
tbr2	*S. tuberosum* ssp. *tuberosum*	TUBc	[14] 77.2102.37	48	-	-	-	1	-
tbr3	*S. tuberosum* ssp. *tuberosum*	TUBc	'Bintje'	48	-	-	-	1	-
adg1	*S. tuberosum* ssp. *andigena* Hawkes	TUBc	B7462	48	-	-	-	10	-
adg2	*S. tuberosum* ssp. *andigena*	TUBc	B24677	48	-	-	-	9	-
ach1	*S. achacachense* Cárdenas	TUBw	B29617	24	Bolivia	-	-	10	10
ach2	*S. achacachense*	TUBw	B53017	24	Bolivia	-	-	10	-
ber1	*S. berthaultii* Hawkes	TUBw	B28009	24	Bolivia	17°55' S	65°55' W	9	-
ber2	*S. berthaultii*	TUBw	B24578	24	Bolivia	19°34' S	65°27' W	10	-
ber3	*S. berthaultii*	TUBw	B10063	24	Bolivia	19°50' S	66°33' W	10	-
brc1	*S. brevicaule* Bitter	TUBw	B18291	24	Bolivia	-	-	10	11
brc2	*S. brevicaule*	TUBw	B28023	24	Bolivia	17°13' S	66°03' W	9	10
brc3	*S. brevicaule*	TUBw	B28038	24	Bolivia	17°19' S	66°22' W	-	10
brc4	*S. brevicaule*	TUBw	B24571	24	Bolivia	17°20' S	66°30' W	10	-
buk1	*S. bukasovii* Rybin	TUBw	B15424	24	Peru	-	-	10	11
buk2	*S. bukasovii*	TUBw	B18294	24	Peru	-	-	10	-
can1	*S. canasense* Hawkes	TUBw	[15] B8105	24	Peru	13°23' S	71°54' W	10	14
can2	*S. canasense*	TUBw	B7162	24	Peru	13°26' S	71°51' W	8	10
can3	*S. canasense*	TUBw	B8012	24	Peru	15°50' S	70°02' W	9	12
cop1	*S. coelestipetalum* Vargas	TUBw	B7942	24	Peru	13°24' S	73°54' W	7	-
cop2	*S. coelestipetalum*	TUBw	B7993	24	Peru	13°39' S	73°23' W	10	10
cop3	*S. coelestipetalum*	TUBw	B7994	24	Peru	13°39' S	73°23' W	10	-
gnd	*S. gandarillasii* Cárdenas	TUBw	B7174	24	Bolivia	-	-	10	-
grl1	*S. gourlayi* Hawkes ssp. *gourlayi*	TUBw	B7180	24	Argentina	23°47' S	65°33' W	10	12
grl2	*S. gourlayi* ssp. *gourlayi*	TUBw	B17338	24	Argentina	24°31' S	66°12' W	9	10
grl3	*S. gourlayi* ssp. *gourlayi*	TUBw	B18529	48	Argentina	23°34' S	65°26' W	10	11
grl4	*S. gourlayi* ssp. *gourlayi*	TUBw	B16837	48	Argentina	23°37' S	65°32' W	10	10
vid1	*S. gourlayi* ssp.*vidaurrei* Hawkes & Hjert.	TUBw	B16831	24	Argentina	22°31' S	65°06' W	10	5
vid2	*S. gourlayi* ssp. *vidaurrei*	TUBw	B18528	24	Argentina	23°12' S	65°27' W	9	-
ktz1	*S. kurtzianum* Bitter & Wittm.	TUBw	B17585	24	Argentina	27°26' S	66°57' W	10	-
ktz2	*S. kurtzianum*	TUBw	B16861	24	Argentina	27°35' S	67°07' W	10	5
ktz3	*S. kurtzianum*	TUBw	B17580	24	Argentina	29°11' S	67°38' W	10	-
lph1	*S. leptophyes* Bitter	TUBw	B7184	24	Argentina	-	-	8	-
lph2	*S. leptophyes*	TUBw	B27176	24	Bolivia	17°34' S	67°09' W	10	9
lph3	*S. leptophyes*	TUBw	B27211	24	Bolivia	18°14' S	66°29' W	10	10
lph4	*S. leptophyes*	TUBw	B27215	24	Bolivia	18°26' S	66°26' W	-	5

Table 1. Continued

Code	Species	Series[1]	Source[2]	Chr. no.	Origin	Latitude	Longitude	[3]# GT AFLP	[4]# GT isoz.
mcd1	*S. microdontum* Bitter	TUBw	B31189	24	Bolivia	17°53' S	64°42' W	10	-
mcd2	*S. microdontum*	TUBw	B24649	24	Argentina	22°08' S	65°02' W	10	-
mcd3	*S. microdontum*	TUBw	B24644	24	Argentina	24°54' S	65°39' W	8	-
mcq1	*S. mochiquense*	TUBw	B32672	24	Peru	-	-	8	-
mcq2	*S. mochiquense* Ochoa	TUBw	B8142	24	Peru	8°07' S	79°02' W	10	-
mlt1	*S. multidissectum* Hawkes	TUBw	B8145	24	Peru	-	-	10	10
mlt2	*S. multidissectum*	TUBw	B15459	24	Peru	12°24' S	75°06' W	10	9
mlt3	*S. multidissectum*	TUBw	B15426	24	Peru	14°10' S	71°06' W	10	4
opl1	*S. oplocense Hawkes*	TUBw	B16868	72	Argentina	21°53' S	66°11' W	10	-
opl2	*S. oplocense*	TUBw	B24650	72	Argentina	22°07' S	65°28' W	10	-
opl3	*S. oplocense*	TUBw	B16879	72	Argentina	23°12' S	65°27' W	10	-
spl1	*S. sparsipilum* Juz. & Bukasov	TUBw	B8209	24	Bolivia	16°33' S	68°09' W	10	-
spl2	*S. sparsipilum*	TUBw	B8150	24	Bolivia	17°34' S	66°21' W	10	-
spl3	*S. sparsipilum*	TUBw	B15455	24	Bolivia	-	-	10	-
spg1	*S. spegazzinii* Bitter	TUBw	B16915	24	Argentina	25°02' S	66°14' W	9	-
spg2	*S. spegazzinii*	TUBw	B24694	24	Argentina	26°40' S	65°49' W	8	-
spg3	*S. spegazzinii*	TUBw	B16905	24	Argentina	28°35' S	68°09' W	10	-
vrn	*S. vernei* Bitter & Wittm. *ssp. vernei*	TUBw	B17542	24	Argentina	26°46' S	65°46' W	10	-
bal	*S. vernei* ssp. *ballsii* Hawkes & Hjert.	TUBw	B17536	24	Argentina	23°36' S	65°08' W	10	-
ver1	*S. verrucosum* Schlechtd.	TUBw	B8255	24	Mexico	19°02' N	98°38' W	10	-
ver2	*S. verrucosum*	TUBw	B8246	24	Mexico	19°24' N	101°36' W	7	-
ver3	*S. verrucosum*	TUBw	B8254	24	Mexico	19°33' N	103°38' W	4	-
chc1	*S. chacoense* Bitter ssp. *chacoense*	YNG	B17034	24	Argentina	24°48' S	65°42' W	10	-
chc2	*S. chacoense* ssp. *chacoense*	YNG	B17018	24	Argentina	32°37' S	64°36' W	7	-
tar1	*S. tarijense* Hawkes	YNG	B17423	24	Argentina	-	-	10	-
tar2	*S. tarijense*	YNG	B8229	24	Argentina	23°00' S	64°34' W	6	-

[1] Series abbreviations according to Hawkes (1990)

[2] PI *Plant Introduction* (Bamberg *et al.* 1996); B *Braunschweig Genetic Resources Collection*; CIP *International Potato Center*, Peru; BOL *PROINPA*, Bolivia; CGN *Center for Genetic Resources*, the Netherlands

[3] Number of genoypes of which a mixed DNA sample was made of, and studied with AFLP

[4] Number of genotypes studied with isozymes

[5] Morphologically aberrant from typical ssp. *aemulans* (see Kardolus 1998)

[6] Wrongly identified as *S. acaule* ssp. *acaule* in Bamberg *et al.* (1996)

[7] Morphologically corresponding *S. albicans*, but not completely similar (see Kardolus 1998)

[8] Synonym *L. hirsutum* Miller; [9] Series *Eriopersicon* A. Child; [10] genebank identification was *S. fendleri*, our identification is *S. hjertingii*; [11] genebank identification was *S. stoloniferum*, our identification is *S. fendleri* ssp. *fendleri*; [12] Synonym *L. esculentum* Miller; [13] Series *Lycopersicon* Wettst.; [14] Described in Van Eck *et al.* (1995)

[15] Resembles cultivated diploid potato species as *S. stenotomum* / *S. phureja* morphologically

To reveal new insights in the biosystematic relations of series *Acaulia* and related potato species we examined a representative sample of *Acaulia* material and species of most series of *Solanum* sect. *Petota*. We combined the AFLP technique with a study on isozyme variation. Isozymes, although revealing fewer polymorphisms than AFLP, have proved to be of great value studying genetic variation. They are codominant and relatively easily detectable, and therefore have been used to unravel the origin of allopolyploids (Smith *et al.* 1970, Sheen 1972, Bryan and Soltis 1987). In general, electrophoretic studies have demonstrated that allopolyploids additively express the allozymes of both diploid parents (Gottlieb 1977, Crawford 1990, Weeden and Wendel 1990).

MATERIALS AND METHODS

Plant material. Seeds (and clones) of in total 171 accessions (Table 1) were obtained from the following *Solanum* germplasm collections: **BGRC/CGN** numbers from CGN-CPRO, Wageningen, the Netherlands (see http://www.bib.wau.nl/cgn/potato); **PI** from NRSP-6, Sturgeon Bay, WI, USA (Bamberg *et al.* 1996); **CIP** from CIP, Lima, Peru via Dr. Ramanna, Dept. of Plant Breeding, Wageningen Agricultural University; **BOL** from the PROINPA Research Station at Toralapa, Bolivia. The accessions represent 53 *Solanum* species, mostly of section *Petota*. Outgroup species in the AFLP analysis were *S. etuberosum* and *S. brevidens* (section *Etuberosum* A. Child) and three species of section *Lycopersicum* Wettst., i.e. *S. sitiens*, *S. lycopersicum* L. (syn. *Lycopersicon esculentum* Miller) and *S. agrimoniifolium* J.F. Macbr. (syn. *L. hirsutum* Miller). The taxonomy of section *Petota* mainly follows Hawkes (1990), except for *S. microdontum* (see Van den Berg and Spooner 1992) and *S. megistacrolobum* (see Giannattasio and Spooner 1994). The taxonomy of the outgroup species is conform Child (1990) and Spooner *et al.* (1993). Identifications of the material are conform their genebank designation, which have been provided by visiting potato taxonomists, and checked by our own observations. For the series *Acaulia* accessions the identifications follow Kardolus (1998). Plants were grown in a greenhouse in 1995 / 1996. Herbarium vouchers of the studied material are deposited at WAG. *S. cajamarquense* could not be included in the study, because no plant material was available.

AFLP procedure. For the AFLP™ analysis a DNA mix of in principle ten genotypes per accession was made. This pool of ten genotypes was the Operational Taxonomic Unit (OTU). In total 141 accessions were examined with the AFLP technique. Exceptions for the number of genotypes representing an OTU are given in Table 1. Nuclear DNA was extracted from young leaves and sprouts using the CTAB-method (Bernatzky and Tanksley 1986). The followed AFLP-protocol has been described in Kardolus *et al.* (1998). Three different primer combinations were used: E+AAC/M+CAC, E+AAC/M+CAG and E+ACA/M+CAC.

AFLP data analysis. The presence or absence of AFLP fragments was scored on the autoradiograms and transferred into a 1 (present) and 0 (absent) matrix over all OTUs. (This AFLP dataset is on request electronically available.) In the first analysis the ordination technique 'nonmetric multidimensional scaling' (Kruskal 1964) was used to get a general overview of the AFLP variation among all OTUs (Fig. 1). Multidimensional scaling (MDS) has the property that the smaller interpoint distances tend to be preserved more faithfully than in a principal component analysis (Rohlf 1993). The nonmetric-MDS was performed with procedure MDSCALE in the program NTSYS-PC (Rohlf 1993). A Dice (1945) similarity matrix was the initial matrix in this procedure. The following parameters were set: three dimensions for the configuration space and 100 as the maximum number of iterations. The OTUs were then plotted in the calculated three-dimensional character space. A minimum-length spanning tree, calculated from the same Dice similarity matrix, was superimposed over the OTUs to help detect local distortions (Rohlf 1975).

An initial neighbor-joining (NJ) analysis (Saitou and Nei 1987) was executed on all 141 OTUs with TREECON 1.2 for Windows (Van de Peer and De Wachter 1994), using Nei and Li's (1979) distance coefficient (= 1-Dice coefficient). Subsequently, OTUs were joined that belonged to the same species and that clustered together in this initial NJ analysis. The dataset was in this way condensed to 81 objects, which made it suitable for cladistic analysis. Characters that were indeterminate (0/1) in the joined objects were replaced by a one, indicating the potential presence of that particular AFLP fragment. Autapomorphic and non-polymorphic characters were excluded from further analysis.

Because of the focus in this study on series *Acaulia*, the 18 *S. acaule* OTUs were joined to six new OTUs based on the formed clusters in the initial NJ analysis. Subsequently, the new dataset was analyzed in TREECON with both a NJ analysis (Fig. 2, with bootstrap values of 500 samples) and the UPGMA cluster criterion (Fig. 4).

The new dataset was then examined with the cladistic package PAUP 3.1.1 (Swofford 1993) using Wagner parsimony (Farris 1970). Most-parsimonious trees were sought using the heuristic procedure Branch Swapping with option TBR and random addition (10 times) of OTUs (Fig. 3). As a control, data were also analyzed with the computer program HENNIG86 (Farris 1988) with options MHENNIG* followed by BB*. Based on the results of chloroplast DNA analyses (Spooner *et al.* 1991, 1993), series *Etuberosa* species *S. etuberosum* and *S. brevidens* served as the outgroup in both the neighbor-joining and the cladistic analyses. To exclude the possible influence of hybrid taxa on the phylogeny reconstruction, the condensed dataset was examined separately with HENNIG86 (Fig. 5) without hypothesized allopolyploid OTUs (Hawkes 1962, 1966, Irikura 1976, Schmiediche *et al.* 1980) and/or polyploid OTUs designated an endosperm balance number (EBN) not corresponding with their ploidy level. The following polyploid taxa were excluded from analysis: *S. acaule*, *S. albicans*, *S. brachycarpum*, *S. chaucha*, *S. demissum*, *S. fendleri*, *S. juzepczukii*, *S. oxycarpum*, *S. paucijugum* and *S. stoloniferum*.

Isozyme electrophoretic procedure. In total 94 accessions were studied with isozymes. Per seed accession three genotypes were analyzed of the predominantly autogamous *S. acaule* ssp. *acaule* and ssp. *punae*, and *S. albicans*. About ten genotypes were analyzed per accession of the diploid and outbreeding species. Also of *S. acaule* ssp. *aemulans* ten genotypes per accession were studied, because of possible intra-accession variation (Echeverria *et al.* 1989). For exact number of genotypes studied per accession see Table 1.

Mature and healthy leaves were crushed and 100 ml sap was immediately added to 70 ml cold extraction buffer (0.1 M Tris-HCl and 1% ß-mercaptoethanol in 60% glycerol; pH 7). Extracts were centrifuged for 10 minutes at 15.000 rpm, at 4°C. Three different dimeric enzymes were examined, revealing in total four loci. Isocitrate dehydrogenase (IDH-NADP, E.C. 1.1.1.42, 1 locus) and phosphoglucose isomerase (PGI, E.C. 5.3.1.9, 1 locus; also called glucose phosphate isomerase) were studied on horizontal starch-gel electrophoresis. Aspartate aminotransferase (AAT, E.C. 2.6.1.1, 2 loci) was studied on a PAGE system. One genotype of *S. pinnatisectum* (**pnt2**) and of *S. tuberosum* (**tbr1**) were run on each gel as a reference for the proper allelic designation of the observed bands.

The horizontal starch gel was made of a 5.0 mM Histidine-HCl.H_2O gel buffer (pH 7), 11% starch (Sigma S-4501) and 4% sucrose (w/v). Whatman™ nr.4 filter paper wicks (17 × 3 mm) were loaded with 7 ml sample. The electrode buffer was a 0.135 M Tris and 0.043 M Citrate buffer (pH 7). Electrophoresis was carried out at constant 25 W (± 90 mA and 300 V) for 0.9 kVh at 4°C. After electrophoresis the gel was cut in slices of 1-1.5 mm thickness.

The vertical discontinuous poly-acrylamide gel (PAGE) was made of a 6.75% acrylamide separating gel with a 0.375 M Tris-HCl buffer with 0.06% TEMED (pH 8.9). The 4.5 cm 5.33% acrylamide stacking gel was made with a 0.062 M Tris-HCl buffer (pH 6.8). The electrode buffer was a 0.025 M Tris and 0.128 M Glycine buffer (pH 8.5). The gel ran at constant 170 mA (± 800 V, ± 145 W) till the front reached the anodal side of the gel (± 2.2 kVh) at 4-12°C.

Gel for IDH was stained at 37°C in 50 ml 0.1 M Tris-HCl pH 7.5 with 50 mg $MgCl_2$, 50 mg DL-isocitrate NA_3 (Sigma I-1252), 5 mg $NADP^+$ (Merck 1.24541), 10 mg MTT and 2 mg PMS; PGI was stained following Wendel and Weeden (1990) with a small modification. The fructose-6-phosphate, Na_2-

salt was added after 20 minutes incubation of the gel in the staining solution. AAT was stained according to Stejskal (1994). The enzymes migrated towards the anode.

Genetic analyses of isozyme loci in *Solanum* have been conducted by Quiros and McHale (1985) and Douches and Quiros (1988). For AAT the zone closest to the front is given a '1' and the slowest zone a '2'. In Fig. 6 the banding patterns and allele designations of the four loci are given. We used a letter-system for the designation of alleles. Per locus the allozyme closest to the anode is designated 'a', the following 'b' etc. In the present experiment more alleles were found than in prior researches studying these enzymes (Martínez-Zapater and Oliver 1984, Staub *et al.* 1984, Oliver and Martínez-Zapater 1984, 1985, Quiros and McHale 1985, Douches and Quiros 1988, Cortés *et al.* 1989, Echeverría *et al.* 1989, Camadro *et al.* 1992).

