INTRASPECIFIC VARIABILITY IN TETRAPLOID VARIETIES (4X) OF POTATO (*Solanum tuberosum* L.)

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ABSTRACT. Six intraspecific crosses, among three commercial varieties of potato -Desirée, Atlantic and Kondor- and four clones coming from the International Potato Center -CIP 23, 110, 114 and 115- were studied during two campaigns (1997/1998, 1998/1999), at the National Institute of Agricultural Sciences (INCA), by means of electrophoretic techniques, with the objective of determining their genetic variability and the possibility of their use in the potato breeding program. Two isozymatic systems, esterases and peroxidases were used. Eighteen samples were taken per each cross, along with the parents, from the first true leaf of the seedlings. A qualitative analysis was carried out for the presence or absence of bands and a quantitative analysis for the calculation of Jaccard's index of similarity. With the total values of the index of similarity for both systems, a test "t" was made to determine any significant differences among the crosses. The greatest variability was found inside each cross than among them, then the crosses Desirée x CIP 23, Desirée x CIP 115 and Desirée x CIP 110 stood out. The esterase system showed a higher polymorphism and detected a bigger variability than the peroxidase system.

Key words: potato, *Solanum tuberosum*, intraspecific hybridization, peroxidase, esterase, electrophoresis, genetic variation

RESUMEN. En el Instituto Nacional de Ciencias Agrícolas (INCA) se estudiaron durante dos campañas (1997/1998, 1998/1999) seis cruzamientos intraespecíficos, entre tres variedades comerciales de papa -Desirée, Atlantic y Kondor- y cuatro clones provenientes del Centro Internacional de la Papa -CIP 23, 110, 114 y 115- mediante técnicas electroforéticas, con el objetivo de determinar la variabilidad genética originada en ellos y la posibilidad de su utilización en el programa de mejoramiento del cultivo. Se utilizaron dos sistemas izoenzimáticos, las esterasas y peroxidasas. Se tomaron dieciocho muestras por cada cruce, conjuntamente con los progenitores, de la primera hoja verdadera de las plántulas. Se realizó un análisis cualitativo por la presencia o ausencia de bandas y un análisis cuantitativo para el cálculo del índice de similitud de Jaccard. Con los valores totales del índice de similitud para ambos sistemas se realizó una prueba "t" para determinar si existían diferencias significativas entre los cruzamientos. Se encontró más variabilidad dentro de cada cruce que entre ellos, destacándose los cruces Desirée x CIP 23, Desirée x CIP 115 y Desirée x CIP 110. Se pudo constatar que el sistema esterasa mostró un elevado polimorfismo, detectando una mayor variabilidad que el sistema peroxidasa.

Palabras clave: papa, *Solanum tuberosum*, hibridación intra-específica, peroxidasa, esterasa, electroforesis, variación genética

INTRODUCTION

Potato cultivars have been historically discriminated and identified through the use of several morphological characteristics, such as: tuber type, leaf type, flower color, bud appearance, growth type and behavior against diseases; however, many of these characters can vary drastically with environmental conditions. Thus, in many cases the environmental effect masks gene effect, so that the phenotype does not represent the real genetic potential of the plant (1, 2).

In this sense, it should be pointed out that electrophoretic techniques make possible the study of genetic variation and the similarities and differences among genotypes to the level of their isozyme or protein composition, independently of the environmental effect (1). It allows to carry out a characterization at a molecular level of the quantity and type of existing genetic variability among closely related species, since in this case the variation of pattern bands can be in general related to the variation in the genetic code for the varying proteins (3).

The genetic variability originated in the combinations among cultivated tetraploid varieties has been studied (4, 5); but it has not been enough to demonstrate that at this level exists a lot of genetic variability to continue being exploited in the potato breeding program.

This variation can be measured through morphoagronomical characters and biochemical techniques; the first ones are limited by the impossibility of equaling genotypes with phenotypes, while the second ones allow to make a molecular characterization of the quantity and kinds of genetic variability among closely related species (2, 6, 7, 8).

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Thus, this work was aimed to demonstrate that in the crosses among tetraploid varieties there exists enough variability to continue our works of potato breeding program.

