Isoenzymatic variability in wild potatoes[1]

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Abstract – Two species of wild potato *Solanum commersonii*, subspecies *commersonii* and *malmeanum*, and *S. chacoense*, subspecies *muelleri* occur in southern Brazil. Their rusticity and easy adaptation to extreme climatic conditions make them valuable for breeding programs. The objective of this work was to analyze the isoenzymatic variability of 113 clones of wild potato subspecies. They were collected and maintained at Embrapa-Centro de Pesquisa Agropecuária de Clima Temperado, at Pelotas, RS, Brazil. Enzymes involved in energetic (group I) or in peripherical (group II) metabolism constituted the material used. Polyacrylamide horizontal gel electrophoresis was used to analyze peroxidase, aspartate transaminase, phosphoglucomutase and isocitrate dehydrogenase isoenzymes. *Solanum* spp. has considerable genetic variability for isoenzymatic patterns. Cluster analysis classified the clones into 51 subgroups, based on electrophoretic variants of group I enzymes, and into 89, when group II enzyme variants were added. Genotypic differentiation of *S. chacoense muelleri* in relation to *S. commersonii commersonii* and *S. commersonii malmeanum* is evident when expressed through similarity and cluster analysis.

Index terms: peroxidases, aminotransferases, isomerases, isocitrate dehydrogenase, genetic variation.

Variabilidade isoenzimática em batata silvestre

Resumo – No sul do Brasil ocorrem apenas duas espécies silvestres de batata, *Solanum commersonii*, com as subespécies *commersonii* e *malmeanum*, e *S. chacoense*, com a subespécie *muelleri*, de interesse aos programas de melhoramento, pela rusticidade e fácil adaptação a condições climáticas extremas. O objetivo deste trabalho foi analisar a variabilidade isoenzimática de 113 clones de batata silvestre. O material foi coletado e mantido na Embrapa-Centro de Pesquisa Agropecuária de Clima Temperado, em Pelotas, RS. Foram usadas enzimas envolvidas nos metabolismos energético (grupo I) e periférico (grupo II). Eletroforese horizontal em gel de poliacrilamida foi empregada para análise de isoenzimas de peroxidase, aspartato transaminase, fosfoglucomutase e isocitrato desidrogenase de folhas. Existe grande variabilidade isoenzimática em clones de *Solanum* spp. A análise de agrupamento permitiu a classificação dos clones em 51 subgrupos, quando baseada em variantes eletroforéticas de enzimas do grupo I, e em 89 subgrupos, quando acrescida de enzimas do grupo II; é evidente a diferenciação genotípica entre *S. chacoense muelleri* em relação ao *S. commersonii malmeanum* e ao *S. commersonii commersonii*, expressa pelas análises de similaridade e agrupamento.

Termos para indexação: peroxidase, aminotransferase, isomerase, isocitrato desidrogenase, variação genética.

Introduction

Wild species are an important source of germplasm for disease and environmental stress resistance. Two wild potato species, series Commersoniana Buk., occur in southern Brazil: *Solanum commersonii* (2n = 2x = 24, 36) and *Solanum chacoense* (2n = 2x = 24, 36). Two subspecies of *S. commersonii* (*commersonii* and

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malmeanum) and *S. chacoense* (*chacoense* and *muelleri*) are known. These species have similar botanical characteristics. Resistance to common scab, bacterial wilt, late blight, drought, heat and frost were observed in *S. commersonii*. *S. chacoense*, however, has more useful characteristics, such as, resistance to common scab, powdery scab, bacterial wilt, black leg, virus (A, X, Y, F and leaf roll), aphids, nematodes, drought, heat and frost (Hawkes & Hjerting, 1969; Centro Internacional de La Papa, 1979, 1981; Montaldo, 1984).

A relatively large number of alleles occur naturally in potatoes, as demonstrated by isoenzymatic markers (Douches & Ludlam, 1991). A mean of 5.7 alleles/locus have been identified in 92 accessions of 40 species of *Solanum* from North and South America, indicating a considerable genetic variability in wild species (Douches et al., 1989). Martinez-Zapater & Oliver (1984) refer to the utilization of isoenzymes in the identification of 67 cultivars of *S. tuberosum*, including those most used in Europe and North America, and to the analysis of phylogenetic relationships among *S. tuberosum* and other species, based on aloenzyme variability.

Polymorphism level varies according to the enzyme function, that is, enzymes involved in energetic metabolism, glycolysis and the citric acid cycle, present lower polymorphism levels (group I) than those involved in peripherical metabolism (group II) (Gillespie & Kojima, 1968). Both monomorphic and polymorphic systems should be investigated, involving the highest possible number of metabolic routes, to obtain a random sample of populations and genomes, in order to avoid over or under estimates.

The objective of this work was to analyze the isoenzymatic variability of 113 wild potato clones.

Material and Methods

Solanum commersonii malmeanum (SCM), *S. commersonii commersonii* (SCC), *S. chacoense muelleri* (SChM) and non-identified (NI) clones were cultivated under greenhouse, screenhouse and/or field conditions at Embrapa-Centro de Pesquisa Agropecuária de Clima Temperado (Figure 1). They were collected in the Brazilian states of Rio Grande do Sul (Table 1) and Santa Catarina (clones 110 - NI, 119 and 120 - SCC), and in Uruguay (clones 07 - SCC and 195 - NI).

