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RFLP analysis of the wild potato species, *Solanum acaule* Bitter (*Solanum* sect. *Petota*)*

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Summary. Intraspecific variation of a wild potato species, *Solanum acaule* Bitt., was analyzed by RFLPs of genomic DNA. One hundred and five accessions were selected throughout the distribution area, including all subspecies, i.e., ssp. *albicans* (hexaploid), ssp. *punae* (tetraploid), ssp. *acaule* (tetraploid) and ssp. *aemulans* (tetraploid). Twenty-seven low-copy DNA clones (probes) were Southern hybridized with *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I digests of total DNA of all accessions. In total, 238 RFLPs were detected from 94 enzyme × probe combinations. Among them, 49 RFLPs were specific to ssp. *albicans*, suggesting that the additional third genome is distinct from its two other genomes. RFLPs between and within subspecies were analyzed by principal component analysis. DNA similarities between subspecies coincided with a former taxonomic treatment in the sense that ssp. *albicans* is the most distantly related to ssp. *acaule* and ssp. *aemulans* is distantly related. Subspecies *acaule* and ssp. *punae* were indistinguishable. In addition, RFLPs could be used to distinguish groups within subspecies. Subspecies *aemulans*, confined to Argentina, was divided into two populations, one from the province of La Rioja and the other from the province of Jujuy. In ssp. *acaule*, some accessions from the southernmost distribution area were clearly distinguishable, while the others varied continuously, showing a geographical cline from Peru to Argentina.

Key words: *Solanum acaule* – Intraspecific variation – RFLP – Principal component analysis – Potato

* Reference to a specific brand or firm name does not constitute endorsement by the US Department of Agriculture over others of similar nature not mentioned

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Introduction

Solanum acaule Bitter is one of the most widely distributed wild potato species. It is adapted to the high altitudes of the Andes, and its distribution ranges from northern Peru to northwestern Argentina (Fig. 1). Frost, potato virus X, potato leaf roll, PSTV, and cyst nematode resistances of *S. acaule* are traits that attract the interest of breeders (Ross 1986). For horticultural and systematic reasons, this species has been relatively well investigated taxonomically (Brücher 1959; Hawkes and Hjerting 1969, 1989; Ugent 1981). Taxonomic treatments of *S. acaule* are presented in Table 1. Hawkes (1963) recognized four subspecies in *S. acaule*, i.e., ssp. *albicans* (6x), ssp. *punae* (4x), ssp. *acaule* (4x), and ssp. *aemulans* (4x) (this taxonomic treatment is tentatively adopted throughout our text). Subspecies *punae*, however, is not separated from ssp. *acaule* by other taxonomists. Brücher (1959) insisted on ssp. *aemulans* being a good species, which, however, is treated as a subspecies or a variety by the others. Subspecies *albicans*, a hexaploid variant from northern Peru, was first described by Ochoa (1960) as *S. acaule* var. *albicans*. Later, he and Hawkes (1990) elevated it to the rank of species.

Solanum acaule is a weed in the fields of Andean native farmers (Johns and Keen 1986) and is a hypothetical parent in the triploid cultivated species *S. juzepczukii* (*S. acaule* × *S. stenotomum*) (Hawkes 1962; Schmiediche et al. 1980). Natural hybrids between *S. acaule* and other wild species (i.e., *S. brevicaule, S. megistacrolobum, S. spegazzinii*, and *S. toralapanum*) have been reported, but are infrequent, since *S. acaule* normally occurs at very high altitudes where other species rarely grow (Hawkes and Hjerting 1969, 1989). Okada and Clausen (1982) reported that hybridization between *S. acaule* and *S. megistacrolobum* is quite frequent in the high cold

Table 1. Classification of *Solanum acaule*

Brücher (1959)	Correll (1962)	Hawkes (1963, 1978)	Hawkes (1990)	Ochoa (1990)
S. acaule	*S. acaule* ———	*S. acaule* ssp. *acaule* —— *S. acaule* ssp. *acaule* *S. acaule* ssp. *punae* —— *S. acaule* ssp. *punae*	——	*S. acaule* var. *acaule*
S. aemulans	*S. acaule* var. *aemulans* *S. acaule* var. *albicans*	*S. acaule* ssp. *aemulans* *S. acaule* ssp. *albicans*	*S. acaule* ssp. *aemulans* *S. albicans* *S.* × *indunii* *S.* × *viirsooi*	*S. acaule* var. *aemulans* *S. albicans*