MATERIALS AND METHODS

During two campaigns (1997/1998, 1998/1999) six intraspecific crosses, among three commercial varieties of potato -Desirée, Atlantic and Kondor- and four clones coming from the International Potato Center -CIP 23, 110, 114 and 115- were studied at the National Institute of Agricultural Sciences (Table I). Crosses were made in the campaign 1996/1997. Seeds were extracted and sowed under greenhouse conditions, where leaves were taken for the electrophoretic studies.

Two izozyme systems, esterases and peroxidases were used to estimate variability. Eighteen samples were taken per each crossing, along with parents, from the first true leaf of the 30 day-old seedlings, planted in nylon bags under greenhouse conditions.

Table I. Crosses studied

No.	Crosses
1	Atlantic x Kondor
2	Desirée x CIP 23
3	Desirée x CIP 115
4	Desirée x Kondor
5	Desirée x CIP 110
6	CIP 114 x Desirée

Preparation of the samples and parameters of electrophoresis assays. Leaf samples of 0.2 g were taken for the preparation of extracts. They were homogenized in buffer Tris-HCL containing 0.05 M to pH 7.5, 10 % sucrose, 5 % PVP, 0.5 % Polyetilenglicol-6000 and 10 % ß mercaptoetanol in proportion 1:1. The extract was centrifuged to 5ºC at 12 500 rpm for ten minutes. The non precipitated rest was used for electrophoretic studies. The electrophoresis running for both izozyme systems was respectively made in polyacrilamide gels to 8.5 and 10 % with buffer Tris-HCL 0.375 M to pH 8.8 in the gel separator and 4 % with buffer Tris-H3BO3 0.16 M-0.035 M to pH 8.9 in Laemmli compartments.

The time of assay was determined in each case, for the displacement of Kolrhauch band until approximately 6 cm from the beginning of the separated gel. The intensity of current used was 10 MA for one hour and later on the assays continued to 20 MA, in camera of vertical electrophoresis Mighty Small II of Pharmacy Biotech. After having made the separation, gels were specifically stained according to Iglesias'methodology (9).

Analysis of the zymograms. The isozyme phenotypes were settled down in connection with band number and the relative position of each stained band, the last one settled down on the base of half the distance of migration obtained, divided among the distance of migration of the bromophenol blue band.

A qualitative analysis was carried out for the presence or absence of bands and a quantitative analysis for the calculation of Jaccard's index of similarity (10) by means of the formula:

SI = Number of common bands/Number of total bands

Variability was detected according to the number of bands presented by each hybrid offspring in connection with the izozyme pattern of parents.

Statistical analysis. With the total values of the similarity index for both systems, transformed to percentages, a test "t" was carried out to determine if there existed significant differences among the crosses.

RESULTS AND DISCUSSION

Isozyme analysis. Peroxidase system. The electrophoretic diagrams obtained after having revealed the specifically stained gels for the isozyme peroxidase, it was allowed to appreciate a high enzymatic activity in the system, because between 8 and 11 bands were observed depending on the analyzed crosses, which were located in three activity places detected -Px_1, Px_2 and Px_3-, and presented a total of four constituent bands common to all the analyzed genotypes.

The electrophoresis peroxidase diagrams (Figure 1) detected a bigger number of hybrid offsprings in the crossings Desirée x CIP 23, Desirée x CIP 110 and Desirée x CIP 115 with four different progenies, followed by Atlantic x Kondor and CIP 114 x Desirée with two hybrid offsprings. However, the crossing Desirée x Kondor only presented the band patterns of either parent.

With regard to isozymatic activity places, it should be highlighted that both were firstly characterized to present a little polymorphism in their band patterns, in general a codominance effect being shown in the segregation of parental alleles. Thus, in Px_1 place, parents showed up to five bands, with ranges of Rf between 0.428 and 0.718; while their hybrid ones showed indistinctly characteristic phenotypes of either parent (Figure 1b, c and f), except the crossing Atlantic x Kondor (Figure 1a), in which band segregation was not appreciated in this area. Px_2 place, on the other hand, presented enzymatic monomorphism in the crossing Desirée x CIP 114 and different phenotypes for the rest of the crossings.

