Diversity in the wild potato species *Solanum chacoense* Bitt.

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Summary

The morphological and isozyme variation was studied in 22 accessions of *Solanum chacoense* from Paraguay and Argentina. Clear geographic groups were identified through the use of multivariate analyses. *S. chacoense* from mountain sites in Argentina could be readily distinguished from plains forms from Paraguay, on the basis of several correlated morphological characters. Three isozyme systems, namely phosphoglucose isomerase (PGI), glutamate-oxaloacetate transaminase (GOT) and peroxidase (PRX) were studied using starch gel electrophoresis. The banding patterns indicated that for each isozyme there were several loci, which were polymorphic. A genetic interpretation of one of the PGI loci was made, and indices of genetic diversity and genetic identity calculated. Principal components analysis, cluster analysis and genetic diversity indicated a close relationship between the geographical groups. These results are discussed in the context of *in situ* genetic conservation.

Introduction

Solanum chacoense Bitt. is considered by Hawkes & Hjerting (1969) to be the commonest, most aggressive and most adaptable of the South American wild potato species. It is found in Argentina, Paraguay, Uruguay and south-eastern Brazil, with some extension into central Bolivia, covering a wide range of phytogeographical regions and altitudes. It has been suggested that *S. chacoense* was once a native species of a much more limited area but that it has extended its range of habitats so that it now occurs as a weed of fields, of roadsides and waste places (Hawkes & Hjerting, 1969). An extensive altitude range is now covered by the species between sea level and 2400 m (Hawkes, 1978), but it may have once been limited to the plains and low foothill areas (to 1000 m), with an increase in the altitude range by secondary extension acquired through gene introgression from species of higher altitudes, such as *S. microdontum* (Hawkes & Hjerting, 1969).

This apparant introgression of alien germplasm has enabled *S. chacoense* to extend its range of biotypes and become adapted to a wider range of ecological conditions. Hawkes & Hjerting (1969) proposed that a varied series of *S. chacoense*-like biotypes were formed in the comparatively recent past which, through natural selection, were able to colonise new areas giving rise to a situation whereby a distinct series of forms exists, differing from the typical *S. chacoense*, yet linked by connecting intermediate forms. Although distinct, no constant combination of characters defines the biotypes or microspecies adequately. A well-marked pattern of variation has apparently arisen, only part of which can be explained by introgression, with characteristic regional forms evident in the north-west

mountain regions of Argentina, distinct forms in the Cordoba and San Luis provinces of Argentina, the more 'typical' form in the plains areas, and a variety of intermediate linking forms in the areas in between.

Although *S. chacoense* has a wide geographical range, the largest number of collections has been made in Argentina, particularly in the north-western provinces that are the most important regions for wild potatoes. Many of these provinces are mountainous with a great range of climatic and ecological conditions. *S. chacoense* is found here in a very wide range of habitats.

Hawkes & Hjerting (1969) and Hawkes (1978) concluded that the morphological variation observed in *S. chacoense*, particularly that between the plains and mountain forms, is largely genetically determined, with the maximum amount of variation to be found in the mountain regions. The taxonomy of *S. chacoense* is well understood, but details of its genetic diversity have not been studied. Wide genetic diversity is of great interest to plant breeders, and *S. chacoense* has itself attracted interest because of its reported resistance to more than 20 pests and diseases (Hawkes & Hjerting, 1969).

Wild potatoes are normally conserved as true seed accessions in gene banks. However, it is becoming increasingly clear that the *in situ* genetic conservation of wild species should also be considered, so that evolution can continue. This is 'dynamic conservation' (Guldager, 1975) as opposed to 'static conservation' in gene banks. The problem is to determine which populations of a species should be conserved *in situ*, based on studies of genetic diversity within species.

The components of genetic diversity include the kinds and numbers of alleles present, the heterozygosity, and correlation of alleles present between loci (Brown, 1978). Consequently, the extent of genetic diversity is difficult to assess accurately. An ideal method would be the direct study of DNA in species populations, but until this is fully possible, other methods have to suffice.