Horizontal starch gel slices were also stained for cathodal/anodal peroxidases (PRX), malate dehydrogenase-NAD^+-form (MDH, E.C. 1.1.1.37), malic enzyme (ME, E.C. 1.1.1.40) and shikimate dehydrogenase (SKD, E.C. 1.1.1.25). 6-Phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44) was studied on PAGE. The banding patterns of these enzymes were not used because too often they were unclear (ME, MDH), too complex to interpret (SKD, 6PGD) or inconsistent (PRX).

Isozyme data analysis. The mean observed heterozygosity H_o was calculated for the outbreeding accessions by the sum of h_o (= fraction of heterozygous individuals per locus) over all loci divided by the total number of loci. OTUs were clustered using the UPGMA cluster-criterion in SAHN with the computer program NTSYS-PC (Rohlf 1993). The genetic distance, Cavalli-Sforza and Edwards' (1967) chord distance, was calculated in this program with procedure SIMGEND. Allele frequencies per accession are calculated following Lu and Pickersgill (1993) to be able to compare diploid and polyploid OTUs in one analysis; the allele frequency of allele a in the tetraploid genotype aabb was counted as 0.5.

RESULTS

AFLP analysis. A dataset of 997 AFLPs was obtained with the three studied primer combinations. Primer combination E+AAC/M+CAG revealed most AFLPs (430). Scored fragments were of a size of circa 130 to 550 nucleotides. Shorter fragments tended to give less sharp bands and longer fragments were not always consistently amplified. The mean number of AFLPs scored per OTU was 114. The diploid OTUs **ifd1**, **rap2**, **sct1** and **phu2** revealed more than 150 AFLPs, as did the polyploid OTUs **juz1, 2,** and **4,** and **oxc** and **pcj.** The (Dice) similarity was rather high (> 0.75) among the OTUs belonging to *S. acaule*, *S. albicans*, *S. demissum*, *S. achacachense*, *S. berthaultii*, *S. oplocense*, *S. verrucosum*, *S. paucissectum*, *S. etuberosum*, *S. brevidens* and *S. pinnatisectum*. Other species showed higher degrees of variation or were studied by one OTU only.

With the multidimensional scaling technique most of the 141 studied OTUs formed a dense cluster (Fig. 1) when plotted in the first two dimensions. In total 19 OTUs fell out of this cluster, belonging to the non-tuber-bearing *Solanum* species and/or 'primitive' potatoes, e.g. *S. pinnatisectum*. The two *S. mochiquense* OTUs were placed above the cluster, all 17 other OTUs were plotted below or at the right of this cluster. The superimposed minimum spanning tree (MST) formed separate branches. The six branches in Fig. 1 were independently connected to different OTUs in the dense cluster, possibly indicating several evolutionary lines. OTUs that belong to the same series clustered together with the MST. *S. sitiens* was connected to the tomato OTUs **esc** and **agr.**

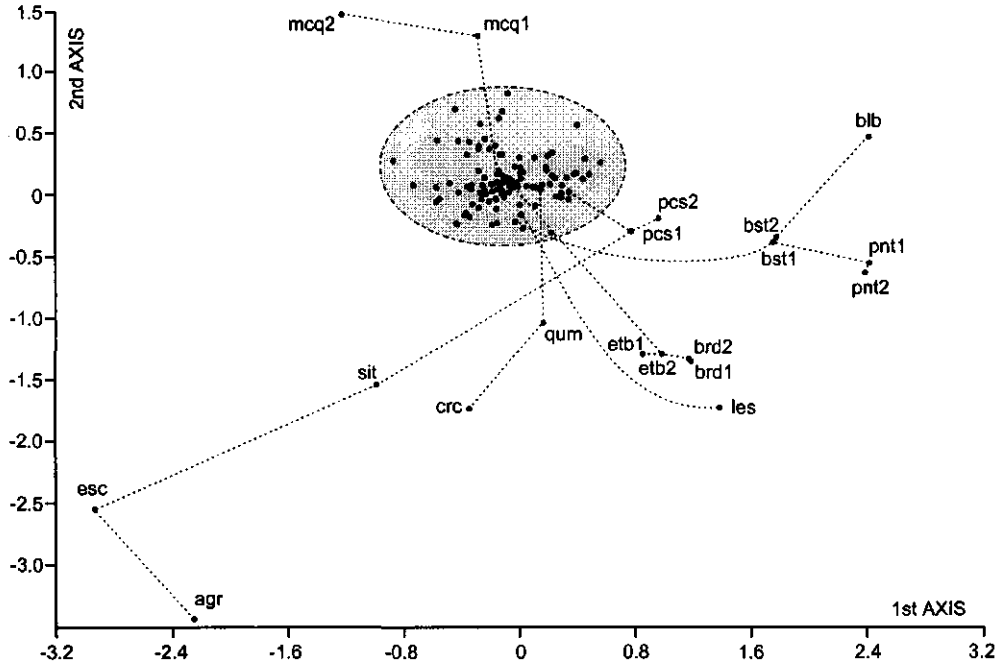


Figure 1. Scatterplot of 141 *Solanum* OTUs on the first two multidimensional scaling axes, calculated from the complete AFLP dataset. A Minimum Spanning Tree is superimposed over this plot.

The initial neighbor-joining analysis (figure not shown), based on the Nei and Li (1979) dissimilarity matrix, clustered most OTUs that belong to one species directly together or relatively close to each other and it was not difficult to condense the dataset from 141 to 81 OTUs. In the trees based on the analysis of this condensed dataset (Figs. 2, 3) eight large groups were appointed, all containing OTUs of several species. With these groups it was possible to describe the most significant trends in the results of the phenetic and parsimony analyses. Some of the groups were equivalent with taxa (e.g. group **A** comprised all series *Acaulia* OTUs), other were based on common origin, such as group **B** with species from Bolivia and Argentina. Three small groups (**L**, **D**, and **J**) accommodated OTUs of only one species each, respectively *S. lignicaule*, *S. demissum*, and *S. juzepczukii*.

Group **P** was located at the base of the Neighbor-Joining tree (Fig. 2). This group **P** (the '**P**rimitive' potatoes and the outgroup species) included all species that in the multidimensional scaling (Fig. 1) plotted in the periphery. Secondly positioned in the NJ-tree was group **H** with polyploid OTUs of series *Conicibaccata*. Group **H** also comprised OTUs **sct1** and **rap2**. These were placed separate from the other *S. sanctae-rosae* and *S. raphanifolium* OTUs, that were placed in a series *Megistacroloba* group (**M**). Furthermore OTU **juz1-2** was grouped in **H**, instead of being grouped with OTUs **juz3-4** and **juz5** in **J**. *S. lignicaule* (**L**) was not part of a cluster. *S. tuberosum* grouped with diploid wild species from Peru (**T**). The species in **R** are now classified in several series, but all have a rather robust habit. These robust species were placed at the base of a group OTUs of series *Tuberosa*, *Cuneoalata*, and *Megistacroloba* from Bolivia and Argentina (**B**). Also species of

Mexico and the USA grouped together (**N**), and formed a sister group with series *Acaulia* material. *S. juzepczukii* OTUs **juz3-4** and **juz5** (in **J**), and the *S. demissum* OTUs (**D**) were placed between groups **N** and **A**.

In the cladistic analyses eight most parsimonious phylograms were found with a length of 5410 (retention index 0.383). PAUP gave five phylograms and three other phylograms were revealed with HENNIG86. All were quite similar to each other. In one of the PAUP phylograms (Fig. 3) a monophyletic group of all *S. juzepczukii* OTUs was present (clade **J**), the other four PAUP phylograms varied only in the position of **juz1-2** and **buk2**. Compared to the NJ analysis (Fig. 2), other differences could be observed. Most remarkable (Fig. 3) was the placement of group **N** (with representatives of the polyploid series *Longipedicellata* and *Demissa*). Group **N** now formed a sister group of **B** (the Bolivian OTUs) and was separated from the OTUs of *S. demissum* and series *Acaulia*. *S. demissum* remained at the base of the clade consisting also the series *Acaulia* OTUs. *S. juzepczukii* was positioned intermediately between the *S. demissum* and series *Acaulia* OTUs. Group **M** (with the series *Megistacroloba* OTUs) was in the cladistic analysis placed at the base of these more advanced polyploids, and contained now also **rap2** and **sct1**.

The topology of the three most parsimonious HENNIG86 phylograms was comparable with those of PAUP, but certain OTUs or groups in the HENNIG86 phylograms were positioned differently: *Solanum lignicaule* was placed at the base of clade **T**, comprising the wild series *Tuberosa* species from Peru and cultivated potatoes. Furthermore, clade **N** with most of the diploid and polyploid OTUs from Mexico/USA, together with *S. gandarillasii*, was in all three HENNIG phylograms placed at the base of clade **B**. The *S. vernei* OTUs were situated at the base of the clade with the *S. maglia* and *S. microdontum* OTUs.

A phenetic analysis, using the condensed dataset, gave a UPGMA dendrogram (schematic representation in Fig. 4) that resembled the results from the cladistic analysis. The most distant groups in the dendrogram were not the species of sect. *Etuberosum*, which were defined as outgroup in the cladistic analysis, but the species of sect. *Lycopersicum*. Furthermore, *S. demissum* clustered first with the *S. acaule* OTUs in this analysis, and *S. albicans* clustered only thereafter with this combined group. A HENNIG86 analysis with only diploid and autopolyploid OTUs resulted in a phylogram (schematic representation in Fig. 5, length 4194) with a similar composition of groups as in Fig. 3.

Isozyme analysis. Twenty-nine putative alleles, encoded by four dimeric enzyme loci, were identified (Fig. 6). The alleles of *AAT1* and *AAT2* overlapped partly in mobility (Fig. 6), but could be distinguished because *AAT2* gave sharper bands. Camadro *et al.* (1992) have interpreted a rather broad band of enzyme activity at *AAT1* as one allele (their Fig. 5), and described ssp. *acaule* as being monomorphic for *AAT1*. *S. acaule* displayed *AAT1* in the present study as three clearly distinct bands, although these were close together. *AAT* is dimeric and this pattern was consequently interpreted as two alleles: *AAT1-b* and *AAT1-c*.

Allele *AAT1-b* was only present in series *Acaulia* material, *S. juzepczukii* and *S. demissum*. Correspondingly, *PGI1-g* was present in the same material as *AAT1-b*, but *S. pinnatisectum* also expressed allele *PGI1-g* (Table 2). Accessions of the inbreeding polyploid species *S. acaule*, *S. albicans*, *S. demissum*, and *S. fendleri* showed fixed heterozygosity at one to four loci. Only six accessions of series *Acaulia* showed within-accession polymorphy. In the outbreeding species the mean heterozygosity varied between 0 and 34%.

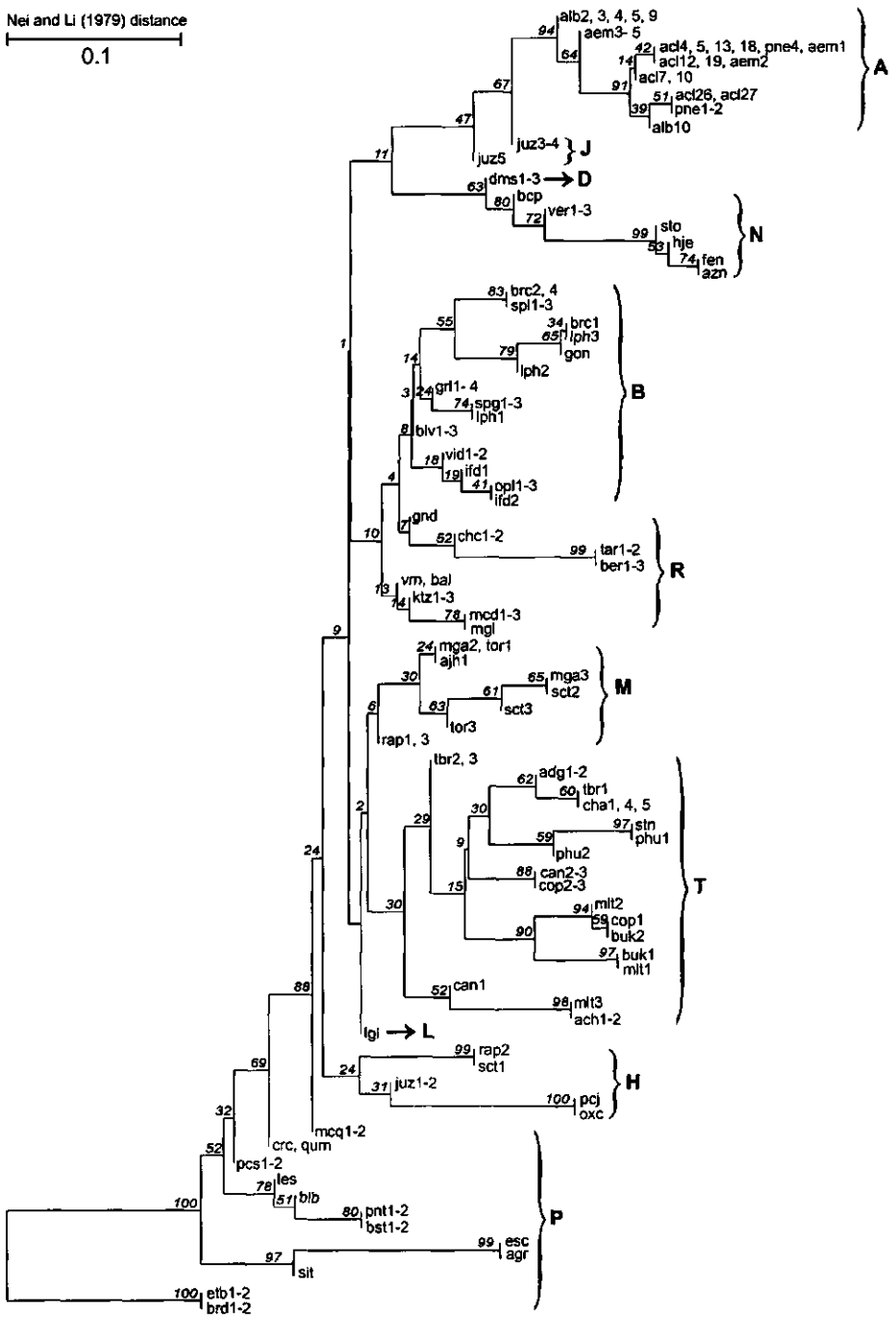


Figure 2. Neighbor-joining analysis of the condensed AFLP dataset, consisting of 81 OTUs and 885 AFLPs, rooted with *S. etuberosum* and *S. brevidens*. The terminal branches are discarded. Bootstrap values (%) are given for each branching.

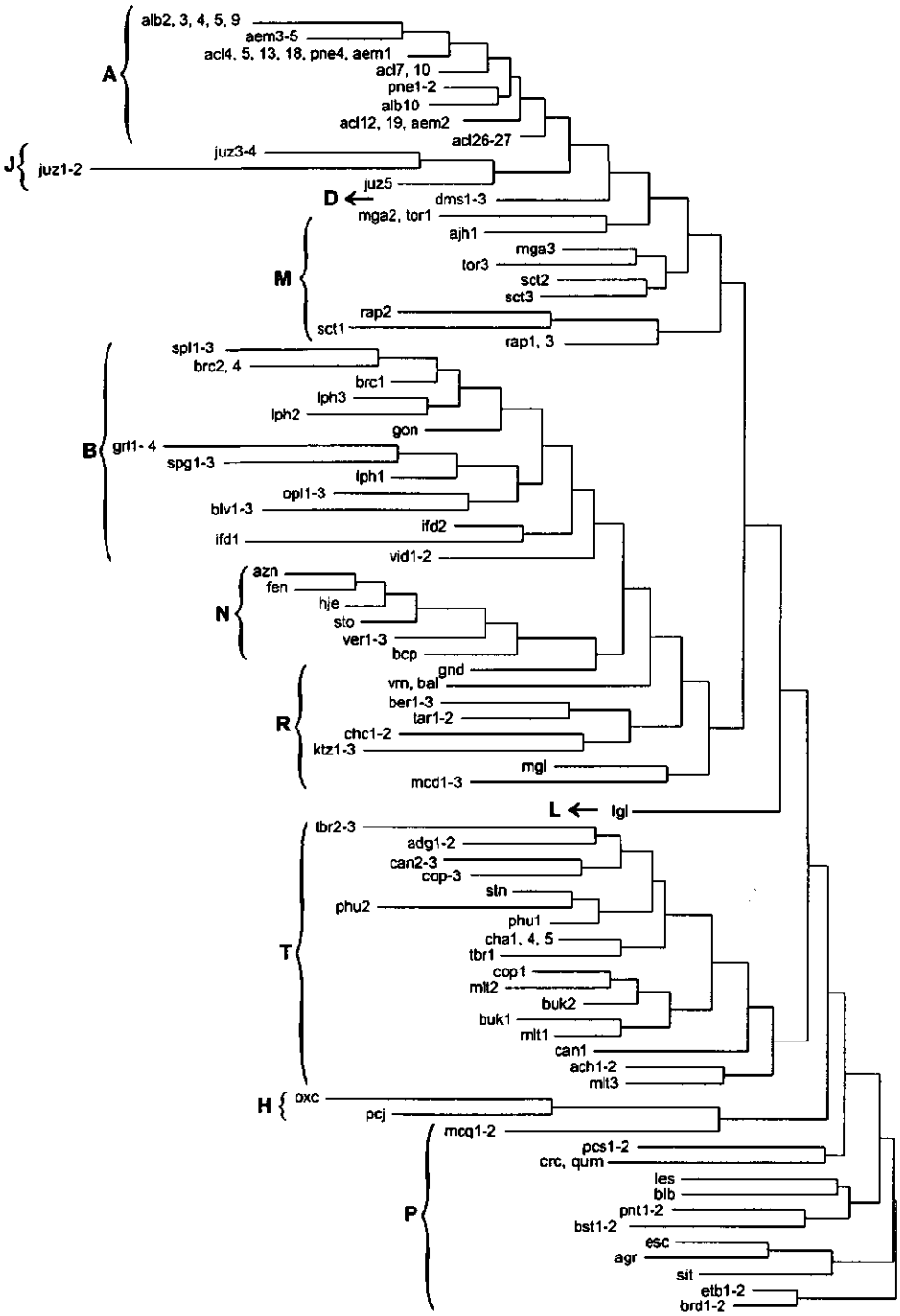


Figure 3. One of the five most parsimonious PAUP-phylograms of the condensed AFLP dataset (see Fig. 2), rooted with *S. etuberosum* and *S. brevidens*.

