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Isozyme markers for measuring genetic erosion in potatoes in Andean farms

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EXECUTIVE SUMMARY: Our objectives were to determine the level of genetic variability of cultivated potato in subsistence fields and regional markets in Cusco, Peru. The implementation of the project involved the use of biochemical markers, the training of Peruvian scientists in the use of this technique and creation of a biochemical marker laboratory at the University of Cusco. The samplings of potato varieties in the field and markets disclosed extensive variability of this crop. We found that the growers had a folk naming system of variety classification based on morphological tuber traits. This system was consistent with variety classification based on isozymes as genetic markers. The folk system was accurate when employed within and across fields for the most common varieties. However, it collapsed across fields for uncommon varieties. In the subsistence fields at 3600 to 3850 m above sea level sampled, commercial varieties were rare. Most of the cultivars planted were native land races thus indicating low erosion of native genetic resources by replacement with improved varieties. In our studies, we found evidence of sexual propagation, which seems to have been instrumental for the development of new genetic combinations resulting in the increase of native land races. High levels of cross-pollination and interspecific hybridization between cultivated species, and these with related weedy species support our findings. An isozyme laboratory for determination of genetic variability and analysis was established at the U. of Cusco for surveying the genetic variability fluctuations of native varieties <u>in situ</u>.

4) **RESEARCH OBJECTIVES:**

The objectives of this project were: 1) to analyze the genetic variability of cultivated potatoes in the Cusco region of the Peruvian Andes, the main center of potato cultivation and evolution 2) to estimate the level of genetic erosion of potato native land races and cultivars in subsistence fields in the Cusco region by determining the displacement of native cutivars by improved commercial varieties 3) to determine the levels of out-crossing and interspecific hybridization of cultivated and related weedy potato species in their center of origin, 4) to train Peruvian scientists in genetic variabity surveys and analysis by use of biochemical markers, in both U. of Cusco and the International Potato Center 5) to implement an isozyme laboratory at the University of Cusco for monitoring the genetic variability of potatoes and other crops in situ.

5) **Methods and Results**

A) Assesment of genetic variability in Andean cultivated pototoes:

(**Publication:** Quiros et al. 1989. Biochemical and Folk Assessment of Variability of Andean Cultivated Potoatoes. Econ. Bot. 44:254-266

Isozyme markers were used to survey the genetic variability of non-bitter potatoes in 10 subsistence Andean fields at 3600 to 3800 m above sea level in a radius of 30Km in Paucartanbo, Cuzco, Peru (Table 1).

Table 1. Field location, altitude, farmer name and number of tubers sampled per field

Farmer	Location	Altitude(m)	Tubers
Unknown	Moqopata Alta	3850	185
Jesus Amao	Colquepata	3650	70
Julio Amao	Colquepata	3600	47
Sebastian Mauri	Sipascancha Alta	3750	101
Santos Chipa	Sispacancha Alta	3700	76
Trinidad	Mollomarca	3680	135
Gomercindo	Mollomarca	3720	45
Augustin	Mollomarca	3750	37
Hipolito Mamani	Carpampa	3830	72
Pablo Mamani	Carpampa	3780	62

After sampling a single tuber from two hundred plants in each field chosen at random, the tubers were bulked together in a pile. Then, the owner of the the field was asked to classifiy and name each tuber into varieties according to his knowledge. Then, the

tubers were washed, placed in paper bags identified by variety and by field. The only exception was the field at Moqepata where Brush classifed the tubers before the farmer named the varieties.

Because of the large number of tubers, when a variety had more than 30 tubers per bag, a representative sample of 10 to 25 tubers was taken for the electrophoretic determination depending on the level of morphological heterogeneity present in the bag.

Isozyme analysis: Horizontal starch gel electrophoresis was employed to assay for 9 enzyme systems disclosing 12 loci useful for genotyping the tubers of each variety (Quiros and McHale 1985, Douches and Quiros 1987). These are phophoglucomutase isomerase 1 (PGI-1), Phosphoglucomutase 1 and 2 (PGM-1 and PGM-2), 6 phosphogluconase isomerase 3 (6PGD-3), isocitrate dehydrogenase 1 (IDH-1), malate dehydrogenase 1 and 2 (MDH-1 and MDH-2), acid phosphatase 1 (APS-1), glutamate oxaloacetate transaminase 1 and 2 (GOT-1 and GOT-2), peroxidase 3 (PRX-3) and shikimic acid dehydrogenase (SKD-1).

For the electrophoretic assay a tuber eye was excised and crushed in 75 ul of 0.1M Tris-HCl pH 7.0 buffer containing 2% glutathione as antioxidant. The extract was soaked into 3M filter paper wicks and subjected to electrophoresis.

Other tuber determinations: The following tuber characteristics were recorded: skin and flesh color, eye depth, skin texture and shape. After the electrophoretic survey, chromosome counts were made for a representative tuber of each electrophoretic phenotype. For this purpose the tubers were wrapped in moisted towels and introduced into covered plastic shoe boxes. Since most of the tubers were already sprouted, they formed roots readily at the base of the sprouts. The root tips were collected fixed in alcohol-acetic acid (3:1) and stained with Feulgen.

Genetic parameters: Two parameters were determined in the Andean tuber population, % heterozygosity (H), total number of alleles and total number electrophoretic phenotypes in a per locus basis. These values were compared to those recorded in 25 North American and European potato cultivars: 'Russet Burbank', 'Norchip', 'Red Pontiac', 'Gold Rush', 'Kathadin', 'Rosa', 'Bintjie', 'Sangre', 'Bel Rus', 'Pioneer', 'Atlantic', 'Monona', 'Targee', 'Nooksack', 'Shepody', 'Superior', 'Agaziss', 'Red Norland', 'Alpha', 'Centenial Russet', 'Bison', 'Lemhi', 'Sebago' and 'Red La Soda'

Ploidy: Sixty seven percent of the varieties were tetraploids corresponding to the species *Solanum tuberosum* spp. *andigena*, 14% were triploids, probably corresponding to the species *S.xchaucha* and 13% were diploids corresponding to the species *S. stenotomum*, *S. phureja* and S. *goniocalyx*.

Table 2. Ploidy distribution and species of the electrophoretic phenotypes sampled in the Paucartambo region

Ploidy	Freq.(%)	Species
Diploids	13% (16/125)	*stenotomum, gonyocalyx, phureja*
Triploids	14% (17/125),	*chaucha*
Tetraploids:	67% (84/125)	*andigena*

The isozyme information served to determine the accuracy of the folk classification system for potatoes in the Andes (Table 3). We found a high degree of correspondence between farmer classification and electrophoretic phenotypes. The accuracy of the folk system in electrophoretic terms depended on the farmer doing the classification. The most common error of missclassification consisted in calling different electrophoretic phenotypes by more than one variety name, leading to a general underestimation of the genetic variability present in the fields.

Table 3. Number of varieties reported and actual number of electrophoretic phenotypes.

Farmer	Sample size	Varieties reported	Phenotypes observed	Dif.	% missed
Moqopata	185	43	36	-7	
JesusAmao	70	11	15	4	5.1
JulioAmao	47	14	16	2	4.2
Sebastian Mauri	101	22	31	9	8.9
Trinidad	135	17	34	17	12.6
Gomercindo	45	8	10	3	6.7
Augustin	37	11	10	-1	
Santos Chipa	76	15	24	9	11.8
Hipolito Mamani	72	14	20	6	8.3
Pablo Mamani	62	16	17	1	1.6

The amount of variability observed in the Andean potato population was superior to that present in North American and European varieties. This was measured in terms of number of alleles, number of electrophoretic phenotypes and % heterozygosity. This finding supports the impression that a substantial amount of yet to be exploited variability remains in Andean potato populations.

B) <u>Increase and regeneration of genetic resources by the Andean farmer: the role of sexual propagation of cultivated varieties.</u>

Publication: Quiros et al 1992. Increase of potato genetic resources in their center of diversity: the role of natural outcrossing and selection by the Andean farmer. Gen. Resources and Crop Evol. 39:107-113

The purpose of this project was to document the amplification of genetic diversity by the Andean farmer through sexual propagation by botanical seed and genotypic selection for the creation of new cultivars.

Three traditional farmers from different communities in Cusco were potato is grown as a subsistence crop were questioned regarding the origin of their potato cultivars,cultural and planting potato techniques, and selection of tubers (Table 4).

Table 4. Farmers in the communities of Cusco surveyed in the study

Community	Province	Altitude(m)	Farmer
Yanacona	Urubamba	3500-4000	Tomasa Quispe
Yanacona	Urubamba	3500-4000	Eugenio Auccapuma
Matinga	Calca	3600	Eduardo Huarancca

Isozyme analysis: Horizontal starch gel electrophoresis was employed to assay for 9 enzyme systems disclosing 12 loci useful for genotyping the tubers of each variety (Quiros and McHale 1985, Douches and Quiros 1987).

The following tuber characteristics were recorded: skin and flesh color, eye depth, skin texture and shape. After the electrophoretic survey, chromosome counts were made for a representative tuber of each electrophoretic phenotype.

Market survey: To determine the genetic makeup of some of the potato cultivars grown and marketed in the area, 542 tubers collected from 18 markers sampled in the Cusco were collected and described on the basis of tuber traits and isozymes.

The survey disclosed a total of 229 different cultivars among diploid, triploid and tetraploid forms of *Solanum tuberosum* L. These could be grouped by isozyme cluster analysis in four major groups, and 6 small groups. However, no discrete distribution for flesh of skin color was observed among the groups. Therefore, it can be concluded that all the genotypes belong to a single, large gene pool due to considerable gene flow between cultivars of different groups. When the samples were arranged by the three most common tuber skin color, namely red/pink (Q'ompis type), purple (Yana Imilla type), and yellowish/brown (Yuraq Kusi type) similar number of allozymes were observed in the three classes (Table 5). The structure of the isozymic phenotypes within each group indicate

that they may have derived as segregants after natural outcrossing of diverse parental types.

Table 5. Common and rare alleles for three potato skin colors:

	Frequent				Rare			
Skin	Pgi-1	Mdh-1	Got-1	Got-2	Pgi-1	Mdh-1	Got-1	Got-2
Pink	1,2	1,3	3	2,4	5		2	
Purple	2,5	1,2,3	3	1,4				2,3
Brown	1,2,5	1,2,3,4	2,3	1,4				

In order to prove further the origin of new types by hybridization, two segregating diploid progenies were generated by crossing purple by yellow skin types. In the resulting F1, most of the tuber phenotypes observed in the Andean varieties were reproduced in these crosses. It can be concluded that the Andean potatoes form a large and plastic gene pool amplified and renovated by outcrossing followed in some cases by human selection of desirable phenotypes.

C) Outcrossing determinations of Andean potatoes

1) Determinations in farmers fields:

In order to assess the level of outcrossing and progeny ploidy, tubers and berries of a number of plants were sampled from three potato fields located at approximately 3500 meters above sea level in the Cusco region. These included mixed plantings of the following cultivated species: *Solanum stenotomum* and *S. goniocalyx* (diploids), *S. chaucha* (triploid) and *S. tuberosum* ssp *andigena* (tetraploid). Outcrossing was estimated by determining specific isozyme markers present in the maternal plant and its progeny. Ploidy was determined by chromosome counts in root tips.

The level of outcrossing in the two diploid species was very different. *S. stenotomum* had a minimum average outcrossing rate of 54.4% whereas no outcrossing was observed in the yellow flesh species *S. goniocalyx*. The plants of triploid *S. chaucha* sampled, often considered sterile, produced a few seeds. Interestingly, the progenies from these plants were all tetraploid and as expected the result of outcrossing. The average outcrossing rate in the tetraploids was approximately 20%, however some plants displayed a rate of 82%. Progenies from diploids were always diplod and those of tetraploids were always tetraploid (Table 6). Therefore we did not find evidence indicating that new forms of ssp *andigena* are being generated by hybridization of diploid cultivated species to

the weed *S. sparsipilum* commonly observed surrounding the cultivated fields. However, it can be considered that new forms of *andigena* originated from the triploid *S. chaucha*.

Table 6. Number of plants sampled for tuber and berries for determination of outcrossing in Andean plots

Species	N of plants Carpampa	N of plants Mollomarca	Outcrossing C	Outcrossing M
S. tuberosum ssp andigena	18	17	12%	23%
S. stenotomum	0	3	67	42
S. gonyocalyx	4	0	0	-
S. chaucha	4	10	79	82

2) Determination in experimental plots:

Publication: Rabinowitz et al. 1990. High levels of interspecific hybridization between *Solanum sparsipilum* and *S. stenotomum* in experimental plots in the Andes. Amer. Pot. J. 67:73-81.

Five experimental plots of 3 X 8 m containing 80 plants each of *S. stenotomum* (60 plants) and *S. sparsipilum* (20 plants), a weedy wild potato species, were established in Granja K'ayra in Cusco. The plantings were intended to mimic small fields of traditional farmers on low fertility soil and steep terrain. Plants of each species were distinguished from another by at least four isozyme loci and by stem pigmentation. Ninety five percent of the seedlings grown from either open pollinated seed of *S. stenotomum* or *S. sparsipilum* were found to be interspecific hybrids. That the majority of the open-poolinated seed were of hybrid orgin suggests that gene flow between weedy and diploid cultivated species is important in generating new potato genotypes in the Andes.

D) Development of Randomly Amplified DNA (RAPDs) markers in potato genetics

Publication: Quiros et al. 1993. Use of RAPD markers in potato genetics:segregations in diploid and tetraploid families. Amer. Pot. J. 70:35-42

The objective of the present study was to evaluate randomly amplified polymorphic DNA (RAPD) as a source of markers for use in investigations in potato genetics.

Plant Material: Two segregating F1 families were employed. The first one consisted of 40 individuals, and was the result of crossing the diploid species *S. gonyocalyx* 'Quello' by breeding line 84S10 of *S. phureja* generated in our potato genetic program. The second one was a tetraploid family of 40 plants obtained by crossing breeding line 89S119 of *S. tuberosum* spp *tuberosum*, from our program by *S. tuberosum* spp. *andigena* 'Mi Peru'.