Morphological polymorphisms have been extensively used to study diversity. The methods of numerical taxonomy using computers have enabled larger amounts of data to be handled and analysed more thoroughly. However, only the expressed part of the genome can be measured. The contribution of individual genes cannot usually be detected, nor can it be ascertained to what extent they vary between individuals. The study of rare characters or the study of a range of more common characteristics governed by many genes that cannot be recognised individually or separated from environmental influence has been the usual method of the population geneticist (Gottlieb, 1981). In this paper, we report the results of a study of the morphological and isozyme variation in *S. chacoense* from Paraguay and Argentina, and the relationship between geographical distribution of the species and its genetic diversity.

Materials and methods

Five seeds each of 22 accessions of *S. chacoense* (Table 1) were germinated under sterile conditions, after surface sterilisation with 2% sodium hypochlorite. Seedlings were grown *in vitro* on Murashige minimal organic medium plus agar (0.8%) and sucrose (2.0%), pH adjusted to 5.6. Once they were well established in culture, the young seedlings were subcultured to provide identical genotypes both for the study of morphological variation in the screenhouse, and for the electrophoretic study of isozymes.

Multivariate analysis. After weaning off culture medium, young plants were transplanted to pots in the screenhouse, in a randomised complete block design with five replicate plants per accession, each group of five plants designated as a family. A total of 19 characters was measured, listed in Table 2, and standardised between plants.

The data were analysed by principal components analysis (PCA) and cluster analysis (euclidean distance plus Ward's method) using the Clustan 2 package (Wishart, 1978). Only 16 of the characters were used in these analyses. Ratios were included, but only one of the component values of each ratio was used.

Starch gel electrophoresis. Plant material from *in vitro* culture is ideal for use in starch gel electrophoresis of isozymes. It is tender and easy to macerate, and furthermore, *in vitro* culture can be used to provide a steady supply of plant material of the same genotype and of more or less the same age. Although sampling of leaves, petioles and stems from single plants gave similar isozyme banding patterns, only new leaf growth was used for comparative analysis between genotypes, since tissue specific differences have been reported (Van Den Berg & Wijsman, 1981). Pollen for isozyme study was obtained from fully, but freshly opened flowers.

In order to obtain consistent and reliable separations of isozymes it was necessary to undertake a preliminary screening for buffer systems, starch concentrations, staining recipes and variation in pH, as well as empirically determining the strength and duration of the electric field to be used for the different combinations of isozyme, buffer and starch concentration. Three isozyme systems, namely phosphoglucose isomerase (PGI), glutamate-oxaloacetate transaminase (GOT) and peroxidase (PRX) were chosen for study because they gave consistent results and required the same gel and electrode buffers. Recipes and electrophoresis conditions were those recommended by Shaw & Prasad (1970), Rick et al. (1977), Shields et al. (1983) and Vallejos (1983).

Samples were extracted in 0.1M Tris buffer (pH 7.2) with the addition of $50\mu l$ of 2-mercaptoethanol per 50ml of the final volume. Up to 25 samples were run in each gel, and extracts of *S. tuberosum* cv. Maris Piper were included as a reference control. Electrophoresis was carried out in 12.8%

Table 1. Provenance data for *Solanum chacoense* accessions used in this study.

PG No.[1]	Family	Country[2]	Province	Department	Altitude (m)	Collectors' No.
0502	3	PAR			c100	Bordas
0506	5	PAR			c100	Bordas 2A
0496	7	PAR			c100	Bordas
0572	9	PAR			c100	Bordas
0426	13	PAR			c100	Bordas
0505	19	PAR			c100	Bordas
0509	21	PAR			c100	Bordas 4
0445	12	ARG	Jujuy	Capital	1750,	HP & R56
0439	15	ARG	Jujuy	Capital	1750	HP & R56
0353	16	ARG	Jujuy	Tilcara	2300	P & H P-349
0564	2	ARG	Salta	Guachipas	1100	HH & R3629
0412	8	ARG	Salta	Guachipas	c1100	
3049	17	ARG	Salta	Chicoana	2400	HH & R3706
0570	18	ARG	Salta	Chicoana	1700	HH & R3700
0519	20	ARG	Salta	Chicoana	1500	HP & R295
0565	4	ARG	Cordoba	Punilla	1550	HHO & R3297
0562	10	ARG	Cordoba	Punilla	850	HH & R3305
0544	14	ARG	Cordoba	San Javier	650	HH & R3198
0561	22	ARG	Cordoba	Cruz de Eje	1700	HH & R3327
0552	1	ARG	San Luis	Chacabuco	900	HH & R3194
0559	6	ARG	San Luis	La Capital	900	HH & R3163
0525	11	ARG	San Luis	La Capital	850	HH & R3158

[1] University of Birmingham Potato Germplasm No.