Group I (EC 5.4.2.2 phosphoglucomutase (PGM), EC 1.1.1.41 isocitrate dehydrogenase (IDH) and EC 2.6.1.1 aspartate transaminase (AT)) and group II enzymes (EC 1.11.1.7 peroxidase-PRX) from leaves of, at least, two plants per clone, were analyzed. Samples were taken from leaflets of the third or fourth leaf, before blooming (AT); of the fourth or fifth leaf, at the beginning of blooming (PGM); and of the fifth or sixth leaf, at full blooming (PRX and IDH). Ten milligrams of each sample were squeezed directly (AT) or in 0.01 mL gel buffer and 0.15% 2-mercaptoethanol (PRX, PGM and IDH). Clone number 186 was used as a control.

Horizontal electrophoresis, with 5% (PRX, PGM and IDH) and 6% (AT) polyacrylamide gels was used with discontinuous buffer systems described by Scandalios (1969) (PRX and AT) and Shields et al. (1983) (PGM and IDH). Staining methods are referred in Scandalios (1969) (PRX); Ayala et al. (1972) (AT); Vallejos (1983) (IDH and PGM).

Genetic similarity and cluster analysis, using Jaccard coefficient unweighted pair group method, arithmetic average (UPGMA) were calculated using the NTSYS – pc version 1.7.

Results and Discussion

No variation was observed in the peroxidase, aspartate transaminase phosphoglucomutase and isocitrate dehydrogenase isoenzymatic patterns of clones grown under different environmental conditions (greenhouse, screenhouse or field).

Number and concentrations of multiple molecular forms of peroxidase vary with plant ontogenesis (Chen et al., 1970). Thirty-four anodic peroxidase polymorphic bands, in sixty-six patterns, were detected at full blooming (Table 2). There were three to eight bands per pattern (Figure 2). According to Bassiri & Adams (1978), systems with higher number of polymorphic bands, such as peroxidase, are the most adequate to identify cultivars. These enzymes (group II) act over a class of molecules which, normally, come from the environment and that can have qualitatively and quantitatively more variables than enzymes (group I) which act on *in vivo* substrates, generally restricted to molecules that are products of a previous internal enzymatic reaction (Kojima et al., 1970).

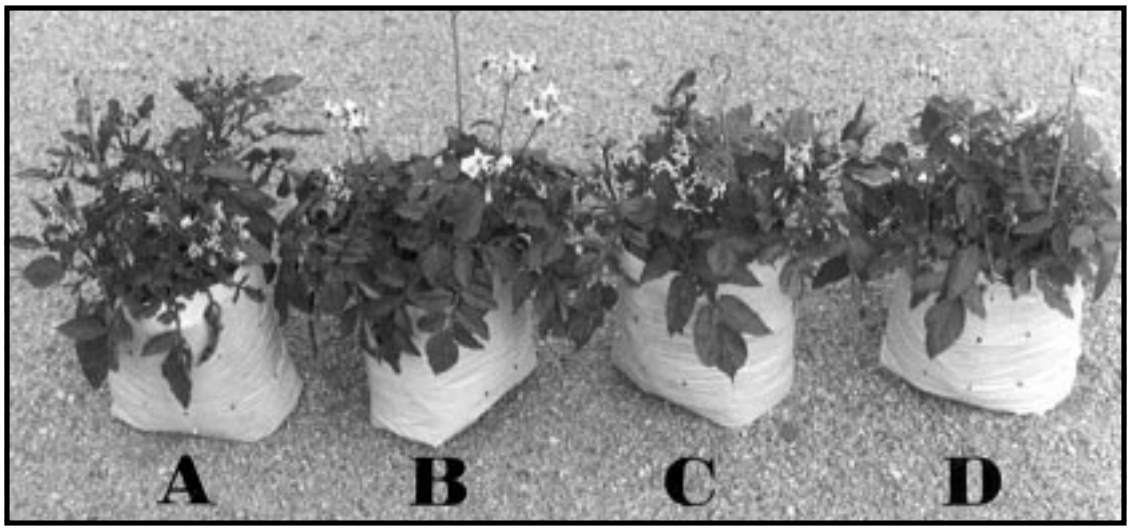

Figure 1. Clones of non-identified species (A and D), *Solanum commersonnii malmeanum* (B) and *Solanum commersonii commersonii* (C) clones.

Table 1. Rio Grande do Sul collection sites for *Solanum* spp. clones.

Site number	Municipality	Subspecies[1]	Clone	Latitude (S)[2]	Longitude (W)[2]	Altitude (m)[2]
1	Uruguaiana	NI	199, 218	29º45'17"	57º5'18"	66
2	Alegrete	NI	200, 202	29º46'59"	55º47'31"	102
3	Santa Maria	SCC	71	29º41'3"	53º48'25"	151
4	Itaqui	NI	222	29º7'31"	56º33'11"	57
5	Jaguari	NI	246	29º29'51"	54º41'24"	112
6	Júlio de Castilhos	SCC	70	29º13'37"	53º40'54"	513
7	São Borja	NI	176	28º39'38"	56º0'16"	123
8	Santo Antônio das Missões	NI	225	28º30'41"	55º13'40"	213
9	São Miguel das Missões	SCM	2	28º33'46"	54º33'15"	305
10	Augusto Pestano	SCC	8	28º31'1"	53º59'32"	385
		NI	10			
		SCM	55			
11	Santo Ângelo	SCM	56, 58	28º17'57"	54º15'47"	281
		NI	113			
12	Giruá	SCM	58	28º1'42"	54º20'59"	429
13	Porto Xavier	SCM	61, 62	27º54'20"	55º8'15"	115
14	Santa Rosa	SCM	59	27º52'15"	54º28'53"	277
15	Tuparendi	SCM	64	27º45'23"	54º28'54"	328
16	Alecrim	SCC	124	27º39'18"	54º45'50"	311
17	Horizontina	NI	21	27º37'33"	54º18'28"	343
		SCM	65			
18	Crissiumal	SCM	67	27º29'59"	54º6'4"	410
19	Três Passos	SChM	68	27º27'20"	53º55'55"	451
20	Tenente Portela	SCM	69	27º22'16"	53º45'30"	390
21	Iraí	SCM	109	27º11'37"	53º15'2"	235
22	Sarandi	SCM	108	27º56'38"	52º55'23"	503
23	Lagoa Vermelha	SCC	150	28º12'31"	51º31'33"	801