DNA extraction: DNA was extracted following the method described by Tanksley et al. 1988 with minor modifications. Freshly harvested young leaves (2-5 grams) were grounded to fine power in a mortar, with 0.38 g of sodium metabisulfite, 0.1 to 0.3 g of acid washed sand and liquid nitrogen. Ten ml of extraction buffer (350 mM sorbitol, 100 mM tris and 5 mM EDTA, pH 8.2) were added to the mortar, and the resulting slurry was transferred to a 50ml centrifuge plastic tube. Ten ml of lysis buffer (200 mM tris, 50 mM of EDTA, 2 M of NaCl and 2% of CTAB (cetyltriethylamine bromide) and 2 ml of 10 % sarkosyl were added to the tube, mixed by vortexing for 20 seconds and incubated at 60 C for 20 minutes. An equivalent volume of chloroform was added and the contents mixed by inverting the tube 3 to 5 times before centrifugation for 20 min in a desktop centrifuge. Then the aqueous phase was transferred into a new tube, and an equivalent volume of isopropanol was added to precipitate the DNA. DNA was pelleted by centrifuging the tubes at approximately 10000 rpm for 5 minutes. The pellet was rinsed with 70% ethanol, dried in a vacuum oven at room temperature for 1 hour, resuspended in 0.75 ml of TE buffer (10 mM of tris-HCl, 1 mM EDTA, pH 8.0) and incubated in presence of 50 ug/ml RNAse at 65 C for 1 hour. DNA content was determined with an spectrophotometer by measuring optical density (OD) at a wavelength of 260 nm. One OD unit at 260 nm equals to 50 ug/ml of DNA. The DNA concentration was adjusted to 0.1 to 0.2 OD units (5 to 10 ng/ul) with dilution buffer (10 mM tris-HCl, 0.1 mM EDTA, pH 8.0) for the RAPD reaction.

DNA amplification: The primers used were arbitrary 10-mer oligonucleotides purchased from Operon Technologies (Alameda, CA). The reaction conditions were based on Williams et al 1990 except for the following modifications: *Taq* polymerase and 10X reaction buffer (1.9mM MgCl2, 50 mM KCl, 10 mM tris-HCl (pH 9.0), 0.1% triton X-100) were purchased from Promega (Madison, WI). Each reaction was prepared by adding the following components to a 600ul microfuge tube: 1X reaction buffer, 0.1mM for each dNTP, 0.5 units of *Taq* polymerase, 10 to 20 ng of genomic DNA and ddH2O to make a final volume of 10 ul. The reaction mix was overlaid with one drop of mineral oil. Amplification was carried out in a Perkin Elmer Cetus DNA Thermal Cycler, which was programed 1 cycle of 94 C 30 sec followed by 45 cycles of 92 C, 30 sec; 35 C, 1 min; and 72 C, 2 min, and then 1 cycle of 72 C, 5 min. After the cycles were completed, the amplified products were separated in 2% agarose gel in 1X TAE buffer by electrophoresis at 90 v for 4 hours and visualized under UV after staining with 10 PPM of ethidium bromide

solution for 30 min. RAPD profiles were recorded on polaroid film 667.

Nomenclature: Loci were named by the primer kit letter and number followed by a dash with the locus number. The sizes of the products are indicated in Tables 7 and 8.

Data Analysis: Goodness of fit and linkages values for the diploid population were calculated with the 1990 version of program Linkage-1 (Suiter et al. 1983). Only values below 35cM were considered probable linkages.

Segregation of 18 loci in diploid *Solanum gonycalyx x S. phureja* and 12 loci in tetraploid *S. tuberosum ssp tuberosum x S. tuberosum ssp andigena* families fitted Mendelian and tetrasomic ratios, respectively (Tables 7 and 8) . Eight loci in the diploid progeny were arranged in three linkage groups. Segregations of these markers fitting expected ratios indicate that they can be effectively used in potato genetics, breeding and evolution.

Table 7. Mendelian segregation for RAPD markers in the diploid F1 progeny *S. gonyocalyx x S. phureja*

Locus	Size bp	Genotype *AA/Aa*	*aa*	Ratio	*X2*	P
A04-1	500	15	23	1:1	1.68	0.19
A04-2	1000	27	11	3:1	0.31	0.57
A05-1	550	24	16	1:1	1.60	0.21
A16-1	1300	18	22	1:1	0.40	0.53
B07-1	900	13	25	1:1	3.78	0.05
B07-2	1400	18	20	1:1	0.11	0.75
B14-1	700	15	22	1:1	1.32	0.25
D03-1	800	18	19	1:1	0.03	0.86
D03-2	1400	21	17	1:1	0.42	0.52
D04-1	600	23	13	3:1	2.37	0.12
D04-2	1400	19	16	1:1	0.26	0.61
E01-1	1400	21	16	1:1	0.68	0.41
E03-1	1600	27	9	3:1	0.00	1.00
E07-1	900	17	23	1:1	0.90	0.34
E08-1	850	20	15	1:1	0.71	0.40
E09-1	400	21	11	3:1	1.50	0.22
E09-2	450	22	12	3:1	1.92	1.66
E09-3	555	23	11	3:1	0.98	0.32

Table 8. Tetrasomic segregation for RAPD markers in the tetraploid progeny *S. tuberosum ssp tuberosum x ssp andigena*

Locus	Size bp	Genotype *A___*	*aaaa*	Ratio	*X2*	P
A04-2	1000	24	16	1:1	1.60	0.19
A16-1	1300	32	6	3:1	1.72	0.18
B08-1	800	35	5	11:1	0.91	0.33
B08-2	850	22	18	1:1	0.40	0.52
E01-1	1400	31	9	3:1	0.13	0.71
E01-2	3300	29	10	3:1	0.01	0.93
E05-1	900	18	21	1:1	0.23	0.63
E07-1	900	18	20	1:1	0.10	0.74
E07-2	1000	26	14	3:1	2.13	0.14
E08-1	850	26	9	3:1	0.01	0.92
E08-2	1100	24	15	1:1	2.07	0.14
E08-3	2000	23	10	3:1	0.49	0.51

E) Training:

Ms. Maria del Rosario Herrera a technician from the International Potato Center spent three months at Davis, training in enzyme electrophoresis. During her stay, she learned all aspects of the technique including gel reading, analysis and data collection. She worked closely with Mr. Pedro Cisneros, a Research Assistant and PhD student also from Peru, hired to work on this project. Ms. Herrera analyzed parental stocks of segregating progenies developed at CIP, and Cusco, tubers of cultivated potatoes collected in Andean farms for a genetic erosion study, and selected tubers of various cultivated and wild species sampled at fields located above 3500 m of altitiude for and introgression study. She went back to CIP where she is using the isozyme technology to characterize and maintain the extensive germplasm collection of her home institution

Ms Ella Schmidt a Peruvian PhD student and research assistant in the group of Dr. Steve Brush spent two months in Peru collecting potatoes from Andean farms an markets in the Cuzco-Paucartanbo area with Ing. Ramiro Ortega of the Universidad San Antonio Abad del Cusco. These were brought to Davis by her and by Ms. M. Herrera, and were analyzed electrophoretically.

Ing. Ramiro Ortega came to Davis in 1990 for 6 months to learn the technique of isozyme markers and its use in genetic analysis and genetic resources evaluation. As part of his training he analyzed the plant material that he developed and collected in several Andean fields for evidence of introgression and interploidy hybridization.

We implemented a starch gel electrophoresis laboratory for Professor R. Ortega at the University of Cusco, with the assistance of Dr. John Dodds from CIP. His laboratory is now fully equiped to use isozyme markers in germplasm evaluation and speciation studies in situ. Besides the necessary equipment of isozyme electrophoresis, a computer was also bought this laboratory for data analysis.

Dr. Carlos Quiros, went to Peru in December 1989 to plan and coordinate the potato project with the Peruvian collaborators. During this visit, Quiros had the chance to vist the Research Center for Andean Crops located at Granja K'ayra, Cusco and to plan the introgression experiment to be carried out by Ortega during the following months.

6) **Impact, relevance and technology transfer:**

Our findings although limited only to the Cusco region of Peru indicates that the extent of potato genetic variability quantified by the number of different land races is still quite extensive. In subsistence fields, the erosion of potato genetic resources (displacement of land races by improved cultivars) is limited. The farmers have an adequate system of classification for their varieties. Furthermore, some of the farmers maintain and create new genotypes intentionally by sexual propagation through botanical seed. This variability is augmented by natural out crossing in fields were different cultivated species are grown together. Spontaneous seedlings from the hybrid seed may be adopted intentionally or unintentionally as new varieties. Although we did not find evidence of genetic erosion for potato germplasm in the region sampled, it is important to realize that this variability must be protected and maintained. Our work indicates that we are still on time to preserve the existing resources and to maintain the systems provided by the farmers to augment and increase the number of genotypes.

The training of two Peruvian professionals, one from CIP and another from U. of Cusco has provided these institutions with the necessary expertise to monitor their genetic resources using isozymes as molecular markers. Furthermore, the implementation of an isozyme laboratory in Cusco by this grant has provided this institution with a research capability that it did not have before. Being this research center at the focus of diversity of potatoes, it is in a unique situation for doing a number of studies <u>in situ</u> in the area of genetic resources, genetics and evolution. Although to our knowledge CIP is using this technique at the present time to manage its genetic resources, the situation seems different at U. of Cusco. The problem is the lack of support for this type of research at that institution. Once that the chemicals and supplies provided by the grant were spent, the researchers do not have the means to buy them again. We were hoping to renew the grant at least to see the laboratory in Cusco fully operational during the following three years, but it did not happen. In order for these grants to have a real impact in the developing countries, funding should be committed for at least 10 years.

I addition to Ortega and Herrera, two Peruvian PhD students were involved in the project as part of their dissertation research, Mr. Pedro Cisneros and Ms. Ella Schmidt.

7) **Project activities/outputs:**

Meetings: American Potato Association: 1989, 1990, 1991

Publications (attached)

Quiros CF, Brush SB, Douches DS, Zimmerer KS and G Huestis. 1989. Biochemical and Folk Assessment of Variability of Andean Cultivated Potoatoes. Econ. Bot. 44:254-266

Quiros CF, Ortega R, van Raamsdonk L, Herrera-Montoya M, Cisneros P, Schmidt E and SB Brush 1992. Increase of potato genetic resources in their center of diversity: the role of natural outcrossing and selection by the Andean farmer. Gen. Resources and Crop Evol. 39:107-113

Quiros CF, Ceada A, Georgescu A, and J Hu 1993. Use of RAPD markers in potato genetics:segregations in diploid and tetraploid families. Amer. Pot. J. 70:35-42

Rabinowitz D, Linder CR, Ortega R, Begazo D, Murguia H, Douches DS and CF Quiros 1990. High levels of interspecific hybridization between Solanum sparsipilum and S. stenotomum in experimental plots in the Andes. Amer. Pot. J. 67:73-81.

8) **Project productivity:**

This project was highly productive. We met the objectives stated in the project, including the application of isozymes to estimate genetic variability and genetic erosion in Andean potatoes, train four Peruvian scientists and established an isozyme laboratory at the U. of Cusco. Furthermore, we prepared the ground for the application of novel molecular markers in potatoes.

9) **Future work:**

Our collaborators in Peru are continuing with their projects dealing with the maintenance and evaluation of potato genetic resources. We at Davis are continuing with the introgression, speciation and cross pollination of Andean potato species.

In the future, we will like to use the isozyme marker laboratory established at the U. of Cusco to evaluate systematically the existing genotypes for agronomic traits and markers for developing a complete data base of the existing germplasm. Also we are interested to continue the sampling of fields for studying introgression and hybridization under cultivation in subsistence fields.

10) **Literature Cited**

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HIGH LEVELS OF INTERSPECIFIC HYBRIDIZATION BETWEEN *SOLANUM SPARSIPILUM* AND *S. STENOTOMUM* IN EXPERIMENTAL PLOTS IN THE ANDES

D. Rabinowitz[1,2], C.R. Linder[2,3], R. Ortega[4], D. Begazo[4], H. Murguia[4], D.S. Douches[5], and C.F. Quiros[5*]

Abstract

Five experimental plots of 3 x 8 m containing 80 plants each, 60 of *Solanum stenotomum* (12 clones) and 20 of *S. sparsipilum* (unknown number of clones) interspaced at random, were established at the Granja K'ayra in Cuzco, Perú. The plantings were intended to mimic small fields of traditional farmers on low fertility soil and steep terrain. Plants of each species were distinguished from another by at least four isozyme loci and by stem pigmentation. Ninety five percent of the seedlings grown from either open-pollinated seed of *S. stenotomum* or *S. sparsipilum* were found to be interspecific hybrids. That the majority of the open-pollinated seed were of hybrid origin suggests that gene flow between weedy and diploid cultivated species is important in generating new landraces of potatoes in the Andes.

Compendio

En la Granja K'ayra, Cuzco, Perú, se establecieron cinco parcelas experimentales de 3 × 8 m conteniendo 80 plantas cada una, 60 de *Solanum stentomum* (12 clones) y 20 de *S. sparsipilum* (número desconocido de clones) interespaciadas al azar. Las siembras intentaron duplicar las condiciones de los pequeños campos de agricultores tradicionales, sobre suelos de baja fertilidad y topografía escarpada. Las plantas de cada especie se distinguieron unas de las otras en por lo menos cuatro loci de isoenzimas así como por la pigmentación del tallo. Se encontró que noventa y nueve por ciento de las plántulas obtenidas de semilla de polinización libre tanto de *S. stenotomum* como de *S. sparsipilum* eran híbridos interespecíficos. El hecho que la mayoría de la semilla de polinización libre fuera de origen híbrido sugiere que el intercambio de genes entre especies no cultivadas y especies cultivadas

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diploides es importante en la generación de nuevas variedades nativas de papa en los Andes.

Introduction

Biosystematic, genetic and molecular evidence indicate that the Andean cultivated tetraploid *Solanum tuberosum* ssp. *andigena* (Juz. et Buk.) Hawkes has arisen many times by hybridization and introgression involving wild and cultivated diploid species (2,7,12). Hawkes (4) identified the species *S. stenotomum* Juz. et Buk. and *S. sparsipilum* (Bitt.) Juz. et Buk. as the most likely ancestors of *S. tuberosum* ssp. *andigena*. The former is a diploid cultigen restricted to the Andean region, while *S. sparsipilum* is an aggressive diploid weed that occurs in and around the cultivated potato fields of Perú and Bolivia (2,13). The biosystematic work of Ugent (13) suggests that the extensive polymorphism of *S. sparsipilum* derives from continuous hybridization of diploid cultivated species with several related diploid wild species. This process may have resulted in stabilization of a large number of diploid hybrid variants, all pooled under the species name *S. sparsipilum*. Moreover, Ugent (13) suggested that *S. sparsipilum* plays an active role in development of new cultivated diploid potatoes by serving as a genetic bridge, allowing the flow of genes from wild species. This constant influx of genes increases the variability of the gene pool, thus permitting constant selection by man for improved cultivars.