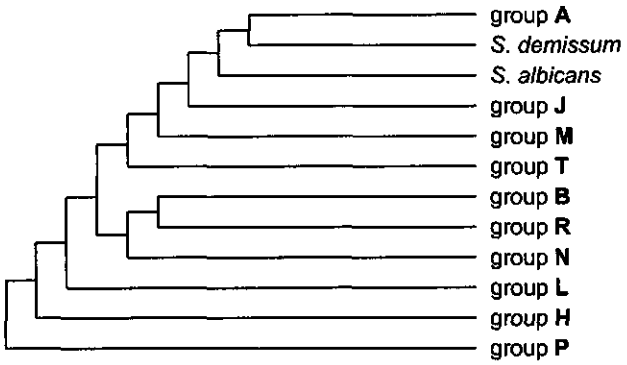


Figure 4. Schematic dendrogram based on an UPGMA cluster analysis of the condensed AFLP dataset and Nei and Li's pairwise distances. For the designation of the groups see Fig. 2.

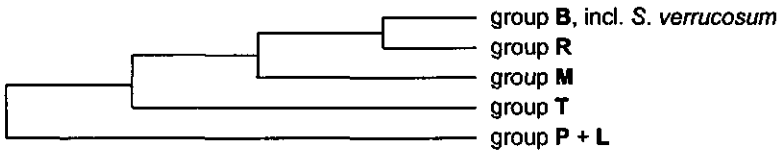


Figure 5. Schematic phylogram based on a Cladistic analysis of the condensed AFLP dataset, minus the polyploid taxa (see Materials and Methods). For the designation of the groups see Fig. 3.

In the cluster analysis the accessions of *S. acaule* and *S. albicans* clustered per species, and together they formed a series *Acaulia* cluster (* in Fig. 7). *S. demissum* was just positioned outside of this cluster. In the series *Acaulia* cluster accession **alb10** grouped with *S. acaule*, just as in the AFLP analysis. Accession **alb10** had allele *AAT1-c*, as did all *S. acaule* accessions (Table 2). In the *S. albicans* accessions, **alb1-alb9,** this allele was absent. Furthermore, **alb10** lacked allele *AAT2-a*, a common allele in *S. albicans* which was not present in *S. acaule* accessions. The subspecies of *S. acaule* did not form three separate clusters, but all three accessions of *S. acaule* ssp. *aemulans* from province La Rioja, **aem3-5**, could be distinguished from other *S. acaule* material in having allele *AAT2-e*. *S. demissum* was positioned next to the series *Acaulia* cluster in Fig. 7.

In the dendrogram was furthermore a large cluster (marked with **) present consisting of wild series *Tuberosa* species, cultivated potatoes, and series *Megistacroloba* species. A third group (***) was composed of Bolivian and Argentinean species of series *Tuberosa*, and *S. boliviense* (series *Megistacroloba*), *S. infundibuliforme* (series *Cuneoalata*) and *S. fendleri* ssp. *arizonicum* (series *Longipedicellata*). Separately from these two larger groups, two accessions of the morphologically distinct series *Circaeifolia* (**cap, crc**) clustered together. *S. pinnatisectum* and *S. kurtzianum* were most distantly placed in the cluster analysis.

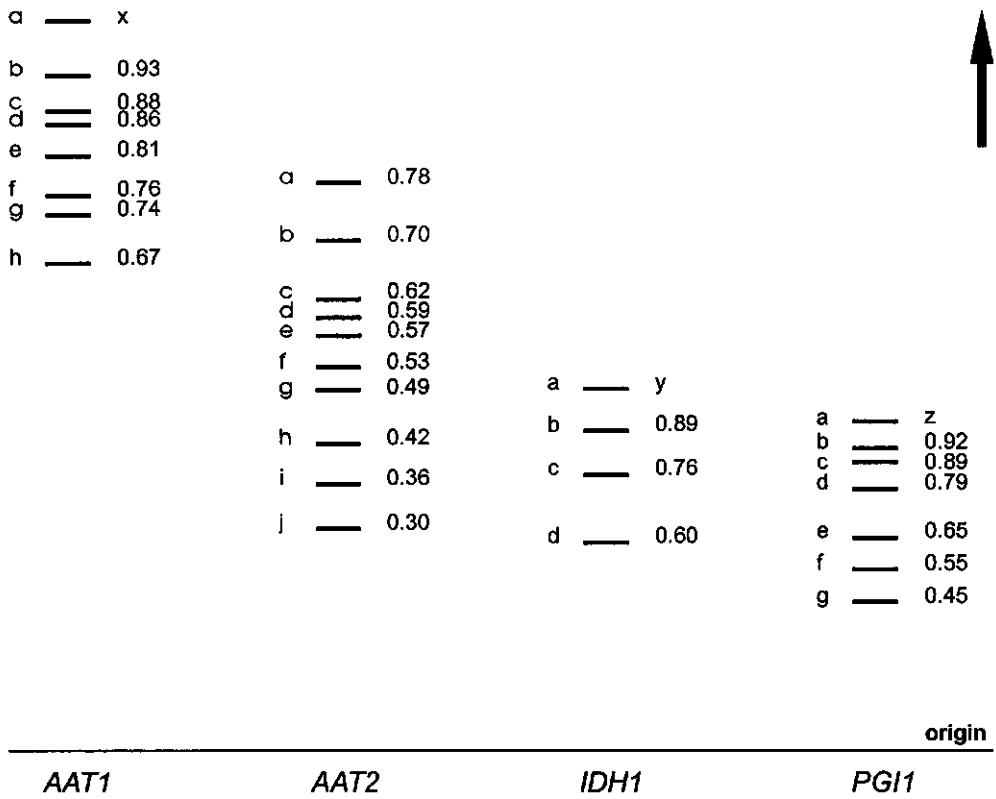


Figure 6. Schematic representation of enzyme patterns. The symbols x, y, z are the distances of the enzymes with the highest mobility of respectively *AAT1*, *IDH1* and *PGI1*. The relative distance of the other enzymes are given next to the alleles. The distance for *AAT2*-alleles are relative to the distance of allozyme *AAT1-a*.

DISCUSSION

The main goal of this study was to unravel the origin of the allopolyploid series *Acaulia*. However, since a representative set of all tuber-bearing *Solanum* species was studied, it seemed possible and desirable to discuss the biosystematic position of section *Petota* and the gross classification of this section. Some noteworthy relations within the section will be considered more extensively. Subsequently, series *Acaulia* and its most related species will be focused on, using both the AFLP and isozyme data. The discussion will be concluded with a description of the variation within series *Acaulia*.

Biosystematic position of *Solanum* sect. *Petota*. The three outgroup species of section *Lycopersicum* proved to be most aberrant in their AFLP pattern, instead of the section *Etuberosum* species which were chosen as outgroup in the NJ and parsimony analysis. This choice was based on a chloroplast DNA study (Spooner *et al.* 1993), in which section *Etuberosum* was a sister-group of the clade containing both section *Lycopersicum* and

section *Petota*. Spooner *et al.* (1993) have pointed to the fact that the basal position of sect. *Etuberosum* was based on only few mutations. Crossing data and a previous cpDNA study of Hosaka *et al.* (1984) have suggested a closer relationship between sections *Etuberosa* and *Petota*. The present study can not conclude on the phylogenetic relatedness of these three sections, because more distantly related species (for character polarity determination) were not examined. With the AFLP technique it may be impossible to make this comparison due to a lack of homology in AFLP markers of these species, since the difference between section *Lycopersicum* and the other examined OTUs is already substantial (Nei and Li-distance circa 0.85).

Based on the AFLP dataset, the taxa of sections *Lycopersicum* and *Etuberosum*, together with some 'primitive' section *Petota* species, were distinguished (Fig. 1) from a group that may be named the 'potato cluster'. This cluster encompassed the wild tuber-bearing species that are relatively closely related to the cultivated potato, *Solanum tuberosum*. The taxa of section *Petota* that do not fall in the 'potato cluster' have an endosperm balance number (EBN, see Johnston *et al.* 1980) of one (Hawkes 1990). Apparently, these taxa are genetically rather distinct from the species of this potato cluster. Two exceptions were found in our analysis: *S. lignicaule* (EBN=1) was positioned in the AFLP analysis within the potato cluster, although rather isolated (**L** in Figs. 2, 3), and *S. paucissectum* (EBN=2) was not in the potato cluster (Figs. 1-3). These groupings match the classification based on chloroplast DNA data (Spooner and Castillo 1997). In their results *S. lignicaule* was located in the clade with *S. tuberosum* and its sister species (the group currently assigned as the potato cluster), and *S. paucissectum* and other species of series *Piurana* Hawkes were placed separately (see Fig. 2 in Spooner and Castillo 1997). Likewise, in the cpDNA analysis *S. mochiquense* (EBN=1, classified in series *Tuberosa*) was not so closely related with species of the potato cluster, and it appeared as a sister-taxon of series *Piurana* species. One notable difference between the cpDNA data and the current AFLP analysis was the placement of series *Circaeifolia* taxa (EBN=1). In the cpDNA analysis (Spooner and Castillo, 1997) *S. capsicibaccatum* of series *Circaeifolia* grouped in the 'potato' clade, though it was discriminated by three unique mutations. The two OTUs of *S. circaeifolium* (**crc** and **qum**) in the AFLP experiment were not positioned in the potato cluster (Figs. 1-3) because of their deviating AFLP pattern.

Biosystematic relationships within *Solanum* sect. *Petota*. Hawkes (1990) has classified the tuber-bearing *Solanum* species in subsection *Potatoe* G. Don, and this subsection was subdivided in 19 series. This classification in series, which suggests a phylogenetic relation between the species classified into a particular series, is based on morphological and/or geographical concordance. Chloroplast DNA divided section *Petota* in only four clades (Spooner and Castillo 1997). The phylogeny reconstruction based on the nuclear DNA marker AFLP corresponded well with these cpDNA data, but the biparentally inherited AFLP markers resolved the phylogeny of section *Petota* in more detail because they are less conservative than cpDNA.

The phylogeny generally resembled a phylogeny based on nuclear RFLPs (Debener *et al.* 1990). These authors studied a comparable but smaller set of material. Series *Tuberosa* is cut into two branches in both their study and the present AFLP study. One branch (**T** in Figs. 2, 3) consists of cultivated potatoes and diploid wild species from Peru and north-west Bolivia (*S. achacachense*), and the other group (**B** in Figs. 2, 3) contains

Tuberosa species from Bolivia and Argentina similar to *S. brevicaule*, and *S. infundibuliforme* (series *Cuneoalata* Hawkes) and *S. boliviense* (series *Megistacroloba*). This split has also been found in morphological studies (Van den Berg *et al.* 1996, 1998). Although these two *Tuberosa* groups are morphologically similar and as a group have been called 'the brevicaule-complex' (Van den Berg *et al.* 1996, 1998), the two branches are not sister groups based on analysis of both AFLP (this study) and RFLP data (Debener *et al.* 1990). In these molecular studies the second group (**B**) is more similar to a group of species, all with a rather robust habit (**R** in Figs. 2, 3), comprising *S. vernei*, *S. microdontum*, *S. berthaultii*, *S. gandarillasii* and *S. kurtzianum* (all of series *Tuberosa*), and species now classified in other series, e.g. *S. tarijense* and *S. chacoense* (both of series *Yungasensia* Correll) and *S. maglia* (series *Maglia* Bitter).

The series classification, as put forward by Hawkes (1990), is in serious need of reconsideration. The assignment of species to series has been questioned (Spooner and Van den Berg 1992a, Spooner *et al.* 1995, 1997) because the series classification often does not reflect the phylogenetic relations well. This is demonstrated by the phylogenetic position of the species in three '*Tuberosa*' groups **T**, **B** and **R** (Figs. 2, 3). They are at present classified in five different series (Hawkes 1990). Spooner and Van den Berg (1992b) have already disputed the classification of the morphologically similar taxa *S. berthaultii* and *S. tarijense* in different series. In the current AFLP study their close phylogenetic affiliation was demonstrated (Figs. 2, 3). Our conclusions regarding a new classification have to be limited because only a subset of all tuber-bearing *Solanum* species was examined. A monographic analysis of all tuber-bearing species, using nuclear molecular markers, is the next step in the understanding of the true biosystematic relations of this group. Such a study might also reveal the necessity to reduce the number of taxa in this group, as was proposed in various recent studies (Spooner and Castillo 1997, Van den Berg *et al.* 1998).

Kardolus *et al.* (1998), studying a limited number of *Solanum* species with the AFLP technique, have shown that phenetic and cladistic analyses of the binary dataset gave comparable results. In the present study the resulting trees of neighbor-joining analysis (Fig. 2) and parsimony analysis (Fig. 3) had once again a similar overall topology, although some noticeable differences could be observed. The NJ and parsimony analyses revealed different aspects of the phylogenetic relationships among tuber-bearing *Solanum* species. In the NJ analysis, and for that matter in the UPGMA analysis as well, certain morphologically rather distinct OTUs were not united with each other. For example, **sct1** and **rap2** (see group **H**, Fig. 2) did not group with other series *Megistacroloba* OTUs (group **M**) with this phenetic method. Likewise, OTUs **ifd1** did not cluster directly with **ifd2** (Fig. 2). All three OTUs **sct1**, **rap2** and **ifd1** were characterized by a high number of AFLPs, probably due to some unexplained heterozygosity among the individual genotypes of which the AFLP sample was composed. In the PAUP phylogram (Fig. 3) **sct1** and **rap2** were placed with related material in group **M**, and both **ifd**-OTUs formed a monophyletic group. Correspondingly, OTU **juz1-2** grouped with the other *S. juzepczukii* OTUs.

S. demissum, which is a member of the Mexican polyploid series *Demissa*, was intermediately positioned between the Mexican/USA most polyploid OTUs (group **N**) and series *Acaulia* (**A**) in the NJ analysis (Fig. 2). With the cladistic analysis no connection was observed between *S. demissum* and the OTUs of group **N**. The closest relationship of *S. demissum* was with series *Acaulia* (Fig. 3). This association was also found with a

UPGMA analysis (Fig. 4), which was based on the same distance matrix as used in the NJ method. Molecular (Debener *et al.* 1990, Kardolus *et al.* 1998) and morphological studies (Spooner *et al.* 1995, Kardolus 1998) have already described the close relationship of *S. demissum* with series *Acaulia*. These recent studies refute the ideas of Ugent (1981). He has disputed assumptions that both groups are phytogeographical equivalents (Correll 1962, Hawkes and Hjerting 1969: p.254; also later described by Ochoa 1990) and even raised the question if *S. demissum* and series *Acaulia* are even distantly related. Ochoa (1990: p. 32) similarly stated that 'they appear to have had an independent evolutionary origin'.

The Neighbor-Joining analysis of AFLP data in the present study revealed that *S. demissum* is, next to its relation with series *Acaulia*, also connected with taxa from Mexico/USA now classified in series *Demissa*, *Longipedicellata* and *Tuberosa*. This finding corresponds with the ideas that the polyploids from this region are related to each other (Marks 1965, Hawkes 1990), and that the diploid *S. verrucosum*, the sole representative of series *Tuberosa* in this region, may have played an important role in their origin (Spooner and Sytsma 1992).

The intermediate position of *S. demissum* could indicate that a hybridization event occurred between series *Acaulia* and some species which have their distribution now in Mexico, resulting in the hexaploid species *S. demissum*. This assumption is supported by the results of interspecific crosses of Kameraz *et al.* (1978). They have conducted test-crossings of *S. acaule* with tetraploid *Longipedicellata* material that often resulted in hexaploid hybrids. McDade (1990) has predicted, based on an empirical study of known hybrids, that a hybrid in a cladistic analysis will be placed as a basal lineage to the clade that includes its most derived parent. This prediction correlates well with the placement of *S. demissum* at the base of the series *Acaulia* clade.

Correspondingly, *S. juzepczukii* was positioned just basal of the series *Acaulia* clade (Fig. 3). This triploid cultigen has been assumed to be a hybrid of *S. acaule* and a diploid cultivated potato, such as *S. stenotomum* (Hawkes 1962). This hypothesis has been reexamined in crossing studies (Schmiediche *et al.* 1982), a prior AFLP study (Kardolus *et al.* 1998), and in a genomic-in-situ-hybridization experiment (Kardolus *et al.* 1997). Our AFLP and isozyme data were concordant with the assignment of *S. acaule* as one of its parents.

Biosystematic position of series *Acaulia*. The AFLP technique revealed many markers and gave a valuable overview of the phylogenetic relations within *Solanum* sect. *Petota*. Nevertheless, it proved to be impossible to assign AFLP markers directly to the different genomes which are present in the series *Acaulia* taxa. Therefore, they do not give much insight in the hybridization events and parental taxa of series *Acaulia*. An isozymic dataset is more suitable to resolve this problem because it reveals co-dominant alleles. A large set of series *Acaulia* material was studied, and compared with the isozymic composition of a sample of putatively closely related species.

