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Allozyme polymorphisms in tetraploid potato gene pools and the effect on human selection

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Abstract The need for broadening a crop's genetic base may be determined by comparing allele frequencies within the gene pools of farmer selections in their centers of diversity with that of modern breeding populations. The genetic structure of Andean and Chilean potato farmer selections was investigated with the aid of nine isozymes, which have been studied in detail and used to characterize North American cultivars and advanced breeding lines. These isozymes are associated with the most-important agronomic or quality characters in the North American gene pool. By comparing these data with previous analyses of the North American gene pool, allozyme frequency changes for nine loci were monitored. Allozyme frequency changes were not always due to genetic drift, but resulted also from directional selection of isozyme marker linked quantitative trait loci (QTLs) affecting agronomic or quality characters. Changes in allozyme frequency can also occur as a consequence of pleiotropy, i.e. the isozyme itself may be involved in the expression of a phenotype. These allozyme frequency changes may reflect the manipulation of the potato genome by breeders. There were allozymes in some North American cultivars that were not observed in the farmer selections from the Andes and Chile. This confirms that breeders have already introgressed exotic genes from wild and other primitive cultivated tuber-bearing *Solanum* species. On this basis, the need for broadening the genetic base for specific chromosomes (or chromosome regions) should be based on analysis with these and other genetic markers available in potato.

Keywords Genetic erosion · Isozymes · Landraces · *Solanum tuberosum*

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Introduction

The potato (*Solanum tuberosum* L.) is one of the major food crops grown world-wide, with an annual world production of around 275-million tonnes and an area of about 18-million hectares . At present, 69% of the global production is in developed countries and 31% in developing countries (CIP 1995). There are in excess of 200 wild and cultivated tuber-bearing *Solanum* species, but the most popular potato belongs to the tetraploid species *S. tuberosum* (Hawkes 1990; Ochoa 1998) There are two subspecies: *S. tuberosum* ssp. *andigena*, which is cultivated throughout the Andes, and *S. tuberosum* ssp. *tuberosum*, which was domesticated in Chile.

The primary center of diversity for cultivated potatoes is located in the highlands of Andean South America. Farmer-selected cultivars of *S. tuberosum* ssp. *andigena* are most widespread in the highlands of Mexico, Guatemala, Venezuela, Colombia, Ecuador, Peru, Bolivia and northern Argentina, where altitudes range from about 2,000 to 4,000 masl. The Chiloé Archipielago of Chile is a secondary center of diversity where hundreds of farmer-selected cultivars of *S. tuberosum* ssp. *tuberosum* exhibit adaptation to long photoperiods and grow under cool environments at or near sea level (Huaman 1998).

The potato was introduced into Europe by the Spaniards in the 16th century, where it was adapted to the long summer days, and was then taken by European immigrants to North America in the 17th century (Simmonds 1995). During the mid-19th century, potato

crops were severely attacked throughout Europe by the fungal disease late blight [*Phytophthora infestans* (Mont.) de Bary], perhaps owing to the crop's genetic uniformity (Brown 1993). This disease was most devastating in Ireland, where a six-fold population growth in around half a century (1790–1845) had been partially sustained by this crop (Brown 1993). One million Irish people starved and two million emigrated as a consequence of the late blight famine of the 1840s. After this epidemic, new resistant cultivars were developed based on the introgression of exotic germplasm into the cultivated gene pool adapted to these northern latitudes on both sides of the Atlantic.

Progress in plant breeding is highly reliant on the effective use of appropriate genetic diversity in the respective crop gene pool. Genetic markers offer a precise means for an analysis of the genetic base of crops. For example, isozymes have been informative markers for assessing diversity in Andean potatoes (Quiros et al. 1992; Zimmerer and Douches 1991) and for determining rates of out-crossing between primitive cultivated potatoes (Rabinowitz et al. 1990). Isozymes have also been used to characterize US potato cultivars available in the 19th century (Douches et al. 1991) and 112 North American cultivars and advanced breeding lines (Douches and Ludlam 1991). In addition, some researchers have reported that certain isozymes are associated with the most important agronomic characters in potato segregating populations (Ortiz et al. 1993; Freyre and Douches 1994; Freyre et al. 1994).