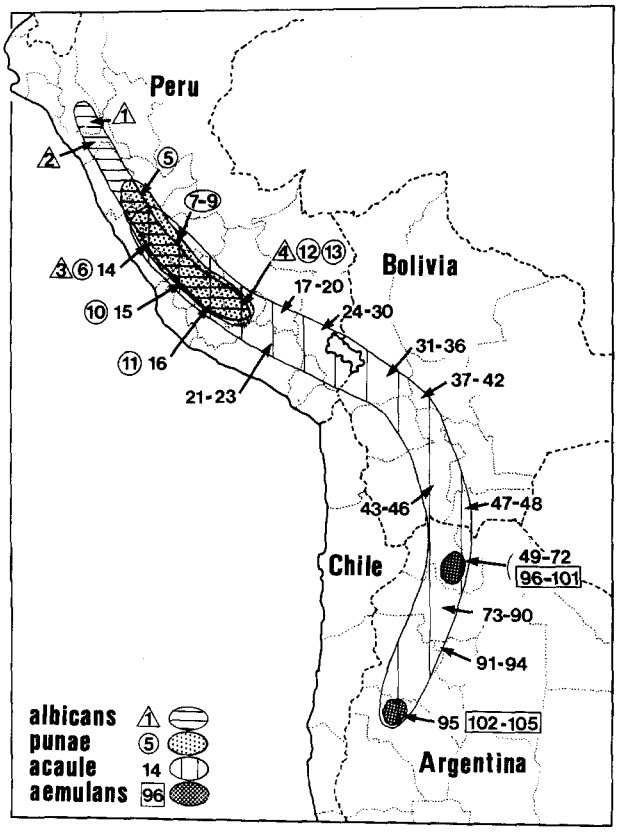

Fig. 1. The distribution area of *Solanum acaule* Bitt. showing collection sites (department or province) of the samples used in this study. Accession identity codes (see Table 2): *1–4* ssp. *albicans*, *5–13* ssp. *punae*, *14–95* ssp. *acaule*, *96–105* ssp. *aemulans*

plateau of northwestern Argentina. They also reported natural hybrids between *S. acaule* and *S. infundibuliforme* (Okada and Clausen 1985) and named these two natural triploid hybrids *S.* × *indunii* and *S.* × *viirsooi*, respectively; both of these designations were adopted by Hawkes (1990) (Table 1). However, these hybrids are all sterile triploids. Hybridization with other South American tetraploids does not normally occur due to endosperm breakdown after fertilization (von Wangenheim 1954; Johnston and Hanneman 1980). Thus, the gene flow into *S. acaule* germplasm seems restricted. In contrast to the wild diploid potato species, most of which are self sterile, *S. acaule* is self fertile and probably self-pollinated, thus retaining its uniqueness as a genetically well-defined group in series *Acaulia* in the tuber-bearing *Solanum* species (Hawkes 1963).

Restriction fragment length polymorphism (RFLP) analysis of nuclear DNA has been a powerful tool to reveal phylogenetic relationships among plants (Song et al. 1988; Hosaka et al. 1990; Miller and Tanksley 1990; Menancio et al. 1990). Debener et al. (1990) analyzed RFLPs between 14 wild and 3 cultivated *Solanum* species, and their results supported previous phylogenetic relationships based on biosystematic studies. In the study presented here, 27 probes derived from random genomic DNA clones of *S. phureja* were used to investigate intraspecific variation of *S. acaule*. The questions underlaid in this paper are the following. (1) Do current taxonomic treatments coincide with DNA similarities? (2) Is there any relationship between geographical distribution and DNA variation within *S. acaule* ssp. *acaule*, which is widely dispersed from central Peru to northwestern Argentina? (3) To what extent is the third genome of *S. acaule* ssp. *albicans* differentiated from its two other genomes?

Materials and methods

Materials

The *S. acaule* accessions used in this study are listed in Table 2. Seeds were supplied by the Inter-Regional Potato Introduction Project (IR-1), Sturgeon Bay, Wisconsin, USA. The identity code of the accessions is numbered serially from north to south in the Andes, and also arranged in order from ssp. *albicans*, to ssp. *punae*, to ssp. *acaule*, and then ssp. *aemulans* (Fig. 1). Seedlings were grown for approximately 70 (50–113 in range) days in soil in Jiffy-pots™ in a greenhouse in Madison, and fresh leaves were bulked from an average of 11.2 (7–12 in range) seedlings for DNA isolation.