Fixed heterozygosity was observed in series *Acaulia*, as has been reported in previous isozyme studies on *S. acaule* (Cortés and Camadro 1989, Camadro *et al.* 1992), but the isozyme composition in *S. acaule* could not directly be interpreted as a summation of two diploid species. About half of the allozymes were unique for series *Acaulia* material (*AAT1-b*) or shared with the outgroup species of series *Circaeifolia* (*AAT2-c*) and

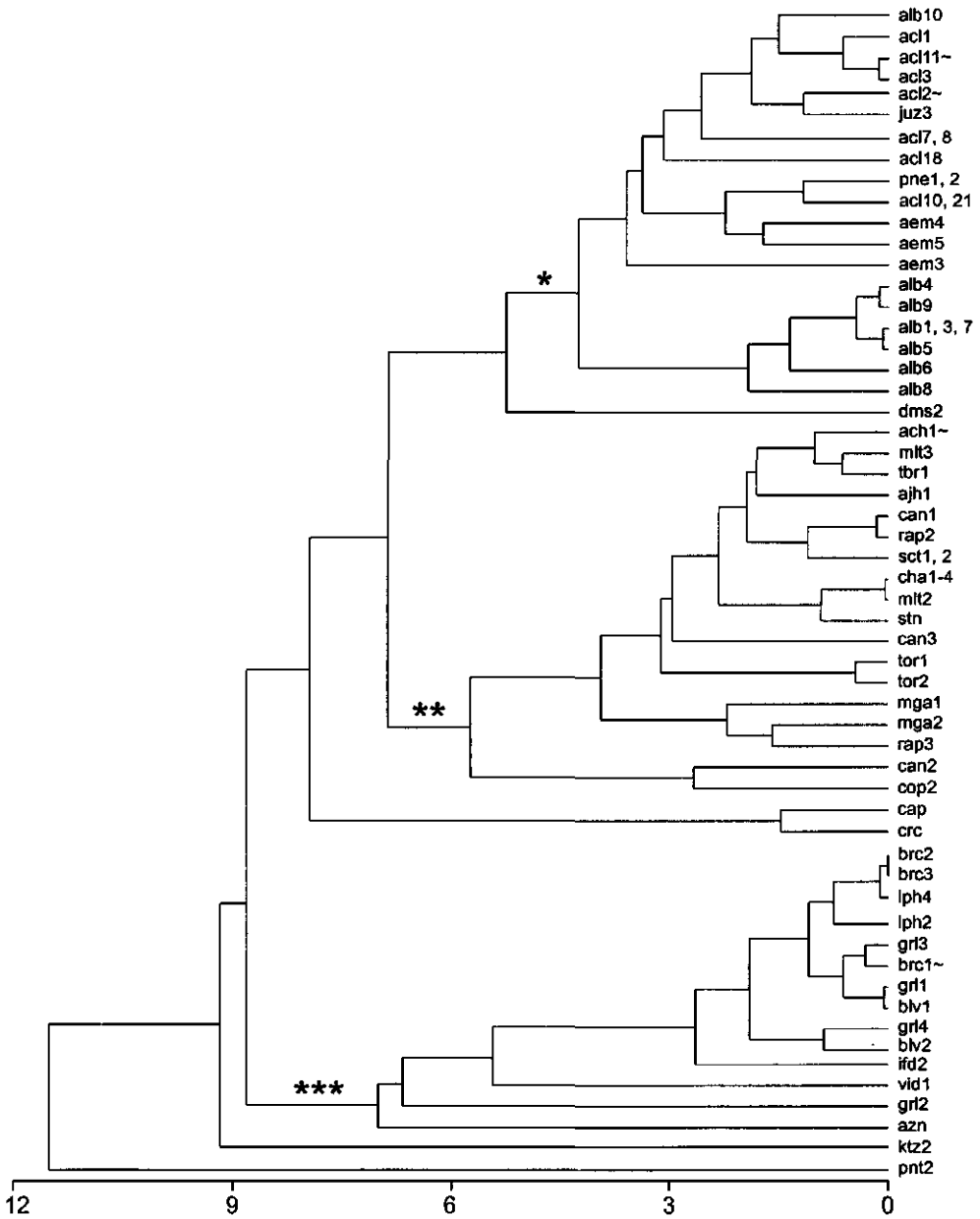


Figure 7. UPGMA dendrogram based on the genetic distance (Cavalli-Sforza and Edwards' (1967) chord distance) between OTUs, calculated from 4 isozyme loci. Only the first OTU of a group of OTUs with the same isozyme composition is placed in the dendrogram and marked with a ~ sign, the other OTUs of such a group can be found in Table 2.

Table 2. Heterozygosity (H_0) and allele presence summarized for 4 isozyme loci in 94 *Solanum* accessions. The vertical lines next to the **code** indicate accessions with the same isozyme composition.

Code [1]	[2]# GT	H_0	AAT1	AAT2	IDH1	PGI1	Code	# GT	H_0	AAT1	AAT2	IDH1	PGI1
alb10	7	1 GT IDH-abc	bc	cf	a(b)c	dg	**ach1**	10	0%	c	f	b	d
acl1	3	2 GT AAT2-bcf	bc	(b)cf	ab	dg	**ajh2**	1	-	c	f	b	d
acl11	3	Fixed	bc	cf	ab	dg	**buk1**	11	0%	c	f	b	d
acl12	3	Fixed	bc	cf	ab	dg	**mlt1**	10	0%	c	f	b	d
acl14	3	Fixed	bc	cf	ab	dg	**sct3**	10	0%	c	f	b	[3] -
acl15	3	Fixed	bc	cf	ab	dg	**mga3**	9	0%	c	f	b	d (f?)
acl16	3	Fixed	bc	cf	ab	dg	**mlt3**	4	13%	ce	f	bc	d
acl17	3	Fixed	bc	cf	ab	dg	[4] **tbr1**	1	-	c	f	bc	d
acl19	3	Fixed	bc	cf	ab	dg	**ajh1**	1	-	ac	f	b	d
acl20	3	Fixed	bc	cf	ab	dg	**can1**	14	25%	c	cf	b	de
acl22	3	Fixed	bc	cf	ab	dg	**rap2**	11	11%	c	cf	b	de
acl23	5	Fixed	bc	cf	ab	dg	**sct1**	10	25%	c	f	b	de
acl24	3	Fixed	bc	cf	ab	dg	**sct2**	8	25%	c	f	b	de
acl25	3	Fixed	bc	cf	ab	dg							
acl26	3	Fixed	bc	cf	ab	dg	**cha1**	1	-	c	f	b	df
acl27	5	Fixed	bc	cf	ab	dg	**cha2**	1	-	c	f	b	df
pne4	3	Fixed	bc	cf	ab	dg	**cha3**	1	-	c	f	b	df
aem1	10	Fixed	bc	cf	ab	dg	**cha4**	1	-	c	f	b (c?)	df
aem2	10	Fixed	bc	cf	ab	dg	**mlt2**	9	5%	c	f	b	df
acl3	3	1 GT AAT1-bc	b(c)	cf	ab	dg	**stn**	13	19%	ac	f	b	df
acl2	3	Fixed	bc	cf	b	dg	**can3**	12	23%	ceg	dfh	bd	d
acl4	3	Fixed	bc	cf	b	dg	**tor1**	10	20%	ceg	f	b	de
acl5	2	Fixed	bc	cf	b	dg	**tor2**	9	27%	cg	f	ab	de
acl6	4	Fixed	bc	cf	b	dg	**mga1**	10	34%	ac	ef	bc	de
acl9	3	Fixed	bc	cf	b	dg	**mga2**	12	15%	c	ef	b	de
acl13	4	Fixed	bc	cf	b	dg	**rap3**	10	13%	c	e	b	de
pne3	3	Fixed	bc	cf	b	dg	**can2**	10	30%	c	h	bd	df
juz3	1	-	bc	cf	bc	dg	**cop2**	10	13%	c	hj	b	d
acl7	4	Fixed	bc	bf	ab	dg	**cap**	2	0%	g	c	b	d
acl8	3	Fixed	bc	bf	ab	dg	**crc**	5	0%	g	bc	b	d
acl18	3	Fixed	ac	cf	ab	dg	**brc2**	10	10%	ce	d	b	d
pne1	3	Fixed	bc	c	b	dg	**brc3**	10	5%	ce	d	b	d
pne2	3	Fixed	bc	c	b	dg	**lph4**	5	15%	ce	d	b	d
							lph2	9	11%	ce	df	b	d
acl10	3	Fixed	bc	c(f?) [3]	ab	dg	**grl3**	11	5%	e	d	b	df
acl21	3	Fixed	bc	c	ab	dg	**brc1**	11	0%	e	d	b	d
aem5	10	3 GT IDH-ab + 3 GT AAT1-bc / 7 GT AAT1-cd	bcd	ce	(a)b	dg	**lph3**	10	0%	e	d	b	d
							gon	10	0%	e	d	b	d
aem4	19	Fixed	bc	ce	b	dg	**blv1**	11	5%	e	d	b	de
aem3	10	Fixed	bc	ef	b	dg	**grl1**	12	10%	e	d	b	de
alb4	8	1 GT AAT2-acf + 7 GT PGI-dg	b	a(c)f	b	d(g)	**grl4**	10	33%	e	d	ab	de
							blv2	10	18%	e	d (a?)	ab	d
alb9	5	Fixed	b	af	b	dg	**ifd2**	7	25%	eh	d	b	acd
alb1	5	Fixed	b	acf	b	dg	**vid1**	5	10%	f	d	b	bd
alb3	6	Fixed	b	acf	b	dg	**grl2**	10	23%	[3] -	dei	b	bd
alb7	3	Fixed	b	acf	b	dg	**azn**	5	Fixed	ce	[3] -	bc	d
alb5	5	3 GT PGI-dg	b	acf	b	d(g)	**ktz2**	5	25%	[3] -	abf	bc	de
alb6	5	Fixed	b	acf	b	d	[4] **pnt2**	1	-	c	c	c	eg
alb8	3	Fixed	b	bcf	b	dg							
dms2	6	1 GT AAT1-bce	b(c)e	cg	bc	dg							

[1] For the accession **code** see Table 1

[2] Number of genotypes studied

[3] Scoring of alleles indecisive

[4] Genotypes **tbr1** and **pnt2** are used as allelic marker during each electrophoresis run

S. pinnatisectum (*AAT2-c*, *PGI1-g*). The other half of the genome composition of most *S. acaule* OTUs, containing alleles *AAT1-c*, *AA2-f*, *IDH1-b* and *PGI1-d*, was present in several *Tuberosa* and *Megistacroloba* OTUs (group ** in Fig. 7). The conclusion that may be drawn from these data is that series *Acaulia* is not a simple hybrid between two diploid species of series *Tuberosa* and *Megistacroloba*, but one of series *Acaulia* ancestors belonged quite certainly to these series.

The second ancestral taxon that gave the distinctive characters, is possibly extinct. Besides being derived from an unknown ancestor, the unique characters could also be caused by mutations after the hybridization. In both cases one might hypothesize that series *Acaulia* is not of recent hybrid origin. Gottlieb (1977) stated that the more ancient the polyploid, the less likely it will retain the alleles it inherited in an unmutated state. On the other hand, an F1-offspring may already reveal novel molecular characteristics (Smith *et al.* 1970). A more definite conclusion on the time of origin and the direct ancestral taxa of series *Acaulia* can not be given yet.

Biosystematic relationships within series *Acaulia*. The hexaploid representative of series Acaulia, *S. albicans*, is a distinct taxon. The OTUs formed a monophyletic group both with AFLPs and in the isozyme experiment. The position of *S. albicans* in relation to *S. acaule* differed between the phenetic and the cladistic analyses, which respectively placed it basally or as advanced in series *Acaulia*. The proposition that this hexaploid taxon is necessarily derived from *S. acaule* because of the lower ploidy level of *S. acaule*, is not unequivocally supported by our data. One would expect that a hybrid polyploid taxon would reveal the genomes of both parents, but *S. albicans* showed (Table 2) only one allele in isozyme *AAT1* (*AAT1-b*) and *IDH1* (*IDH1-b*), whereas *S. acaule* generally had two (*AAT1-bc* and *IDH1-ab*). This isozyme composition suggests that *S. albicans* is the most primitive taxon in series *Acaulia*, which is a novel idea for *Solanum* taxonomy.

S. acaule was represented by in total 36 OTUs in our experiment belonging to the three subspecies *acaule*, *punae* and *aemulans*. These subspecies are morphologically rather well distinguishable (Kardolus 1998). Nevertheless, the molecular divergence of the three groups is not substantial. OTUs of ssp. *acaule* and *punae* did not form monophyletic subspecific groups based on AFLPs (Figs. 2, 3) or isozymes (Fig. 7). However, the most northern representatives of ssp. *punae* (**pne 1, 2**) clustered consistently together as a subspecific unit (Figs. 2, 3 and 7). Due to large overall AFLP similarity in *S. acaule*, a study of more AFLP primer combinations and more OTUs would reveal the relations better.

The *S. acaule* ssp. *aemulans* OTUs (**aem3-5**) collected in the province La Rioja in Argentina, which is the most southern distribution of *S. acaule*, were separated from the other *S. acaule* OTUs in the AFLP analysis (Figs. 2, 3). This is concordant with an extensive RFLP study (Hosaka and Spooner 1992) in which subspecies *aemulans* has been demonstrated to be the most deviating unit of *S. acaule*. Next to the separate position in the AFLP analysis, these OTUs also have a special allozyme, *AAT2-e*. *AAT2-e* was furthermore present in OTUs in **grl2**, **mga1, 2** and **rap3**. The presence of this particular allozyme in ssp. *aemulans* could indicate a hybridization event of *S. acaule* ssp. *acaule* with some species of series *Megistacroloba* or *Tuberosa*. Moreover, *S. acaule* material with a 'normal' ssp. *acaule*-constitution (OTU **acl27**, Figs. 2, 3 and 7) has been collected at the locality of OTUs **aem3-5**. *S. megistacrolobum* and *S. gourlayi* do not occur in province La Rioja. However, *S. spegazzinii*, the sister-taxon of *S. gourlayi* (see Fig. 3), is found in La

Rioja. Hawkes and Hjerting (1969) have described probable hybrids between *S. acaule* and *S. spegazzinii*, which supports the possibility of *S. spegazzinii* playing a role in the ancestry of ssp. *aemulans* from La Rioja.

Okada and Clausen (1982) have concluded that ssp. *aemulans* material from the province Jujuy is a fertile hybrid derivative from *S. megistacrolobum* with ssp. *acaule*. Therefore, Hawkes (1990) only retained material from province La Rioja as *S. acaule* ssp. *aemulans*. However, accessions from Jujuy are morphologically similar to accessions of ssp. *aemulans* from La Rioja (Kardolus 1998). According to Hosaka and Spooner (1992), ssp. *aemulans* can be divided into two populations based on RFLP data, one cluster of material from La Rioja and one from Jujuy. They have also shown a third group of distinct ssp. *acaule* accessions from Argentina, mostly from the provinces Salta and Tucumán (in their Fig. 3b; data on these accessions were given by K. Hosaka, pers. comm.), but did not discuss their separate position. Kardolus (1998) has described accessions of province Tucumán and Salta as ssp. *aemulans*, but also indicated that their morphology did not fall exactly in the variation of ssp. *aemulans*. In the present study two of these accessions were incorporated as **aem1** and **aem2**. Both fell within the isozymic and AFLP variation of ssp. *acaule* OTUs. Their previous classification as ssp. *aemulans* (Kardolus 1998) was proven incorrect. *S. acaule* ssp. *aemulans* material from Jujuy was not included in the present study, so no final conclusions on the taxonomic and phylogenetic status of ssp. *aemulans* material can be drawn. Still it is clear that hybridization events in the most southern part of the distribution of *S. acaule*, i.e. in Argentina, may have played an important role in the rich variational pattern of *S. acaule* in this region.

The hexaploid OTU **alb10** is phylogenetically most closely related to tetraploid *S. acaule* OTUs, rather than to *S. albicans* (Figs. 2, 3). OTU **alb10** resembled *S. albicans* morphologically more than *S. acaule* (Kardolus 1998) and is in the latest gene-bank inventory identified as *S. albicans* (Bamberg *et al.* 1996). Due to its molecular concordance with *S. acaule* (Table 2, Figs. 2, 3), this accession might constitute a hexaploid cytotype of *S. acaule*. Its taxonomic status is further discussed in chapter 6 of this thesis.

Conclusions. Series *Acaulia* is a unique group within *Solanum* section *Petota* because it features both autapomorphic molecular markers (isozymes and AFLPs) and morphological characters, such as the modified pedicel articulation. The variation within the series has been partitioned in two species, the hexaploid species *S. albicans* and the tetraploid species *S. acaule*. *S. acaule* ssp. *aemulans* material from La Rioja was the most distinct entity within *S. acaule* in the present study. Hexaploid series *Acaulia* material of Ecuador fell in the variation of *S. acaule*, so the taxonomy of *S. acaule* will be reconsidered in Chapter 6 of this thesis.

Series *Acaulia* had strong relationships with both series *Tuberosa* and *Megistacroloba*, and with series *Demissa*. Its hypothesized amphiploid origin is not in conflict with the present results, but could only be partly unveiled. Through the combined use of the phenetic neighbor-joining method and a cladistic parsimony analysis more phylogenetic information was revealed than both methods did individually, especially on mutual relationships of the polyploid series *Acaulia*, *Demissa* and *Longipedicellata*. The current series classification of *Solanum* sect. *Petota* is in need of adjustment and an AFLP analysis of a more complete sample could elucidate the phylogeny of all tuber-bearing *Solanum* species.

CHAPTER 6

A new series *Acaulia* taxon from Ecuador (*Solanum* sect. *Petota*)

***Solanum acaule* Bitter subspecies *palmirense* Kardolus subsp. nov.**
Type: *Spooner, Castillo and López 5070* (PTIS holo; NA, CIP iso).

Solanum albicans auct. non Ochoa: Kardolus (1994, 1998), Kardolus and Groendijk-Wilders (1998), Kardolus *et al.* (1998)

LATIN DIAGNOSIS
Herbaceum, tuberiferum. Differt a *S. acaule* subspp. *acaule*, *punae* et *aemulans* habitu robustiore, et foliis, corollis, antherisque maioribus. Pedicelli articulati vix manifeste. *Solanum albicans* similis est, cum pilis albis, longis, sed indumento foliarum sparsiore, foliis foliolis pluribus, calycibus longioribus et baccis maioribus. Pedicellus violaceus, non viridis.