To test Ugent's hypothesis concerning the role of *S. sparsipilum* as a genetic bridge, we planted *S. stenotomum* (2x) and *S. sparsipilum* in experimental fields designed to mimic traditional farmers' fields and then determined the level of interspecific hybridization between them.

Materials and Methods

Five experimental plots of 3 x 8 m and located approximately 250 m from one another were established in Granja K'ayra, Cuzco, Perú at 3300 m altitude (13°35'S, 70°51'W). Each plot contained 80 plants, 60 of *S. stenotomum* (stn) (8 clones) and 20 of *S. sparsipilum* (spl) (number of clones unknown), interspaced at random in rows approximately 1 m apart. Species abbreviations follow the recommendations proposed by Huaman and Ross (8). These accessions represented a small part of the diversity in the species. The stn accessions were supplied by Centro de Investigaciones de Cultivos Andinos, Cuzco. The spl accessions were supplied by the International Potato Center, Lima. Plantings were intended to duplicate small subsistence fields on steep terrain commonly cropped with potatoes by Andean traditional farmers. The nearest cultivated potato fields were at least 1 km away, and no populations of wild potato species were found in the immediate vicinity.

One plot (#3) was used for controlled hand pollinations. For each species, pollen was collected from numerous individuals by vibrating mature anthers with a tuning fork. This pollen was then used to pollinate emasculated flowers. Hand pollinations were performed within 3 hrs. of pollen collection, and the flowers were covered immediately with paper bags to prevent open pollination. Intra- and interspecific control crosses were performed in this fashion. Once berries began to form, the paper bags were replaced with mesh bags.

Berries from open pollination, in the other four plots, were placed in mesh bags as each inflorescence matured.

All plants of both species were harvested at the end of the growing season. Seeds from a total of 18 plants of three stn clones and 7 plants of spl were sampled for the determination (Tables 2 and 3). Selection of stn clones 2, 4 and 8 was based on seed availability from control crosses. After extraction, the seed was treated with 1000 ppm of gibberelic acid to break dormancy. Seedlings were then grown under standard greenhouse conditions.

The phenotype of each seedling was determined for at least two enzyme loci by starch gel electrophoresis of young, healthy leaf tissue (3,12). The two species included in the plots had electrophoretic phenotypes that differentiated seedlings derived from either intra- or interspecific crosses. The diagnostic loci were *Mdh-1*, *Prx-2*, *Got-1* and *Got-2*, which were homozygous for different allozymes in the plants sampled from both species. Thus, interspecific hybrids were identified by presence of heterozygous genotypes for the isozyme loci (Fig. 1A). The inheritance of these isozymes has been reported elsewhere (3,12). In addition to the enzyme loci, it was possible to identify interspecific hybrid seedlings from open-pollinated spl seed by the presence of purple pigmentation in the hypocotyl and leaf nodes. After finding perfect association between lack of pigmentation and presence of spl specific isozyme markers in the first 100 seedlings evaluated, the morphological trait was used to quantify the number of interspecific hybrids for spl open-pollinated progeny. Analysis of variance was employed to compare the level of interspecific hybridization among plots for both species. Percentages were transformed to arcsine values before analysis.

Results and Discussion

Seed Yields from Controlled and Open-Pollinated Plots — Intra- and interspecific control crosses differed markedly in their ability to set berries (Table 1) and to produce viable seed. Stn x stn crosses yielded very few berries as compared to spl x spl and interspecific crosses performed in both directions. While it was not possible to assess berry set in open-pollinated plots, seed set for open-pollinated berries provides a rough point of comparison with control crosses. Open-pollinated berries collected from stn plants yielded

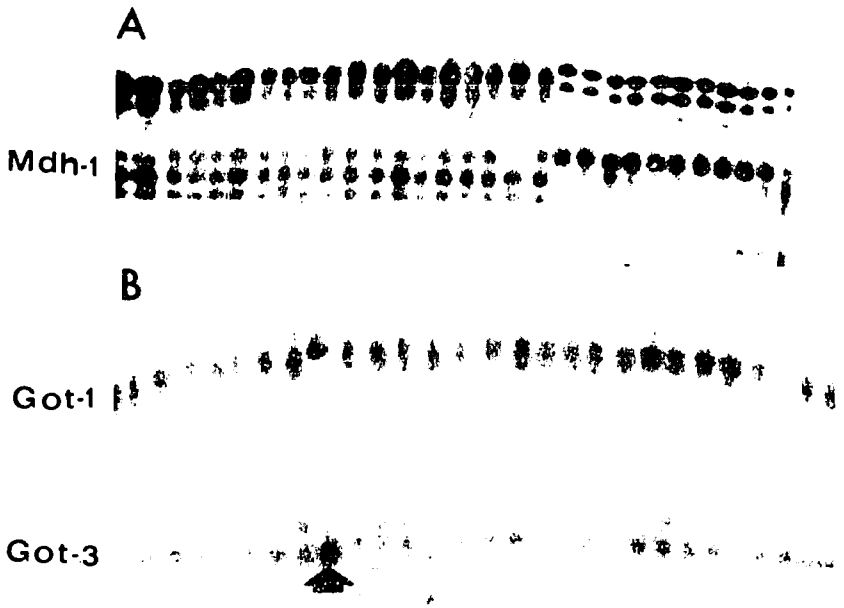

FIG. 1. Zymograms for potato seedlings derived from controlled and open pollinations. Anode is above. A. Discrimination of interspecific from intraspecific seedlings by the locus *Mdh-1*. Interspecific spl x stn hybrids display a three banded heterozygous genotype (lines 1 to 19 from the left), whereas *S. stenotomum* seedlings lack allele $Mdh\text{-}1^2$ of lowest migration (lines 20 to 29). Seedlings derived from controlled crosses. B. Phenotypes of an open-pollinated progeny obtained from a berry of *S. stenotomum*. Only one individual resulted from an stn x stn cross (single bands for both loci, *Got-1* and *Got-3*, arrow). The rest are interspecific hybrids.

TABLE 1. — *Frequency of berry formation for control crosses of* Solanum stenotomum *(stn) and* S. sparsipilum *(spl) at Granja Kayra, Cuzco, Perú.*

Control ♀ × ♂	Number of flowers pollinated	Berries	
		Number set	(%)
stn × stn	36	3	8.3
spl × spl	59	26	44.1
stn × spl	54	26	48.1
spl × stn	86	82	95.3

2 to 15 viable seeds/berry, whereas berries collected from spl plants yielded between 10 and 450 viable seeds/berry. Most of the spl seed looked plump and fully developed, whereas the stn seed often was smaller than normal and shrivelled. These observations and treatment of the seeds with gibberelic acid make it unlikely that seed dormancy was a factor influencing seed ger-

mination of the different seed types. Sterility was probably a factor affecting seed yield in some crosses since four of 10 hybrid plants derived from stn open-pollinated berries had mostly nonviable pollen. Sterility in these plants was due to chromosomal aberrations resulting in multivalents in diakinesis and laggards in anaphase I. Thus, the low seed set in the stn clones might be due to accumulation of chromosomal aberrations affecting both male and female fertility and probably also to self-incompatibility. The low seed set of stn plants was reflected in the small number of progeny they generated for analysis of interspecific hybridization (Table 2).

Open-pollinated stn Progenies — The level of interspecific hybridization per plant (average of % interspecific hybridization/berry of all the berries collected in a plant) was not significantly different among plots (95% confidence), indicating that pollinator activity was uniform throughout the experimental fields. The small number of progeny sampled (1 to 13 seedlings per berry) gave a wide frequency range of interspecific hybrids per berry, spanning from zero to 100% (Fig. 2). The lowest values were observed for berries that produced no interspecific hybrids, and this was observed for

TABLE 2. — *Percent interspecific hybridization in open-pollinated progenies obtained from three clones of* S. stenotomum *based on % hybrids/berry.*

Clone	Plant	Number of berries	No. of Seedlings Tested		Interspecific Hybridization (%)
2	1-7	3	16		100
2	2-8	4	5		75
2	2-10	1	3		100
2	4-3	9	16		100
Subtotal		17	40	$\bar{x}$	94 ± 6.2
4	1-9	7	25		98
4	1-10	2	5		88
4	2-1	1	3		100
4	2-2	4	17		88
4	2-4	2	4		100
4	2-7	2	9		75
4	4-1	1	1		100
Subtotal		19	64	$\bar{x}$	93 ± 3.6
8	1-1	2	7		100
8	1-8	3	28		92
8	2-3	1	2		100
8	2-6	2	4		100
8	4-10	1	2		100
8	4-11	1	4		100
8	4-12	1	2		100
Subtotal		11	49	$\bar{x}$	99 ± 1.1
Total		47	153	Combined Average	95 ± 1.99

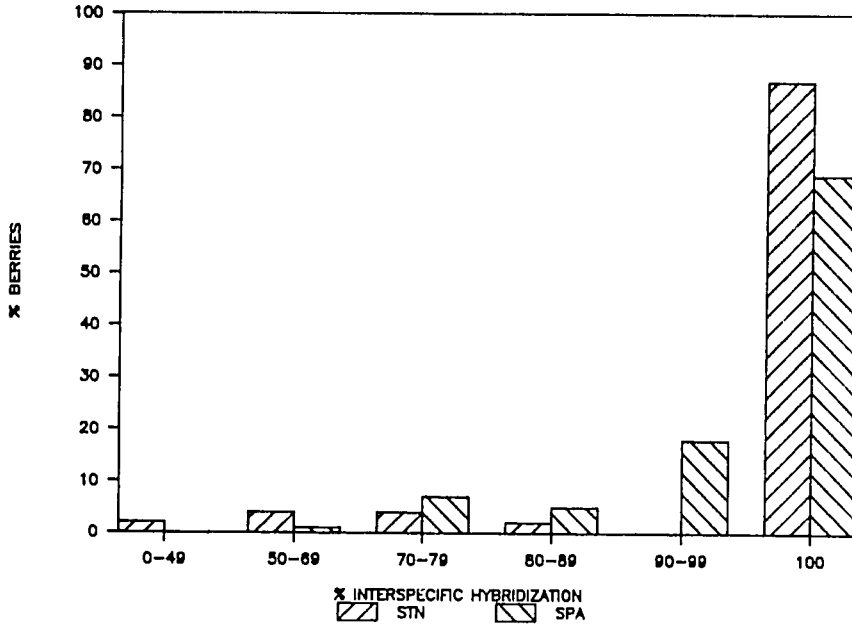

FIG. 2. Frequency distribution of % interspecific hybridization for berries derived from open pollination in *S. stenotomum* and *S. sparsipilum*.

a berry having only a single seedling progency. The next lowest value observed was 50% interspecific hybridization in two berries yielding only two seedlings each. The rest of the berries yielded progenies that were from 70 to 100% interspecific hybrids. The range of interspecific hybridization per plant was from 75 to 100%, with more than half of the plants having only hybrid progency. The overall mean for the stn open-pollinated progenies on a per berry basis was 95% interspecific hybridization (Table 2, Fig. 1B).

Open-pollinated spl Progenies — The same situation observed for the stn open-pollinated progenies was found for progenies derived from open-pollinated berries of spl. However, in this case, seed germination was very good, allowing samples of 18 seedlings per berry on average.

The level of interspecific hybrids per berry, which ranged from 50 to 100% (Fig. 2), was better estimated in spl because of the larger number of progeny (21 to 382 seeds/berry). The range of interspecific hybridization per plant was from 92 to 100% (Table 3). Again, no significant differences for interspecific hybridization were observed among plots (95% confidence). Interestingly, the overall interspecific hybridization mean was 96%, practically the same value observed for the stn progenies. This finding reveals that interspecific hybridization occurs in both directions and at similar intensities, albeit more successfully when spl is the female parent.

TABLE 3. — *Percent interspecific hybridization in open-pollinated progenies from seven* S. sparsipilum *plants based on % hybrids/berry.*

Plant	Number of berries	Progeny	Interspecific Hybridization (%)
1-5	10	133	92.3
1-7	1	21	100.0
4-3	22	318	95.4
4-8	22	382	95.4
5-4	10	335	95.7
5-5	1	23	96.0
5-6	8	95	99.1
Total	74	1307	
Combined Average			96.3 ± 1

Pollination in the experimental plots was mediated by bees of the species *Thygater melanotricha* and *Lonchopria* sp. While *Lonchopria* sp. (formerly *Leioproctus* sp.) has previously been observed pollinating potatoes (9). *T. melanotricha* has not been reported in the literature. Other work performed by us in Tungasuca, Perú (approximately 125 km south of Cuzco in the Rio Vilcanota drainage, altitude 3800 m) (unpublished data), and work by Johns and Keen (9) on the Bolivian altiplano indicate that there are more species of potato pollinators than were first suggested (1a, 3a), the species varying by location and altitude. Our observations make it clear that the high rate of hybridization observed in the present study can be attributed to *T. melanotricha* and *Lonchopria* sp.

Since our plots were isolated from other potato fields, it is safe to assume that the vast majority, if not all, of the pollen interchange took place between the plants in our experiment. It is possible, however, that the level of interspecific hybridization observed was enhanced by our particular planting scheme: interplanting of *S. stenotomum* and *S. sparsipilum* in the same rows. Field studies conducted in Tungasuca, Perú found that spl was relatively common in the disturbed margins of cultivated fields, but was rarely found within the borders of the fields (unpublished data). This evidence suggests that natural hybridization of spl and stn will tend to take place near the borders of cultivated potato fields and with decreasing freqency toward the center of fields. This stands in contrast to work on *S. ajanhuiri* (9,10) where hybrid swarms of *S. ajanhuiri* (weed) were found growing in potato fields with *S. ajanhuiri* (crop) and *S. stenotomum*.