The aims of our research were (1) to determine the allozyme frequency for nine isozyme loci in the *andigena* and *tuberosum* gene pools, and, (2) to monitor the effects of potato breeding in North America using these genetic markers by comparing the allozyme frequency in these gene pools with that of North American cultivars whose allozyme frequencies have been determined previously (Douches and Ludlam 1991; Douches et al. 1991). This information will help to determine the need for broadening the genetic base of potato breeding in North America.

Material and methods

A total of 2,379 Andean farmer-selected *andigena* cultivars collected throughout Latin America and 120 *tuberosum* cultivars native to the Archipielago of Chiloe in Chile, were included in this study. These cultivars are preserved in the field genebank of the International Potato Center (CIP), near Huancayo (3,200 m) in Perú, and duplicate sets of tubers are held in cold storage at 4°C at La Molina (near Lima, Perú). Additional duplicates of these genetic resources are conserved in La Molina and in Quito (Ecuador) as in vitro collections.

Allozyme diversity in these cultivars was determined using horizontal gel electrophoresis and two buffer systems (Histidine-citrate at pH 5.7 and Lithium-borate at pH 8.3). The procedures for tissue processing, electrophoresis, gel staining and allozyme scoring were those of Douches and Quiros (1988). Nine isozyme loci of six enzyme systems (Table 1) were resolved without progeny testing following the protocol of Douches and Ludlam (1991).

Most of these isozyme loci (except *Mdh-2)* have been mapped in potato. The gene-centromere mapping distances have also been reported for *Idh-1*, *Pgm-2*, *Mdh-1*, *Pgi-1* and *Got-1*, by half-tetrad analysis. *Got-1* and *Pgm-2* are close to the centromere (0.9 and 1.96 cM, respectively), while *Idh-1* and *Pgi-1* are farther from the centromere (18.36 and 26 cM, respectively). *Mdh-1* appears to be at 33.5 cM from the centromere, and should exhibit chromatid segregation (Douches and Quiros 1988; Ortiz 1998).

Allozyme frequencies (q) were calculated for each isozyme locus in both the entire and core collections (Weir 1996). Standard errors (SEs) for the frequency of the most-common allozymes for each isozyme locus were calculated for each gene pool as $SE=[q\times(1-q)/4\ N]^{0.5}$ where N is the number of accessions scored. Statistical differences between the most-common allozymes were determined by non-overlapping confidence intervals at P=0.05. The homogeneity of the allozyme frequency distributions between gene pools was determined by χ^2 tests.

Results

There were more allozymes in the Andean cultivars than in the other potato gene pools (Table 1). The number of allozymes according to their gene frequency was significantly different among some gene pools as determined by χ^2 tests of homogeneity between samples (Fig. 1). There were significant differences between the 19th century US cultivars and farmer selections in the Andes (χ^2=12.565; P=0.006) or Chile (χ^2=8.562; P=0.035), as well as be-