ENGLISH DIAGNOSIS
Herb, tuber-bearing. *Solanum acaule* ssp. *palmirense* is a hexaploid cytotype (2n=72) of *S. acaule* (series *Acaulia* Juz.). Subspecies *palmirense* has a high DNA-homology with the tetraploid (2n=48) subspecies of *S. acaule*, i.e. ssp. *acaule*, ssp. *punae* (Juz.) Hawkes & Hjerting and ssp. *aemulans* (Bitter & Wittm.) Hawkes & Hjerting. It differs from these in a more robust habit and larger leaves, corollas and anthers. Subspecies *palmirense* has an incompletely differentiated floral abscission zone in the pedicel. In this character and in overall morphology *S. acaule* ssp. *palmirense* is more similar to *S. albicans* (Ochoa) Ochoa, likewise a hexaploid taxon. The leaves of ssp. *palmirense* are less pubescent than those of *S. albicans*, but the hairs are also white, long and spreading. Furthermore, the leaves of ssp. *palmirense* are more dissected, the calyx including the acumens of the lobes is longer and the mature berries are larger. The colour of the pedicel is purple instead of green as in *S. albicans*.

EXAMINED MATERIAL

Herbarium material: Ecuador, Province Chimborazo. Latitude 02°09' South, Longitude 78°43' West, altitude 3750 m. May 11, 1991. Locality: Palmira. On Loma Moyocancha, at Estación Experimental Páramo Moyocancha, 11.4 km East of Tixán-Palmira road, 3.3 km South of Cocán; collected in the wild. Growing among *Stipa ichu* Kunth. Plants in all stages of maturity from flowers to mature fruit. *D.M. Spooner, R. Castillo and L.E. López 5070* (PTIS holotype; NA, CIP isotypes).
Possibly *S. acaule* ssp. *palmirense*: Ecuador, Province Chimborazo. Faldas del Cerro Colorado, altitude 3600 m. June 4, 1979. 2n=72. *C. Ochoa 13395* (CIP). Ochoa (1993) has given more precise locality data of presumably this collection. He has collected in Cerro Quilua on the route from Cerro Colorado to Carihuayrazo, Prov. Chimborazo, but did not cite the collection number of this material.

Living material examined: PI 561642 (germplasm collection from *SCLp 5070*, see Bamberg *et al.* 1996). This material has been studied in a greenhouse at Wageningen, the Netherlands (Fig. 1). Voucher material is deposited at WAG: *R.G. van den Berg 777* - herbarium specimen, alcohol material and spread preparations + photos of chromosomes; *Kardolus 001* - alcohol material; *Kardolus s.n.* - alcohol material, several photos and colour slides. At Sturgeon Bay, USA, the same material has been studied in a field plot. Voucher specimen: PI 561642 (deposited at PTIS).

PLANT DESCRIPTION (see Fig. 1)

Habit: herb, rosette [1]{[to erect, up to 45 cm tall]}.

Pubescence: plant sparsely pubescent with conspicuous spreading white hairs, up to 3 mm long, mostly on stem, rachis and veins on the lowerside of the leaflets. The younger parts of the plants are most prominently pubescent. Peduncle and pedicels glabrous, calyx pubescent.

Tubers: small, global to ovate [up to 18 × 27 mm; skin colour yellowish white (RHS 158A, of Royal Horticultural Society (1986) colour chart); flesh colour creamy white (RHS 158B)].

Stem: ± 3 [-8] mm in diameter; [wings 0-1.2 mm broad; stem colour green with brownish-red anthocyanin colouring, markedly less anthocyanin at nodes and in the young parts of the plant].

Leaf: imparipinnate; 4-6 [-8] pairs of lateral leaflets and 4-10 [-22] interjected leaflets; length 50-160 [-320] mm, width 25-55 [-85] mm, widest point mostly at the second laterals, or at the first pair of laterals, the third and subsequently laterals decreasing in size; lateral leaflet margins undulate at the base to rather straight at the apex; pseudostipules very inconspicuous or absent; [medium green and shining above, lighter green below].

Terminal leaflet: 25-50 [-75] mm long (including the petiolule), 15-32 [-42] mm wide; ovate to ob-ovate; apex obtuse usually with a ± 1 mm short acumen; base cuneate; petiolule 3-10 [-17] mm.

Largest lateral leaflet: 18-27 [-55] mm long, 12-16 [-30] mm wide; ovate-elliptic; apex making a ± 90° angle; base (slightly cordate to) truncate (to ± cuneate); shortly (1-2 mm) petiolulate to slightly decurrent on the rachis.

Interjected leaflets: mostly decurrent on the rachis.

Inflorescence: inflorescence bearing 4-7 [-15] flowers; peduncle 0-15 [-62] mm long; pedicel 12-25 [-58] mm long; [small leaves can be present in the inflorescence, placed mainly at the furcation of pedicels].

Pedicel articulation: not visible in herbarium specimens. In living material the articulation is observable at anthesis in part of the pedicels as a light green band on the purple pedicel, 1-2.5 mm below the calyx. In pedicels with young fruits the articulation is not visible anymore because the green band is coloured by anthocyanin like the rest of the pedicel. When the fruits ripen, the pedicel may grow out at the articulation (see fig. 1, mature fruits). This extension is variable, even within one plant.

Calyx: 6-9 mm long; lobes 4-5 mm long, triangular with tips elongated into acumens (up to 3 mm long).

Corolla: rotate with 1.3-1.9 mm long acumens; diameter ±18 [-27] mm; {medium purple outside (RHS 94C), somewhat lighter purple inside (RHS 92B), with a light green star in the center}.

Stamen: anther 3.5-5 mm long [orange]; filament jointed at the base (Fig. 1), free part 1-1.5 mm long.

Pistil: ± 6 mm long (without ovary); style cellular-papillose below the middle; exsertion out of anther column ± 0.5-1.5 mm; stigma ovoid to globose and darker green compared to the light green style and ovary.

Fruit: ovoid to globose, ± 9 {-23} mm long, ± 9 {-23} mm wide; [medium green].

Chromosome number: 2n=72

[1] Between square brackets: observations on accession PI 561642 grown in 1997 in a greenhouse in Wageningen, The Netherlands. Between accolades: observations in a field plot in Sturgeon Bay, WI, USA (see Kardolus 1998).

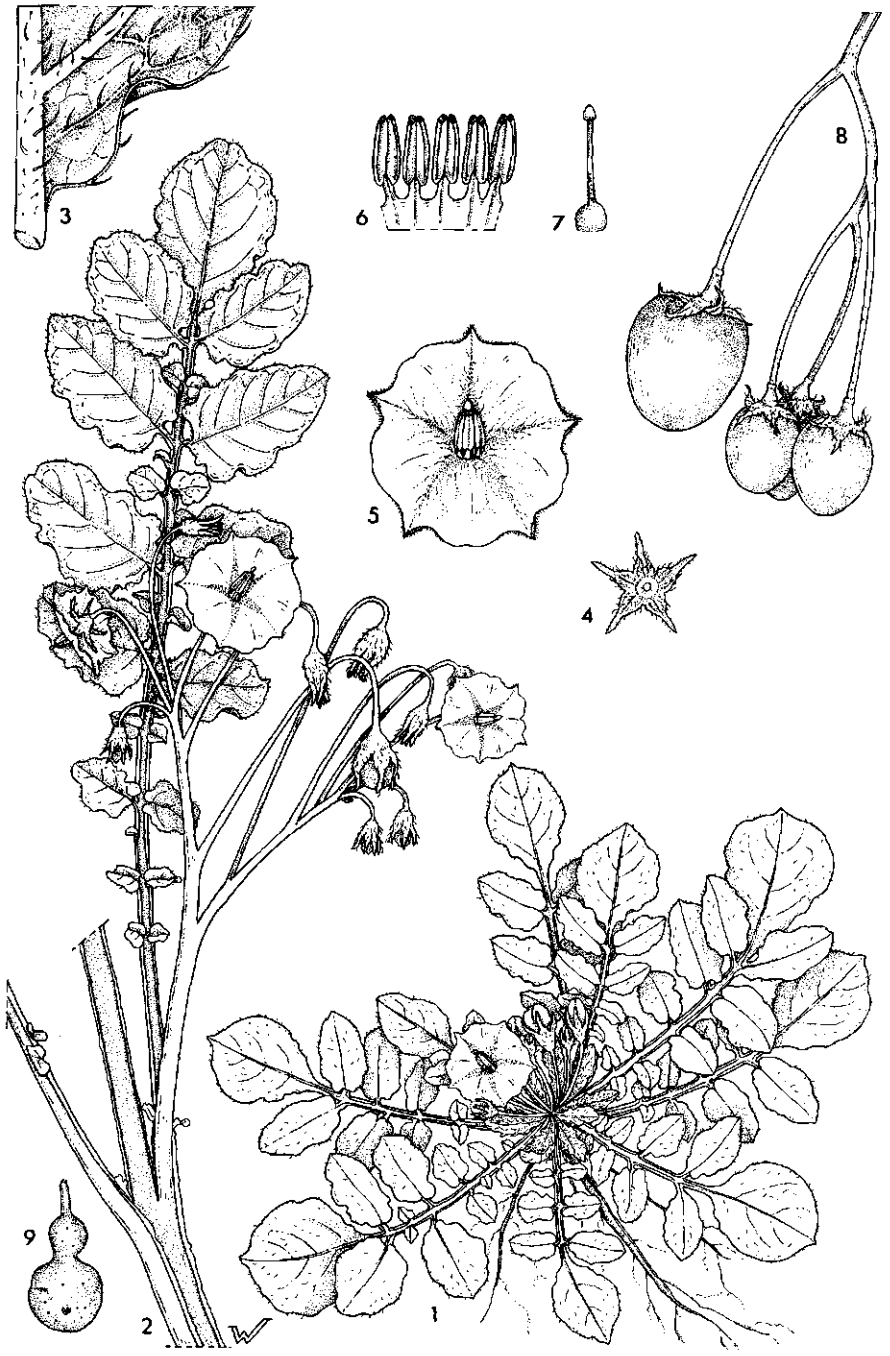


Fig. 1. *Solanum acaule* ssp. *palmirense* Kardolus. - 1: habitus (× $^2/_3$); 2: part of flowering branch (× $^2/_3$); 3: detail of leaf, lower side (× 3); 4: calyx, spread out (× 1); 5: flower (× 1); 6: stamens, ventral view (× 2); 7: pistil (× 2); 8: mature fruits (× $^2/_3$); 9: tuber (× $^2/_3$). 1: *Spooner, Castillo and López 5070* (NA-isotype), collected in Ecuador; 2-9: *Kardolus 001* (WAG), plant grown in greenhouse.

NOTES

In 1991 the potato taxonomist David M. Spooner collected together with Raúl Castillo and Luis López under number *SCLp 5070* a population of plants which they identified as *S. acaule* Bitter (Spooner *et al.* 1992). They found these plants at the Moyocancha farm (altitude 3750 m) of the Universidad Politecnica del Chimborazo in Palmira, province Chimborazo, Ecuador. Spooner *et al.* (1992) remarked about this collection that it combined the characters of the flat rosettes of *S. acaule* ssp. *punae* and the short hairs of ssp. *acaule*. In the 1993 genebank inventory (Bamberg and Martin 1993) germplasm from this collection was identified as *S. acaule* ssp. *acaule*.

Examining the morphology of series *Acaulia* at the genebank in Sturgeon Bay in 1993, Kardolus (1998) included *SCLp 5070* in the set of studied material because of its deviating locality. Until then no collections of *S. acaule* or the related species *S. albicans* from series *Acaulia* were known from Ecuador (Hawkes 1990), although Ochoa (1990) reported that *S. albicans* is distributed from northern Peru to Ecuador. Studying *SCLp 5070* in the field-plot at Sturgeon Bay, the habit was much more elongated and robust than of both *S. acaule* ssp. *acaule* and ssp. *punae*. The leaves of the plants were sparsely pubescent, although on the stem, rachis and veins conspicuous white, rather long spreading hairs were visible. On older plant parts these tend to disappear which probably was the reason for describing the 'short hairs' in the original collection (Spooner *et al.* 1992).

Kardolus (1998) described *SCLp 5070* as morphologically most similar to *S. albicans*, and to *S. acaule*. On a total of 34 mostly quantitative characters, 14 to 18 characters were significantly different between *SCLp 5070* and the three tetraploid subspecies of *S. acaule*, i.e. ssp. *punae*, ssp. *acaule* and ssp. *aemulans*. Between *SCLp 5070* and *S. albicans* only nine characters were significantly different. Furthermore, the pedicel-articulation of *SCLp 5070* is rather similar to material of *S. albicans* (Kardolus and Bezem submitted). Chromosome counts (Kardolus 1998) revealed that this collection was hexaploid (2n=72), as is *S. albicans*. The accession was provisionally classified as *S. albicans* (Bamberg *et al.* 1996), but Kardolus (1994, 1998) already suggested to give it a separate taxonomic status.

In molecular studies (Kardolus *et al.* 1998, Chapter 5 this thesis), using both isozymes and the AFLP fingerprinting technique (Vos *et al.* 1995), accession *SCLp 5070* clearly belonged to series *Acaulia*. However, it differed substantially from *S. albicans* and was closer related to *S. acaule*. *SCLp 5070* had, compared to all eight studied accessions of *S. albicans*, isozyme AAT1-*allele c* and lacked AAT2-*allele a*, as did all examined accessions of *S. acaule* (Chapter 5 this thesis). Studying AFLPs, *SCLp 5070* was phylogenetically placed in the *S. acaule* clade (Kardolus *et al.* 1998, Chapter 5 this thesis). The hexaploidy of accession *SCLp 5070* may have caused the morphological convergence with the typical *S. albicans* accessions. However, its molecular constitution places it right in *Solanum acaule* (series *Acaulia* Juzepczuk) and it seems most appropriate to give it a subspecific status in this species. The new subspecies will be named *palmirense*, derived from the type-locality Palmira. The standard abbreviation (see Hawkes 1990) of this new subspecies will be: **pal**.

The occurrence of a hexaploid cytotype of *S. acaule* may indicate the need to re-evaluate the recognition of *S. albicans* at the species level, having originally been a variety of *S. acaule* (Ochoa 1960) and later a subspecies of *S. acaule* (Hawkes 1963).

The type *SCLp 5070* was found at a high-altitude farm of the Universidad Politecnica del Chimborazo. The workers at the farm stated that the plants appeared without human interference. The research of this experimental station has mainly been on animals: Alpaca and Vicuña. Research on plants was restricted to some production plots of broad bean, potatoes and other Andean tubers. No breeding activities take or have taken place at the Moyocancha station (pers. comm. Raúl Castillo), so there are no grounds for a theory that these plants are recent hybrid derivatives. Furthermore, the plants are fertile and produce large berries with many viable seeds. They are fully self-compatible and the off-spring after at least two generations of generative reproduction (one in the Sturgeon Bay genebank, and one in Wageningen) is homogeneous.

Ochoa has collected two hexaploid collections in Ecuador which he identified as *S. albicans* (Ochoa 1993). One collection, *Ochoa 16173*, was collected in the Province Tunguraha, Ecuador (herbarium specimen of original collection not seen). Only a herbarium specimen of *Ochoa 16173* was available of a plant that had been grown in a field plot in Huancayo, Peru. This specimen is clearly *S. albicans* because of its small, white corollas and leaf composition (few interjected leaflets and margin of lateral leaflets straight). The other collection, *Ochoa 13395*, is from the same province as the type collection of *S. acaule* ssp. *palmirense*, but only consists of two very small, non-flowering rosettes. This makes the identification difficult. However, the leaf margins are somewhat undulate as in the type specimens of ssp. *palmirense*. This accession could possibly be ssp. *palmirense*.

SUMMARY AND CONCLUDING REMARKS

SERIES *ACAULIA*, A MORPHOLOGICALLY DISTINCT GROUP

Multivariate phenetic analyses revealed that series *Acaulia* (*Solanum* sect. *Petota*), comprising the polyploid species *S. acaule* and *S. albicans*, is a well-defined group (Chapter 1). These two species are well distinguished from other tuber-bearing potato species. Nevertheless, quite a striking similarity could be demonstrated between *S. albicans* and *S. demissum* (series *Demissa*) from Mexico. The tetraploid species *S. acaule* and the hexaploid *S. albicans* can be well separated within series *Acaulia*.

A hexaploid accession from series *Acaulia* has been collected at La Palmira in Province Chimborazo, Ecuador. Spooner, Castillo and López determined this material as *S. acaule* (Spooner *et al.* 1992). It differs from all three *S. acaule* subspecies in ploidy level and morphology, and is morphologically most similar to *S. albicans* (Chapter 1). However, in AFLP and isozyme composition this collection fell within the variation range of *S. acaule*, and was quite unlike typical *S. albicans* (Chapter 5). Therefore, it has been described as a new subspecies of *S. acaule*, *S. acaule* ssp. *palmirense* Kardolus subspec. nov. (Chapter 6). Other taxa within *S. acaule*, viz. subspecies *punae*, *acaule* and *aemulans*, are morphologically rather distinct entities. However, some accessions of sspp. *acaule* and *aemulans* are not identifiable to one of the two subspecies because they show overlap in characteristics (Chapter 1). This may be explained by hybridization events in the most southern part of the distribution of *S. acaule*, which could have played a role in the rich variational pattern of *S. acaule* in Argentina (see Chapter 5).

INFLORESCENCE FEATURES OF *SOLANUM* SECT. *PETOTA* SPECIES

The species of *Solanum* sect. *Petota* generally display a dichasial inflorescence, and less frequently a monochasium. Variation in the composition of inflorescences, even within one genotype, makes it difficult to use this inflorescence architecture taxonomically. In this study (Chapter 2) a special and so far unrecorded inflorescence type is described in the polyploid series *Acaulia* and compared to those of other tuber-bearing *Solanum* species. This inflorescence can be characterized as a monochasium with a strongly reduced peduncle and one or two 'extra' flowers in the axil of the subtending leaf. In *Solanum* series *Tuberosa* this type of inflorescence has never been described. Only in *S. megistacrolobum* this type of inflorescence can be found occasionally. In this species and in *S. demissum* monochasia are observed that have separately placed flowers on the peduncle, but these single 'extra' flowers form part of the inflorescence.

The inflorescence architecture of wild tuber-bearing *Solanum* species is correlated with their habitat and breeding behavior. Inbreeding species such as *S. acaule* and *S. albicans*, with a compact habit and relatively short peduncles and small corollas, do survive at high altitudes in the Andes.