It continues...

Table 1. Continuation.

Site number	Municipality	Subspecies[1]	Clone	Latitude (S)[2]	Longitude (W)[2]	Altitude (m)[2]
24	Carazinho	SCC	107	28º17'2"	52º47'11"	603
25	Não-Me-Toque	SCC	129	28º27'33"	52º49'15"	514
26	Soledade	SCC	131	28º49'6"	52º30'37"	726
27	Nova Prata	SCC	128	28º47'2"	51º36'36"	662
28	Sobradinho	NI	112	29º25'17"	53º1'43"	427
29	Caxias do Sul	SCC	85	29º10'5"	51º10'46"	817
30	Torres	SCC	117, 118	29º20'7"	49º43'37"	16
31	Candelária	SCC	139	29º40'9"	52º47'20"	57
32	Vera Cruz	SCC	140	29º42'53"	52º30'20"	68
33	Santa Cruz do Sul	SCC	141	29º43'3"	52º25'33"	73
34	Venâncio Aires	SCC	142	29º36'23"	52º11'31"	46
35	Lageado	SCC	54	29º28'1"	51º57'41"	31
36	Nova Petrópolis	NI	127	29º22'35"	51º6'52"	579
37	Portão	NI	148	29º42'6"	51º14'31"	45
38	São Francisco de Paula	SCC	143	29º26'53"	50º35'1"	907
39	Esteio	SCC	73	29º51'41"	51º10'45"	11
40	Capão da Canoa	SCC	116	29º44'44"	50º0'35"	6
41	São Jerônimo	SCC	133	29º57'33"	51º43'20"	29
42	Eldorado do Sul	SCC	72	30º5'2"	51º36'58"	19
43	Viamão	SCC	75, 76	30º4'52"	51º1'24"	111
44	Osório	SCC	122	29º53'12"	50º16'11"	16
45	Pantano Grande	SCC	136, 137	30º11'29"	52º22'25"	100
46	Butiá	SCC	134	30º7'11"	51º57'44"	71
47	Barra do Ribeiro	SCC	74	30º17'28"	51º18'4"	5
48	Encruzilhada do Sul	SCC	154, 156	30º32'38"	52º31'19"	432
		NI	211			
49	Dom Feliciano	SCC	157	30º42'15"	52º6'27"	154
50	Tapes	SCC	40, 77	30º40'24"	51º23'45"	7
51	Amaral Ferrador	SCC	161	30º52'42"	52º15'27"	140
52	Camaquã	SCC	39, 158	30º51'4"	51º48'44"	39
53	Cristal	SCC	34, 35, 159	30º59'59"	52º2'54"	50
54	São Lourenço do Sul	SCC	15, 32, 78	31º21'55"	51º58'42"	19
55	Pelotas	SCC	3, 12, 13, 25, 26, 30	31º46'19"	52º20'33"	17
		NI	53			
56	Pedro Osório	NI	185	31º51'51"	52º49'24"	31
57	São José do Norte	SCC	167, 168	32º0'53"	52º2'30"	4
58	Rio Grande	SCC	18, 31, 36, 45, 86, 92, 96, 165, 166	32º2'6"	52º5'55"	5
59	Santa Vitória do Palmar	SCC	97	33º31'8"	53º22'5"	23
60	Herval	NI	186	32º1'25"	53º23'44"	287
61	Pinheiro Machado	SCC	183	31º34'42"	53º22'52"	439
62	Bagé	NI	179	31º19'53"	54º6'25"	212
63	Dom Pedrito	NI	188	30º58'58"	54º40'23"	141
64	Santana da Boa Vista	NI	153, 214	30º52'19"	53º6'55"	306
65	Caçapava do Sul	SCC	11	30º30'44"	53º29'29"	444
66	Quaraí	NI	194, 196	30º23'15"	56º27'5"	112
67	Rosário do Sul	NI	203	30º15'30"	54º54'51"	125
68	São Gabriel	NI	204	30º20'11"	54º19'12"	114
69	São Sepé	NI	104, 105	30º9'38"	53º33'55"	85

[1]SCC: *Solanum commersonii commersonii*; SCM: *Solanum commersonii malmeanum*; SChM: *Solanum chacoense muelleri*; NI: non-identified clones.[2]Source: IBGE (1998).

Table 2. Peroxidase isoenzymatic patterns detected in 113 *Solanum* spp. clones.