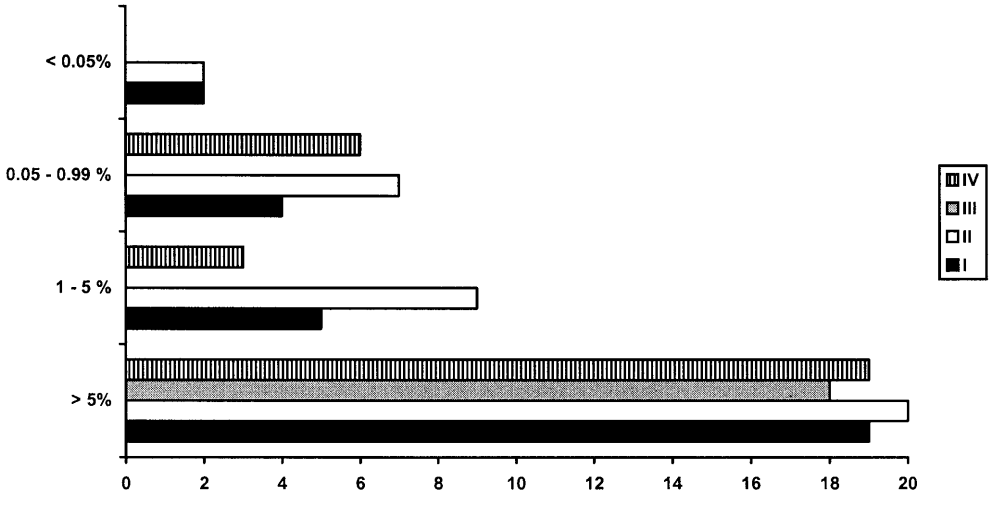

Fig. 1 Allozyme number according to gene frequency (in percentage) among Chilean (*S. tuberosum* ssp. *tuberosum*) (*I*) and Andean (*S. tuberosum* ssp. *andigena*) (*II*) farmer-selected tetraploid potatoes, tetraploid potato cultivars grown by US farmers in the 19th century (*III*), and currently available North American tetraploid cultivars and advanced breeding lines (*IV*)

Table 1 Isozyme loci assayed in tetraploid potatoes from the Andes, Chile and North America.

Buffer system	Isozyme	Genetic symbol	Chromosome
Histidine-Citrate (pH 5.7)	Isocitric acid dehydrogenase 1	*Idh-1*	I
	Malate dehydrogenase 1	*Mdh-1*	VII
	Malate dehydrogenase 2	*Mdh-2*	Unknown
	Phosphoglucose isomerase 1	*Pgi-1*	XII
Tris-borate (pH 8.3)	Diaphorase 1	*Dia-1*	V
	Glutamate oxaloacetate transaminase 1	*Got-1*	VIII
	Glutamate oxaloacetate transaminase 2	*Got-2*	VII
	Phosphoglucomutase 1	*Pgm-1*	III
	Phosphoglucomutase 2	*Pgm-2*	IV

Table 2 Allozyme frequencies for nine isozyme loci among Chilean (*S. tuberosum* ssp. *tuberosum*) and Andean (*S. tuberosum* ssp. *andigena*) farmer selected tetraploid potatoes, tetraploid potato cultivars grown by US farmers in the 19th century, and currently available North American tetraploid cultivars and advanced breeding lines. Data for the 19th century US and North American potato cultivars was previously published elsewhere (Douches and Ludlam 1991; Douches et al. 1991). Most common allozyme frequencies followed by the same letter within the same row were not significantly different as determined by their overlapping confidence intervals at 95% (or P=0.05)

Isosyme	Allele	*tuberosum*	*andigena*	USA 19th Century	North America
Idh-1	*1*	0.6833 b	0.8408 a	0.630 b	0.644 b
	2	0.3145	0.1451	0.370	0.351
	3		0.0002		
	4	0.0021	0.0138		0.005
Mdh-1	New[a]		0.0013		
	1	0.2125	0.3308	0.175	0.116
	2	0.6958 a	0.6182 b	0.650 ab	0.634 b
	3	0.0354	0.0257	0.100	0.093
	4	0.0562	0.0240	0.075	0.157
Mdh-2	*1*	0.1146	0.1851		0.013
	2	0.8854 c	0.8087 d	1.000 a	0.964 b
	3		0.0062		0.023
Pgi-1	*1*	0.0083	0.0043		0.003
	2	0.8542 c	0.8012 d	1.000 a	0.966 b
	3	0.0083	0.0082		
	4	0.1292	0.1861		0.026
	5		0.0002		0.005
Dia-1	*0*	0.0375	0.0162		0.005
	1	0.6875 bc	0.6472 c	0.800 ab	0.837 a
	2	0.2750	0.3331	0.200	0.158
	3		0.0035		
Got-1	*1*	0.0062	0.0860		
	2		0.0102		
	3	0.8417 a	0.8077 a	0.650 b	0.670 b
	4	0.1250	0.0631	0.350	0.330
	5	0.0271	0.0320		
	6		0.0010		
Got-2	*2*				0.008
	3	0.1292	0.0628	0.250	0.292
	5	0.8375 a	0.8302 a	0.750 ab	0.700 b
	7	0.0333	0.1070		
Pgm-1	New	0.0062	0.0431		
	1	0.2646	0.0870	0.350	0.376
	2	0.1312	0.2087		0.106
	3	0.5979 b	0.6612 a	0.650 ab	0.515 b
	4				0.003
Pgm-2	*1*		0.0166		
	2	0.9688 a	0.9714 a	0.880 ab	0.830 b
	3	0.0021	0.0101	0.120	0.170
	4	0.0291	0.0019		