The pedicels of the species of the genus *Solanum* usually possess a floral abscission zone. The place where this zone is located, is designated 'articulation' or 'joint'. Series *Acaulia* is characterized by an unclear or completely absent articulation (Chapter 3). In *Solanum acaule* the abscission zone proves to be absent in pedicels without an articulation. The absence of the floral abscission zone is a recessive trait. *Solanum acaule* ssp. *aemulans* has articulated pedicels and a floral abscission zone. The hexaploid *Solanum albicans* of series *Acaulia* has an anatomically modified abscission zone. These special features of pedicel articulation in series *Acaulia* are discussed in relation to comparable 'jointless'-mutations in tomato (*Solanum* section *Lycopersicum*).

The position of the articulation on the pedicel has often been used for the classification of potatoes, but without a precise definition. In this study the relative trait 'articulation-position' is combined with an absolute measure of the pedicel length. It is demonstrated that the position is often quite variable. Especially when the articulation is about half way the pedicel, which is the case in many potato species, the position is less useful for the characterization of potato species than has been suggested in prior taxonomic work on section *Petota*.

THE POTENTIAL OF THE AFLP TECHNIQUE FOR TAXONOMY

Using the AFLP™ technique highly informative DNA fingerprints were generated (Chapters 4 and 5). Both phenetic and cladistic analyses were conducted from the individual genotypic level to the species level in *Solanum* subgenus *Potatoe*. An AFLP fingerprint, using a combination of suitable AFLP primers, generated 12 to 71 scorable fragments per genotype which was sufficient for taxonomic interpretation. The classifications based on the molecular marker AFLP were generally in agreement with current taxonomic opinions, but new relations were revealed. Potatoes (*Solanum* section *Petota*) and tomatoes (*Solanum* sect. *Lycopersicum*) still have homologous bands in common. The comparison of even less related groups may prove to be impossible because of a too low degree of band-homology.

The AFLP technique provided a more detailed insight in the phylogeny of section *Petota* than the study of chloroplast DNA restriction site variation has given (Spooner and Castillo 1997), and its results can be compared with that of previous RFLP studies (Debener *et al.* 1990). AFLP is an efficient and reliable technique to generate biosystematic data and therefore a promising tool for evolutionary studies of related groups.

THE CLASSIFICATION OF TUBER-BEARING *SOLANUM* SPECIES INTO SERIES

Solanum section *Petota* is now (Hawkes 1990) subdivided in 19 series. The examination of taxa of 14 of these series by AFLPs (Chapter 5) revealed, as has been expressed in various recent studies (Spooner and Van den Berg 1992a, Spooner *et al.* 1995, Spooner and Castillo 1997), that this classification into series only partly depicts the relationships within this group well. Various species seem to have been classified into the wrong series, and the status of certain series themselves is doubted by the results presented in this thesis.

S. boliviense is classified (Hawkes 1990) in series *Megistacroloba* and is morphologically *Megistacroloba*-like with its generally simple leaves. However, based on its molecular constitution it bears stronger affinities with taxa of series *Tuberosa*, a series in which it has been classified formerly (Hawkes 1958, Correll 1962). Another example is series *Cuneoalata*, of which *S. infundibuliforme* was examined. This series, which accommodates in total three species, has been characterized by its typical pinnatifid leaves with lanceolate to linear-lanceolate lateral leaflets, the rachis with narrow wedge-shaped decurrent wings between each pair of leaflets (Hawkes 1990). The separate maintenance of series *Cuneoalata* may be questioned because of the close relatedness of *S. infundibuliforme* with *S. gourlayi* ssp. *vidaurrei* and other taxa of series *Tuberosa*. Furthermore, species of series *Yungasensia* and *Maglia* also fell within the variation range of *Tuberosa*, as based on the molecular data presented in Chapter 5. A new classification could be inferred in a future AFLP-study that focuses on all series and species of which living (genebank) material is available. This classification will reflect the natural relations of the species better and will be more practical for plantbreeders and other users of tuber-bearing *Solanum* species.

A NEW GENOME FORMULA FOR *SOLANUM DEMISSUM*

The neighbor-joining analysis of AFLP data from a large set of species (Chapter 5), revealed that *S. demissum* is likely to be closely related to both series *Acaulia* and series *Longipedicellata* and feasibly a hybrid descendant of these series. The genome formulae that have been given to *S. demissum* do not reflect this theory well. Hawkes (1958) has put forward the following formulae: $A_2A_2A_3A_3$ for *S. acaule*, $A_1A_1A_4A_4B_1B_1$ for *S. demissum*, and A_4A_4BB for series *Longipedicellata*. These formulae imply only a clear-cut connection between *S. demissum* and series *Longipedicellata*. Matsubayashi (1991) has given *S. demissum* a formula of $AADDD^dD^d$, *S. acaule* AAA^aA^a and the tetraploid *Longipedicellata* species AABB, which reflect little of the possible relationships of *S. demissum* with series *Acaulia* and series *Longipedicellata*. Matsubayashi has based the formula of *S. demissum* on meiotic pairing patterns in pentaploid hybrids of *S. demissum* × *S. stoloniferum* (series *Longipedicellata*). The most common pairing observed (see Matsubayashi 1991) was 1 trivalent + 20 or 21 bivalents + 15 or 17 univalents.

Matsubayashi's formulae for *S. acaule* and series *Longipedicellata* may be retained, and a new formula for *S. demissum*, $AAA^aA^aB^dB^d$, is proposed. This formula reflects the relationships of *S. demissum* with both series *Acaulia* and *Longipedicellata* better, and can as well explain the pairing pattern in the *S. demissum* × *S. stoloniferum* hybrid. The genome constitution of the pentaploid hybrid will now be AAA^aB^dB. The bivalents are formed between the two A genomes but also between some chromosomes of the B^d and B genomes and the univalents are mainly derived from A^a as well as from B^d and B.

ORIGIN OF *SOLANUM ACAULE*

Series *Acaulia* is the most advanced group in the cladistic analysis of AFLP data-sets of section *Petota* (Chapter 4 and 5). Morphologically, series *Acaulia* bears characteristics similar to various diploid taxa of series *Tuberosa* (leaf composition) and *Megistacroloba* (rosette-habit, inflorescence architecture, see Chapter 2). However, it is concluded in this study that series *Acaulia* is not a simple hybrid derivative of a crossing of these two series, because of various unique molecular (Chapter 4 and 5) and morphological features, such as the modification of the floral abscission zone or even the absence of this zone (Chapter 3). One of its ancestors is postulated to belong to the *Megistacroloba / Tuberosa* affiliation. The other ancestor that contributed to series *Acaulia* is possibly extinct, at least it was not included in the set of studied material.

SAMENVATTING

DOEL EN OPZET VAN HET ONDERZOEK

De aardappel behoort tot de belangrijkste voedselgewassen ter wereld, na tarwe, rijst en maïs. Het is één botanische soort, *Solanum tuberosum*, waaruit door plantenveredelaars vele rassen zijn geselekteerd. Bekende rassen zijn 'Bintje', 'Eigenheimer', 'Doré' en 'Santé'. Daarnaast zijn er speciale rassen voor de produktie van chips (zoals 'Lady Rosetta'), zetmeel en frites. Oorspronkelijk komt de aardappel uit de Nieuwe Wereld, waar ook de wilde verwanten van de aardappel voorkomen. Uit Zuid- en Midden-Amerika zijn ongeveer 200 wilde knoldragende soorten beschreven die vaak onkruidachtig langs de randen van wegen en akkers voorkomen; enkelen treffen we aan in onverstoorde milieus, bijvoorbeeld als epifyt in nevelbossen. De wilde aardappelsoorten hebben meestal kleine knolletjes die in het algemeen niet voor consumptie geschikt zijn. Vanwege resistenties tegen velerlei ziekten en plagen worden ze gebruikt in veredelingsprogramma's.

Solanum acaule, het onderwerp van studie in dit proefschrift, is een van de meest gebruikte wilde soorten in de veredeling. Naast resistent tegen diverse ziekten is *S. acaule* ook vorsttolerant; ze groeit namelijk op grote hoogte in het Andes-gebergte, tot op 4500 meter. *S. acaule* is de meest wijdverbreide wilde soort en komt voor van het zuiden van Ecuador, via Peru en Bolivia, tot in Argentinië. De naam 'acaule' duidt aan dat de stengel (Lat. '*caulis*') ontbreekt; de habitus van de plant is namelijk rozetvormig.

Net als de cultuuraardappel *S. tuberosum* is *S. acaule* tetraploïd (2n=48). Beide polyploïde soorten hebben vier sets van 12 chromosomen, in tegenstelling tot 'normale' diploïde soorten die twee sets chromosomen hebben. Een groot verschil tussen de cultuuraardappel en *S. acaule* ligt in het feit dat *S. tuberosum* een autotetraploïde soort is en dat *S. acaule* een allotetraploïd is. Bij een autotetraploïd zijn de vier sets chromosomen grotendeels gelijk, wat wordt aangegeven met de genoom-formule AAAA.

Bij een allotetraploïd paren de chromosomen tijdens de meiose in bivalenten waardoor er van uitgegaan wordt dat er twee keer twee sets chromosomen zijn. Dit levert de genoom-formule AABB op. Allopolyploïde soorten zijn ontstaan door een hybridisatie van twee verschillende soorten, A en B. De hybridisatie gaat samen met een verhoging van het ploïdie-niveau, waarbij ongereduceerde gameten vaak een belangrijke rol spelen. Van alle aardappelsoorten is ongeveer een kwart polyploïd, terwijl voor vele andere plantengroepen een gelijk of zelfs hoger percentage polyploïden is geschat. Amphiploïdie, zoals het gecombineerde proces van hybridisatie/polyploïdisatie ook wel wordt genoemd, wordt gezien als een belangrijk soortsvormingsproces.

Het doel van het onderzoek was om uit te zoeken of het mogelijk was de voorouder-soorten van *S. acaule* aan te wijzen en de taxonomische relaties van wilde knoldragende *Solanum* soorten te verhelderen. Op grond van morfologische overeenkomsten werden vertegenwoordigers uit de taxonomische series *Megistacroloba* en *Tuberosa* genoemd als mogelijke voorouder soorten van *S. acaule*. Deze hypothese was echter nog nooit getoetst door verwantschapsonderzoek op basis van een objectieve analysemethode.

SERIE *ACAULIA*, EEN MORFOLOGISCH AFWIJKENDE GROEP

Solanum acaule vormt samen met de hexaploïde soort *Solanum albicans* de taxonomische serie *Acaulia*. Gebaseerd op een morfometrische analyse (hoofdstuk 1) blijkt deze serie een duidelijk gedefinieerde groep die afwijkt van andere knoldragende soorten. *S. albicans*, die in Peru voorkomt, vertoont wel een opvallende gelijkenis met de uit Mexico afkomstige *Solanum demissum*. Ook wordt in dit hoofdstuk een recent verzamelde hexaploïde collectie uit Ecuador beschreven die qua morfologie het meest lijkt op *S. albicans*, maar voor wat betreft AFLPs en isozymen in de variatie van *S. acaule* valt. Op basis hiervan is besloten dit materiaal als nieuwe ondersoort van *S. acaule* te beschrijven in hoofdstuk 6 van dit proefschrift.

Hoofdstuk 2 beschrijft een onderzoek naar de opbouw van bloeiwijzes. Serie *Acaulia* kenmerkt zich door het voorkomen van een speciaal type bloeiwijze dat nog niet eerder in de literatuur vermeld was, namelijk een monochasium met een verkorte peduncel (bloeiwijze-steel) en één of twee 'extra' bloemen in de oksel van het ondersteunende blad. Dit type bloeiwijze en andere kenmerken van de bloeiwijze, zoals de dimensies van de bloemen en bloemstelen, zijn bediscussieerd in relatie tot de taxonomie van de knoldragende *Solanum* soorten, hun leefomgeving en voortplantingswijze (zelf/kruisbevruchter).

Het onderscheid tussen veel aardappelsoorten berust op kwantitatieve, continue kenmerken. Dit soort kenmerken is vaak variabel, wat leidt tot problemen met de afbakening van groepen. Serie *Acaulia* is te determineren op een kwalitatief kenmerk. Het punt waar normaal de niet-bevruchte bloemen of rijpe bessen afbreken, de articulatie van de pedicel, is in deze groep afwezig of nauwelijks ontwikkeld. Hoofdstuk 3 beschrijft een anatomisch onderzoek van deze articulatie. In niet-gearticuleerde pedicels in *Solanum acaule* blijkt de abscissielaag geheel afwezig, terwijl in *S. albicans* deze laag gemodificeerd is. Deze speciale kenmerken worden in de discussie vergeleken met de zogeheten 'jointless'-mutaties in tomaat.

HET GEBRUIK VAN DE AFLP-TECHNIEK IN DE TAXONOMIE

Met de AFLP™-techniek zijn zeer informatieve 'DNA-fingerprints' verkregen die bruikbaar zijn voor taxonomische interpretatie (hoofdstukken 4 en 5). Analyses zijn uitgevoerd met zowel de fenetische methode (groepering op basis van similariteit) als de cladistische methode. Bij deze laatste methode is de groepering gebaseerd op de gecombineerde aanwezigheid van 'afgeleide' kenmerken, de zogeheten 'synapomorfiën'. Analyses zijn uitgevoerd van het individuele genotype niveau tot op het niveau van soorten binnen het geslacht *Solanum*. De huidige taxonomische indeling van aardappelsoorten is deels door de AFLP-techniek bevestigd, maar ook nieuwe verbanden zijn aangetoond. De AFLP-techniek is goed bruikbaar tot een niet al te hoog taxonomisch niveau. Aardappelsoorten (*Solanum* sectie *Petota*) en tomatensoorten (*Solanum* sectie *Lycopersicum*), twee tamelijk nauw verwante groepen, zijn nog juist vergelijkbaar omdat ze enige overeenkomstige AFLP-banden bezitten. Minder verwante groepen lijken niet met de AFLP-techniek te kunnen worden vergeleken vanwege onvoldoende bandhomologie.

DE CLASSIFICATIE VAN KNOLDRAGENDE *SOLANUM* SOORTEN

In dit proefschrift zijn 14 van de 19 taxonomische series met knoldragende soorten (*Solanum* sectie *Petota)* bestudeerd. De huidige indeling in series geeft de relaties binnen deze groep niet altijd goed weer. Deze stelling is in recente studies geponeerd en wordt ook in dit proefschrift onderbouwd. Diverse soorten zijn in een serie ingedeeld waarin ze op grond van genetische verwantschap niet thuis horen. Gebaseerd op de resultaten van hoofdstuk 5 mag tevens de status van bepaalde taxonomische series in twijfel worden getrokken. Een toekomstig AFLP-onderzoek, waarbij alle soorten worden bestudeerd waarvan genenbankmateriaal aanwezig is, zal kunnen leiden tot een nieuwe classificatie die de natuurlijke relaties tussen de soorten beter weerspiegelt en die praktischer is voor plantenveredelaars en andere gebruikers van knoldragende *Solanum* soorten.

Op basis van een 'neighbor-joining' analyse van de AFLP-gegevens (hoofdstuk 5) is de conclusie getrokken dat de hexaploïde soort *Solanum demissum* (uit Mexico) zeer waarschijnlijk nauw verwant is met zowel de Zuid-Amerikaanse serie *Acaulia* als de Centraal-Amerikaanse polyploïde serie *Longipedicellata*. Het is mogelijk een hybride-nakomeling van vertegenwoordigers van deze twee series.

HET ONTSTAAN VAN *SOLANUM ACAULE*

Serie *Acaulia* is de meest afgeleide groep in de cladistische analyses van de AFLP-data-sets. Morfologisch lijkt deze groep zowel op diploïde soorten van serie *Tuberosa* (opbouw van het blad) en *Megistacroloba* (rozetvormige habitus, opbouw van de bloeiwijze). Toch is in dit onderzoek geconcludeerd dat *Solanum acaule* geen directe hybride is uit een kruising tussen deze twee series vanwege de diverse unieke moleculaire (AFLPs / isozymen) en morfologische kenmerken, zoals de modificatie van de pedicel-articulatie. Een van de voorouders wordt verondersteld afkomstig te zijn uit de *Megistacroloba/Tuberosa* groep, die tamelijk nauw aan elkaar verwant zijn. De andere voorouder die heeft bijgedragen tot het ontstaan van serie *Acaulia*, is mogelijk uitgestorven.

REFERENCES

Averett J.E. (1980) Polyploidy in plant taxa: summary. In: Lewis W.H. (ed.) Polyploidy: biological relevance. Proceedings of the international conference on polyploidy. Washington University, St.Louis, Missouri, May 24-27, 1979. Plenum Press, New York: 269-273.

Bachmann K. (1992) Nuclear DNA markers in angiosperm taxonomy. Acta Bot. Neerl. 41: 369-384.

Bachmann K. (1995) Progress and pitfalls in systematics: cladistics, DNA and morphology. Acta Bot. Neerl. 44: 403-419.

Ballvora A., Hesselbach J., Niewöhner J., Leister D., Salamini F. and Gebhardt C. (1995) Marker enrichment and high-resolution map of the segment of potato chromosome VII harbouring the nematode resistance gene *Gro1*. Molec. Gen. Genet. 249: 82-90.

Bamberg J.B. and Martin M.W. (1993) Inventory of tuber-bearing *Solanum* species. Catalog of potato germplasm. Potato Introduction Station, NRSP-6, Sturgeon Bay (WI).

Bamberg J.B., Martin M.W. and Schartner J.J. (1994) Elite selections of tuber-bearing *Solanum* species germplasm. Based on evaluations for disease, pest and stress resistance. Potato Introduction Station, NRSP-6, Sturgeon Bay, WI, USA.

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

Becker J., Vos P., Kuiper M., Salamini F. and Heun M. (1995) Combined mapping of AFLP and RFLP markers in barley. Molec. Gen. Genet. 249: 65-73.

Bernatzky R. and Tanksley S.D. (1986) Genetics of actin regulated sequences in tomato. Theor. Appl. Genet. 72: 314-321.

Biaiñ de Elizalde M.M. (1980) Histology of the abscission zones of tomato flowers and fruit and some effects of 2-chloroethylphosphonic acid application. Phyton 38: 71-79.