Total DNA extraction

Bulked leaves (1–20 g fresh weight) were crushed and ground in liquid nitrogen with a mortar and pestle. The fine powder was homogenized in 5–10 ml of warmed 2 × CTAB isolation buffer (Doyle and Doyle 1987) and placed at 60 °C for 1 h. The homogenate was mixed with an equal volume of chloroform-isoamyl alcohol (24:1) and centrifuged by a JA-13.1 rotor (Beckman) at 10,000 rpm for 10 min. The clear supernatant was filtered through one layer of Miracloth (Calbiochem®) and dripped into a 50-ml tube containing 15 ml isopropanol and a

Table 2. *Solanum acaule* accessions used in this study

Code	PI number	2n	Locarity[a]
Ssp. *albicans*			
1	266381	72	P, Cajamarca
2	365376	72	P, La Libertad
3	365306	72	P, Lima
4	365305	72	P, Apurimac
Ssp. *punae*			
5	365312	48	P, Huanuco
6	246571	48	P, Lima
7	210031	48	P, Junin
8	266386	48	P, Junin
9	473443	48	P, Junin
10	473481	48	P, Huancavelica
11	473440	48	P, Ayacucho
12	473434	48	P, Apurimac
13	473436	48	P, Apurimac
Ssp. *acaule*			
14	473485	48	P, Lima
15	473483	48	P, Huancavelica
16	473439	48	P, Ayacucho
17	205507	48	P, Cuzco
18	473432	48	P, Cuzco
19	473433	48	P, Cuzco
20	473444	48	P, Cuzco
21	473486	48	P, Arequipa
22	473487	48	P, Arequipa
23	473488	48	P, Arequipa
24	473518	48	P, Puno-Cuzco
25	210033	48	P, Puno
26	230493	48	P, Puno
27	246504	48	P, Puno
28	473313	48	P, Puno
29	473327	48	P, Puno
30	473514	48	P, Puno
31	473323	48	B, La Paz
32	473324	48	B, La Paz
33	473325	48	B, La Paz
34	473512	48	B, La Paz
35	473516	48	B, La Paz
36	473517	48	B, La Paz
37	310923	48	B, Cochabamba
38	473315	48	B, Cochabamba
39	473316	48	B, Cochabamba
40	473317	48	B, Cochabamba
41	498082	48	B, Cochabamba
42	498083	48	B, Cochabamba
43	310924	48	B, Potosi
44	473319	48	B, Potosi
45	473321	48	B, Potosi
46	498066	48	B, Potosi
47	210029	48	B, Tarija
48	473322	48	B, Tarija
49	255501	48	A, Jujuy
50	472637	48	A, Jujuy
51	472641	48	A, Jujuy
52	472646	48	A, Jujuy
53	472651	48	A, Jujuy
54	472664	48	A, Jujuy
55	472668	48	A, Jujuy
56	472672	48	A, Jujuy
57	472680	48	A, Jujuy
58	472684	48	A, Jujuy
59	472686	48	A, Jujuy
60	472702	48	A, Jujuy
61	472706	48	A, Jujuy
62	472710	48	A, Jujuy
63	472722	48	A, Jujuy
64	472747	48	A, Jujuy
65	472751	48	A, Jujuy
66	472764	48	A, Jujuy
67	472768	48	A, Jujuy
68	472776	48	A, Jujuy
69	472779	48	A, Jujuy
70	472791	48	A, Jujuy
71	473510	48	A, Jujuy
72	500011	48	A, Jujuy
73	320276	48	A, Salta
74	472643	48	A, Salta
75	472655	48	A, Salta
76	472687	48	A, Salta
77	472689	48	A, Salta
78	472691	48	A, Salta
79	472693	48	A, Salta
80	472695	48	A, Salta
81	472716	48	A, Salta
82	472719	48	A, Salta
83	472731	48	A, Salta
84	472733	48	A, Salta
85	472735	48	A, Salta
86	472740	48	A, Salta
87	472742	48	A, Salta
88	472754	48	A, Salta
89	472772	48	A, Salta
90	472777	48	A, Salta
91	472755	48	A, Tucuman
92	472756	48	A, Tucuman
93	472757	48	A, Tucuman
94	472758	48	A, Tucuman
95	472801	48	A, La Rioja
Ssp. *aemulans*			
96	472793	48	A, Jujuy
97	472794	48	A, Jujuy
98	472795	48	A, Jujuy
99	472796	48	A, Jujuy
100	500018	48	A, Jujuy
101	500047	48	A, Jujuy
102	472798	48	A, La Rioja
103	472800	48	A, La Rioja
104	472802	48	A, La Rioja
105	472803	48	A. La Rioja

[a] P, Peru; B, Bolivia; A, Argentina

small ball of glass wool. All of the components were mixed by inverting the tube, and the solution was then decanted. The glass wool-DNA aggregate was washed by placing it in 75% ethanol with 10 m*M* ammonium acetate for 30 min, and then in 75% ethanol for another 30 min. The glass wool-DNA aggregate was placed in 5 ml of 50 m*M* Tris-HCl buffer (pH 8.0) and 20 m*M* EDTA for 3 h to resuspend DNA. The DNA was then collected by ethanol precipitation.