[2] PAR Paraguay; ARG Argentina.

starch gels with a Tris-citric acid buffer (pH 7.8), at a constant current of 20mA for $5^{1}/_{2}$ hours at 4°C in the refrigerator. Staining was carried out following the methods of Vallejos (1983) for GOT and PGI, and Shaw & Prasad (1970)for PRX. After staining, the gels were washed in tap water and placed in 50% v:v glycerol. Banding patterns were compared, but for PGI only was a genetic interpretation possible. For this isozyme, the genetic diversity (He) within accessions and the genetic identity (I) amongst the families (Nei, 1972) were calculated.

Results

Morphology. Mean values, coefficients of variation (CV%), and variances for each character are shown in Table 2. There were significant differences between families for all characters ecxept leaflet length. The results of the principal components analysis for family means are shown in Fig. 1. The first five eigen vectors accounted for 82.6% of the variation, with Components 1 and 2 accounting for 37.5% and 19.7% respectively. The most important character on Component 1 was plant height (variance of 0.370), but there were significant contributions from pedicel articulation position (−0.358), the pedicel ratio (−0.346), the leaflet length/breadth ratio (0.344) and leaflet breadth (−0.337). On Component 2 the main characters were leaf length (0.454), calyx lobe length (0.394), corolla lobe breadth (0.363), calyx acumen length (0.338) and days to flower (−0.336). Several groups of families were indicated by this analysis.

The cluster analysis gave four main groups, with the lowest of the subdivisions at a dissimilarity coefficient of 5.405; these groups are plotted on

Table 2. Simple statistics and variances of 19 quantitative characters studied in 22 accessions of *Solanum chacoense* (n = 110). Morphological characters measured in cm.

Character	Mean	C.V.%	Variance between	within accessions	F-ratio Significance
			(21 d.	(88 d.f.	
Days to flowering	69.18	6.68	106.900	45.800	* * *
Pedicel articulation	1.00	20.22	0.207	0.037	* * *
Pedicel ratio	0.49	29.42	0.103	0.011	* * *
Corolla lobe length	1.08	13.29	0.103	0.040	* * *
Corolla lobe breadth	1.06	16.68	0.172	0.049	* * *
Corolla lobe l/b	1.06	11.72	0.078	0.040	* *
Calyx acumen length	0.09	56.99	0.117	0.001	* * *
Calyx lobe length	0.30	21.93	0.213	0.004	* * *
Calyx lobe breadth	0.25	13.95	0.006	0.001	* * *
Calyx lobe l/b	1.23	19.83	0.298	0.082	* * *
Stigma extrusion	0.40	26.60	0.057	0.007	* * *
Leaf length	16.15	13.47	23.700	10.600	* * *
Leaf breadth	7.95	33.68	5.060	2.680	* *
Leaf l/b	2.05	12.30	0.318	0.054	* * *
Leaflet length	3.54	10.57	0.700	0.511	NS
Leaflet breadth	1.65	20.80	0.589	0.153	* * *
Leaflet l/b	2.23	17.20	0.741	0.084	* * *
Petiolule length	0.37	39.32	0.108	0.033	* * *
Plant height	56.02	42.62	2850.000	213.000	* * *

* *   Significant at 5% level.
* * * Significant at 1% level.
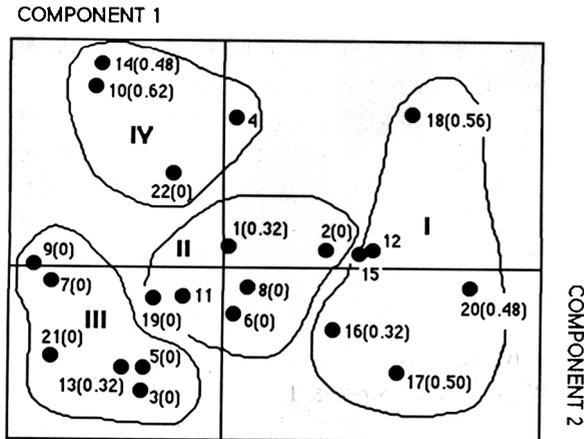NS Not significant.