Bitter G. (1912) Solana nova vel minus cognita V. Feddes Repert. 11: 349-394.

Breyne P., Boerjan W., Gerats T., Van Montagu M. and Van Gijsel A. (1997) Applications of AFLP in plant breeding, molecular biology and genetics. Belg. J. Bot. 129: 107-117.

Bryan F.A. and Soltis D.E. (1987) Electrophoretic evidence for allopolyploidy in the fern *Polypodium virginianum*. Syst. Bot. 12: 553-561.

Butler L. (1936) Inherited characters in the tomato. J. Heredity 27: 25-26.

Camadro E.L., Masuelli R.W. and Cortés M.C. (1992) Haploids of the wild tetraploid potato *Solanum acaule* ssp. *acaule*: generation, meiotic behavior, and electrophoretic pattern for the aspartate aminotransferase system. Genome 35: 431-453.

Cavalli-Sforza L.L. and Edwards A.W.F. (1967) Phylogenetic analysis: models and estimation procedures. Evol. 21: 550-570.

Chase M.W., Soltis D.E., Olmstead R.G., Morgan D., Les D.H., Mishler B.D., Duvall M.R., Price R.A., Hills H.G., Qiu Y.-L., Kron K.A., Rettig J.H., Conti E., Palmer J.D., Manhart J.R., Sytsma K.J., Michaels H.J., Kress W.J., Karol K.G., Clark W.D., Hedren M., Gaut B.S., Jansen R.K., Kim K.-J., Wimpee C.F., Smith J.F., Furnier G.R., Straus S.H., Xiang Q., Plunkett G.M., Soltis P.S., Swenson S.M., Williams S.E., Gadek P.A., Quinn C.J., Eguiarte L.E., Golenberg E., Learn G.H., Graham S.W., Barrat S.C.H., Dayanandan S. and Albert V.A. (1993) Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. Ann. Missouri Bot. Gard. 80: 528-580.

Chavez R. (1984) The use of wide crosses in potato breeding. Thesis for the faculty of science and engineering. University of Birmingham.

Child A. (1979) A review of branching patterns in the Solanaceae. In: Hawkes J.G., Lester R.N. and Skelding A.D. (eds.) The biology and taxonomy of the Solanaceae. Academic Press, London: 345-356.

Child A. (1990) A synopsis of *Solanum* subgenus *Potatoe* (G. Don) D'Arcy (*Tuberarium* (Dunal) Bitter (s.l.)). Feddes Repert. 101: 209-235.

Child A. and Lester R.N. (1991) Life form and branching within the Solanaceae. In: Hawkes J.G., Lester R.N., Nee M. and Estrada N. (eds.) Solanaceae III: Taxonomy, Chemistry, Evolution. Royal Botanical Gardens, Kew: 151-160.

Cockerham G. (1970) Genetical studies on resistance to potato viruses X and Y. Heredity 25: 309-348.

Correll D.S. (1962) The Potato and its wild relatives. Texas Research Foundation, Renner.

Cortés M.C. and Camadro E.L. (1989) Análysis isoenzymático de poblaciones naturales de *S. acaule* ssp. *acaule* Bitt. Indico de heterocygosis fija para de aspartato aminotransferase. In: XIV Reunión de la Asoc. Lat. de la Papa. Mar del Plata, Bs.As., Argentina, March 6-11: 69.

Cortés M.C., Echeverría M.M. and Camadro E.L. (1989) Variación isoenzymático de aspartato aminotransferase (AAT) en dos subespecies de *S. acaule* Bitt. In XIV Reunión de la Asoc. Lat. de la Papa. Mar del Plata, Bs.As., Argentina, March 6-11: 70.

Crawford D.J. (1990) Enzyme electrophoresis and plant systematics. In: Soltis D.E. and Soltis P.S. (eds.) Isozymes in plant biology. Chapman and Hall, London: 146-164.

Cutter E.G. (1978) Structure and development of the potato plant. In: Harris P.M. (ed.) The potato crop. Chapman and Hall, London: 70-152.

D'Arcy W.G. (1972) Solanaceae studies 2: typifications of subdivisions of *Solanum*. Ann. Missouri Bot. Gard. 59: 262-278.

D'Arcy W.G. (1991) The Solanaceae since 1976, with a review of its biogeography. In: Hawkes J.G., Lester R.N., Nee M. and Estrada N. (eds.) Solanaceae III: Taxonomy, Chemistry, Evolution. Royal Botanical Gardens, Kew: 75-137.

Danert S. (1957) Über den Sprossaufbau und die Blattentwicklung bei der Kartoffel. Der Züchter 27: 22-23.

Danert S. (1967) Die Verzweigung als infragenerisches Gruppenmerkmal in der Gattung *Solanum* L. *Kulturpflanze* 15: 275-292.

Debener T., Salamini F. and Gebhardt C. (1990) Phylogeny of wild and cultivated *Solanum* species based on nuclear restriction fragment length polymorphisms (RFLPs). Theor. Appl. Gen. 79: 360-368.

Den Nijs T.P.M. and Peloquin S.J. (1977) Polyploid evolution via 2N gametes. Amer. Potato J. 54: 377-386.

Dice L.R. (1945) Measures of the amount of ecologic association between species. Ecology 26: 297-302.

Dorsey M.J. (1919) A note on dropping of flowers in the potato. J. Heredity 10: 226-228.

Douches D.S and Quiros C.F. (1988) Additional isozyme loci in tuber-bearing Solanums: inheritance and linkage relationships. J. Heredity 79: 377-384.

Echeverría M.M., Cortés, M.C. and Camadro E.L. (1989) Analysis de la variación isoenzymático en *Solanum acaule*. In: Congrese Argentino de Genética 20° Aniversario, Bahía Ilanca, Argentina, September 17-20, 1989: 60.

Estrada R.N. (1980) Frost tolerance potato hybrids via *Solanum acaule* Bitter diploid-tetraploid crosses. Amer. Potato J. 57: 609-619.

Estrada R.N. (1984) Acaphu: a tetraploid, fertile breeding line, selected from a *S. acaule* × *S. phureja* cross. Amer. Potato J. 61: 1-7.

FAO (1995) FAO production yearbook 1995. Vol. 49. FAO, Rome.

Farris J.S. (1970) Methods for computing Wagner trees. Syst. Zool. 19: 83-92.

Farris J.S. (1988) HENNIG86 reference, version 1.5. University of Stoney Brook, New York.

Felsenstein J. (1985) Confidence limits on phylogenies: an approach using bootstrap. Evolution 39: 783-791.

Folkertsma R.T., Rouppe van der Voort J.N.A.M., De Groot K.E., Van Zandvoort P.M., Schots A., Gommers F.J., Helder J. and Bakker J. (1996) Gene pool similarities of potato cyst nematode populations assessed by AFLP analysis. Molec. Plant-Microbe Interact. 9: 47-54.

Giannattasio R.B. and Spooner D.M. (1994) A reexamination of species boundaries between *Solanum megistacrolobum* and *S. toralapanum* (*Solanum* sect. *Petota*, series *Megistacroloba*): morphological data. Syst. Bot. 19: 89-105.

Gottlieb L.D. (1977) Electrophoretic evidence and plant systematics. Ann. Missouri Bot. Gard. 64: 161-180.

Grant V. (1971) Plant speciation. Columbia University Press, New York.

Grun P. (1990) The evolution of cultivated potatoes. Econ. Bot. 44: 39-55.

Harlan J.R. and De Wet J.M.J. (1975) On Ö. Winge and A. Prayer: the origins of polyploidy. Bot. Review 41: 361-390.

Hawkes J.G (1958) Potatoes: taxonomy, cytology and crossability. In: Kappert, H. and Rudorf W. (eds.) Handbuch der Planzenzüchtung, 2nd ed., Chapter 1, Vol. 3. Paul Parey, Hamburg: 10-43.

Hawkes J.G. (1962) The origin of *Solanum juzepczukii* Buk. and *Solanum curtilobum* Juz. et Buk. Z. Pflanzenzücht. 47: 1-14.

Hawkes J.G. (1963) A revision of the tuber-bearing Solanums (2nd ed.). Scot. Pl. Breed. Sta. Rec.: 76-181.

Hawkes J.G. (1966) Modern taxonomic work on the *Solanum* species of Mexico and adjacent countries. Am. Potato J. 43: 81-103.

Hawkes J.G. (1972) Evolutionary relationships in wild tuber-bearing *Solanum* species. Symposia Biologica Hungarica 12: 65-69.

Hawkes J.G. (1990) The Potato. Evolution, biodiversity and genetic resources. Belhaven Press, London.

Hawkes J.G. and Hjerting J.P. (1969) The potatoes of Argentina, Brazil, Paraguay and Uruguay. Oxford University Press, Oxford.

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

Hayward H.E. (1938) The structure of economic plants. Macmillan Company, New York.

Hermsen J.G.Th. (1966) Crossability, fertility and cytogenetic studies in *Solanum acaule* × *Solanum bulbocastanum*. Euphytica 15: 149-155.

Hermsen J.G.Th. and Ramanna M.S. (1969) Meiosis in different F1-hybrids of *Solanum acaule* Bitt. × *S. bulbocastanum* Dun. and its bearing on genome relationships, fertility and breeding behavior. Euphytica 18: 27-35.

Hill M., Witsenboer H., Zabeau M., Vos P., Kesseli R. and Michelmore R. (1996) PCR-based fingerprinting using AFLPs as a tool for studying genetic relationships in *Lactuca* ssp. Theor. Appl. Genet. 93: 1202-1210.

Hosaka K. and Spooner D.M. (1992) RFLP analysis of the wild potato species, *Solanum acaule* Bitter (*Solanum* sect. *Petota*). Theor. Appl. Genet. 84: 851-858.

Hosaka K., Ogihara Y., Matsubayashi M. and Tsunewaki K. (1984) Phylogenetic relationship between the tuberous *Solanum* species as revealed by restriction endonuclease analysis of chloroplast DNA. Jap. J. Genet. 59: 349-369.

Huys G., Coopman R., Janssen P. and Kersters K. (1996) High-resolution genotypic analysis of the genus *Aeromonas* by AFLP fingerprinting. Int. J. Syst. Bacteriol. 46: 572-580.

Irikura Y. (1976) Cytogenetic studies on the haploid plants of tuber-bearing Solanum species. II. Cytogenetical investigations on haploid plants and interspecific hybrids by utilizing haploidy. Res. Bull. Hokaido Nat. Agric. Exp. Station 115: 1-80.

Ishimura C.I., Lapitan N.L.V., Van Buren A., Fenwick A. and Pedas K. (1994) Id. of parents suitable for molecular mapping of immunity and resistance genes in *Solanum* species. Am. Potato J. 71: 517-533.

Janssen G.J.W. (1997) Resistance to root-knot nematodes, *Meloidogyne* spp., in potato. Thesis Wageningen.

Janssen P., Coopman R., Huys G., Swings J., Bleeker M., Vos, P., Zabeau, M. and Kersters K. (1996) Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. Microbiology 142: 1881-1893.

Johnston S.A., Den Nijs T.P.M., Peloquin S.J. and Hanneman R.E. (1980) The significance of genic balance to endosperm development in interspecific crosses. Theor. Appl. Genet. 57: 5-9.

Kameraz A.Y., Vavilova M.A., Zhitlova N.A. and Ivanova V.N. (1978) Interspecific hybridisation of the Potato involving wild species of the Series Acaulia Juz. Trudy Po Prikl. Bot., Genet. i Sel. 62: 115-138

Kardolus J.P. (1994) Morphological variation within series *Acaulia*. Solanaceae Newsletter 4: 23-24.

Kardolus J.P. (1998) Morphological variation within series *Acaulia* (*Solanum* sect. *Petota*). In: Nee M., Simon D., Jessop J.P. and Hawkes J.G. (eds.) Solanaceae IV: progress in biology and utilisation. Royal Botanical Gardens, Kew (in press).

Kardolus J.P. and Bezem N. (submitted) The floral abscission zone in series *Acaulia* and related taxa of *Solanum* section *Petota*.

Kardolus J.P. and Groendijk-Wilders N. (1998) The inflorescence architecture of *Solanum acaule* and related taxa of *Solanum* section *Petota*. Acta Bot. Neerl. 47 (2) (In press).

Kardolus J.P. and Van den Berg R.G. (submitted) A biosystematic analysis of the polyploid series *Acaulia* and related potato species (Solanaceae).

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. Pl. Syst. Evol. (In press).

Kardolus J.P., Zevenbergen M. and Van den Berg R.G. (1997). On the origin of the triploid cultivated potato *Solanum juzepczukii*. In: Hollingsworth P., Bateman R. and Gornall R. (eds.) Advances in plant molecular systematics. An international meeting of the Systematics Association and the Linnean Society. August 13-15, 1997. Conference abstracts. University of Glasgow, Glasgow.

Karp A., Seberg O. and Buiatti M. (1996) Molecular techniques in the assessment of botanical diversity. Ann. Bot. 78: 143-149.

Kendall J.N. (1918) Abscission of flowers and fruits in the Solanaceae, with special reference to *Nicotiana*. Botany 5: 347-428.

Kramer C.Y. (1956) Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 12: 309-310.

Lindhout P., Pet G. and Van der Beek H. (1994) Screening wild *Lycopersicon* species for resistance to powdery mildew (*Oidium lycopersicum*). Euphytica 72: 43-49.

Lu J. and Pickersgill B. (1993) Isozyme variation and species relationships in peanut and its wild relatives (*Arachis* L. - Leguminosae). Theor. Appl. Genet. 85: 550-560.

Marks G.E. (1955) Cytogenetic studies in tuberous *Solanum* species. I. Genomic differentiation in the group *Demissa*. J. Genet. 53: 262-269.

Marks G.E. (1965) Cytogenetic studies in tuberous *Solanum* species. III. Species relationships in some South and Central American species. New Phytol. 64: 293-306.

Martínez-Zapater J.M. and Oliver J.L. (1984) Genetic analysis of isozyme loci in tetraploid potatoes (*Solanum tuberosum* L.). Genetics 108: 669-679.

Matsubayashi M. (1991) Phylogenetic relationships in the potato and its related species. In: Tsuchiya T. and Gupta P.K. (eds.): Chromosome engineering in plants: genetics, breeding, evolution. Part B: 93-118. Amsterdam: Elsevier.

McDade L. (1990) Hybrids and phylogenetic systematics. I. Patterns of character expression in hybrids and their implications for cladistic analysis. Evolution 44: 1685-1700.

Meksem K., Leister D., Peleman J., Zabeau M., Salamini F. and Gebhardt C. (1995) A high-resolution map of the vicinity of the *R1* locus on chromosome V of potato based on RFLP and AFLP markers. Molec. Gen. Genet. 249: 74-81.

Miller J.T., Van den Berg R.G., Ugarte M.L., Kardolus J.P., Villand J. and Spooner D.M. (1996) Morphological and molecular systematics of the wild potato *Solanum brevicaule* complex (abstract). Amer. J. Bot. 83: 179.

Mueller U.G., Lipari S.E. and Milgroom M.G. (1996) Amplified fragment length polymorphism (AFLP) fingerprinting of symbiotic fungi cultured by the fungus-growing ant *Cyphomyrmex minutus*. Molec. Ecol. 5: 119-122.

Nei M. and Li, W.-H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269-5273.

Norušis M.J. (1990a) SPSS/PC+. Statistics 4.0. SPSS Inc., Chicago.

Norušis M.J. (1990b) SPSS/PC+. Advanced Statistics 4.0. SPSS Inc., Chicago.

Ochoa C.M. (1983) A new taxon and name changes in *Solanum* (Sect. *Petota*). Phytologia 54: 391-392.

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

Ochoa C.M. (1993) Karyotaxonomic studies on wild Ecuadorian tuber-bearing *Solanum* sect. *Petota*. Phytologia 75: 422-431.

Okada K.A. (1973) Colección de papas silvestres, variedades nativas cultivadas e híbridos interespecíficos artificiales. Lista de semillas No. 2. Instituto Nacional de Tecnología Agropecuaria, Balcarce, Argentina. 47 pp.

Okada K.A. and Clausen A.M. (1982). Natural hybridization between *Solanum acaule* Bitter and *S. megistacrolobum* Bitter in the province of Jujuy, Argentina. Euphytica 31: 817-835.

Oliver J.L. and Martínez-Zapater J.M. (1984) Allozyme variability and phylogenetic relationships in the cultivated potato (*Solanum tuberosum*) and related species. Pl. Syst. Evol. 148: 1-18.

Oliver J.L. and Martínez-Zapater J.M. (1985) A genetic classification of potato cultivars based on allozyme patterns. Theor. Appl. Genet. 69: 305-311.

Olmstead R.G. and Palmer J.D. (1994) Chloroplast DNA systematics: a review of methods and data analysis. Amer. J. Bot. 81: 1205-1224.

Philouze J. (1978) Comparison of the effects of the j and j-2 genes responsible for the jointless character in tomato, and epistatic relationship between j and j-2 in lines of the same varietal type. Ann. Amélior. Plantes 28: 431-445.

Pijnacker L.P. and Ferwerda M.A. (1984) Giemsa C-banding of potato chromosomes. Canad. J. Genet. Cytol. 26: 415-419.

Pimentel A.P. and Riggins R. (1987) The nature of cladistic data. Cladistics 3: 201-209.

Quiros C.F. and McHale N. (1985) Genetic analysis of isozyme variants in diploid and tetraploid potatoes. Genetics 111: 131-145.

Rick C.M. (1956) Genetic and systematic studies on accessions of *Lycopersicon* from the Galapagos islands. Amer. J. Bot. 43: 687-696.

Rickett H.W. (1954) Materials for a dictionary of botanical terms - II. Bull. Torrey Bot. Club 81: 188-198.

Rohlf F.J. (1975) Generalization of the gap test for the detection of multivariate outliers. Biometrics 31: 93-101.

Rohlf F.J. (1993) NTSYS-PC. Numerical Taxonomy and multivariate analysis system. Version 1.80. Applied Biostatistics Inc., New York.

Ross H. (1986) Potato Breeding - problems and perspectives. Verlag Paul Parey, Berlin.