Probes

A random genomic DNA library was constructed from *Eco*RI digests of total DNA of *S. phureja* clone 1.22 by the procedures described by Hosaka et al. (1990). The 27 clones chosen randomly were: P10, P43, P122, P135, P140, P159, P209, P215, P247, P256, P278, P279, P292, P298, P304b, P307, P352, P368, P374, P392, P403, P417, P434, P473, P477a, P562, and P648. P352 is a few-copy DNA; P140, P256, P279, and P473 are double-copy DNA, and the others are single-copy DNA in the genome of *S. phureja* clone 1.22. The DNA clone was digested by *Eco*RI and electrophoresed on a low-melting point agarose gel. The insert DNA was dissected from the gel and labelled by [^{32}P]-dCTP using the method of Feinberg and Vogelstein (1984). The radiolabelled probe was used without removal of the unincorporated nucleotides.

Southern hybridization

Total DNA (5 μg) digested with *Eco*RI, *Eco*RV, *Hin*dIII, or *Xba*I restriction endonucleases was transferred to a nylon membrane (Zeta-Probe™, Bio-Rad) by alkaline transfer (Reed and Mann 1985). The hybridization buffer consisted of 0.25 *M* $NaHPO_4$ (pH 7.2), 0.25 *M* NaCl, 7% SDS, 10% polyethylene glycol 8000, 0.5% nonfat powdered milk, and 1 m*M* EDTA (Amasino 1986). Two membranes, with the DNA binding surfaces to the outside, were sandwiched between a sheet of Miracloth, and up to eight membranes were stacked; these were then put into a heat-sealable plastic bag with the hybridization buffer. After at least 3 h of pre-hybridization, a denatured probe was injected into the bag. Hybridization was performed at 65 °C overnight at a probe concentration of 0.5 ng/ml. The membranes were washed in 1% SDS, 2× SSC (0.3 *M* sodium chloride, 0.03 *M* sodium citrate), and 0.1% tetrasodium pyrophosphate for 15 min at room temperature, then for 30 min in the same solution at 65 °C, followed by two washings at 65 °C for 30 min each in 1% SDS, 0.1 × SSC, and 0.1% tetrasodium pyrophosphate solution. The washed membranes were autoradiographed using Lightning Plus™ intensifier screens (Du Pont) either at −80 °C or at room temperature either overnight or for up to 2 days depending on the strength of the signals.

RFLP analysis

Only visibly reliable and variable bands were scored and converted to 1-0 type data. From this raw data matrix (samples × characters), the Euclidian distances were calculated. Principal component analysis (PCA) was applied with a treatment of the Euclidian distance matrix (samples × samples) as a raw data matrix instead of the generally used samples × characters matrix. The eigen values were calculated from a correlation coefficient matrix. Calculations of Euclidian distances and PCA were performed using a main computer (Acos-6, NEC) at the Kobe University Information Processing Center with program packages STATPAC-6 and CLUSTER-6 (both from NEC).

Results

One hundred and five accessions of *S. acaule* from throughout its distributional area were analyzed for RFLPs with 27 probes that were mostly single-copy DNAs. Four different restriction endonucleases, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I, were used, generating the informational RFLP data from the 94 probe × enzyme combinations. Some bands were extremely variable in intensity between samples. As bulked leaves were used for DNA isolation, those variable bands probably resulted from genetic segregation within and/or between accessions. In many cases, very high molecular weight bands were skewed by contaminants in the DNA sample such as polysaccharides or proteins, which often made it difficult to compare them. Thus, those ambiguous bands and also the bands showing no variation between samples were not scored. In total, 238 bands were scored, and these distinguished most of the accessions. However, the following accessions could not be distinguished from each other: 20 and 31, 6 and 29, 41 and 42, and 91, 92, and 93. Probe and enzyme efficiencies for the detection of variation will be discussed elsewhere.