COMPONENT 1

Fig. 1. Scatter diagram obtained from the first two components of principal components analysis, expressing the morphological variation in 22 families of *Solanum chacoense,* based on mean values. The groups I-IV were determined through cluster analysis. Points on the scatter diagram are identified by the family number, and values in brackets are indices of genetic diversity, He, based on allozyme variation at the PGI-B locus.

Fig. 1, and confirm the groups from principal component analysis. Group I consists of high altitude families (above 1500 m) from the provinces of Salta and Jujuy in Argentina. All plants had mean heights over 70cm, possessed short pedicels with the articulation above the mid-point, and had long calyx acumens. The families from Group II came from Paraguay as well as the San Luis and Salta provinces of Argentina. However, none of these families came from sites higher than 900 m. It is interesting to note that Group III consists only of those families whose origin is Paraguay; all have mean heights less than 45cm, and all but Family 5 were shorter than 35cm. In addition they had pedicel lengths in excess of 1cm, and articulations below the mid-point of the pedicel. All had short, almost rudimentary calyx acumens. Group IV consists of those families from the Cordoba province of Argentina, found at a range of altitudes up to 1700 m, and possessing a wider range of character variation than the two previous groups.

Isozymes

Phosphoglucose isomerase (PGI)

Staining for PGI produced three anodal zones of activity. The analysis of PGI in pollen indicated a dimeric structure, confirming the results of other workers in several different species (Weeden & Gottlieb, 1979; Gottlieb, 1981; Gottlieb & Greve, 1981; Staub et al., 1984). A fast-migrating band with a mobility of 0.57 was deemed to be equivalent to the PGI-A locus of cultivars reported by Martinez-Zapater & Oliver (1986). This appears to be a plastid enzyme (Weeden & Gottlieb, 1979; Martinez-Zapater &Oliver, 1986). The Rf values of the seven bands in *S. chacoense,* and those of the bands from cv. Maris Piper coincided generally with those reported by Martinez-Zapater & Oliver (1986). Four of these were electromorphs with Rf values of 0.45, 0.39, 0.33 and 0.28, and the three other bands were presumed heterodimeric bands with Rf values of 0.41, 0.36 and 0.31. The reactivity of this zone of bands, designated PGI-B, was uniform and easily studied. A third set of bands (PGI-C) was sometimes observed, but not studied due to poor resolution on many of the gels.

Five allozyme phenotypes were indicated by interpretation of the different banding patterns observed for PGI-B (Fig. 2 and Fig. 3). This interpretation can be confirmed positively through crossing experiments and analysis of segregation of banding patterns, but it provides a working hypothesis on which to base the following observations. The five allozyme variants can be designated as genotypes *aa, ab, bb, bc* and *ad.* If the definition of a polymorphic locus is taken to be one at which the most common allele has a frequency less than 0.99 (Gottlieb, 1981), then eight of 17 families were polymorphic for the PGI-B locus. However, the number of families polymorphic for this locus varied with geographical area. In the plants from Paraguay, for example, only one family from six (17%) showed polymorphism at this locus, whereas the figure rises to 60% for the families from Salta. For plants from Cordoba, the figure is higher still at 66%.