In addition to the dilution of hybridization by the presence of spl primarily in field borders, further dilution would result in farmers' fields since many other species of potatoes would usually be planted with *S. stenotomum* (1,11). It is expected that the probability of pollinators mediating a spl x stn hybridization is in inverse proportion to the number of non-*S. stenoto-*

mum potatoes planted in fields, assuming a haphazard planting regime for various potato species and cultivars (1). However, it is possible that spl will also hybridize in these fields to other related cultivated species. The precise nature of crop/weed hybridization is undoubtedly a function of the ecology of the particular species involved, their pollination biology and the cropping system in use, so field studies of naturally occuring *S. sparsipilum/S. stenotomum* complexes are called for to fully elucidate where hybrids occur and in what quantity. Examples of natural hybrids among other species have been reported by Hawkes and Hjerting (5), Johns and Keen (9) and Johns et al. (10).

Ugent (14) suggested that introgression in the opposite direction occurs from *S. tuberosum* to wild species via *S. sparsipilum*, allowing the establishment of newly generated recombinants which undergo additional cycles of natural hybridization with cultivated potatoes. Since these species share similar natural environments, it is unlikely that ecological barriers will prevent the survival of their natural hybrids (5). The high level of variability generated in the diploid gene pool by interspecific hybridization is likely to cause the influx of meiotic mutant genes determining 2n or unreduced gamete formation, which will eventually result in polyploidization (6). This would permit the establishment of highly polymorphic tetraploid potatoes such as *S. tuberosum* ssp. *andigena*. This hypothesis is consistent with chloroplast DNA data (7) indicating that cultivated tetraploid potatoes of the Andean region arose from cultivated diploid × wild species hybridizations taking place in either direction. The high frequency of interspecific hybridization observed in the present study between spl and stn demonstrates that gene exchange between these two species is a common event when they grow in close proximity to one another and provides experimental evidence supporting Ugent's hypothesis (5,13). The high level of hybridization reported here probably plays an important role in generating new variability, thus helping to account for the large number of varieties (landraces) observed in the Andean region.

Although our study was confined to only two species of potato, interbreeding is likely to occur between a larger number of cultivated and wild species (9,10) which are commonly found in Andean fields. Additional in situ studies are needed to determine the level of interploidy hybridization and to elucidate the natural origins of triploid and pentaploid species.

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USE OF RAPD MARKERS IN POTATO GENETICS: SEGREGATIONS IN DIPLOID AND TETRAPLOID FAMILIES

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Abstract

The objective of the present study was to evaluate random amplified polymorphic DNA (RAPD) as a source of markers for use in investigations in potato genetics. Segregation of 18 loci in diploid *Solanum goniocalyx* x *S. phureja* and 12 loci in tetraploid *S. tuberosum* ssp. *tuberosum* x *S. tuberosum* ssp. *andigena* families fitted Mendelian and tetrasomic ratios, respectively. Eight loci in the diploid progeny were arranged in three linkage groups. Segregations of these markers fitting expected ratios indicate that they can be effectively used in potato genetics, breeding and evolution.

Compendio

El ADN polimórfico amplificado al azar (RAPD) fue evaluado como fuente de marcadores para ser usados en investigaciones sobre genética de papa. La segregación de 18 loci en familias diploides de *Solanum goniocalyx* × *S. phureja* y de 12 loci en familias tetraploides de *S. tuberosum* ssp. *tuberosum* × *S. tuberossum* ssp. *andigena* se ajustaron a las proporciones mendelianas y tetrasómicas respectivamente. Ocho loci de la progenie diploide estaban arreglados en tres grupos de enlace. La segregación de estos marcadores, que están de acuerdo con las proporciones esperadas, indican que pueden ser usados en forma efectiva en estudios de genética, mejoramiento y evolución de la papa.

Introduction

Isozymes (5, 6) and restriction fragment length polymorphisms (RFLPs) (1, 3) are well established chromosome markers in potato. The main limitation of the isozyme technique is the small number of loci it discloses. Although RFLP is able to generate a large number of polymorphisms, it is laborious and lengthy. Randomly amplified polymorphic DNA (RAPD) is a recent technique proposed for generating molecular markers (12, 13). Although this technique usually discloses dominant markers, its advantages are simplicity, rapidity and need of minute amounts of DNA. Because of their versatility, RAPD markers have a wide application in plant

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genetics. For example, we have used them to characterize alien addition-lines (8) and for cultivar identificatioin in *Brassica* crops (4). We are also starting to apply these markers to study the phylogeny and evolution of tuber bearing species (2).

The objective of the present communication is to evaluate RAPDs as markers for genetical studies in potato. For this purpose their segregation has been followed in diploid and tetraploid families.

Materials and Methods

Plant Material

Two segregating F1 families were employed. The first one consisted of 40 individuals, and was the result of crossing the diploid species *S. goniocalyx* "Quello" by breeding line 84S10 of *S. phureja* generated in our potato genetic program. The second one was a tetraploid family of 40 plants obtained by crossing breeding line 89S119 of *S. tuberosum* spp. *tuberosum*, from our program by *S. tuberosum* spp. *tuberosum* x ssp. *andigena* hybrid variety "Mi Peru".

DNA Extraction

DNA was extracted following the method described by Tanksley *et al.* (11) with minor modifications. Freshly harvested young leaves (2-5 g) were ground to fine powder in a mortar, with 0.38 g of sodium metabisulfite, 0.1 to 0.3 g of acid washed sand and liquid nitrogen. Ten ml of extraction buffer (350 mM sorbitol, 100 mM tris and 5 mM EDTA, pH 8.2) were added to the mortar, and the resulting slurry was transferred to a 50 ml centrifuge plastic tube. Ten ml of lysis buffer (200 mM tris, 50 M of EDTA, 2 M of NaCl and 2% of CTAB (cetyltriethylamine bromide) and 2 ml of 10% sarkosyl were added to the tube, mixed by vortexing for 20 sec and incubated at 60 C for 20 min. An equivalent volume of chloroform was added and the contents mixed by inverting the tube 3 to 5 times before centrifugation for 20 min in a desktop centrifuge. The aqueous phase was then transferred into a new tube, and an equivalent volume of isopropanol was added to precipitate the DNA. DNA was pelleted by centrifuging the tubes at approximately 10,000 rpm for 5 min. The pellet was rinsed with 70% ethanol, dried in a vacuum oven at room temperature for 1 hr, resuspended in 0.75 ml of TE buffer (10 mM of tris-HCl, 1 mM EDTA, pH 8.0) and incubated in presence of 50 μg/ml RNAse at 65 C for 1 hr. DNA content was determined with a spectrophotometer by measuring optical density (OD) at a wavelength of 260 nm. One OD unit at 260 nm equals to 50 μg/ml of DNA. The DNA concentration was adjusted to 0.1 to 0.2 OD units (5 to 10 ng/μl) with dilution buffer (10 mM tris-HCl, 0.1 mM EDTA, pH 8.0) for the RAPD reaction.

DNA Amplification

The primers used were arbitrary 10-mer oligonucleotides purchased from Operon Technologies (Alameda, CA). The reaction conditions were

based on Williams *et al.* (13) except for the following modifications: *Taq* polymerase and 10X reaction buffer (1.9 mM $MgCl_2$, 50 mM KCl, 10 mM tris-HCl (pH 9.0), 0.1% triton X-100) were purchased from Promega (Madison, WI). Each reaction was prepared by adding the following components to a 600 μl microfuge tube: 1X reaction buffer, 0.1 mM for each dNTP, 0.5 units of *Taq* polymerase, 10 to 20 ng of genomic DNA and ddH2O to make a final volume of 10 μl. The reaction mix was overlaid with one drop of mineral oil. Amplification was carried out in a Perkin Elmer Cetus DNA Thermal Cycler, which was programmed 1 cycle of 94 C 30 sec followed by 45 cycles of 92 C, 30 sec; 35 C, 1 min; and 72 C, 2 min, and then 1 cycle of 72 C, 5 min. After the cycles were completed, the amplified products were separated in 2% agarose gel in 1X TAE buffer by electrophoresis at 90 v for 4 hrs and visualized under UV after staining with 10 ppm of ethidium bromide solution for 30 min. RAPD profiles were recorded on Polaroid film 667.

Nomenclature

Loci were named by the primer kit letter and number followed by a dash with the locus number. The sizes of the products are indicated in Tables 1 and 2.

Data Analysis

Goodness of fit and linkage values for the diploid population were calculated with the 1990 version of Linkage-1 program (10). Only values below 25 cM were considered probable linkages.

TABLE 1.—*Mendelian segregation for RAPD markers in the diploid F1 progeny S. goniocalyx x S. phureja.*

Locus	Size bp	Progeny Genotype *AA/Aa*	Progeny Genotype *aa*	Ratio	x^2	p
A04-1	500	15	23	1:1	1.68	0.19
A04-2	1000	27	11	3:1	0.31	0.57
A05-1	550	24	16	1:1	1.60	0.21
A16-1	1300	18	22	1:1	0.40	0.53
B07-1	900	13	25	1:1	3.78	0.05
B07-2	1400	18	20	1:1	0.11	0.75
B14-1	700	15	22	1:1	1.32	0.25
D03-1	800	18	19	1:1	0.03	0.86
D03-2	1400	21	17	1:1	0.42	0.52
D04-1	600	23	13	3:1	2.37	0.12
D04-2	1400	19	16	1:1	0.26	0.61
E01-1	1400	21	16	1:1	0.68	0.41
E03-1	1600	27	9	3:1	0.00	1.00
E07-1	900	17	23	1:1	0.90	0.34
E08-1	850	20	15	1:1	0.71	0.40
E09-1	400	21	11	3:1	1.50	0.22
E09-2	450	22	12	3:1	1.92	0.17
E09-3	555	23	11	3:1	0.98	0.32

TABLE 2.—*Tetrasomic segregation for RAPD markers in the tetraploid progeny S. tuberosum ssp. tuberosum x "Mi Peru".*

Locus	Size bp	Progeny Genotype *A...*	*aaaa*	Ratio[a]	x^2	p
A04-2	1000	24	16	1:1	1.60	0.19
A16-1	1300	32	6	3:1	1.72	0.18
B08-1	800	35	5	11:1	0.91	0.33
B08-2	850	22	18	1:1	0.40	0.52
E01-1	1400	31	9	3:1	0.13	0.71
E01-2	3300	29	10	3:1	0.01	0.93
E05-1	900	18	21	1:1	0.23	0.63
E07-1	900	18	20	1:1	0.10	0.74
E07-2	1000	26	14	3:1	2.13	0.14
E08-1	850	26	9	3:1	0.01	0.92
E08-2	1100	24	15	1:1	2.07	0.14
E08-3	2000	23	10	3:1	0.49	0.51

[a]Putative parental genotypes for segregation ratios assuming chromosomal segregation: 1:1 (*Aaaa* x *aaaa*); 3:1 (*Aaaa* x *Aaaa*); 11:1 (*AAaa* x *Aaaa*).

Results and Discussion

Diploid Progeny

Of the 16 primers tested in this family, 12 disclosed segregating bands that could be assigned to 18 loci. Thus, the amount of polymorphism disclosed by this technique agrees with that observed by isozymes (7) and RFLPs (3). Only four of the primers disclosed multiple loci. The segregating bands were either present or absent in the F_1 plants, thus behaving like dominant markers. This agrees with previous reports on RAPD markers in other species (4, 11, 12). All of the segregations observed at the 18 loci fitted mendelian ratios of 1:1 or 3:1 (Table 1, Fig. 1). These ratios were expected for heterozygous by homozygous (band absence or null allele) and heterozygous by heterozygous crosses, respectively. Although the phenotype of the *S. goniocalyx* parent could not be determined because of the lack of DNA availability, it could be inferred from the resulting segregating ratios. However, the phenotype of the *S. phureja* parent agreed with the observed ratios. Eight of the loci were arranged in three linkage groups, the remainder segregated independently (Table 3). The order of the markers for group II, consisting of four loci was:

E01-1 E03-1 D03-1 E09-2

Tetraploid Progeny

Seven primers disclosed 12 loci in the tetraploid progeny. Approximately one-half of the primers disclosed more than one locus (Fig. 2). The

FIG. 1. Mendelian segregation for *B14-1* (700bp, 1:1 ratio) in diploid *S. goniocalyx* x *S. phureja* F1 family. P = *S. phureja* parent, M = molecular standard.

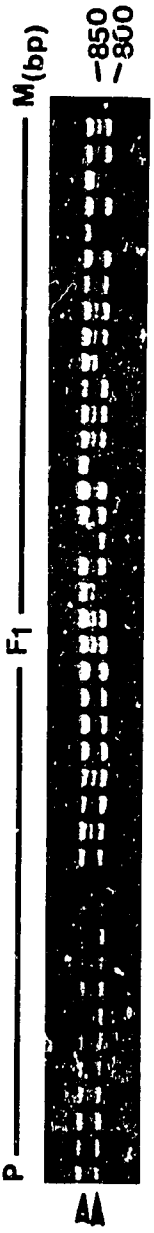

FIG. 2. Tetrasomic segregation for *B08-1* (800bp, 11:1 ratio) and *B08-2* (850bp, 1:1 ratio) in tetraploid *S. tuberosum* spp. *tuberosum* x "Mi Peru" F1 family. P = *S. tuberosum* spp. *tuberosum* parent, M = molecular standard.

TABLE 3.—*Linked loci detected in the diploid population of S. goniocalyx x S. phureja.*

Loci	Linkage Group	Distance (cM)	S.E.
A05-1/E09-1	I	12.30	10.90
D03-1/E03-1	II	10.93	10.10
D03-1/E09-2	II	16.82	12.18
D03-1/E01-1	II	12.12	10.67
A04-2/A16-1	III	21.84	12.07

RAPD loci segregated in tetrasomic fashion for three expected ratios, 1:1, 3:1 and 11:1, corresponding to the following expected parental genotypes *Aaaa* x *aaaa*, *Aaaa* x *Aaaa* and *AAaa* x *Aaaa*, respectively. Although it is likely that multiple alleles were present at some of the RAPD loci, their sequences most likely were not amplified, thus resulting in null phenotypes. This is a limitation of the RAPD technique, which usually detects only one allele per loci (9). The tetrasomic ratios observed, assuming chromosomal segregation, agree with previous reports (5, 6) indicating that tetraploid cultivated potato behaves genetically as an autotetraploid. Five of the 12 loci in the tetraploid progeny segregated also in the diploid progeny.