[a] New allozymes exhibiting migration speeds distinct to those previously reported in a specific locus whose genetic designation awaits review by potato geneticists

tween these 19th century US cultivars and the cultivars currently available for cultivation in North America (χ^2=7.193; P=0.006). However, there were non-significant differences in the number of allozymes according to their gene frequency among *andigena*, *tuberosum* from Chile, and the whole North American gene pool.

The frequency of the most-common allozymes was statistically similar between *andigena* and Chilean *tuberosum* for four isozyme loci (*Dia-1*, *Got-1*, *Got-2*, and *Pgm-2*). In contrast, only the frequency of the most-common allozyme for the *Mdh-1* locus was similar between *andigena* and the North American cultivars (Table 2).

Table 3 Allozyme polymorphisms associated with significant variation in agronomic or tuber quality characters in segregating potato populations

Isozyme/ allozyme	Significant morphological variation putatively associated with the genotype locus in a respective isozyme locus
Idh-1^2	Earliness in long-day environments
Idh-1^3	Rare allozyme in *andigena* lost from other gene pools of higher latitudes, thus suggesting an irrelevant role for crop adaptation to these locations
Mdh-1	Overdominance of this locus associated with high tuber yield. Likewise a QTL for chipping quality (or tuber reducing sugars) mapped to the same chromosome VII
Mdh-2^2	Virtually fixed in North American potatoes, which suggests either enhanced adaptation or founder effect
Pgi-1	On chromosome XII, which also bears genes for resistance to potato virus×(*Rx*), cyst nematode (Gpa_2), and the QTL for glykoalkaloid solasodine
Dia-1	On chromosome V, which also bears a cluster of resistance genes for late blight (R_1), potato virus×(*Nb*), golden nematode (H_1), and the QTL for insect resistance
Dia-1^1	High tuber dry matter
Got-1	Overdominance at this locus associated with high tuber yield
Got-1^1	Short tuber dormancy
Got-1^4	Production of 2n pollen by parallel spindles (*ps*)
Got-2^7	Low tuber dry matter and short tuber dormancy
Pgm-1^1	High tuber dry matter in North American cultivars
Pgm-2	Tuber yield and weight associated with overdominance at this locus
Pgm-2^3	Long tuber dormancy

The frequency of the most-common allozymes for *Idh-1* and *Pgm-1* were statistically similar for *tuberosum* from Chile and the North American gene pool. As expected, the frequency of the most-common allozyme was similar for seven isozyme loci between the US cultivars of the 19th century and the whole North American gene pool included in this investigation. Significantly different most-common allozyme frequencies were observed between 19th century and the sample of North American cultivars for the two isozyme loci that did not show polymorphism in the 19th century cultivars. The most-common allozyme frequency of the *Got-2* locus was similar among the 19th century US cultivars and the farmer selections from Latin America. Likewise, the most-common allozymes for *Pgm-2* and *Mdh-1* had similar frequencies between Andean cultivars and 19th century US cultivars.

By comparing our data with previous reports (Table 2) we were able to monitor changes in *q* for nine loci. The genetic base of the North American gene pool at the beginning of potato breeding in the 19th century appears to have been narrow, based on the number of alleles per polymorphic locus and the frequency of the most-common allozymes (Table 2). Two isozyme loci, *Pgi-1* and *Mdh-2*, have fixed genotypes in the examined North American cultivars of the 19th century, while there was a significant increase of the most-common allozyme frequency in the *Dia-1* locus as compared to that of the Latin America gene pools (Table 2). Similarly, the most-common allozyme frequency in the other five isozyme loci diminished significantly in the North American gene pool. Only, the most-common allozyme frequency in *Mdh-1* locus remained constant between Andean and North American potatoes.