Table 3. Summary of a subspecies- or a particular group-specific bands among 238 scored bands generated by 94 enzyme × probe combinations using 27 probes

Specificity[a]	Probe	Number of enzyme-probe combinations	Specific band		
			Gain	Loss	Total
Ssp. *albicans*	22	38	44	5	49
Ssp. *punae*	0	0	–	–	0
Ssp. *acaule*	0	0	–	–	0
J-*aemulans*	3	4	4	2	6
L-*aemulans*	6	11	10	2	12
J- & L-*aemulans*	0	0	–	–	0
Ssp. *albicans* & J-*aemulans*	1	1	0	1	1
Ssp. *albicans* & L-*aemulans*	2	2	2	0	2
No specificity	1	14	–	–	168

[a] J and L stand for the province of Jujuy population and the province of La Rioja population, respectively

Of the 238 bands scored 49 (21%) were specific to ssp. *albicans* (Table 3). In this paper, a "specific" band is defined as a band that appeared or disappeared (gain or loss in Table 3) in all of the accessions of a given taxon (or in the case of ssp. *aemulans*, the province of Jujuy or province of La Rioja populations), but never in others. For instance, in Fig. 2, band 4 was specific to ssp. *albicans*. Two band differences, i.e., the loss of band 1 and the gain of band 3, were specific to ssp. *aemulans* from the province of Jujuy (accessions 96–101). Four other bands (bands 2, 5, 6, and 7) were variable between accessions (Fig. 2). Such specific bands were also observed for other taxa (Table 3). Six bands were specific to the accessions of ssp. *aemulans* from the province of Jujuy (referred to as J-*aemulans*), and 12 bands to those from the province of La Rioja (L-*aemulans*). One band was specific to both ssp. *albicans* and J-*aemulans*, and 2 bands to both ssp. *albicans* and L-*aemulans*. The rest of the bands (168 bands) were not specific to a particular subspecies or

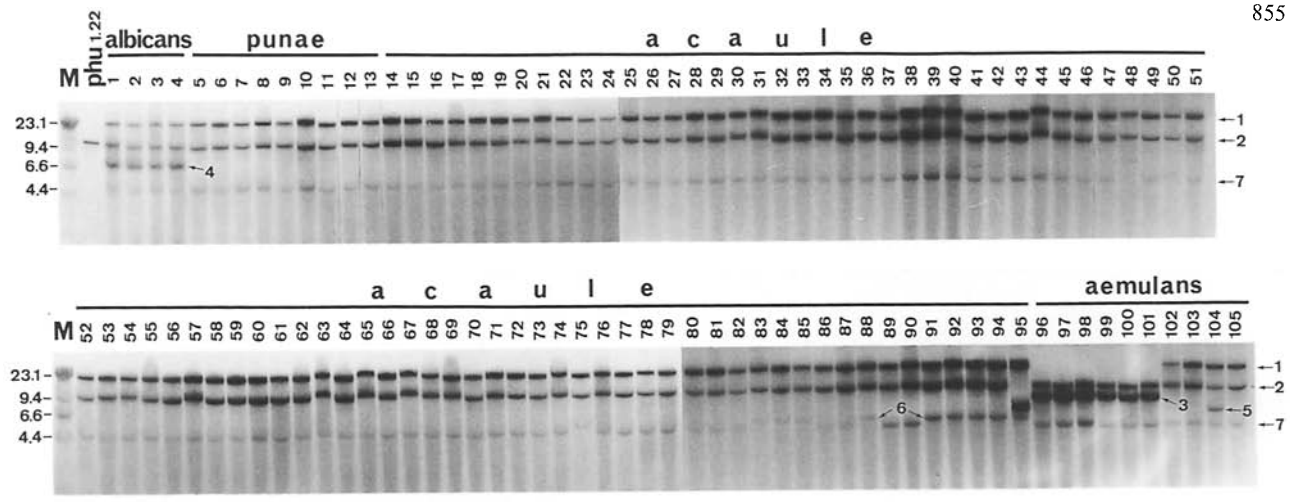

Fig. 2. An autoradiograph showing 7 different RFLPs (*arrows*) detected by the probe P292 in the *Eco*RV digests of *S. acaule*. The *second lane* contains DNA of *S. phureja* 1.22, the source of the probe. See Table 2 for an accession identity code of each lane. The radiolabelled lamda DNA was included in the hybridization buffer at a concentration of 1.25 pg/ml to light up a lamda DNA *Hin*dIII marker (*M*). The molecular size is shown on the *left* in kilobase pairs