Pattern	Relative mobilities	Subspecies[1]	Clone	Pattern	Relative mobilities	Subspecies[1]	Clone
1	0.79, 0.76, 0.71, 0.68, 0.65	SCC	129	30	0.81, 0.76, 0.70, 0.62	SCC	157
		NI	196, 200	31	0.77, 0.74, 0.72, 0.69	SCM	59
2	0.79, 0.77, 0.73	SCC	7, 8			SCC	128
3	0.79, 0.75, 0.69	SCC	13, 30, 107	32	0.92, 0.89, 0.86, 0.82, 0.78, 0.76, 0.70	SCM	109
		NI	10	33	0.81, 0.78, 0.76, 0.69, 0.64, 0.59	SCC	159
4	0.85, 0.83, 0.75, 0.71, 0.68	SCC	3, 12, 18, 26, 32, 47	34	0.89, 0.85, 0.83, 0.80, 0.74, 0.69, 0.63	NI	153
		NI	112	35	0.77, 0.71, 0.69, 0.66	SCC	154
5	0.84, 0.80, 0.71, 0.68, 0.65	SCC	117, 118, 120	36	0.81, 0.76, 0.74, 0.70, 0.68, 0.62	SCC	25
6	0.92, 0.85, 0.82, 0.72, 0.65	SCC	31	37	0.92, 0.89, 0.86, 0.83, 0.73, 0.69, 0.65	SCM	108
		NI	105, 110			SCC	72
7	0.92, 0.90, 0.87, 0.84, 0.79, 0.76, 0.69, 0.65	SCM	67, 69	38	0.88, 0.84, 0.81, 0.74	SCM	2
		SCC	39	39	0.85, 0.82, 0.78, 0.75, 0.72, 0.65	SCM	56
8	0.85, 0.82, 0.78, 0.75, 0.68, 0.63	SCM	55	40	0.86, 0.83, 0.80, 0.74, 0.70, 0.68	SCC	97, 161
9	0.83, 0.81, 0.77, 0.72, 0.68	SCC	36, 75, 116, 122	41	0.86, 0.82, 0.79, 0.76, 0.73, 0.69, 0.65	NI	21
		NI	186	42	0.84, 0.80, 0.75, 0.72, 0.69, 0.65	SCC	15
10	0.81, 0.77, 0.70, 0.65	SCC	183	43	0.80, 0.77, 0.74, 0.69, 0.65, 0.62, 0.56	SCC	150
		NI	179, 195, 202	44	0.81, 0.78, 0.76, 0.70, 0.65, 0.59	SCC	76, 156
11	0.78, 0.76, 0.74, 0.66, 0.63	SCC	167, 168	45	0.87, 0.83, 0.78, 0.74, 0.70, 0.67, 0.61	SCC	96
12	0.77, 0.74, 0.71	SCM	62	46	0.79, 0.76, 0.70, 0.66, 0.63, 0.60	SCC	131
		NI	53, 225	47	0.92, 0.88, 0.82, 0.79, 0.76, 0.74, 0.70, 0.66	SCM	65
13	0.81, 0.77, 0.74, 0.72, 0.63, 0.60	SCC	34, 35, 136	48	0.92, 0.90, 0.87, 0.84, 0.70, 0.67	SChM	68
14	0.88, 0.85, 0.82, 0.80, 0.77, 0.71, 0.68	SCC	54, 137	49	0.86, 0.83, 0.78, 0.74, 0.72, 0.68, 0.63	SCC	70
15	0.87, 0.84, 0.81, 0.78, 0.76, 0.72, 0.63	SCC	140	50	0.83, 0.80, 0.77, 0.75, 0.71, 0.69, 0.65	SCC	74
16	0.81, 0.77, 0.72, 0.65, 0.62	SCC	158	51	0.83, 0.81, 0.78, 0.68, 0.63	SCC	73
		NI	127	52	0.82, 0.79, 0.71, 0.68, 0.63	NI	222
17	0.85, 0.82, 0.79, 0.73	SCC	125	53	0.85, 0.82, 0.78, 0.76, 0.70, 0.60	NI	211
		NI	113	54	0.84, 0.81, 0.79, 0.76, 0.72, 0.69, 0.66, 0.62	NI	218
18	0.76, 0.73, 0.70, 0.67	SCC	139, 141	55	0.81, 0.77, 0.73, 0.72, 0.69, 0.61	NI	204
19	0.85, 0.81, 0.77, 0.74, 0.68	NI	188	56	0.84, 0.81, 0.77, 0.72, 0.68, 0.65, 0.62, 0.59	NI	203
20	0.86, 0.83, 0.80, 0.76, 0.73, 0.69	SCC	86	57	0.79, 0.75, 0.71, 0.67, 0.64, 0.56	NI	214
21	0.86, 0.83, 0.80, 0.77, 0.71, 0.67	SCC	40, 77, 78, 119	58	0.84, 0.79, 0.73, 0.71, 0.67, 0.65	SCC	45
22	0.87, 0.84, 0.80, 0.74, 0.63	SCC	85	59	0.86, 0.82, 0.79, 0.73, 0.66, 0.62	NI	148
23	0.76, 0.72, 0.69, 0.61	NI	194	60	0.76, 0.71, 0.63	SCC	11
24	0.78, 0.75, 0.69, 0.63	SCM	58	61	0.90, 0.86, 0.83, 0.80, 0.76, 0.73, 0.69	NI	246
		SCC	124	62	0.87, 0.82, 0.78, 0.74, 0.69	SCC	92
		NI	199	63	0.80, 0.76, 0.65	SCC	134
25	0.77, 0.74, 0.69, 0.65	SCC	142, 143	64	0.80, 0.75, 0.65, 0.62, 0.59	SCC	133
26	0.82, 0.77, 0.73, 0.70, 0.59	SCC	71	65	0.83, 0.80, 0.76, 0.71, 0.66, 0.63	SCC	147
27	0.78, 0.75, 0.72, 0.64	NI	176	66	0.92, 0.86, 0.83, 0.73, 0.69, 0.64	SCM	64
28	0.78, 0.73, 0.70, 0.65	SCC	165, 166			NI	104
29	0.85, 0.82, 0.78, 0.71, 0.67, 0.62	SCM	61				
		NI	185				