Mendelian and tetrasomic segregations of the RAPD markers in diploid and tetraploid progenies, respectively, indicates that these DNA based markers could be effectively used in potato genetics, breeding and evolution. The greatest advantage of the RAPD technique is the large number of markers that can be reliably generated in a short time. These can be integrated to existing maps consisting of morphological traits, isozyme and RFLP markers. In spite of their dominant nature, which has to be considered in the calculation of the mapping functions, these markers have been used to rapidly construct saturated maps in other organisms (9).

Acknowledgments

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Biochemical and Folk Assessment of Variability of Andean Cultivated Potatoes[1]

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Isozyme markers were used to survey the genetic variability of non-bitter potatoes in 10 subsistence fields of Andean farmers at 3600–3850 m above sea level. Sixty-seven percent of the varieties were tetraploids corresponding to the species Solanum tuberosum *ssp.* andigena, *14% were triploids, probably corresponding to the species* S. × chaucha, *and 13% were diploids corresponding to the species* S. stenotomum, S. phureja, *and* S. goniocalyx. *The isozyme information served to determine the consistency of the folk naming system. We found a high degree of correspondence between farmer identification and electrophoretic phenotypes. The consistency of the folk system in electrophoretic terms depended on the farmer who was interviewed. The most common incongruity consisted of calling different electrophoretic phenotypes by the same variety name, leading to a slight underestimation of genetic variability present in the fields. The amount of variability observed in the sample of the Andean potato population was superior to that present in North American and European varieties. This was measured in terms of number of alleles, number of electrophoretic phenotypes and percent of heterozygosity. This finding supports the impression that a substantial amount of yet unexploited variability remains in Andean potato populations.*

Evaluación bioquímica y popular de la variabilidad interespecífica de las papas cultivadas en los Andes. *En este trabajo se da a conocer los resultados de un estudio genético sobre variedades de papa dulce en los Andes, realizada por medio de marcadores isoenzimáticos en parcelas de subsistencia localizados entre 3600 y 3850 metros sobre el nivel del mar. Se encontró que 67% de las variedades muestreadas eran tetraploides de la especie* S. tuberosum *ssp.* andigena, *14% triploides probablemente de la especie* S. × chaucha, *y 13% diploides de las especies* S. stenotomum, S. phureja *and* S. goniocalyx. *La información isoenzimática fue útil en la evaluación de la precisión del sistema folklorico para identificar variedades. Se encontró un alto grado de asociación entre el sistema de clasificación usado por el campesino para denominar sus variedades, y los fenotipos electroforéticos. La precisión del sistema de identidad folklorico en términos electroforéticos dependió del campesino entrevistado. La discrepancia mas frecuente entre los dos sistemas de nomenclatura consistió en llamar diferentes fenotipos electroforéticos con el mismo nombre varietal, lo que resultó en una subestimación de la variabilidad genética presente en los campos. El nivel de variabilidad observado en la muestra de papas de la población andina fue superior al observado en variedades norteamericanas y europeas. La variabilidad se midió en base al número de alelos, número de fenotipos electroforéticos y porcentaje de heterocigosidad. Estos resultados están de acuerdo con la impresion general de que todavía existe mucha variabilidad en variedades de papa andinas que no ha sido aún explotada.*

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Great intraspecific diversity of cultivated plants is one of the hallmarks of agricultural evolution, but this diversity presents numerous scientific issues. Why is there so much intraspecific diversity of cultivated species, and why is this exaggerated in certain regions? What is the contribution of ecological, agronomic, and human factors to the origin and maintenance of this diversity? What is the best way to describe and analyze this diversity? How is diversity affected by agricultural, economic and other changes?

Two primary steps in answering these questions are to understand the genetic base of the crop and to describe the ways in which farmers perceive and select crops so as to affect diversity. Investigating how and why farmers in centers of crop evolution maintain diversity can usefully begin with folk taxonomies and their relation to the genetics of the crop (Brush 1986). For whatever reason that diversity is maintained, farmers must have a specific means to accomplish this, and a folk nomenclature and taxonomy can be helpful toward this end. Folk nomenclature and taxonomies create labels and keys to distinguish morphological differences. The work of Boster (1985) on Aguaruna taxonomy of *Manihot esculenta* Cranz shows that folk taxonomy of great intraspecific variability can be understood as a means to maintain diversity and that diversity is sought for its own sake and not for a specific reason. Folk taxonomy allows Aguaruna cultivators to make distinctions that have no apparent utility, although culinary and pharmacological differences are noted between some folk varieties. Most important, there are no recognized agronomic characteristics incorporated into the Aguaruna folk taxonomy.

The fact that folk taxonomies are designed to satisfy cultural rather than agronomic reasoning forces the question of their utility for understanding the biological dynamics of a crop. It has been repeatedly shown that interspecific folk taxonomies are biologically accurate (Alcorn 1984; Berlin et al. 1974), but does this accuracy extend to the intraspecific level? Do folk identification and classification provide us with guides to estimating genotypic diversity? These questions are important because crop germplasm collections are often based to some degree on folk taxonomies. Geneticists at the International Potato Center have reduced the number of accessions in the Center's clonally propagated collection because of their conviction that extensive duplication existed. The idea of duplication derives, in part, from the notion that the folk taxonomies that underlie the original accession list are unreliable and tend toward overclassification.

Our article examines the identification of the cultivated potato (*Solanum tuberosum* L., Solanaceae) in reference to its cultivation in the Andes, its center of domestication. It reviews the means to classify the great intraspecific diversity of potatoes employed by farmers and geneticists. The article presents the use of biochemical markers as a way to cope with the great intraspecific diversity encountered in the Andean potato crop, and it correlates the identification of folk varieties and those determined by biochemical means.

VARIETAL CLASSIFICATION OF THE CULTIVATED POTATO

The diversity of the cultivated potato has posed an intriguing and challenging taxonomic problem for several generations of potato geneticists (Correll 1962; Hawkes 1979). Four ploidy levels ($2n = 2x = 24$ to $2n = 5x = 60$) comprise the

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cultivated potatoes. Under the taxonomic system of Hawkes (1979), which is currently the most widely used, these four ploidy levels divide into eight cultivated species (four diploids, two triploids, one tetraploid, and one pentaploid). In Andean potato fields, the tetraploid *S. tuberosum* ssp. *andigena* (Juz. et Buk.) Hawkes is by far the most prominent, accounting for more than two thirds of the planted potatoes (Brush et al. 1981).

Ploidy and species level classification are of somewhat limited value for treating intraspecific diversity at the field level. While some plants in a typical field may be of different species or ploidy levels, most of them are of a single subspecies (*andigena*). Visual and agronomic distinctiveness may be greater within this subspecies than across the eight cultivated *Solanum* species. Because of the potato's predominantly asexual propagation, clonal distinctiveness is particularly important. This propagation works against the survival of products of genetic exchange and hybridization both between and within species. Somatic mutation is common and will not be revealed at the species or ploidy level. Polyploidization and hybridization may create different species and ploidies out of morphologically similar potatoes.

While the geneticist may be interested in describing potato populations, where species and ploidy level are meaningful, the farmers who actually manage the crop are concerned with the individual plant and variety. The selection, exchange, and maintenance of tubers is done at the variety level. This suggests that an understanding of population dynamics of the Andean potato must somehow include a more sophisticated treatment of the crop at the variety level than is allowed in the current taxonomic tools. However, varietal identification is plagued by such problems as somatic mutation, hybridization, and a daunting amount of data to be analyzed. The elaborate naming system of farmers is often viewed with skepticism by geneticists, partly because the specific knowledge base that determines folk classification is not formalized, and there is no easy way to estimate how consistent folk taxonomies are between individual farmers or between communities.

Nevertheless, we ignore varietal diversity and its identification by farmers at our own scientific peril since the actual dynamics of the potato crop are significantly determined at the varietal level. It has been firmly established for the potato and for other crops that folk identification and taxonomy are the keys to understanding the behavioral patterns that affect crop evolution (Boster 1984; Brush et al. 1981; Johns 1985). The Andean folk classification system for potatoes is based on tuber characteristics, cultivation, edibility, processing and frost resistance. Potato folk taxonomy helps determine cultivation practices that make the Andean potato fields dynamic evolutionary systems where new varieties and perhaps new species are generated by cross hybridization and introgression from related wild species (Jackson et al. 1977; Johns and Keen 1986).

Concern for the varietal level requires that new taxonomic techniques be mobilized, since the conventional ones used for the species and ploidy level description miss most of the intraspecific diversity. Further, the data requirements of using conventional morphological markers for variety identification are so large as to be essentially impossible. We have found that biochemical markers, specifically isozymes, provide effective tools for describing this great diversity.

APPLYING BIOCHEMICAL MARKERS TO ANDEAN POTATOES

Recent research in the Peruvian Andes by Brush indicates that a high degree of diversity is still maintained by peasant cultivators. Inventories of potatoes stored in households after harvest revealed that households in the Paucartambo area east of Cusco maintain an average of 9.6 distinct named varieties. Some households were found to maintain as many as 26 varieties, while others kept as few as a single variety. The traditional planting procedure in southern Peru fields is to sow as many as five small tubers in a single hole opened by the footplow. These tubers might comprise different varieties and even species of different ploidies. One reason for planting heterogeneous fields is to enhance the diet by having a mixture of flavors, textures, shapes, and colors. The great diversity and heterogeneity of these fields pose interesting problems of variety identification and classification by the farmers in order to maintain and conserve the original variability. A folk nomenclature based primarily on tuber morphology has been previously described (Brush et al. 1981).

In the last few years a number of biochemical markers useful for potato clone identification have been reported (Oliver and Zapater 1984; Quiros and McHale 1985; Stegemann and Loeschcke 1977). In particular, isozyme markers are most useful as biochemical markers because of their known genetic basis and co-dominant expression (Quiros and McHale 1985). These have been used to follow up segregations in interploidy matings and to generate experimental data on the transmission of heterozygosity by the diploid strains with the ability to produce $2n$ gametes (Douches and Quiros 1987, 1988).

The objective of this article is to survey with isozyme markers the actual genetic variability of non-bitter potatoes in subsistence Andean fields at 3600–3850 m above sea level. This information serves to determine the accuracy of the folk classification system for potatoes in the Andes.

MATERIALS AND METHODS

Field sampling

Ten fields in a radius of 30 km and located between 3600 to 3850 m above sea level were sampled in the Province of Paucartambo, Department of Cusco, Peru (Table 1). After we sampled a single tuber from 200 plants in each field chosen at random, the tubers were bulked together in a pile according to tuber similarity. The farmer was asked to examine each pile, to regroup piles according to his criteria, and to name each unique group or individual tuber. Then, the tubers were washed and placed in paper bags identified by variety and by field. The only exception was the field at Maqopata (field number 10) where the farmer classified the tubers as they were harvested.

Because of the larger number of tubers, when a variety had more than 30 tubers per bag, a representative sample of 10–25 tubers was taken for the electrophoretic determination depending on the level of morphological heterogeneity present in the bag.

Isozyme analysis

Horizontal starch gel electrophoresis was employed to assay for nine enzyme systems disclosing 12 loci useful for genotyping the tubers of each variety (Douches and Quiros 1987; Quiros and McHale 1985). These are phosphoglucoisomerase 1 (PGI-1), phosphoglucomutase 1 and 2 (PGM-1 and PGM-2), 6 phosphogluconase dehydrogenase 3 (6PGD-3), isocitrate dehydrogenase 1 (IDH-1), malate dehydrogenase 1 and 2 (MDH-1 and MDH-2), acid phosphatase 1 (APS-1), glutamate oxaloacetate

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TABLE 1. FIELD LOCATION, ALTITUDE, FARMER NAME AND NUMBER OF TUBERS FOR *SOLANUM* SPECIES SAMPLED PER FIELD (PAUCARTAMBO, CUSCO, PERU).

Farmer	Location	Altitude (m)	Tubers
1 Jesus Amao	Colquepata	3650	70
2 Julio Amao	Colquepata	3600	47
3 Sebastian Mauri	Sipascancha Alta	3750	101
4 Trinidad Sandi	Mollomarca	3680	135
5 Gomercindo Champi	Mollomarca	3720	45
6 Augustin Turpo	Mollomarca	3750	37
7 Santos Chipa	Sispacancha Alta	3700	76
8 Hipolito Mamani	Carpapampa	3830	72
9 Pablo Mamani	Carpapampa	3780	62
10 Faustino Illa	Moqopata Alta	3850	185

transaminase 1 and 2 (GOT-1 and GOT-2), peroxidase 3 (PRX-3), and shikimic acid dehydrogenase (SKD-1).

For the electrophoretic assay a tuber eye was excised from a tuber and crushed in 75 μl of 0.1 M Tris-HCl pH 7.0 buffer containing 2% glutathione as antioxidant. The extract was soaked into 3M filter paper wicks and subjected to electrophoresis.

Other tuber determinations

The following tuber characteristics are recorded: skin and flesh color, tuber eyes (color, depth, shape), skin texture, and shape. After the electrophoretic survey, chromosome counts were made for a representative tuber of electrophoretic phenotype. For this purpose the tubers were wrapped in moistened towels and introduced into covered plastic shoe boxes. Since most of the tubers were already sprouted, they formed roots readily at the base of the sprouts. The root tips were collected, fixed in alcohol-acetic acid (3:1), and stained with Feulgen.

Genetic parameters

Three parameters were determined in the Andean tuber population, percent heterozygosity (H), total number of alleles, and total number electrophoretic phenotypes in a per locus basis. These values were compared to those recorded in 24 North American and European potato cultivars: 'Russet Burbank', 'Norchip', 'Red Pontiac', 'Gold Rush', 'Kathadin', 'Rosa', 'Bintje', 'Sangre', 'Bel Rus', 'Pioneer', 'Atlantic', 'Monona', 'Targee', 'Nooksack', 'Shepody', 'Superior', 'Agassiz', 'Red Norland', 'Alpha', 'Centennial Russet', 'Bison', 'Lemhi', 'Sebago', and 'Red La Soda'.

Species identification

In an attempt to electrophoretically identify by specific allozymes the various species cultivated in the fields, six to 18 accessions of the species *S. stenotomum* Juz. et Buk., *S. goniocalyx* Juz. et Buk., *S. phureja* Juz. et Buk., *S.* × *chaucha* Juz. et Buk., and *S. tuberosum* ssp. *andigena* were electrophoretically surveyed. Also, 16 accessions of the bitter species *S.* × *juzepzukii* Buk. (3×) and 4 of *S.* × *curtilobum* Juz. et Buk. (5×) were included in the survey.