Taking all the plants as one population, only one allele, *d,* can be considered as rare, occurring at a
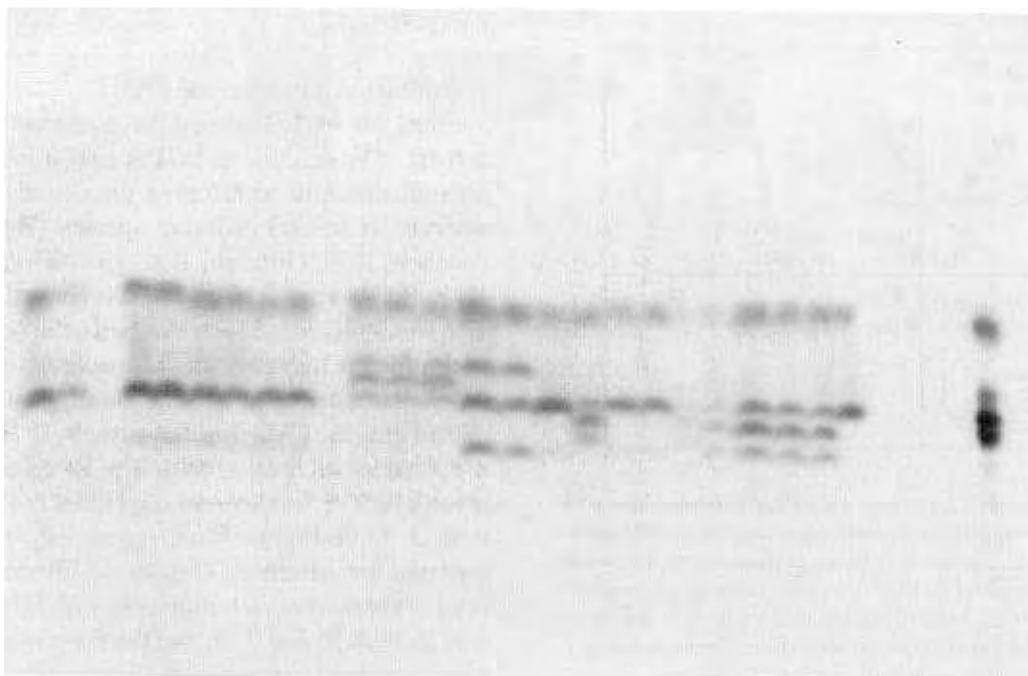
Fig. 2. Allozyme phenotypes at the PGI-B locus in accessions of *S. chacoense* from Argentina. These are diagrammatically shown in Fig. 3. Each accession is represented by allozyme profiles from five individual plants. Two profiles have not stained sufficiently for photographic purposes. The anodal front is at the top of the figure, and the monomorphic PGI-A locus is closest to the anodal front. The reference bands from cv. Maris Piper can be seen as a separate profile to the right hand side. The allozyme phenotypes in this figure represent genotypes *ab, ad, bb* and *bc*. From left to right, the first eight profiles are *bb*, the next three are *ab*, the next two are *ad*, followed by *bb, bc* and two *bb*. All the rest are *bc*, except the last profile which is *bb*.

frequency of 0.01. It was identified in plants from the Cordoba region, at a frequency of 0.07. Only the *b* allele had a frequency greater than 0.8. The least diversity was shown by plants from Paraguay (He≤0.32), and as a geographical group the diversity was He = 0.13. The highest values shown by individual families were He = 0.62 (Family 10 from Cordoba), and 0.56 and 0.50 for Families 17 and 18 respectively from Salta. These two areas also had the highest values for geographical groups of He = 0.55 and 0.44 respectively. The genetic diversity values are also plotted in Fig. 1.

The index of genetic identity (I) can range from one (complete genetic identity) to zero (complete genetic differentiation) according to Nei (1972). Amongst the families included in this study, I = 0.96 for PGI-B, a value comparable to I among populations of a single species (Gottlieb, 1981).

Glutamate-oxaloacetate transaminase (GOT) and Peroxidase (PRX)

For both these isozymes, the resolution of the banding patterns was variable, and their genetic control is not known. A tentative interpretation for GOT indicates that there are two loci, and that each is polymorphic. Pollen was analysed in an attempt to understand the allelic situation but either the pollen did not contain GOT or the staining system failed to resolve any of the GOT bands. PRX had the most complex yet least useful patterns for the purposes of this study. However, it is clear that there were several polymorphic loci.