[1]SCC: *Solanum commersonii commersonii*; SCM: *Solanum commersonii malmeanum*; SChM: *Solanum chacoense muelleri*; NI: non-identified clones.

Peroxidase electrophoretic patterns showed high intraspecific variability. Thirteen *S. commersonii malmeanum* clones and 30 non-identified clones reflected the highest number of individual patterns: twelve and 25, respectively. In 69 clones of *S. commersonii commersonii*, 44 patterns were observed. Juned et al. (1988) found various peroxidase, aspartate transaminase and phosphoglucoisomerase polymorphic bands, indicating great variability in *S. chacoense* from Paraguay and Argentina.

Fifteen anodic bands of aspartate transaminase were found in the fourteen patterns detected (Table 3). One to five bands per pattern were observed (Figure 2). As expected, the intraspecific variability found was lower than for peroxidase. Thirteen *S. chacoense malmeanum* clones and 30 non-identified clones

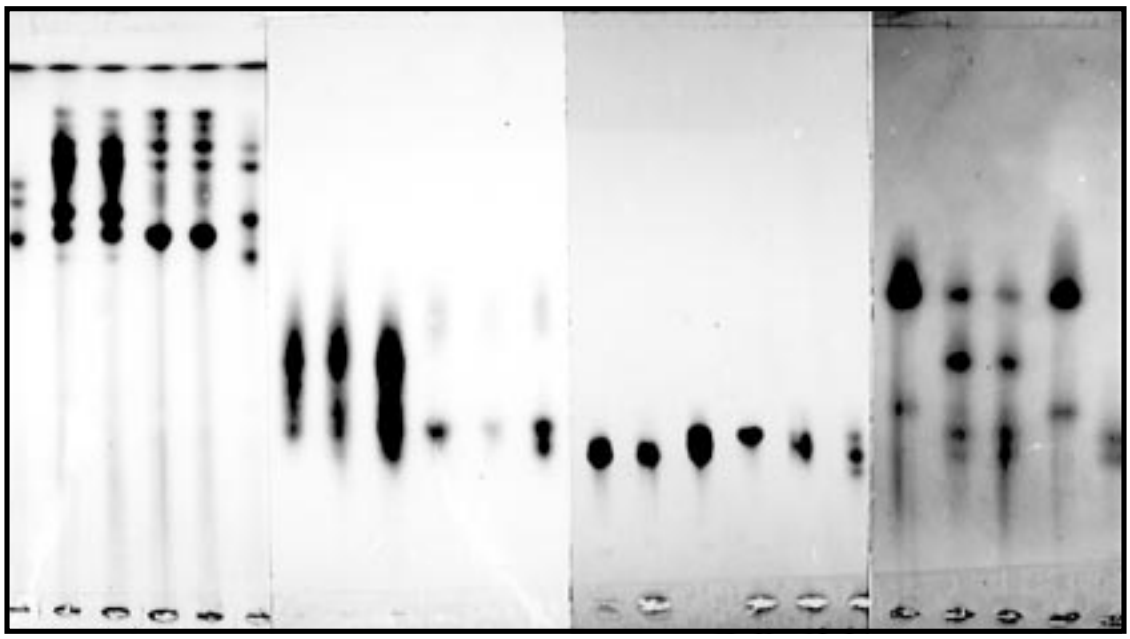

Figure 2. Isoenzymatic patterns of peroxidase, phosphoglucomutase, isocitrate dehydrogenase and aspartate transaminase in leaves of *Solanum* spp. clones.

showed, respectively, four and eleven patterns, while the *S. commersonii commersonii* clones presented eight patterns. All bands were polymorphic.

Seven phosphoglucomutase isoenzyme patterns were observed based on five anodic and polymorphic bands (Table 4). One to four bands were found in each pattern (Figure 2). No enzymatic activity was detected in some clones. Desborough (1983) stated that three distinct forms of phosphoglucomutase were found in potatoes. Suurs et al. (1989), however, observed two to five molecular forms in species of *Solanum* and *Lycopersicon*. Using locus Pgm-2, present only in the used as male parent species, Chien-An & Douches (1993) identified 50% of *S. tuberosum tuberosum* x *S. phureja* hybrids. This system, added to the other 12, was also used by Douches & Ludlam (1991) to characterize 112 potato cultivars and advanced lines obtained through hybridization, from North America. They concluded that electrophoresis is an efficient method to distinguish sexually originated genotypes.