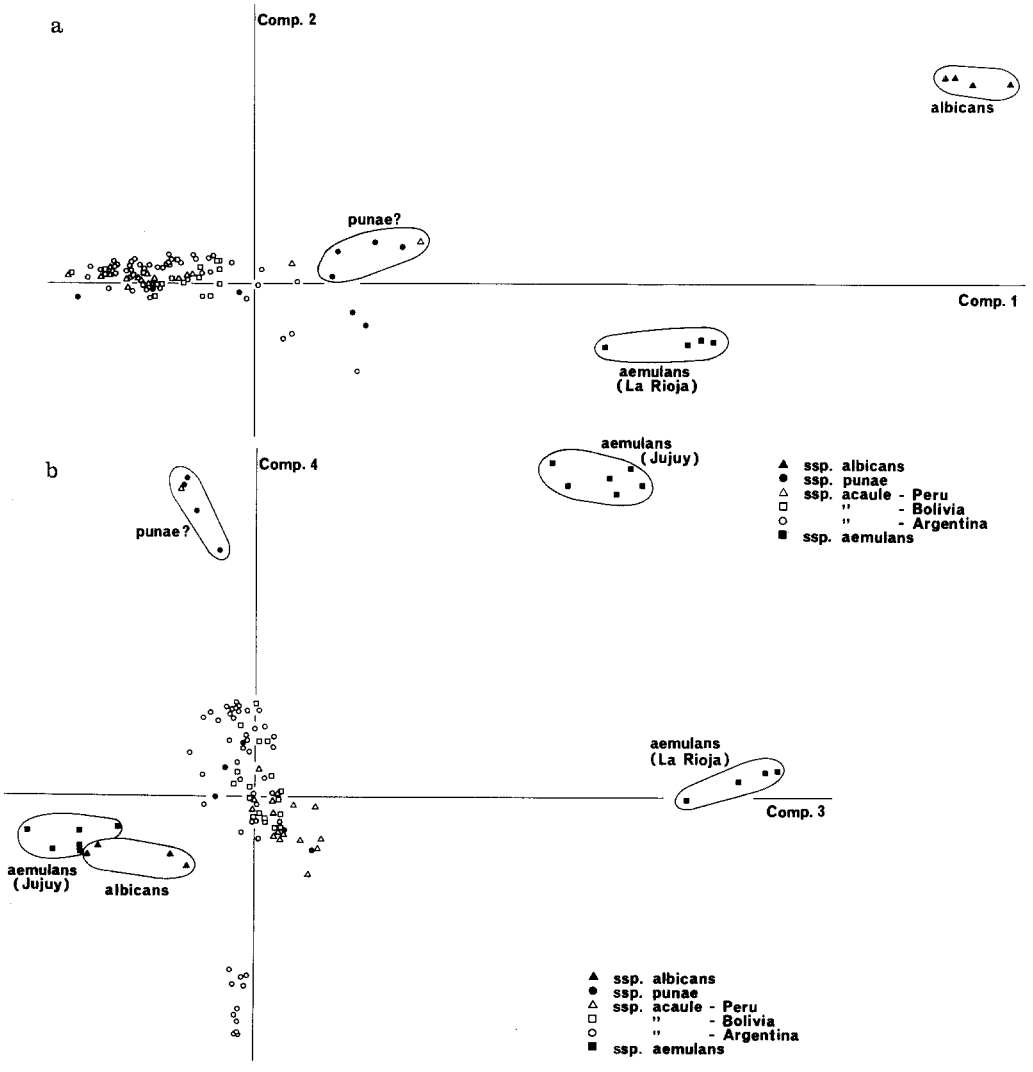

Fig. 3a, b. Scatter diagrams showing variation between accessions of the four subspecies of *S. acaule* based on principal component analysis of RFLPs. The first and second component scores and the third and fourth component scores of each accession were plotted in **a** and **b**, respectively. For ssp. *punae*, a likely population inferred from **b** is indicated in **a** with a *question mark*

group, but variable between and/or within subspecies. None of the bands was specific to ssp. *punae*.

Specific bands of ssp. *albicans* were often detected in 2 or more enzyme digests with the same probe. Accessions 41 and 42 did not show any hybridization signals with probe P307 in any of the enzyme digests. Such RFLPs likely resulted from insertion/deletion. Some of RFLPs might be the result of cosegregation, which provides 2 or more cosegregating restriction fragments in the same enzyme digest with the same probe. To avoid overestimating dissimilarities, we considered those bands which correlated perfectly with other bands detected with the same probe (a correlation coefficient of either 1.0 or −1.0) to be one band, resulting in 168 bands that were used for a further analysis.