Discussion

Changes in allozyme frequency reflect the manipulation of the potato genome by plant breeders in North America. They started with a narrow genetic base, which consisted mainly of a few well-adapted genotypes, perhaps some northern cultivars that survived the late blight epidemics, plus exotic germplasm brought especially from Chile. In this century, a few wild species were introgressed or incorporated into the northern-hemisphere breeding populations in order to develop cultivars resistant to diseases and pests (Ross 1986; Ortiz 1998).

Selection for specific characters also accounts for changes in allozyme frequency (Table 3). For example, the *Pgm-1* locus has been associated with dry matter and chipping ability in potato tubers, whereas changes in the genotype of the *Idh-1* locus are correlated with variation for maturity in the long day length environments of the northern hemisphere (Ortiz et al. 1993). Similarly, a heterozygous genotype for *Mdh-1* appears to be associated with an increase in tuber yield (Ortiz et al. 1993), which may explain why the most-common allozyme frequency does not change at this locus owing to the influence of overdominance, i.e. selection favouring heterozygotes. Likewise, the change in *Dia-1* may be associated with breeders' manipulation of chromosome V that bears a cluster of genes conferring resistance to late blight, potato virus X, nematodes and insects (Ortiz 1998).

Examination of the total number of alleles across potato gene pools shows that 12 out of 38 alleles observed in *andigena* potatoes were lost from the North American gene pool (Fig. 1).This genetic erosion occurred in North American cultivars when the allozyme frequency was mostly below 0.05 (or 5%), except for *Got-1*^{1} and *Got-2*^{7}, whose *q* values are respectively 0.09 and 0.11 in the Andean gene pool. Surprisingly, two allozymes not observed in the Andean or Chilean gene pools in the *Got-2* and *Pgm-1* loci were found among North American cultivars, although at a low frequency (<0.01 or 1%). These two unexpected allozymes could have been incorporated during the intro-

gression of chromosome segments from other wild tuber-bearing *Solanum* species. This incorporation of diploid *Solanum* genetic resources by sexual tetraploidization may also account for the sudden increase of *Got-1*^{4}, an allozyme that has been linked to the parallel spindle gene (*ps*) that controls the production of 2n pollen in potato (Ortiz et al. 1993). These gametes with a sporophytic chromosome number are needed to obtain tetraploids after tetraploid-diploid crosses in potato (Ortiz 1998).

Genes for tuber characters such as dry matter and size appear to be close to the centromere (Tai and De Jong 1997). It has been suggested that loci for tuber yield are scattered between the centromere and the site of maximum recombination, though heterotic loci for high yield are close to the centromere (Tai and De Jong 1997) or between centromeres and proximal crossovers (Buso et al. 1999a, b; Peloquin et al. 1999). In this regard, overdominance at two loci close to the centromere (*Got-1* and *Pgm-2*), and at another showing chromatid segregation (*Mdh-1*), have been reported to be associated with high tuber yield (Ortiz et al. 1993). The changes in the most-common allozyme frequency between gene pools may also have influenced the distance between the centromere and respective isozyme loci. For example, the most-common allozyme frequency for loci close to the centromere was higher in the Latin American gene pools than in the North American cultivars, while this frequency was the same for the *Mdh-1* locus, which was mapped 33.5 cM from its centromere on chromosome VII.

These surveys of allozyme diversity in potato gene pools show that a ”genetic bottleneck”, as measured by the number of allozymes per locus, could have occurred to some extent in certain chromosomes (e.g. around the *Pgi-1* locus in chromosome XII) of the potato breeding pool adapted to the northern hemisphere. However, we do not know whether some of these allozymes (or genes linked to them), especially those with $p<1\%$, enhance the adaptation of potato to these northern latitudes. Allard (1996) has suggested that rare alleles appear to be of little value anywhere. He indicates that plant breeding may lead to a reduction in allelic diversity owing to purifying selection rather than to erosion of useful genetic variation.

We suggest that potato breeders should more-actively pursue the incorporation of allelic diversity from landrace and wild genetic resources into their locally adapted populations. Broadening the genetic base of potato production using diverse germplasm resistant to the most-common pests and diseases will provide a means to sustain and improve the tuber yield of this important vegetatively propagated crop.

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