The isocitrate dehydrogenase zimograms revealed eight anodic and polymorphic bands. One to three bands were detected in each pattern (Figure 2). This reduced number allowed the detection of only 12 patterns (Table 5). Douches et al. (1991) used this enzymatic system, as well as nine others, to examine phylogenetic relationships in 10 cultivars from North America, of historical importance in the last century. Twenty-seven alleles were found in 13 loci. Eleven heterozygotic loci were detected. Locus Idh-1, with two alleles, was monomorphic in just one of the analyzed genotypes, presenting a frequency of 63% for Idh-1[1].

Cluster analysis of the 113 wild potato clones, based on group I enzymatic systems, allowed their classification into 51 subgroups (Figure 3). Two groups, one consisting of just one clone, number 68 (*S. chacoense muelleri*) and another large group composed of two subgroups were observed. All but one access of *S. commersonii malmeanum*, seven of *S. commersonii commersonii* and 13 of the non-identified clones formed the smaller subgroup. The other subgroup was subdivided into two. One consisted of clone number 107 of *S. commersonii commersonii* and the other had 79 clones, including 61 of *S. commersonii commersonii*, 17 of the non-identified group and clone 108 (*S. commersonii*

Table 3. Aspartate transaminase isoenzymatic patterns detected in 113 *Solanum* spp. clones.

Pattern	Relative mobilities	Subspecies[1]	Clone
1	0.80, 0.68, 0.57, 0.51	SCM	2, 56, 65, 67, 69
		NI	21
2	1.00, 0.80, 0.57, 0.51	SCC	13, 30, 139, 141
		NI	194, 195
3	1.00, 0.77, 0.71, 0.65	SCC	39, 70, 72, 107, 128, 129, 131, 133, 136, 137, 142, 143, 159
		NI	148
4	1.00, 0.96, 0.92	SCC	3, 12, 18, 25, 26, 32, 36, 47, 54, 78, 116, 122
		NI	104, 112
5	1.00, 0.65	SCC	31, 34, 35, 45, 71, 73, 74, 75, 76, 85, 86, 92, 96, 97, 119, 125, 134, 140, 147, 150, 154, 156, 161, 165, 166, 167, 168, 183
		NI	179, 185, 188, 202, 203, 211, 214, 218, 246
6	0.57, 0.51	SCM	55, 58, 59, 61, 62
		SCC	7, 8, 124
		NI	10, 113, 176, 196, 199, 200, 204, 222, 225
7	1.00, 0.77	SCC	11, 15, 40, 77, 157
		NI	153
8	0.92, 0.86, 0.80, 0.71	SCM	108
		NI	105, 110
9	0.61, 0.51	NI	53
10	1.02, 0.92, 0.80, 0.61	SCC	117, 118, 120
11	1.00, 0.77, 0.71	NI	186
12	0.92, 0.74, 0.57	SCM	64, 109
13	0.92	SChM	68
14	1.00, 0.90, 0.80, 0.71, 0.68	SCC	158
		NI	127

[1]SCC: *Solanum commersonii commersonii*; SCM: *Solanum commersonii malmeanum*; SChM: *Solanum chacoense muelleri*; NI: non-identified clones.

malmeanum). Various clones formed subgroups which showed maximum similarity. The largest subgroup was composed of 15 accesses of *S. commersonii commersonii* and seven non-identified clones. Jaccard coefficients varied from 0.05 to 1.00.

Cluster analysis, based on all enzymatic systems studied, allowed the classification of the 113 clones into 89 subgroups (Figure 4). Two groups were found. The first one was composed of the majority of the *S. commersonii malmeanum*, eight *S. commersonii commersonii* and 13 non-identified clones. The other was subdivided into two, a small one composed of clone numbers 108 (*S. commersonii malmeanum*), 105, 110, 127 (non-identified), and 158 (*S. commersonii commersonii*) and a larger one which grouped 65.5% of total clones analyzed. The second group was consisted of only clone numbers 64 and 109 (*S. commersonii malmeanum*), and 68

Table 4. Phosphoglucomutase isoenzymatic patterns detected in 113 *Solanum* spp. clones.

Pattern	Relative mobilities	Subspecies[1]	Clone
1	1.22, 1.08	SCM	59, 62, 67
		SCC	36, 78, 107, 116, 122
2	1.14	SCC	11, 34, 35, 45, 85, 86, 134, 140, 157
		NI	10, 153, 185, 211
3	1.14, 1.00	SCM	108
		SCC	3, 12, 15, 18, 25, 26, 31, 32, 39, 40, 47, 54, 70, 71, 72, 73, 74, 75, 76, 77, 92, 96, 97, 117, 118, 119, 120, 125, 128, 131, 133, 136, 137, 142, 143, 147, 150, 154, 156, 158, 159, 161, 165, 166, 167, 168, 183
		NI	105, 110, 112, 127, 148, 179, 184, 186, 188, 202, 203, 214, 218, 246
4	1.32, 1.14	SCC	13, 30
		NI	195
5	1.32, 1.22, 1.14	SCM	55, 58, 65
		SCC	7, 8
		NI	196, 200
6	1.32, 1.22, 1.14, 1.00	SCC	139, 141
		NI	53, 176, 199
7	1.22, 1.14, 1.00	SCM	61
		SCC	124, 129
		NI	104, 113, 204, 222, 225

[1]SCC: *Solanum commersonii commersonii*; SCM: *Solanum commersonii malmeanum*; SChM: *Solanum chacoense muelleri*; NI: non-identified clones.

Table 5. Isocitrate dehydrogenase isoenzymatic patterns detected in 113 *Solanum* spp. clones.