To visualize differences between accessions, the data were analyzed by principal component analysis. The proportion of the variability accounted for by the first four principal components was 74.5%, 6.7%, 3.9%, and 3.3%, respectively, for a total of 88.4%. Scores of the first and second components for each of the accessions are plotted in Fig. 3a, which accounted for 81.2% of the total variation. Because out of 168 bands compared, 22 were ssp. *albicans* specific, the first component was accounted for predominantly by the differences between ssp. *albicans* and others. The variation range of ssp. *punae* overlapped that of ssp. *acaule* (the ssp. *acaule-punae* complex), although most accessions of ssp. *punae* tended to occupy the right half of the variation range of the complex. J-*aemulans* and L-*aemulans* clustered separately by the second component, and both clustered between the ssp. *albicans* and the ssp. *acaule-punae* complex. Though their cumulative contribution ratio was rather small (7.2%), the third and fourth components are also informative (Fig. 3b). As the specificity of ssp. *albicans* was explained mainly by the first component, the third and fourth components did not differentiate it from ssp. *acaule*. In turn, the distinctiveness of L-*aemulans* and ssp. *punae* and the variation within ssp. *acaule* were disclosed. Four accessions of ssp. *punae* (accessions 5, 7, 8, and 10) and 1 Peruvian accession of ssp. *acaule* (accession 22) were clearly distinct from the others as shown by a question mark in Fig. 3b (and also in Fig. 3a). The remaining accessions of ssp. *punae* (accessions 6, 9, 11, 12, and 13) were included in the variation range of ssp. *acaule*. Within ssp. *acaule*, many accessions from the southernmost part of the distribution area (accessions 50, 75, 83–85, 88, 91–95) were distinctly separated by the fourth component, while the others were located in a single cluster in which 5 accessions of ssp. *punae* were included. In this cluster, Peruvian ssp. *acaule* tended to occupy the lower right, Argentine ssp. *acaule* occupied the upper left, and the Bolivian ssp. *acaule* was at an intermediate position between them; however they overlapped to a large extent, and geographical partitioning was not possible.

Discussion

Subspecies albicans

Subspecies *albicans* is hexaploid, having an additional set of genomes (Ochoa 1960). As expected, it was clearly separated by many RFLP markers (22 probes resulting in 49 ssp. *albicans*-specific bands). From a morphological point of view, Hawkes (1963) hypothesized ssp., *albicans* to be an amphiploid hybrid of *S. acaule* with a diploid wild species from another series. Hybridization between *S. acaule* and other tetraploid species generally fails due to endosperm breakdown after fertilization (von Wangenheim 1954; Johnston and Hanneman 1980). The same Endosperm Balance Number (EBN) is assigned to those species giving normal seed set in their crosses regardless of their ploidy levels (Johnston et al. 1980). Most of the South American species are 2x(2EBN) and 4x(4EBN). However, ssp. *albicans* is 6x(4EBN), while other tetraploid subspecies of *S. acaule* are 4x(2EBN) (Johnston and Hanneman 1980). A mean chromosome pairing frequency of a trihaploid of ssp. *albicans* at metaphase I was $1.95_{III} + 9.67_{II} + 10.80_{I}$ per cell (Matsubayashi and Ochoa unpublished data), indicating that ssp. *albicans* is an allohexaploid with two similar genomes and a third genome that is distinct from the first two (Matsubayashi 1991). These data suggest that ssp. *albicans* is of amphiploid origin between tetraploid *S. acaule* and an unknown 2EBN diploid species having a distinct genome.

Out of 49 ssp. *albicans*-specific bands, 44 were additional bands (Table 3), most of which were possibly derived from the third genome. However, RFLPs of ssp. *albicans* were not accounted for by the simple addition of both ssp. *acaule*- or ssp. *punae*-common bands and the possible third genome-derived bands because 5 bands were commonly lost in ssp. *albicans* (Table 3). Those bands characterizing ssp. *albicans* might have resulted from chromosome rearrangement after amphiploidization. Although ssp. *albicans* occupies the northern end and ssp. *aemulans* occupies the southern end of the distributional area of *S. acaule*, ssp. *albicans* showed a closer relationship to ssp. *aemulans* than to either ssp. *acaule* or ssp. *punae* (Fig. 3). This is because some bands appeared or disappeared similarly in ssp. *albicans* and ssp. *aemulans*, but not in ssp. *acaule* and ssp. *punae* (Table 3). The presence of those bands conserved in the geographically isolated subspecies might be a primitive character later changed in both ssp. *acaule* and ssp. *punae*. Therefore, it is suggested that an amphiploidization event occurred not at the stage after the three other subspecies differentiated, as thought by Hawkes (1990), but at the very primitive stage of subspecies differentiation of *S. acaule*.