On the contrary, region 3 (Px_3) revealed a high degree of genetic polymorphism in all the evaluated crossings; thus, characteristic bands of the parents were not visualized in the crossings Desirée x CIP 110 (Figure 1b), Desirée x CIP 23 (Figure 1c), Desirée x CIP 115 (Figure 1e) and CIP 114 x Desirée (Fig. 1f), maybe due to the heterocigocity of the parental loci, followed by disorders in the meiotic segregation (11); likewise an extra band was observed in the crossing Desirée x CIP 110 (Figure 1b), that can be justified for the depression of an allele in the new genetic constitution of the hybrid ones.

Figure 1a) Atlantic x Kondor

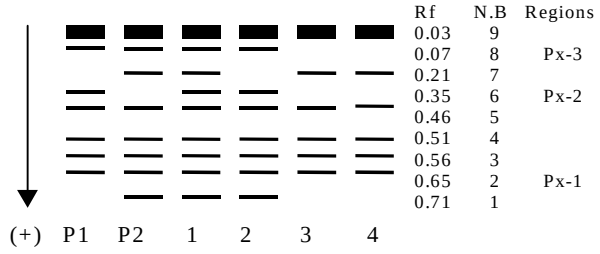

Figure 1b) Desirée x CIP 110

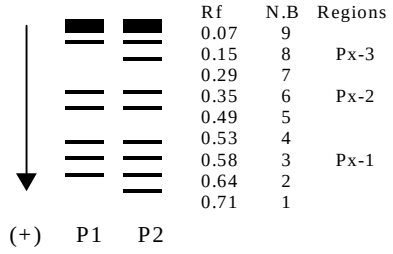

Figure 1c) Desirée x CIP 23

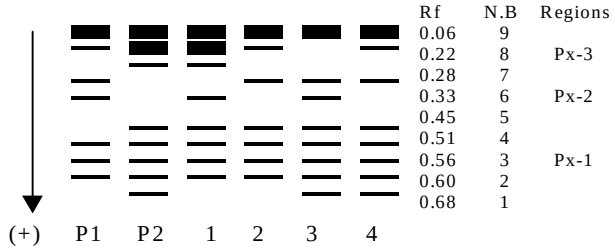

Figure 1d) Desirée x Kondor

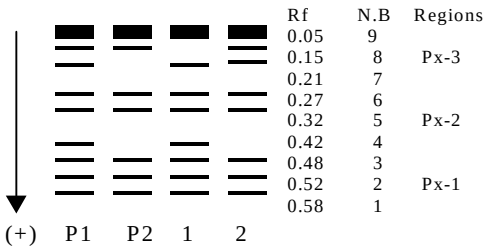

Figure 1c) Desirée x CIP 115

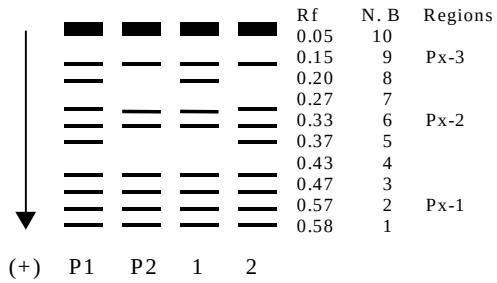

Figure 1c) CIP 114 x Desirée

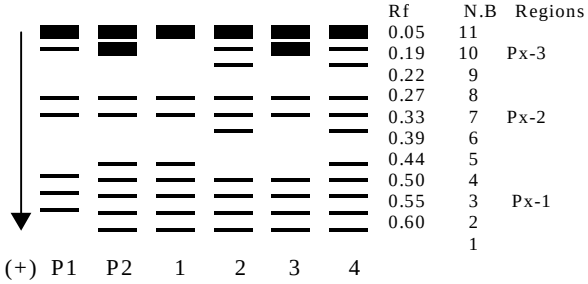

Figure 1. Zymograms of the peroxidase enzyme in the crosses studied

Now then, these results are according with those obtained by diverse authors (3, 11, 12) that found genetic polymorphism in this enzymatic system. In this sense, others (11) found three activity regions for this enzyme when analyzing hybrids of an interspecific crossing and hybrids of S. *tuberosum* with *S. Tarijense, S. Demissum* and *S. chacoense* respectively, where region Px_3 was the most polymorphic. It should also be pointed out, with regard to this region, that it is a polymorphic place in the cultivation included in the genetic map of species, in the chromosome 2 (1) and it can be related with the total yield of tubers (12).