Pattern	Relative mobilities	Subspecies[1]	Clone
1	0.87	SCM	55, 56, 59, 61, 64, 67, 69, 108, 109
		SCC	13, 30, 124
		NI	10, 21, 110, 113, 176, 204
2	0.94	SCC	35, 136, 137, 158
		SChM	68
		NI	127
3	1.00	SCC	70, 71, 73, 74, 75, 76, 85, 86, 96, 97, 125, 128, 129, 131, 133, 134, 140, 142, 143, 154, 156, 157, 159, 161, 165, 166, 183
		NI	104, 153, 179, 185, 186, 188, 202, 203, 211, 214, 218, 246
4	0.92, 0.87	SCM	2, 62
		SCC	3, 12, 18, 25, 26, 32, 47
		NI	112, 199
5	0.94, 0.87	SCC	167, 168
		NI	195, 196, 200, 222, 225
6	0.97, 0.87	SCM	58, 65
		SCC	7, 8, 107
		NI	53
7	1.00, 0.92	SCC	11, 15, 31, 34, 36, 39, 54, 116, 117, 118, 119, 120, 122
		NI	148
8	1.08, 0.94	SCC	40, 77, 78
9	1.00, 0.87, 0.74	SCC	150
10	1.00, 0.87, 0.77	SCC	92
11	1.00, 0.94, 0.87	SCC	45, 139, 141, 147
		NI	105, 194
12	1.08, 1.00, 0.92	SCC	72

[1]SCC: *Solanum commersonii commersonii*; SCM: *Solanum commersonii malmeanum*; SChM: *Solanum chacoense muelleri*; NI: non-identified clones.

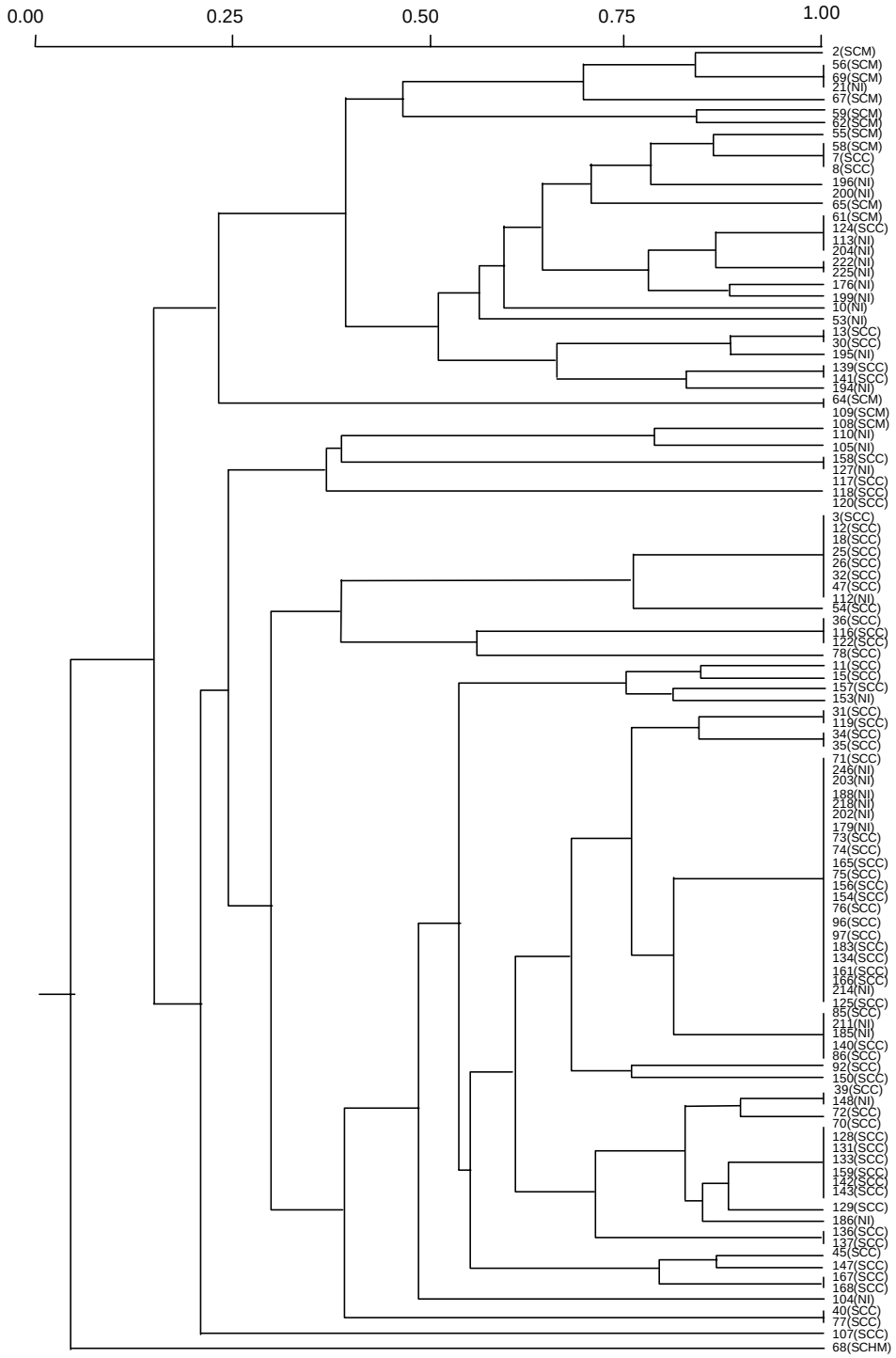

Figure 3. Dendogram based on electrophoretic patterns of group I enzymes (aspartate transaminase, phosphoglucomutase and isocitrate dehydrogenase) obtained from leaves of 113 *Solanum* spp. clones.