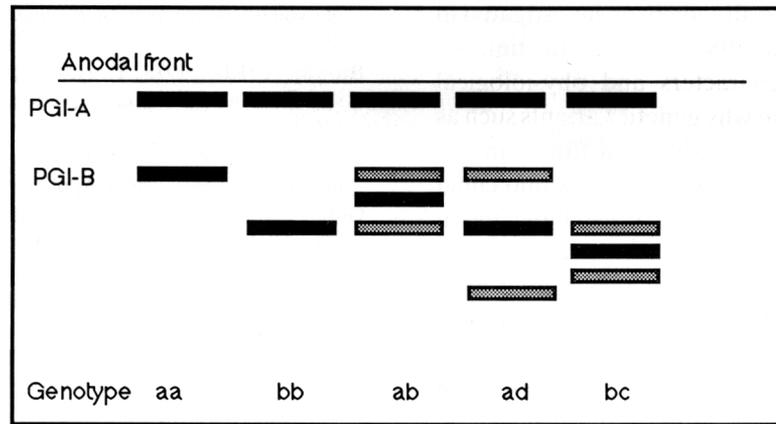### Discussion

In this study of *S. chacoense*, morphological and

Fig. 3. Diagram of the five allozyme phenotypes at the PGI-B locus in *S. chacoense* from Argentina and Paraguay, together with their proposed genotypes.

isozyme data have been used to study the extent of genetic diversity within a sample of germplasm from Argentina and Paraguay. Although the materials studied here may not be representative of *S. chacoense* throughout its entire distribution in South America, the results do show that the diversity in this species is not distributed uniformly.

The taxonomy of *S. chacoense* has been treated in depth by Hawkes & Hjerting (1969) who described 11 microspecies. Furthermore, the considerable morphological variation was explained in terms of the introgression into *S. chacoense* by other wild potatoes, such as *S. microdontum,* so that high altitude forms can be distinguished from non-introgressed plains forms by a number of well-marked features, yet remain linked by a range of intermediaries. Certainly the families from Salta in this study can be distinguished from those from Paraguay on the basis of plant height, position of the pedicel articulation, length of the petiolule and length of calyx acumen, as also shown by the work of Hawkes (1962).

The theme of introgression cannot explain, however, the range of morphological variation in the area of Cordoba, Argentina. Hawkes & Hjerting (1969) state that the distinct forms from Cordoba were a natural development of the species and not due to introgression, as were the forms from San Luis. However, in the present study, forms from Cordoba were distinctly grouped, whilst plants

from San Luis were intermediate in their characteristics. If introgression cannot explain all the variation seen in morphology, is there any other explanation? Hawkes & Hjerting (1969) again have a possible solution, which is that *S. chacoense* found in these areas may represent the most primitive part of the species derived from an ancestral form in the north-west provinces of Argentina. They postulated that the primitive form was retained in the Cordoba and San Luis regions, but was lost as the species became adapted to the conditions of north-eastern Argentina and Paraguay. Furthermore, *S. chacoense* occurs in the Cordoba and San Luis regions as a plant of natural vegetation, whereas elsewhere the species is generally a ruderal or weed of cultivation.

Analysis of the morphological variation in *S. chacoense* in this study produced groupings which resembled those described above. The isozyme variation is therefore of particular interest. Although analysis of the isozymes was limited, geographical groupings were indicated. Plants from Paraguay had the lowest measure of genetic diversity at the PGI-B locus, with the greatest variation in plants from Salta and Cordoba. The relationship between the PCA scatter and the genetic diversity (He), shown in Fig. 1, is interesting and worthy of further investigation. An association between the PGI-B locus and certain quantitative characters such as plant height may be indicated.

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Such associations have already been investigated in wild plants (Van Dijk, 1984). Any connection between biochemical characters and physiological characters may explain why genetic variants such as microspecies seem to have different fitness in different environments. Regional diversity and clinal variation according to altitude are also indicated in this study.

The distribution of genetic diversity may have consequences for the *in situ* conservation of *S. chacoense*, should it ever be possible, or deemed necessary. It is unfortunate, but generally the situation that not all of the areas where a wild species of importance is found can be preserved. Data concerning the pattern of genetic diversity within the species, and its correlation with environment, as shown by the work of Nevo and his colleagues working on wild barley in Israel (Nevo et al., 1979) are necessary for the formulation of sound conservation strategies. It is also important to consider the processes whereby continuing adaptive changes can occur, as in dynamic conservation (Guldager, 1975). Much more needs to be known about the precise reasons for genetic variation in the different populations of *S. chacoense*. This must be based on fieldwork, and the analysis of much larger population samples. Nevertheless, the present study has provided an interesting insight into the nature and distribution of diversity in this species, which might serve as a basis for future studies of the variation in wild species of potatoes.

Acknowledgements

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