Comparison between folk and isozyme identification

An agreement index was developed to express a ratio of the proportion of tubers represented by the most numerous electrophoretic phenotype multiplied by the proportion of farms in the sample whose naming agrees with this phenotype. It is arrived at by comparing the number of tubers in the dominant sub-group with the total number of tubers in the group and the number of farmers agreeing on name with the total number of farmers who had that electrophoretic phenotype. A score of one indicates perfect congruence (six cases) and a score of zero indicates that there was no congruence across farms for the electrophoretic phenotypes found under a common name (11 cases).

TABLE 2. NUMBER OF VARIETIES REPORTED AND ACTUAL NUMBER OF ELECTROPHORETIC PHENOTYPES OBSERVED FOR *SOLANUM* SPECIES SAMPLED (PAUCARTAMBO, CUSCO, PERU).

Farmer	Sample size	Varieties reported	Phenotypes observed	Difference
1 Jesus Amao	70	11	15	4
2 Julio Amao	47	14	16	2
3 Sebastian Mauri	101	22	31	9
4 Trinidad Sandi	135	17	34	17
5 Gomercindo Champi	45	8	10	3
6 Augustin Turpo	37	11	10	−1
7 Santos Chipa	76	15	24	9
8 Hipolito Mamani	72	14	20	6
9 Pablo Mamani	62	16	17	1
10 Faustino Illa	185	43	36	−7

RESULTS AND DISCUSSION

Correspondence of folk names and electrophoretic phenotype

Two major results are evident from this research. First, there is an overall high degree of correspondence between farmer identification and electrophoretic phenotype. Second, the accuracy of the folk naming system in electrophoretic terms depended on the farmer doing the classification.

Two types of incongruity were evident within each field. The first type consisted in calling different electrophoretic phenotypes by the same variety name while the second type consisted in calling the same electrophoretic phenotypes by more than one variety name. The most common error of inconsistency was of the first type, leading to a general underestimation of the genetic variability present in the fields. In only two farms (numbers 6 and 10) more varieties than genotypes were observed. Table 2 summarizes the data on tuber identification from the 10 fields. The range of inconsistency in tuber identification went from 3% to 22%. The highest number of underestimated varieties (17 or 50%) was also observed in the same field (Trinidad Sandi, #4), while the lowest was observed for Pablo Mamani (#9) (1 or 5.9%), excluding the fields of Augustin Turpo (#6) and Faustino Illa (#10) where the number of varieties was overestimated. This wide variation represents, in part, lack of enthusiasm or desire by the farmer to spend the time to do an accurate identification, but it also indicates the wide range of skill and knowledge among farmers. Nevertheless, the inconsistency was justifiable in cases where the tubers were morphologically very similar as far as skin color and shape was concerned. However, slicing of the tubers revealed flesh color differences, which were further substantiated by electrophoretic differences.

A good example of this situation was observed for the variety *Yana Imilla* where three phenotypes were detected (Fig. 1–3). Although the distinction to us is enough to separate the three as different varieties, it is possible that the range of variation within *Yana Imilla* was not enough for the Andean farmer to consider them different entities. The consistency of variety names among fields was much lower than within fields, but the level of agreement is still about half. That is in cases where two farmers give the same name for tubers, they are naming similar

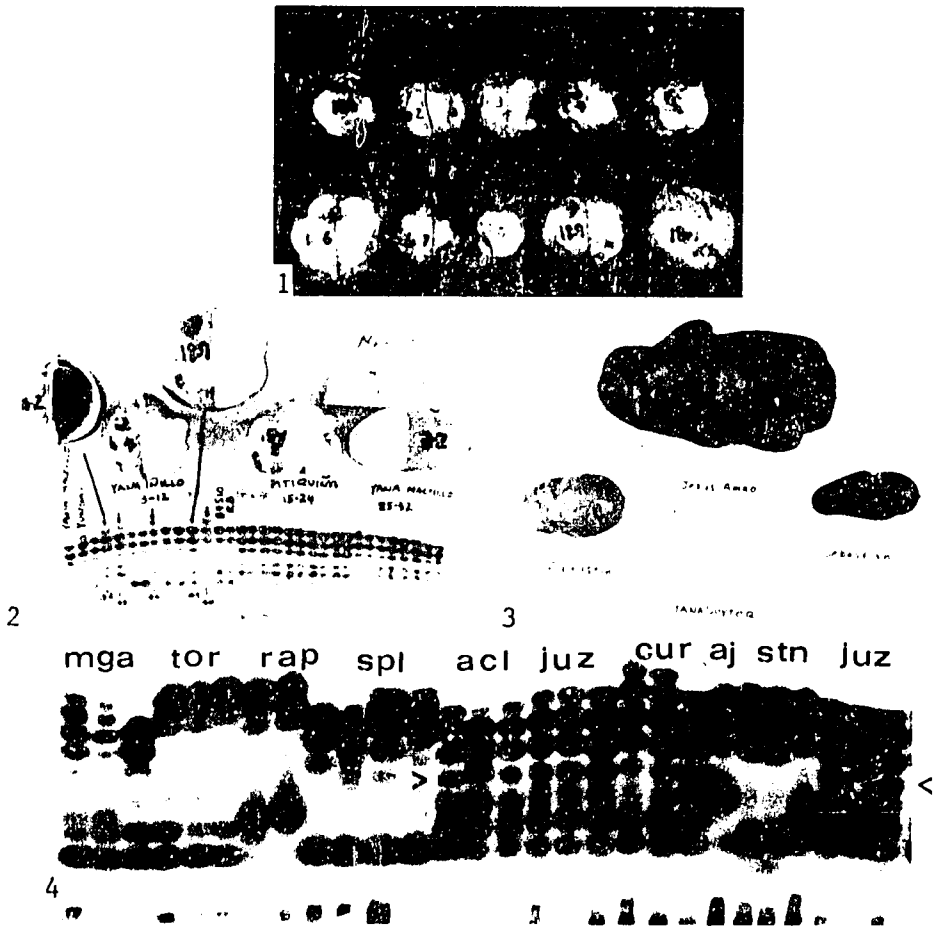

Fig. 1-4. Andean cultivated potatoes. **Fig. 1.** Example of inconsistent classification within *Solanum tuberosum* ssp. *andigena* collected in Paucartambo, Cusco, Peru. Morphologically similar tubers classified as *Yana Imilla*. **Fig. 2.** Example of inconsistent classification within *S. tuberosum* ssp. *andigena* collected in Paucartambo, Cusco, Peru. Sample *Yana Imilla* tubers disclosing three different phenotypes for flesh color and for the enzyme MDH. **Fig. 3.** Example of inconsistency in classification across three fields for the variety *Yana Suyt'u*. Three different morphotypes of *Solanum* species from Paucartambo, Cusco, Peru, with same name. **Fig. 4.** Discrimination of bitter from non-bitter potato species on the basis of the enzyme MDH. The bitter species *S.* × *juzepzukii* (juz) and *S.* × *curtilobum* (cur) carry the unique enzyme Mdh-2[3] also present in their ancestral species *S. acaule* (acl) (arrows). The non-bitter cultivated species and other wild species lack this unique isozyme [mga = *megistacrolobum*, tor = *toralaparium*, rap = *raphanifolium*, spl = *sparsipilum*, aj = *ajanhuiri*, stn = *stenotomum* (Huaman and Ross 1985)]. Three accessions per species are displayed, except for *ajanhuiri*, which includes only one accession.

electrophoretic phenotypes 50% of the time. Comparison between farms is difficult because of the common presence of different electrophoretic phenotypes within a single name. Among 32 named varieties that were found on more than one farm, 19 had more than one electrophoretic phenotype on at least one farm. The average for these multiple electrophoretic phenotypes was 2.9. A common pattern of inconsistency between farms was for agreement on a dominant electrophoretic

TABLE 3. AGREEMENT ACROSS FARMS ON IDENTIFICATION OF LIKE ELECTROPHORETIC PHENOTYPES OF *SOLANUM* SPECIES SAMPLED (PAUCARTAMBO, CUSCO, PERU).

A Variety	B No. tubers in dominant sub-group	C Total no. tubers	D No. farms in agreement	E Total no. farms w. phenotype	F Agreement index*
1	18	30	2	4	0.300
2	6	21	2	5	0.114
3	12	12	2	2	1.000
4	2	4	2	3	0.333
5	13	13	2	2	1.000
6	3	9	2	2	0.333
7	17	21	0	2	0.000
8	2	5	0	2	0.000
9	2	2	2	2	1.000
10	11	11	2	2	1.000
11	1	2	0	2	1.000
12	19	22	3	3	0.864
13	7	17	0	2	0.000
14	11	12	2	2	0.917
15	12	14	2	3	0.571
16	11	21	2	3	0.347
17	1	3	0	2	0.000
18	10	11	0	2	0.000
19	17	23	3	3	0.739
20	1	3	0	2	0.000
21	29	45	6	6	0.644
22	10	13	0	2	0.000
23	10	10	2	2	1.000
24	7	14	0	2	0.000
25	1	2	0	2	0.000
26	10	11	2	2	0.909
27	10	16	0	3	0.000
28	43	54	2	5	0.319
29	33	34	5	5	0.319
30	16	16	2	2	1.000
31	51	70	4	6	0.486
32	2	3	0	2	0.000
	Avg. agreement index			0.43279304	
	St. Dev.			0.41865658	

* Agreement Index (F) = (B/C)(D/E).

phenotype but disagreement on less dominant tubers. Eleven names with accessions from different farms had no multiple electrophoretic phenotypes, and in these cases there was congruence between the name and electrophoretic phenotype on six. Table 3 gives the results of a comparison between names and 32 electrophoretic phenotypes found across our sample of farms. This comparison expresses the dominance of a single electrophoretic phenotype and congruence between farms. An agreement index of one means that the same electrophoretic phenotype was given the same name on all farms where it was found. An index of zero indicates that a different name was given on each farm. Scores between zero and one result when only some farmers agreed on the name or when some electrophoretic phenotypes were given two or more names by the same farmer.

TABLE 4. TUBER NAMES BY ELECTROPHORETIC PHENOTYPE FOR *SOLANUM* SPECIES SAMPLED (PAUCARTAMBO, CUSCO, PERU).

	Name	No. tubers	Skin color
Phenotype 1			
Farm 10	*Yuraq ("white") K'usi*	76	white
	Yana ("black") K'usi	34	black
	Huayro	1	?
Farm 8	*Yuraq K'usi*	10	black
Phenotype 2			
Farm 10	*Soqowaqoto*	4	white
Farm 3	*Soqowaqoto*	2	white
Farm 4	*Yana Ch'eqepphoro*	6	black
	Yuraq Ch'eqepphoro	10	white
	W'allicha	1	?
Farm 6	*Ch'eqepphoro*	1	?
Phenotype 3			
Farm 10	*Yana K'usi*	29	black
Phenotype 4			
Farm 10	*Yuraq W'aqoto*	1	?
Farm 4	*Alqa Qompis*	4	red
	Qompis	7	red
Phenotype 5			
Farm 3	*Onor Qompis*	8	red
Farm 4	*Alqa W'armi*	2	red
Farm 5	*Alqa Qompis*	9	red
	Qompis	6	red
Farm 6	*Qompis*	10	red

In the comparison of names and electrophoretic phenotypes across farms, two types of inconsistencies were, again, evident: the calling of different varieties by the same name and naming a variety by more than two names. This latter inconsistency may be hard to distinguish from synonymy, which is not an error. Synonyms are recognized by farmers and cannot be considered misclassifications.

The previous discussion began with a potato name found on more than one farm and then examined the electrophoretic phenotypes associated with that name. By beginning with the electrophoretic phenotypes and looking for associated names, a pattern of synonymy and some splitting is revealed. Table 4 shows the names for five electrophoretic phenotypes. For phenotype 2, for instance, the names *Soqowaqoto* and *Yuraq Ch'eqepphoro* appear to be synonyms. Farmer 4 split this phenotype into two names according to color, and he apparently erred in the *W'allicha* case.

Our examination of naming in comparison to electrophoretic phenotypes both on single farms and between farms clearly indicates that farmers err in naming principally by underestimating the degree of genetic diversity in their collections. There is far more lumping of different genotypes than splitting of like ones. However, an unexplained fact is that, although we found 104 electrophoretic phenotypes, farmers gave 100 names. This list includes synonyms and overesti-

TABLE 5. PLOIDY DISTRIBUTION AND SPECIES OF THE ELECTROPHORETIC PHENOTYPES OF *SOLANUM* SPECIES SAMPLED (PAUCARTAMBO, CUSCO, PERU).

Ploidy	Frequency (%)	Species
Diploids	13% (16/125)	*stenotomum, gonycalyx, phureja*
Triploids	14% (17/125)	*chaucha*
Tetraploids	67% (84/125)	*andigena*

mation errors of farmers #6 and 10. Our estimate of the actual number of discrete farmer names would be between 80 and 90. This suggests that there is less lumping than indicated in Table 3.

The system of folk identification is expected to be somewhat ambiguous because it is based in part on subjective descriptions. For example, the variety *Yana Kuwi Sullu* means literally "black guinea pig fetus." So depending on the criteria and imagination of the farmer, many of the black skinned tubers could be classified under the same variety name. A good example of this type of inconsistency can be appreciated in the variety *Yana Suyt'u* (Fig. 2). The prefix "yana" refers to black skin color, so Jesus Amao (#1) and Sebastian Mauri (#3) might have been right when they classified the tubers in the figure as *Yana Suyt'u*, although the morphological differences between the two are very distinct. The third farmer, Augustin (#6), might have been truly mistaken since he classified a pink skin tuber as black. However, "suyt'u," means long, which was an accurate description for all three tubers. This second name, of course, might have been used by Augustin as the main criterion to classify his tubers under this variety.