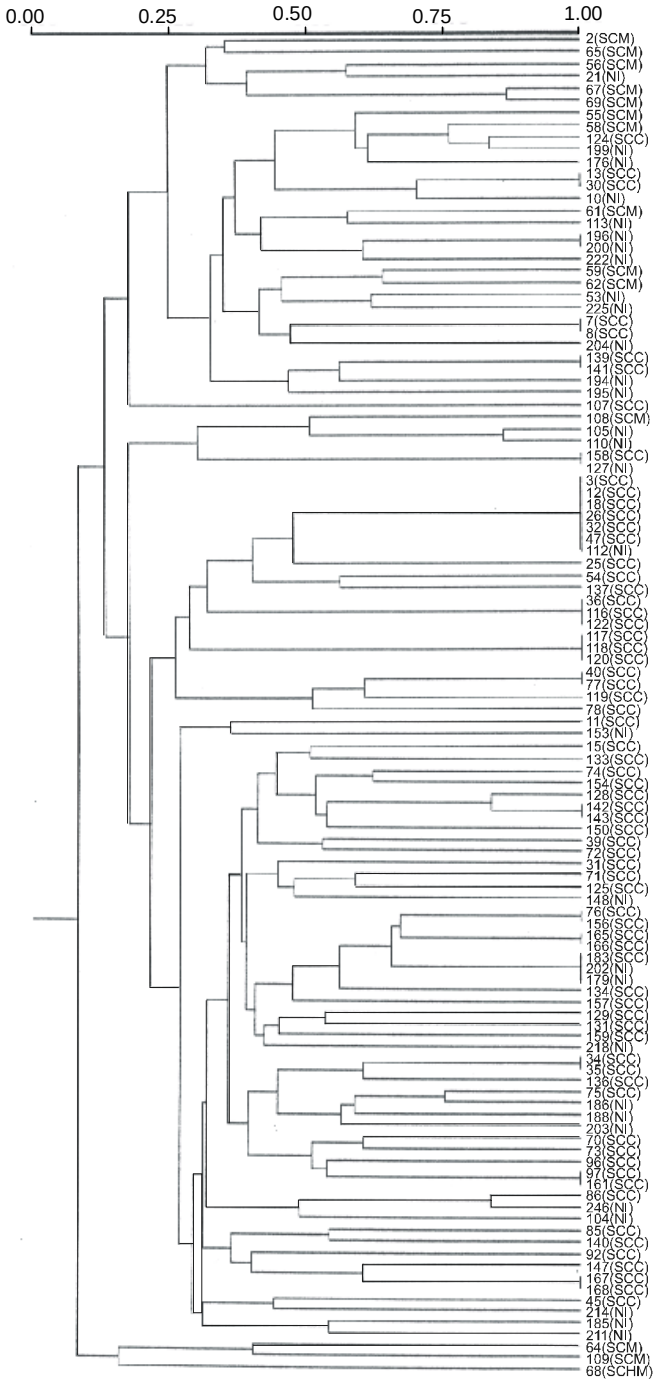

Figure 4. Dendogram based on electrophoretic patterns of group I and group II enzymes (peroxidase, aspartate transaminase, phosphoglucomutase and isocitrate dehydrogenase) obtained from leaves of 113 *Solanum* spp. clones.

(*S. chacoense muelleri*). Sixteen subgroups presented maximum similarity. Jaccard coefficients varied from 0.08 to 1.00.

Analysis of genetic similarity revealed a great variability in the potato clones, even when based only on group I enzymes. Jaccard coefficients varied from 0.49 to 0.97 when Samec & Nasinec (1996), using RAPD technique, identified and classified 42 wild and cultivated *Pisum sativum* genotypes. The authors concluded that some cultivars have common ancestors. In the wild potato clones analyzed in this work, coefficients varied from 0.05 to 1.00. Furthermore, the number of loci studied was lower, since isoenzymic analysis was used.

Douches & Ludlam (1991) were able to separate 112 potato cultivars into 95 groups, based on electrophoretic patterns. However, they used 13 enzymatic systems, including the ones used in this work.

As expected, a higher number of subgroups were formed when enzymes of group II were included in the cluster analysis (Figures 3 and 4). In addition, when peroxidase isoenzymes were considered in the analysis, clone numbers 64, 109 (*S. commersonii malmeanum*) and 68 (*S. chacoense muelleri)* were all included in the same group. Preferential clustering was maintained, with most of the *S. commersonii malmeanum* and *S. commersonii commersonii* clones included, respectively, in the upper and intermediate parts of the dendograms. *S. chacoense muelleri* clone remained in the lower part.

Conclusions

1. *Solanum* spp. has considerable genetic variability for isoenzymatic patterns.

2. Enzymes involved in peripherical metabolism present higher polymorphism level in wild potatoes than enzymes involved in energetic metabolism, glycolisis and citric acid cycle.

3. There is a great genotypic differentiation of *S. chacoense muelleri* in relation to *S. commersonii commersonii* and *S. commersonii malmeanum*.

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