Hawkes (1990) has very recently elevated ssp. *albicans* to the species rank as *S. albicans* (Ochoa) Ochoa, as previously treated by Ochoa (1983). This taxonomic treatment is supported by the present study.

Subspecies punae

Subspecies *punae* and ssp. *acaule* could not be well distinguished in this study. Four accessions of ssp. *punae* together with 1 accession of ssp. *acaule* were clearly separated from ssp. *acaule*, whereas 5 other ssp. *punae* accessions clustered with ssp. *acaule*. Misidentification might have happened because ssp. *punae* has such a morphological similarity to ssp. *acaule* that taxonomists other than Hawkes did not separate them into different taxa (Table 1). The lack of genetic separation between ssp. *punae* and ssp. *acaule* disclosed in this study correlates with the lack of morphological distinctiveness between them, suggesting that ssp. *punae* is synonymous with ssp. *acaule*.

Subspecies acaule

Subspecies *acaule* is widely distributed from central Peru to northern Argentina, from which one may expect a wide range of morphological and genetic variation. However, all accessions clustered well except for a small fraction of ssp. *acaule* from the southern end of its distributional area. *S. acaule* is adapted to the high altitudes where other related species rarely grow (Hawkes and Hjerting 1969, 1989). Endosperm breakdown in the crosses of *S. acaule* with other tetraploid species is a common phenomenon (von Wangenheim 1954; Johnston and Hanneman 1980). Thus, it seems likely that, despite of its wide distribution, ecological and reproductive isolations restricted gene flow from other species and a high preference of self-pollinations retained its genetic uniqueness, resulting in coherent genetic diversity.

In the variation of ssp. *acaule*, however, a certain geographical cline from Peru to Argentina was found, suggesting geographical differentiation. This finding provides a basis for the hypothesis that closely distributed accessions are also genetically close. Most accessions were distinguished from each other by RFLP markers, but some were not. Accessions 20 and 31 and accessions 6 and 29 were collected from different departments in Peru or Bolivia, whereas accessions 41 and 42 and accessions 91–93 were collected in the same department and province, respectively. Thus, it is possible that the latter are duplicate collections, although it might be possible to differentiate them using more RFLP markers.

Subspecies aemulans

Interestingly, ssp. *aemulans* was separated into two groups by RFLP markers; that is, the one from the province of La Rioja (L-*aemulans*) and another from the province of Jujuy (J-*aemulans*). The La Rioja populations are about 600 km apart from the Jujuy populations, and no collections of ssp. *aemulans* are known from the intervening areas. Subspecies *acaule* also occurs throughout these areas. However, both ssp. *aemulans* populations were clearly different from ssp. *acaule* (Fig. 3), although L-*aemulans* was more distinct from ssp. *acaule* than J-*aemulans*.

Correll (1962) proposed that ssp. *aemulans* was of hybrid origin (*S. acaule* × *S. megistacrolobum*). However, Hawkes and Hjerting (1969) have suggested that ssp. *aemulans* is the most primitive of the four subspecies, followed by ssp. *acaule*, ssp. *punae*, and ssp. *albicans*, since pedicel articulation becomes more and more obscure as one passes northwards from Argentina and the subspecies themselves become more distinctive and less like any other wild potato species. Hawkes and Hjerting (1969) thought J-*aemulans* to be an intermediate type between ssp. *acaule* and typical ssp. *aemulans* (L-*aemulans*). Okada and Clausen (1982) investigated natural triploid hybrids between *S. acaule* (4x) and *S. megistacrolobum* (2x) that occurred in the province of Jujuy. As the J-*aemulans* and the ssp. *acaule* × *S. megistacrolobum* hybrids have some characters in common, they postulated that J-*aemulans* was not a primitive form of *S. acaule* as thought by Hawkes and Hjerting (1969), but rather a fertile hybrid derivative of ssp. *acaule* × *S. megistacrolobum* through the functioning of 2n gametes (Okada and Clausen 1982). Hawkes and Hjerting (1989) accepted this latter hypothesis. Okada and Clausen (1982) did not further speculate on the relationship between L-*aemulans* and J-*aemulans* besides a brief description that if L-*aemulans* is of hybrid origin, it must have been derived from a cross of ssp. *acaule* with some other parental species because *S. megistacrolobum* does not occur in that region. The taxonomic findings coincide with the present data in the sense that J-*aemulans* and L-*aemulans* are different. Some specifically common bands were found in ssp. *aemulans* and ssp. *albicans* as mentioned in the previous section; however, this does not necessarily indicate that ssp. *aemulans* is more primitive than the other subspecies.

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