Esterase system. The esterase electrophoretic diagrams were shown equally polymorphic (Figure 2), but they allowed to differentiate a bigger number of hybrid offsprings, the most variable being the crossings Desirée x CIP 23 (Figure 2c) and Desirée x CIP 115 (Figure 2e) with eight and seven new genotypes respectively, followed by Atlantic x Kondor (Figure 2a), Desirée x CIP 110 (Figure 2b) and CIP 114 x Desirée (Figure 2f) with five hybrid offsprings each one, while the crossing Desirée x Kondor (Figure 2d), like the previous system, presented the smallest number of different genotypes.

Concerning esterase system, 10 (Figure 2a, b and e) and 11 (Figure 2c, d and f) bands were observed, out of which only one was present in all genotypes. The presence of new bands were observed with electrophoretic mobility of 0.72, for the crossings Desirée x CIP 110 (Figure 2b) and 0.79 for the cross Desirée x CIP 23 (Figure 2c), which can be a product of the segregation of the parental alleles.

In this sense, it should be pointed out that in most crops, esterase isozymes are constituted by a complex group of proteins associated with specific intra-cell proteins that play a vital role in the photosynthesis (10), which is related with the great number of opposing bands.

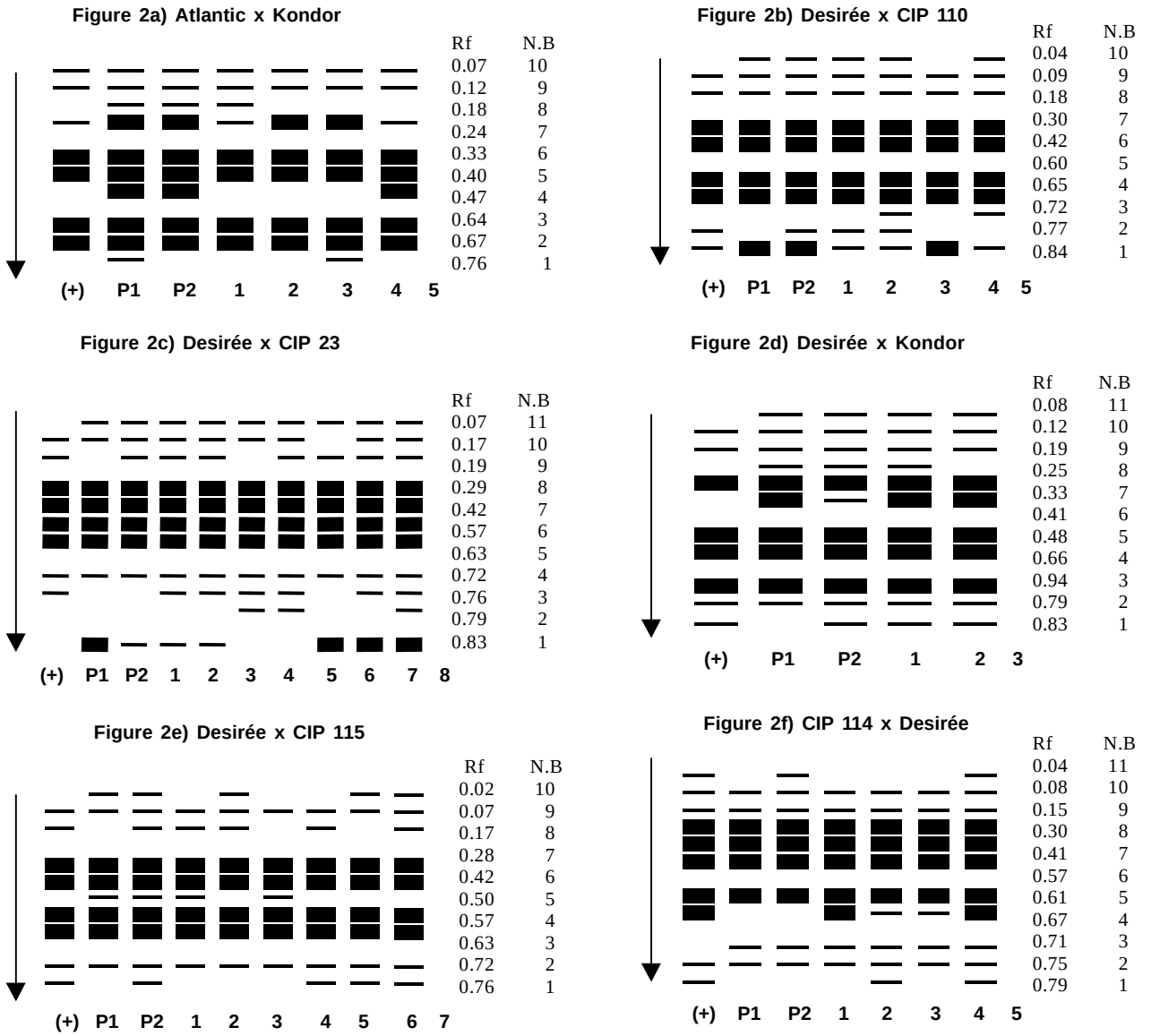

Figura 2. Zymograms of the esterase enzyme in the different crosses studied

The complexity of most patterns of esterase bands in potato was investigated (13); they proposed a model to prove the existence of three loci each one coding for a subunit. Supposing the presence of three subunits, each one coded by a different locus, those bands that can associate and determine the enzymatic activity are supposed to be a tetrameric or homotetrameric enzyme. Thus, according to this model, up to 15 bands can be present, but the patterns showed as maximum eleven bands, which suggests that some of them comigrated; likewise, the variation of band number in the different crosses, even in parents with the same number of bands (Figure 2b, c and e) suggests (11) the presence of a polymorphic allele in some loci that had not been kept in mind in the previous pattern presented.