Rouppe van der Voort J.N.A.M., Van Zandvoort P.M., Van Eck H.J., Folkertsma R.T., Hutten R.C.B., Draaistra J., Gommers F.J., Jacobsen E., Helder J. and Bakker J. (1996) Use of allele specifity of comigrating AFLP markers to align genetic maps from different potato genotypes. Molec. Gen. Genet. 255: 438-447.

Royal Horticultural Society (1986) R.H.S. color chart, in association with the flower council of Holland. London - Leiden.

Saitou N. and Nei M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molec. Biol. Evol. 4: 406-425.

Schmiediche P.E., Hawkes J.G. and Ochoa C.M. (1980) The breeding of the cultivated potato species *S.* × *juzepczukii* Buk. and *S.* × *curtilobum* Juz. and Buk. I. A study of natural variation of *S.* × *curtilobum* and their wild progenitor *S. acaule*. Euphytica 29: 685-704.

Schmiediche P.E., Hawkes J.G. and Ochoa C.M. (1982) The breeding of the cultivated potato species *S.* × *juzepczukii* Buk. and *S.* × *curtilobum* Juz. and Buk. II. The resynthesis of *S.* × *juzepczukii* and *S.* × *curtilobum*. Euphytica 31: 695-707.

Sharma S.K., Knox M.R. and Ellis T.H.N. (1996) AFLP analysis of diversity and phylogeny of Lens and its comparison with RAPD analysis. Theor. Appl. Genet. 93: 751-758.

Sheen S. (1972) Isozymic evidence bearing on the origin of *Nicotiana tabacum*. Evolution 26:143-154.

Smith J.J., Scott-Craig J.S., Leadbetter J.R., Bush G.L., Roberts D.L. and Fulbright D.W. (1994) Characterization of random amplified polymorphic DNA (RAPD) products from *Xanthomonas campestris* and some comments on the use of RAPD products in phylogenetic analysis. Molec. Phylogenet. Evol. 3: 135-145.

Smith R.S., Hamill D.E., Weaver E.A. and Thompson K.H. (1970) Multiple molecular forms of peroxidases and esterases among *Nicotiana* species and amphiploids. J. Heredity 61: 203-212.

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

Soltis P.S., Soltis D.E. and Doyle J.J. (1992) Molecular systematics of plants. New York, London: Chapman and Hall.

Spooner D.M. and Bamberg J.B. (1991) The Inter-Regional Potato Introduction Project (IR-1), US center for potato germplasm. Diversity 7: 32-35.

Spooner D.M. and Castillo R.T. (1997) Reexamination of series relationships of South American wild potatoes (Solanaceae: *Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation. Amer. J. Bot. 84: 671-685.

Spooner D.M. and Sytsma K.J. (1992) Reexamination of series relationships of Mexican and Central American wild potatoes (*Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation. Syst. Bot. 17: 432-448.

Spooner D.M. and Van den Berg R.G. (1992a) An analysis of recent taxonomic concepts in wild potatoes (*Solanum* sect. *Petota*). Gen. Res. Crop Evol. 39: 23-37.

Spooner D.M. and Van den Berg R.G. (1992b) Species limits and hypothesis of hybridization of *Solanum berthaultii* Hawkes and *S. tarijense* Hawkes: morphological data. Taxon 41: 685-700.

Spooner D.M., Anderson G.J. and Jansen R.K. (1993) Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (Solanaceae). Amer. J. Bot. 80: 676-688.

Spooner D.M., Castillo R.T. and López L.J. (1992) Ecuador, 1991 potato germplasm collecting expedition: taxonomy and new germplasm resources. Euphytica 60: 159-169.

Spooner D.M., Sytsma K.J. and Conti E. (1991) Chloroplast DNA evidence for genome differentiation in wild potatoes (*Solanum* sect. *Petota*: Solanaceae). Amer. J. Bot. 78: 1354-1366.

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.

Staub J.E., Kuhns L.J. and May B. (1984) Genetic bases of isozyme variation for alkaline phosphatase and glucosephosphate isomerase in *Solanum*. Theor. Appl. Genet. 67: 505-513.

Stejskal J. (1994) Aspartate aminotransferase isozymes in plants: Comparison of two staining methods in polyacrylamide gels. Biologia Plantarum 36: 359-364.

Stuessy T.F. (1990) Plant taxonomy: the systematic evaluation of comparative data. Columbia Univ. Press, New York.

Swaminathan M.S. (1954) Nature of polyploidy in some 48 chromosome species of the genus *Solanum* section *Tuberarium*. Genetics 39: 59-76.

Swaminathan, M.S. and H.W. Howard (1953) The cytology and genetics of the potato and related species. Bibliographia Genetica 16. Martinus Nijhoff, The Hague.

Swofford D.L. (1993) PAUP: phylogenetic analysis using parsimony, version 3.1. Computer program. Illinois Natural History Survey, Champaign.

Troll W. (1957) Praktische Einführung in die Pflanzenmorphologie. Gustav Fisher Verlag, Jena.

Tukey J. (1953) A problem with multiple comparisons. Princeton University, Princeton.

Ugent D. (1966) Hybrid weed complexes in *Solanum*, section *Tuberarium*. Ph.D. Dissertation, University of Wisconsin, USA.

Ugent D. (1981) Biogeography and origin of *Solanum acaule* Bitter. Phytologia 48: 85-95.

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. Applic. Biosci. 10: 569-570.

Van den Berg R.G. and Groendijk-Wilders N. (1998) Numerical analysis of the taxa of series *Circaeifolia* (*Solanum* sect. *Petota*). In: Nee M., Simon D., Jessop J.P. and Hawkes J.G. (eds.) Solanaceae IV: Progress in biology and utilization. Royal Botanical Gardens, Kew (In press).

Van den Berg R.G. and Spooner D.M. (1992) A reexamination of infraspecific taxa of a wild potato, *Solanum microdontum* (*Solanum* sect. *Petota*: Solanaceae). Pl. Syst. Evol. 182: 239-252.

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 (Solanaceae: section *Petota*). Amer. J. Bot. 85 (In press).

Van Eck H.J., Rouppe van der Voort J., Draaistra J., Van Zandvoort P., Van Enckevort E., Segers B., Peleman J., Jacobsen E., Helder J. and Bakker J. (1995) The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. Molec. Breeding 1: 397-410.

Velenovsky J. (1910) Vergleichende Morphologie der Pflanzen. Teil 3. Verlagsbuchhandlung, Prag.

Vos P., Hogers R., Bleeker M., Reijans M., Van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. and Zabeau M. (1995) AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23: 4407-4414.

Vulkova-Achkova Z. (1982) The inheritance of two jointless tomato fruit stem types in the hybrids *Lycopersicon esculentum* Mill. × *L. pimpinellifolium* f. *galapagos* Rick. Genetika i Selekcija 15: 456-461.

Weberling F. (1989) Morphology of flowers and inflorescences. Cambridge University Press, Cambridge.

Weeden N.F. and Wendel J.F. (1990) Genetics of plant isozymes. In: Soltis D.E. and Soltis P.S. (eds.) Isozymes in plant biology. Chapman and Hall, London: 46-72.

Wendel J.F. and Weeden N.F. (1990) Visualization and interpretation of plant isozymes. In: Soltis D.E. and Soltis P.S. (eds.) Isozymes in plant biology. Chapman and Hall, London: 4-45.

Whitkus R., Doebley J. and Wendel J.F. (1994) Nuclear DNA markers in systematics and evolution. In: Phillips R.L. and Vasil I.K. (eds.) DNA-based markers in plants, pp. 118-141. Kluwer, Dordrecht.

Williams C.E. and St.Clair D.A. (1993) Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum*. Genome 36: 619-630.

Wing R.A., Zhang H.-B. and Tanksley S.D. (1994) Map-based cloning in crop plants. Tomato as a model system: 1. Genetic and physical mapping of jointless. Mol. Gen. Genet. 242: 681-688.

Wittenbach V.A. and Bukovac M.J. (1972) An anatomical and histochemical study of abscission in maturing sweet cherry fruit. J. Amer. Soc. Hort. Sci. 97: 214-219.

NAWOORD

Mijn proefschrift is af! Een deel van mijn leven heb ik afgesloten met de afronding van dit werk. Natuurlijk heb ik mijn onderzoek niet kunnen uitvoeren zonder de medewerking van velen. Hierbij wil ik alle medewerkers van Plantentaxonomie bedanken voor de geboden praktische hulp en voor het nuttige commentaar op mijn manuscripten.

Speciaal wil ik mij in dit nawoord richten op de 'aardappel'-groep. Vooral zij hebben mij gemotiveerd en bijgestaan in de vele proeven in het laboratorium, de kas en op het proefveld. Als eerste noem ik Ronald van den Berg. Hij heeft mijn onderzoek geïnitieerd en onder zijn supervisie wordt het taxonomisch onderzoek aan wilde aardappelsoorten uitgevoerd.

Ronald, bedankt voor je prettige en motiverende begeleiding. Je bekeek kritisch heel wat stukken en hebt ze van onmisbaar commentaar voorzien. Door de vele gesprekken die we voerden kristalliseerden de oorspronkelijke onderzoeksvragen uit tot de inhoud van dit proefschrift. Natuurlijk heeft mijn onderzoek niet alle vragen opgelost. Er zijn zaken blijven liggen, en er zijn nieuwe vragen en onderzoeksmethoden naar voren gekomen. Genoeg mogelijkheden voor interessant onderzoek in de komende jaren. Tenslotte wil ik nog de twee inspirerende studiereizen naar Wisconsin noemen die we samen hebben ondernomen; voor mij een sprekend voorbeeld van onze goede samenwerking.

Nynke Groendijk-Wilders, Martin Zevenbergen, Wim Koopman en Nanda Bezem bedank ik voor de prettige samenwerking, het werk dat jullie voor mij hebben gedaan en de discussies die we hebben gevoerd. Het was een goede tijd!

I want to acknowledge David Spooner (University of Wisconsin, USA) for his inspiring contribution to my PhD-project and for his valuable comments on my manuscripts. Furthermore, I would like to thank him and his wife Ann for their hospitality during my two visits to Wisconsin and the pleasurable time we had together.

Marion Mulder, Carina Trompetter, Marco van Veller, Theresa Barbat, Marianne Schreuder: jullie hebben direkt of indirekt deelgenomen aan mijn promotie-onderzoek. Allen hartelijk dank!

Mijn dank gaat ook uit naar de medewerkers van Unifarm. Jullie hebben ervoor gezorgd dat al mijn proeven in de kas goed zijn verlopen. Voor het foto- en tekenwerk in dit proefschrift bedank ik Sijbout Massalt en Wil Wessel-Brand.

Ook buiten Plantentaxonomie hebben een groot aantal mensen mede zorg gedragen voor een succesvol verloop van mijn onderzoek. Vooral wil ik een aantal medewerkers van Plantenveredeling noemen vanwege de aansporing om de AFLP-techniek te gaan toepassen. Ik kreeg de mogelijkheid om bij hen te werken, ik was verzekerd van hun hulp in het laboratorium, en ik ontving commentaar op mijn schrijfsels. Herman van Eck, Prof. Evert Jacobsen, Dr. M.S. Ramanna, Fien Meijer-Dekens, Petra van den Berg, Pim Lindhout en

alle anderen: bedankt. Verder heeft Menno Schildhuizen mij meermalen geholpen met het computerprogramma PAUP. Rory Post, Peter Wittich en Rob den Outer hebben mijn manuscripten ook van nuttig commentaar voorzien. Hiervoor mijn dank.

Van diverse genenbanken ontving ik uitgangsmateriaal voor mijn proeven. Een belangrijk deel kreeg ik uit de CGN-aardappelcollectie die door Roel Hoekstra wordt beheerd. Roel, ook bedankt voor je gastvrijheid in Braunschweig, Duitsland. Verder heb ik genenbank-materiaal mogen ontvangen uit Sturgeon Bay, Wisconsin, USA. John Bamberg, thank you and your staff for providing material and the good cooperation. Tevens heb ik gebruik kunnen maken van materiaal van het CIP (Peru) en van het PROINPA (Bolivia).

Tenslotte, lieve Caroline, familie en vrienden: bedankt voor jullie waardering en interesse voor mij en mijn werk.

Jouke

CURRICULUM VITAE

Jouke Pieter Kardolus was born on 17 December 1966 in Hengelo (Overijssel), the Netherlands. In 1985 he obtained his VWO-B certificate from the Ichthus College at Enschede, and started in the same year with a study Plant Breeding at Wageningen Agricultural University. As part of his course, he spent a practical training period at the department 'Biologia Ambientale' of the University of Siena, Italy. This period was funded by the European Erasmus-program. Furthermore, he worked as part of his study program for three months at the plant breeding company 'Goldsmith' in Andijk, The Netherlands. In September 1991 he graduated 'cum laude' at Wageningen Agricultural University with specializations in Plantcytology and -morphology, Plant Breeding and Plant Taxonomy. During his military service period he was artillery observer.

From January 1993 to January 1997 he conducted a Ph.D. research at the Department of Plant Taxonomy of Wageningen Agricultural University. This research was embedded in the graduate school Experimental Plant Sciences. The main goal of the Ph.D. project was the biosystematic analysis of the tetraploid species *Solanum acaule*. During his Ph.D. he made two study trips to Wisconsin (USA). Here he had the possibility to work together with Dr. David Spooner, USDA potato taxonomist and professor of the University of Wisconsin, Madison. At the research stations of Wisconsin University in Sturgeon Bay and Hancock genebank material was studied. These journeys were granted by the Netherlands Organization for Scientific Research (NWO). At the moment he is appointed as researcher in the ECOSYN-project, a project on the biodiversity of West Tropical Africa.

Jouke Pieter Kardolus werd op 17 december 1966 geboren te Hengelo (Overijssel). Na het behalen van het diploma VWO-B aan het Ichthus College te Enschede, studeerde hij vanaf september 1985 Plantenveredeling aan de Landbouwuniversiteit Wageningen. Tijdens deze studie is een gecombineerd stage/afstudeervak uitgevoerd in het kader van het Europese Erasmus-programma op de vakgroep 'Biologia Ambientale' aan de universiteit van Siena, Italië. Tevens heeft hij drie maanden stage gelopen bij het veredelingsbedrijf Goldsmith te Andijk. Afstudeervakken deed hij in de Plantencytologie en -morfologie (embryozak isolatie bij *Lilium* en mannelijke steriliteitsprocessen bij *Aloe*), Plantenveredeling (transformatie en regeneratie van aardappel) en Plantentaxonomie (het '*Solanum brevicaule*-complex'). In september 1991 studeerde hij cum laude af aan de Landbouwuniversiteit. De dienstplicht vervulde hij vervolgens bij de 'Gele Rijders' in Arnhem. Tijdens deze periode was zijn functie artillerie-waarnemer.

Van januari 1993 tot januari 1997 was hij aangesteld als AIO bij de vakgroep Plantentaxonomie van de Landbouwuniversiteit Wageningen. Deze aanstelling viel binnen de onderzoeksschool Experimentele Plantenwetenschappen. Bij deze onderzoeksschool heeft hij onder andere cursussen over genoom-analyse gevolgd en deel uitgemaakt van de AIO-raad.

Tijdens het 4-jarige AIO-project verrichtte hij een biosystematisch onderzoek aan de tetraploïde soort *Solanum acaule*. Een zeer breed scala aan technieken en analyse-

methoden, waaronder de AFLP™ 'DNA-fingerprint'-techniek, is aangewend om een beter inzicht te verkrijgen in de evolutionaire processen van (wilde) aardappelsoorten. Gedurende dit onderzoek zijn twee studiereizen gemaakt naar Wisconsin (VS) om het aldaar aanwezige genenbankmateriaal te bestuderen en samen te werken met David Spooner, aardappeltaxonoom van het Ministerie van Landbouw (USDA) en professor aan de Universiteit van Wisconsin te Madison. Deze reizen werden mede mogelijk gemaakt door STIR-beurzen van NWO. Vanaf december 1997 heeft hij een tijdelijke aanstelling als onderzoeker bij het ECOSYN-project. Dit project is gericht op de biodiversiteit van tropisch West-Afrika.

PUBLICATIONS

Kardolus J.P. (1994) Morphological variation within series *Acaulia*. Solanaceae Newsletter 4: 23-24.

Kardolus J.P. (1994) Morphological variation within series *Acaulia* (*Solanum* sect. *Petota*). In: Jessop J.P. and Eadsforth M. (eds.) Abstracts IV International Solanaceae Conference (1994): 25.

Kardolus J.P. (1998) Morphological variation within series *Acaulia* (*Solanum* sect. *Petota*). In: Nee M., Simon D., Jessop J.P. and Hawkes J.G. (eds.) Solanaceae IV: progress in biology and utilisation. Royal Botanical Gardens, Kew (In press).

Kardolus J.P. and Bezem N. (submitted) The floral abscission zone in series *Acaulia* and related taxa of *Solanum* section *Petota*.

Kardolus J.P. and Groendijk-Wilders N. (1998) The inflorescence architecture of *Solanum acaule* and related taxa of *Solanum* section *Petota* (Solanaceae). Acta Botanica Neerlandica 47 (2) (In press).

Kardolus J.P. and Van den Berg R.G. (submitted) A biosystematic analysis of the polyploid series *Acaulia* and related potato species (Solanaceae).

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. Plant Systematics and Evolution (In press).

Kardolus J.P., Zevenbergen M. and Van den Berg R.G. (1997). On the origin of the triploid cultivated potato *Solanum juzepczukii*. In: Hollingsworth P., Bateman R. and Gornall R. (eds.) Advances in plant molecular systematics. An international meeting of the Systematics Association and the Linnean Society. August 13-15, 1997. Conference abstracts. University of Glasgow, Glasgow.

Miller J.T., Van den Berg R.G., Ugarte M.L., Kardolus J.P., Villand J. and Spooner D.M. (1996) Morphological and molecular systematics of the wild potato *Solanum brevicaule* complex (abstract). American Journal of Botany 83: 179.

Van den Berg R.G., Groendijk-Wilders N. and Kardolus J.P. (1996) The wild ancestors of cultivated potato: the brevicaule-complex. Acta Botanica Neerlandica 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 (Solanaceae: section *Petota*). American Journal of Botany 85 (In press).

Wagner V.T., Kardolus J.P. and Van Went J.L. (1989) Isolation of the lily embryo sac. Sexual Plant Reproduction 2: 219-224.