The chromosome counts revealed that the majority of the genotypes cultivated in the fields sampled at Paucartambo were tetraploids, most likely corresponding to *S. tuberosum* ssp. *andigena*. Cultivated diploid and triploid genotypes shared about the same frequency (Table 5). Although it was not possible to find specific allozymes to differentiate the species of the group tuberosum, it is safe to assume that the diploids included the species *S. stenotomum*, *S. phureja*, and *S. goniocalyx*, while the triploids corresponded exclusively to *S.* × *chaucha*. On the other hand, specific allozymes were observed for the bitter species *S. acaule* Bitt., *S.* × *juzepzukii*, and *S.* × *curtilobum*. For example, the allozyme *Mdh-2*[3] was observed in these three species supporting the hypothesis that the former species is ancestral to both the triploid *S.* × *juzepzukii* and the pentaploid *S.* × *curtilobum* (Hawkes 1979) (Fig. 4). So, on the basis of this unique marker, it was possible to conclude that non-bitter triploids were present in the fields sampled at Paucartambo.

No studies have been done in potatoes on the possible associations of specific isozyme loci and genes determining morphological traits such as tuber flesh or skin color. We did not observe in our survey any evidence of these associations for the isozyme loci and traits investigated. These traits include skin and flesh color and tuber shape. For example, the varieties *Yuraq K'usi* and *Yana K'usi* in Maqopata (#10) had the same electrophoretic phenotype for eight loci tested; however, the first is triploid and its skin is white, and the second is black and tetraploid. Therefore, it is likely that one might have been derived from the other by somatic mutation and chromosome doubling. A similar situation was observed for *Yuraq Ch'eqepphoro* and *Yana Ch'eqepphoro*, where the first one is a white

TABLE 6. GENETIC VARIABILITY IN THE ANDEAN POPULATION AND IN NORTH AMERICAN/EUROPEAN CULTIVARS OF *SOLANUM* SPECIES.

	Andean population			North American/European cultivars		
Loci	Alleles	Phenotypes	% H	Alleles	Phenotypes	% H
Pgi-1	3	4	36	2	2	20
Pgm-1	2	2	74	2	3	46
Pgm-2	3	4	4	3	2	52
6pgd3	3	5	81	2	2	92
Idh-1	3	3	44	—	—	—
Mdh-1	4	8	94	4	6	80
Mdh-2	2	2	25	2	2	8
Aps-1	3	4	39	—	—	—
Got-1	3	4	3	2	3	24
Got-2	5	7	2	—	—	—
Prx-3	3	4	58	3	3	52
Sdh-1	4	5	64	—	—	—
Total[a]	23	33	47%	20	24	44%

[a] Excluding *Idh-1*, *Aps-1*, *Got-2*, and *Sdh-1* in the Andean population since these loci were not surveyed in the North American/European population.

tetraploid, and the second one is a black diploid. Another case was observed in two tetraploids of identical electrophoretic phenotype, *Yana Ruki* with black skin and *Yuraq Ruki* with white skin. "Ruki" is the local term for bitter varieties, probably indicating that these samples were misidentified. These observations indicate that skin color is independent of the eight isozyme loci studied.

There were also several cases where identical electrophoretic and morphological phenotypes for tubers of different ploidies were observed, indicating that these varieties might have originated directly by self polyploidization. Other situations included almost identical tubers and very similar electrophoretical phenotypes, making it very difficult to distinguish two or three varieties. For example, the tubers of *Qompis*, *Alqa Qompis*, and *Alqa Warmi* are very similar, and their genotypes are identical for 10 loci but different for the *Prx-1* or *Sdh-1* loci. *Qompis* and *Alqa Qompis* are heterozygous while *Alqa Warmi* is homozygous for these loci. Often the tubers of these varieties were classified as a single variety that was designated by one of the three names, indicating that the farmers understand them as synonyms.

In general we observed that morphologically distinguishable tubers differed in at least one isozyme locus, except in situations where somatic mutations for skin color or polyploidization were evident. In these situations, of course the isozyme technique was superfluous as an identification tool. However, the electrophoretic technique was useful to distinguish tubers that otherwise could be classified as belonging to the same variety. It provided an additional criterion for variety identification. The capability of the technique is expected to increase with the number of isozyme loci surveyed.

Genetic variability in Andean versus North American/European varieties

Although the sample size for both populations was highly unequal—24 North American/European clones against 830 Andean clones—it allowed us to obtain

a rough estimate of the variability in both populations. Furthermore, it is likely that the cultivars from the North American/European population covers pretty much the variability existing in that group, while the Andean population in spite of the larger sample covers only a fraction of all the variability present in the Andes. In any event, the amount of variability observed in the Andean population was superior for the three parameters measured: total number of alleles, total number of electrophoretic phenotypes, and percent of heterozygosity (% H) (Table 6). The largest difference was observed for the number of electrophoretic phenotypes. This increased variability in the primitive population was evident even when considering that 33% was composed by diploid and triploid tubers, while the other population was formed exclusively by tetraploids. An interesting finding in the Andean population was the high frequency of specific alleles at loci *Pgm-2* (allele 2), *Got-1* (allele 3), and *Got-2* (allele 4), reflected in a large number of homozygous genotypes. On the other hand, this was only observed for allele 3 of *Got-1* in the North American/European population. Additional research including a larger survey will be necessary to understand the significance of this observation.

The larger number of alleles and electrophoretic phenotypes in the Andean population, some of which are not represented in the North American/European populations, supports the impression that a substantial amount of yet unexploited variability remains in the primitive potato populations.

CONCLUSIONS

The use of isozymes as a basis for potato classification is advantageous to conventional taxonomies using plant morphological and general protein in several ways. It more closely approximates the genetic base of the potato. It is more easily quantified. It allows for a fuller treatment of intraspecific diversity. It provides a clearer picture of the relation between cultivated species and between cultivated and non-cultivated species.

Folk naming systems of the Andean potato are based on tuber characteristics and rarely include keys from other plant markers. The use of isozyme electrophoresis as a classification tool indicates that farmer identification corresponds with a high degree of accuracy to the actual biological diversity of Andean potato fields. There is, of course, a range of consistency among different farmers, depending on their interest and devotion to knowledge about their potato collection. While consistency within a particular household in classifying its potato collection has been demonstrated previously (Brush et al. 1981), this research suggests that interhousehold confusion over duplicate potatoes is not a major problem. Out of a relatively small sample of 10 fields, we identified 104 electrophoretic phenotypes, and among the 10 different collections there were 100 different names given by our informants. A comparison of electrophoretic and folk identification both within and between fields reveals that farmers may slightly underestimate the diversity of their fields. This finding bears on the debate of the degree of duplication found in the accessions collected from Andean potato fields. Our work leads us to conclude that duplication among accessions is not common. When duplication is found, it might be the result of sampling methods that are less proximate to the ultimate source of Andean potatoes. For instance, collections in markets, rather than in fields or in farmers' storage bins, might produce more duplication

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and lead to an underestimation of the actual amount of diversity still extant in Andean potato agriculture.

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Increase of potato genetic resources in their center of diversity: the role of natural outcrossing and selection by the Andean farmer

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Summary

The Andean farmer conserves and maintains the existing genetic diversity of potato cultivars by means of clonal propagation of tubers. However, surveys of traditional farms showed that botanical seed propagation was used for disease elimination, stock rejuvenation and the creation of new cultivars. Electrophoretic surveys based on 542 tubers collected from 18 markets sampled in the Cusco area disclosed a total of 229 different cultivars from diploid, triploid and tetraploid forms of *Solanum tuberosum* L. These could be classified by isozyme cluster analysis into four major groups and six minor groups. However, they did not agree with groups based on flesh or skin color. It is therefore concluded that all genotypes belong to a single, large gene pool with considerable gene flow between cultivars of different groups. When the samples were grouped by the three most common tuber skin colors, namely red/pink ('Q'ompis type'), purple ('Yana Imilla' type), and yellowish/brown ('Yuraq Kusi' type), similar allozymes were observed in all three classes. The structure of the isozymic phenotypes within each group indicate that they may have been derived as segregants after outcrossing of diverse parental types. In order to provide further evidence for the origin of new types by hybridization, two segregating diploid progenies were generated by crossing purple by yellow skin types. In the resulting F_1, most of the tuber phenotypes observed in the Andean varieties were reproduced in these crosses. It can be concluded that the Andean potatoes form a large and plastic gene pool amplified and renovated by outcrossing followed in some cases by human selection of desirable phenotypes.

Introduction

The Andean region is one of the most important centers of diversity and domestication for many crops. Undoubtedly, the domestication of native plants has involved a long period of selection by Farmers. They have developed and conserved very great diversity of potato germplasm to the extent that probably 2000 to 3000 different cultivars are now grown in the Andes (Brush et al., 1981). These include seven species of four ploidy levels (Hawkes, 1990).

The traditional potato planting procedure under subsistence agriculture in Andean fields maintains the genetic diversity of this crop (Jackson et al., 1980; Brush et al., 1981). The planting of heterogeneous fields, including different varieties, species and ploidies, can be considered to serve at least three purposes: (1) to enhance the diet by providing a mixture of flavors, textures, shapes and colors, (2) to assure the survival and harvest of at least a few plants in case of diseases or other damages, (3) to employ specific cultivars for consumption in different ways such as: soups[1], chuño[2] and moraya[3], watia[4] or for ceremonial rituals. The great diversity of these fields is managed effectively by a system of folk taxonomy able to classify and identify existing varieties (Jackson et al., 1980; Quiros et al., 1990).

The methods of potato cultivation in the Andes provides a dynamic evolutionary system where new varieties and perhaps new species are generated by cross hybridization and introgression from related wild species (Jackson et al., 1977; Johns & Keen, 1986; Rabinowitz et al., 1990). In order to conserve and maintain existing genetic diversity, the Andean farmer has simply carried out clonal tuber propagation of existing cultivars. The broadening of diversity by the creation and adaptation of new genotypes undoubtedly has involved sexual propagation through botanical seed and deliberate selection of desirable individuals derived from this process (Malagamba & Monares, 1988).

The purpose of this paper is to document the increase of genetic diversity of potatoes by the Andean farmer through sexual propagation and phenotypic selection for the creation of new cultivars.

[1]Soups: Varieties with low dry matter which conserve tuber integrity after boiling are used for this purpose.

[2]Chuño: Term used for a product derived from freeze dried potatoes by natural freezing and sun exposure. For this purpose, small and slightly damaged tubers of mostly non-bitter potatoes are used.

[3]Moraya: Similar to chuño, except that medium to large tubers of bitter potatoes (*S. × juzepczukii* and *S. × curtilobum*) are used to elaborate this product. Another difference is that after freezing, before sunrise, the frozen tubers are placed in pools of running water where they are washed for 15 to 18 days to eliminate the toxic alkaloids.

[4]Watia: Potatoes are baked on rustic ovens made of soil clods after harvest. Only non-bitter potatoes with high dry matter are used for this purpose.

Materials and methods

Market sampling. To determine the genetic make-up of some of the potato cultivars grown and marketed in the area, 542 tubers from 18 local markets were collected and described on the basis of tuber high heritability traits such as tuber skin and flesh colors and isozymes. Skin color is determined by three loci; flesh color is also determined by three loci (De Jong, 1987; Dodds & Long, 1955; Howard, 1970). The markets sampled comprise most of the centers of potato growing in the department of Cusco. Five markets were sampled in the city of Cusco, the remaining markets were sampled in six potato production areas which supply the city: Ancahuasi, Chincheros, Pisac, Huancarani, Urcos and Urubamba. In each market, two tubers per variety were collected from a representative vendor who named each variety. Based on tuber traits and isozyme analysis, it was determined that this collection included diploid, triploid and tetraploid forms of *Solanum tuberosum* L. (Dodds, 1962).

Isozyme analysis. Horizontal starch gel electrophoresis was employed to assay for three enzyme systems disclosing four loci useful for genotyping the tubers of each variety (Quiros & McHale, 1985; Douches & Quiros, 1987). These loci were *Pgi-1* (phosphoglucomutase isomerase), *Mdh-1* (malate dehydrogenase), *Got-1* and *Got-2* (glutamate oxaloacetate transaminase). For the electrophoretic assay a tuber bud was crushed in 75 μl of 0.1 M Tris-HCl pH 7.0 buffer containing 2% glutathione as antioxidant. The extract was soaked into 3M filter paper wicks and subjected to electrophoresis. Representatives of all available genotypes and skin/flesh color combinations were subjected to principal component and cluster analyses using the program IRIS (Raamsdonk, 1988).

Multivariate analysis. Agglomerative cluster analysis was based on a matrix with Euclidean distances between accessions using Unweighted Arithmetic

Table 1. Farmers in the communities of Cusco surveyed in this study

Community	Province	District	Altitude (m)	Farmer
Yanacona	Urubamba	Chinchero	3500-4000	Tomasa Quispe
Yanacona	Urubamba	Chinchero	3500-4000	Eugenio Auccapuma
Matinga	Calca	Paray	3600	Eduardo Huaraneca

Average (UPGMA) as cluster criterion. Principal component analysis was carried out by means of eigenvalue and eigenvector extraction from a matrix with correlation coefficients between variables (Raamsdonk, 1988).

Field surveys. In an attempt to explain the extensive tuber variation found in the markets, three traditional farmers from two communities in the Department of Cusco, Peru, where the potato is grown as a subsistence crop, were questioned regarding the origin of their potato cultivars, cultural and planting techniques, and selection of tubers (Table 1). These farmers were selected because they were known to manage a highly variable stock of cultivars and were also known for selling potato seed tubers to other traditional farmers.

Results and discussion

A total of 229 different combinations of isozyme and skin/flesh color phenotypes were found among the 592 tubers collected in the markets. Mono-allelic to tri-allelic, but no tetra-allelic individuals were observed in the isozyme survey. By cluster analysis 218 combinations were grouped in four discrete, major groups (Table 2), whereas the rest were clustered in six small heterogenous groups.

Group 1 (176 accessions). Absence of allele $Pgi\text{-}1^5$. This allele was also absent in 19 accessions of group 2 and in 12 of group 4, but present in all other accessions. Alleles $Pgi\text{-}1^1$ and $Pgi\text{-}1^2$ were mostly present together. Only mono- and di-allelic individuals were observed in this group. Representative cultivars: 'Ccompis', 'Cusi' and 'Suito'.

Group 2 (104 accessions). Predominant absence of $Pgi\text{-}1^2$. The presence of this allele clustered in a subgroup 5 accessions of this group. It was also absent in a subgroup (31 accessions) of group 1 and in 22 accessions of group 4. Mono-, di- and several tri-alleleic individuals were present. All accessions of 'Mactacha' and most of 'Bole' clustered in this group.