The index of similarity (Table II) as an approach of sensitivity of each enzymatic system to determine the variability among the crossings was far from 1 in both systems. It is according with investigations (3, 10, 14) previously carried out, where this one is recommended to value the hybrid nature of *Solanum* species like other polyploid crops. Nevertheless, a bigger variation was evident for the esterase system when presenting lower percentages of similarity for all crossings with regard to peroxidase system.

Table II. Polymorphism of the peroxidase and esterase systems for the determination of variability in the crosses made

Crosses	Peroxidases			Esterases			Total		
	NBC	TB	SI	NBC	TB	IS	NBC	TB	SI
Atlantic x Kondor	8	10	0.80	6	10	0.60	14	20	0.7
Desirée x CIP 23	6	9	0.67	6	11	0.55	12	20	0.61
Desirée x CIP 115	7	10	0.70	6	10	0.60	13	20	0.65
Desirée x Kondor	7	8	0.88	7	11	0.64	14	19	0.76
Desirée x CIP 110	7	10	0.70	6	10	0.60	13	20	0.65
CIP 114 x Desirée	7	9	0.78	7	11	0.64	14	20	0.71

NCB-Number of common bands TB-Total bands SI-Similarity index

The most variable cross was Desirée x CIP 23, which presented the smallest index of similarity for both systems with 0.61, followed by the crosses Desirée x CIP 115 and Desirée x CIP 110 with 0.65 each one. The cross Desirée x Kondor was the least variable reaching the biggest index similarity for both systems with 0.76 and the nearest value at 1 with 0.88 for the peroxidase system, the highest value among all crosses.

The results obtained with test "t" of comparison of percentages, for the values of the index of similarity, using both systems (Table III) showed no significant differences among crossings, which would indicate that the degree of genetic variability inside each cross, based on isozymatic studies, is similar for the different crosses. Also, we must keep in mind that although Desirée variety was used in five of the six crosses, there was a great segregation detected by these systems.

Table III. Results from the test "t" of comparison of percentages for the similarity index value

Crosses	1	2	3	4	5	6
1	1.00					
2	0.327 ns	1.00				
3	0.665 ns	0.338 ns	1.00			
4	0.327 ns	0.00 ns	0.338 ns	1.00		
5	0.665 ns	0.589 ns	0.256 ns	0.589 ns	1.00	
6	0.443 ns	0.112 ns	0.229 ns	0.112 ns	0.485 ns	1.00

1- Atlantic x Kondor
2- Desirée x CIP 23
3- Desirée x CIP 115
4- Desirée x Kondor
5- Desirée x CIP 110
6- CIP 114 x Desirée

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