Group 3 (43 accessions) Absence of allele $Pgi\text{-}1^1$. This allele was present in the three other groups. Mono-allelic, di- and some tri-allelic individuals were observed. Representative cultivars: 'Cohuisuyo', 'Huaman una'.

Group 4 (57 accessions). Allele $Mdh\text{-}1^2$ was almost always present (only 2 exceptions) whereas allele $Mdh\text{-}1^3$ was always absent in this group. Allele $Mdh\text{-}1^2$ was present in one accession of group 3, allele $Mdh\text{-}1^3$ was absent in 30 accessions of group 1 (mostly 'Suito' cultivars), and absent in 5 accessions of group 2. This group consisted mostly of tri- and tetra-allelic individuals. Probably misclassified 'Ccompis' 'Suito' and 'Bole' cultivars clustered in this group.

The flesh and skin colors and all other allozymes did not show a discrete distribution among the groups. Practically all tuber phenotypes were found in each of the four groups. The grouping did not correspond to the markets from which the material was sampled. One of the six remaining groups (1 specimen) clustered against all other groups for lacking the $Got\text{-}2^4$ allele, which is present in all other individuals. Three other groups (4 specimens) deviated by the predominant occurrence of $Pgi\text{-}1^3$, $Pgi\text{-}1^6$ and $Got\text{-}1^4$. The last two groups (6 specimens) were more or less connected to group 3 with some slight differences. Alleles $Pgi\text{-}1^3$ (5 specimens), $Pgi\text{-}1^6$ (3 specimens), $Got\text{-}1^4$ (3 specimens) and $Got\text{-}2^4$ (6 specimens) were almost completely lacking from the main groups.

Table 2. Characteristic allozymes clustering Andean cultivars in 4 groups. 0 = absence of specific allozyme, 1 = presence: In brackets number of accessions with specific allozyme characteristic of the group

Group	N	$Pgi\text{-}1^1$	$Pgi\text{-}1^2$	$Pgi\text{-}1^5$	$Mdh\text{-}1^2$	$Mdh\text{-}1^3$
1	176	1 (176)	1 (145)	0	0	1 (146)
2	104	1 (176)	0 (5)	1 (85)	0 (1)	1 (99)
3	43	0 (3)	1 (43)	1	0 (1)	1 (42)
4	57	1 (56)	1 (35)	1 (45)	1 (55)	0

It can be concluded from the cluster analysis that all genotypes belong to a single, large gene pool. A considerable gene flow between cultivars of different groups may occur by crossing of different genotypes. This opportunity may be enhanced by tuber transportation to the markets, where potatoes from different locations are pooled together.

Because the number of named cultivars in the sample is large, most likely due to the extensive number of markets and vendors surveyed, we selected three samples comprising three main tuber skin colors for further analysis. In the first group consisting of 'Q'ompis' type potatoes, two main phenotypes were observed: pink skin, cream flesh and pink brownish skin, cream flesh. These two tuber phenotypes, however, included 13 different isozymic phenotypes (Table 3).

The second group included purple skin potatoes of the type known as 'Yana Imilla'. Three flesh colors were observed in this group (Table 4).

The third group, included yellow to brown skin tubers of the type 'Yuraq Kusi'. Three flesh colors could be distinguished in this group (Table 5).

Although in the present study we did not attempt to separate the varieties by ploidy levels, it is safe to assume that the majority of the potatoes sampled were tetraploid. This assumption was based on a previous cultivar survey in the region, on tuber traits and electrophoretic profiles (Quiros et al., 1990). Furthermore, field surveys by other investigators indicates that tetraploid varieties are the most common of all grown in Andean fields (Jackson et al., 1980; Bush et al., 1981). Therefore, most of the varieties classsified in the three skin color groups can be considered tetraploid.

Within each group, the number of isozymic phenotypes ranged from 13 for Q'ompis type to 6 for 'Yuraq Kusi' type. However, the number of alleles among groups was similar. The majority of the alleles were common to all three skin color groups (Table 6). The main difference among isozymic phenotypes was due to homozygosity or heterozygosity of the various loci. Thus, the structure of the phenotypes within each group indicates

Table 3. Frequency of isozymic phenotypes for 'Q'ompis' type potatoes sampled in 18 markets in the Cusco area. The two main groups in the first column were established on the basis of tuber skin and flesh colors

Tuber traits	*Pgi-1*	*Mdh-1*	*Got-1*	*Got-2*	f
Pink skin, cream flesh	1,2	3	3	4	0.33
	1	3	3	4	0.07
	1	1,3	3	2,4	0.05
	1,2	1,3	3	2,4	0.03
	1,5	1	2,3	4	0.03
	1	3	3	2,4	0.02
	1	1,3	3	2,4	0.02
	1,5	2	3	4	0.02
	1,5	3	2,3	4	0.02
	1	2	3	4	0.02
Pink/brown skin, cream flesh	1,2	1,3	3	4	0.33
	1,2	3	2,3	4	0.03
	1	1,3	3	2,4	0.03
			Total	60	

Table 4. Frequency of isozymic phenotypes for 'Yana Imilla' type potatoes sampled from 18 markets in the Cusco area. The three main groups in the first column were established on the basis of tuber skin and flesh colors

Tuber traits	*Pgi-1*	*Mdh-1*	*Got-1*	*Got-2*	f
Purple skin, cream purple flesh	1,2	1	3	4	0.41
	1,2,5	2	3	1,4	0.13
	1,5	1,3	3,4	1,4	0.02
Purple skin, yellow flesh	1,5	3	3	2,4	0.10
	1,5	3	3	1,4	0.08
Purple skin, yellow purple flesh	2,5	1,3	3	4	0.05
	1,5	2	3	1,4	0.18
	1,2,5	1,3	3	1,3,4	0.02
			Total	39	

Table 5. Frequency of isozymic phenotypes for 'Yuraq Kusi' type potatoes sampled from 18 markets in the Cusro area. The three main groups in the first column were established on the basis of tuber skin and flesh colors

Tuber Traits	*Pgi-1*	*Mdh-1*	*Got-1*	*Got-2*	f
Brown skin, white flesh	1,2	1,3	3	4	0.25
Brown skin, cream flesh	1,2	1,3,4	3	1,4	0.17
	1,2,5	2,4	2,3	1,4	0.19
	1	1	3	1,4	0.11
	1	1,3	3	4	0.05
Brown skin, yellow flesh	1,2	3	3	4	0.22
			Total	36	

Table 6. Common and rare alleles of eight isozyme loci for three potato skin colors in all the groups of potatoes sampled from 18 markets in the Cusco area

Skin	Frequent				Rare			
	Pgi-1	*Mdh-1*	*Got-1*	*Got-2*	*Pgi-1*	*Mdh-1*	*Got-1*	*Got-2*
Pink red	1,2	1,3	3	2,4	5	2	2	
Purple	1,2,5	1,2,3	3	1,4			4	2,3
Brown	1,2,5	1,2,3,4	2,3	1,4				

they may have derived as segregants after natural outcrossing among diverse parental phenotypes. Outcrossing most likely takes place at two levels. At the first level, between varieties of contrasting tuber traits. This will generate extensive segregation for skin and tuber colors as well as the introgression of rare alleles. At the second level, between varieties of similar tuber traits. This will result in individuals with sufficiently uniform tuber traits to be considered a single variety, however, they will differ in homozygosity for various isozyme loci. Human selection of new types combining different skin and flesh color and shape, as well as better and uniform types will result in the creation of new genotypes. The study of Rabinowitz et al. (1990) demonstrates that outcrossing among Andean potatoes is very high, resulting many times in interspecific hybridization between closely related species, including weedy and cultivated species

The activities of the farmers surveyed served to explain at least in part the extensive variability observed in the market samples. All three growers surveyed undertook botanical seed propagation. They recalled using this technique in their community for at least 40 to 50 years. It was used in small scale only when the yields decreased due to tuber 'tiring' and 'degeneration'. This deterioration is most likely due to virus disease accumulation in the tubers. Propagation by botanical seeds will eliminate several of these diseases which are not seed transmitted, resulting in 'clean tubers' (Pallais, 1991). The preferred cultivar for sexual propagation is 'Q'ompis', which is the most popular, followed by 'K'usi' and 'Bole', (Quiros et al., 1990). These tetraploid cultivars are chosen also because of their ability to produce abundant berries ('tamboroccotos'). The fruits are collected from the plants when they are ripe, and from the furrows before the tuber harvest. They are left to rot in ceramic containers to facilitate the seed extraction with water. The seed is planted in beds of well prepared soils. The seedlings are either transplanted into the field or left in place until tuberization. The resulting mini-tubers are re-planted later on in the field.

Selection of the best tubers is based on size, number and quality. The selected tubers are used as seed in their farm and also sold to other farmers to rejuvenate their potato crop (Malagamba & Monares, 1988). In two of the communities, three of these selections have become known new cultivars, 'Olones' and 'Wiraccocha', in Yanacona, and 'Q'ompis blanca in Matinga. 'Olones', oldest of these varieties, is 15 to 20 years old.

Although the survey only included a few farmers, it is possible that others may also perform botanical seed propagation and seedling selection. For example anecdotal information was obtained about the use of this practice in the neighboring communities of Calca and Paucartambo. Although the intentional use of botanical seed cannot be considered a common practice, there is plenty of opportunity for unintentional use. This is based on tuber selection from volunteers originating by botanical seed from spontaneous berries dropping to the ground (Johns & Keen, 1986).

In order to prove further the origin of new types by hybridization, we crossed two diploid varieties of purple skin, white/purple flesh to two yellow skin, yellow flesh varieties.

In the resulting F_1s, most of the different tuber phenotypes observed in the Andean varieties were reproduced in these crosses (Fig. 1; Table 7). Similarly, 6 isozyme loci segregating in these two families generated close to the expected 64 isozyme combinations (2 phenotypes per locus). Thus these simple crosses generate an ample number of phenotypes for selection of different types. Certainly at the tetraploid level, one would expect that crossing

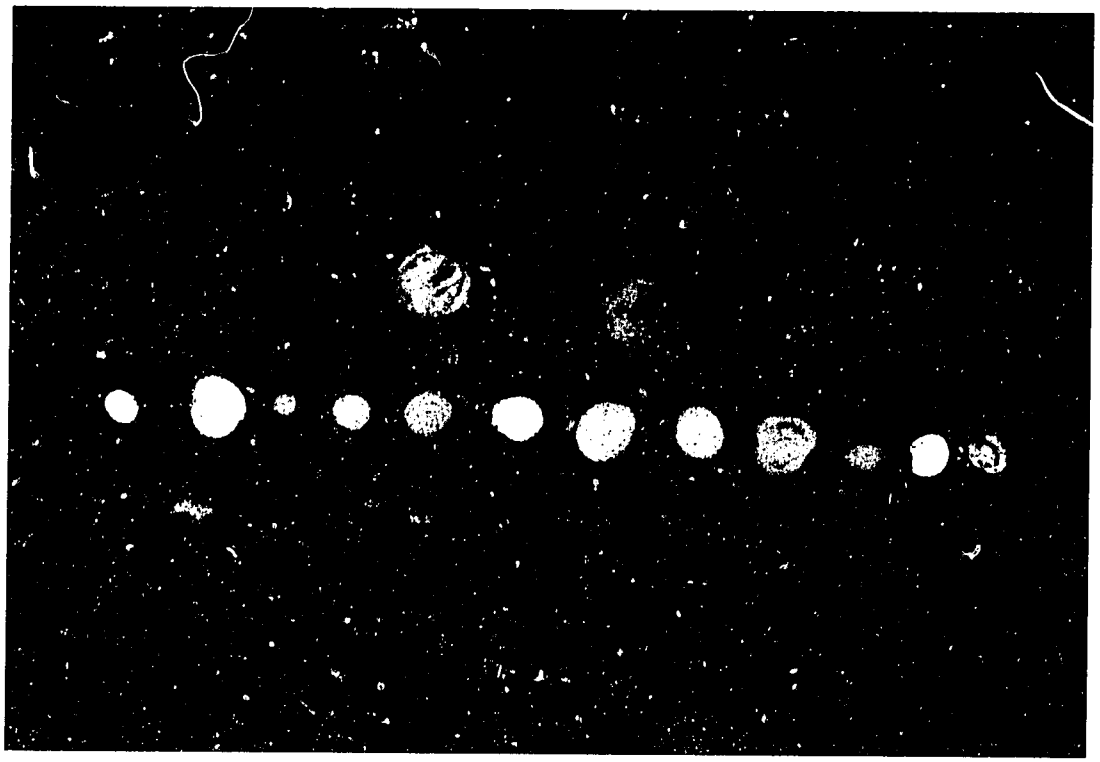

Fig. 1. Segregating F_1 diploid progeny generated by crossing two varieties of yellowish flesh, brown skin and white flesh, purple skin. Tubers of the parental plants are shown in the top two rows.

of plants with contrasting tuber traits will generate a wider range of variation in the resulting progeny than in diploid crosses. This is because the possibility of tri- and tetra-allelic loci in tetraploids versus di-allelic loci in diploids (Douches & Quiros, 1987).

Table 7. Segregation for tuber phenotypes in two F_1 progenies generated by crossing two contrasting diploid potato strains. The tubers of one parent were yellow flesh, brown skin and round shape, those of the other parent were white flesh with purple blush and eliptic shape (see Fig. 1)

Phenotype	N	
Skin color		
Purple	65	
Red pink	64	
Brown white	155	
Flesh color		
White	143	
Yellow	116	
Pigmented (purple red blush)	223	
Non-pigmented	69	
Shape		
Round	219	
Eliptic long	73	Total progeny = 292

In conclusion, the present survey strongly indicates that the Andean potato is a large and plastic gene pool being increased and renovated by outcrossing followed in some cases by human selection of desirable phenotypes. Considering that the peculiarity of the Andean potatoes is its great variability, it is likely that the intentional or unintentional exploitation of botanical seed propagation is intimately linked to the domestication of this crop. Thus in addition to the conservation by the farmers of the existing cultivars, the creation of new cultivars from seedlings followed by selection is a parallel route that may have existed since the early beginnings of this crop but perhaps unconsciously. Furthermore, the transportation and local commercialization of potatoes in the market serves to pool together varieties from different regions which, by selection, propagation and outcrossing, will contribute to the enhancement of the gene pool.

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