

A genetic classification of potato cultivars based on allozyme patterns

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Summary. A total of 25 potato isozymes were characterized by the numbers and relative mobilities of their allozymes, the subunit number, the subcellular localization, and the patterns of tissue expression. Using hierarchically ordered phenotype arrays at 9 of these isozymes, we were able to construct a dichotomous classification table for a total of 74 potato varieties, including those of most agronomical interest in Europe and North America.

Key words: *Solanum tuberosum* – Potato – Varietal classification – Isozymes

Introduction

Allozymes (= allelic isozymes) have proven to be a very useful tool in deciphering the evolutionary relationships within different groups of plant and animal organisms. As genetic markers, they have been also successfully used for cultivar identification in many food crops and related plants (Desborough and Peloquin 1968; Fedak 1974; Ntarella and Sink 1975; Almgard and Clapham 1975, 1977; Wehner et al. 1976; Wolfe 1976; Bassiri and Rouhani 1977; Valizadeh et al. 1977; Werner and Sink 1977; Kuhns and Fretz 1978; Santamour and Demuth 1980; Salinas et al. 1982).

In a previous paper (Martínez-Zapater and Oliver 1984a) we reported the preliminary results obtained in the isozyme identification of potato cultivars (*Solanum tuberosum* group Tuberosum). We report here the characterization (number and relative mobilities of the allozymes, subunit number, subcellular localization, and pattern of tissue expression) of a total of 25 potato isozymes. The hierarchically ordered phenotype arrays at 9 of these isozymes allow us to construct a dichotomous classification table for a total of 74 potato varieties, 68 of which were entirely distinguished.

Materials and methods

A total of 74 potato varieties were analyzed (Table 3). Almost 30% of them were from more than one source (Estación de Mejora de la Patata, Vitoria, Spain; Instituto Nacional de Semillas y Plantas de Vivero, Madrid, Spain; Government Institute for Research on Varieties of Cultivated Plants, Wageningen, Holland; Potato Introduction Station, Madison, Wisconsin, USA). The potato tubers were normally maintained in a lighted room to avoid sprouting, and put under dark conditions when sprouts were required. Roots, leaves, and flowers were obtained from tubers grown in pots or in hydroponic culture with a standard nutritive solution.

Samples of different organs of the plant (central zone of tuber, basis and tip of the sprout, leaf, root, petal, anther, pollen grains, ovary, and calyx) were analyzed. Once the tissue expression pattern of each isozyme was determined, we selected those isozymes expressed in the tuber and/or the sprout for the program of varietal identification. All but POX-F selected isozymes were analyzed in the tuber. Both tuber and sprout are the most easily available organs when varietal identification studies are attempted. Special care was taken to analyze the different organs in a similar developmental stage in all the varieties.

In order to prevent browning, the enzyme extraction was accomplished by crushing the plant material in a buffered solution of several reducing agents at 1:2 w/v (Valizadeh 1977). For PGD and PGM enzymes, glycerol (10%) was added to the extraction buffer (Roose and Gottlieb 1980). The enzyme extracts were adsorbed directly onto 3×8 mm paper wicks (Whatman no. 3) and subjected to horizontal starch gel electrophoresis, with LiOH/Borate (pH 8.1) electrode buffer and Tris/Citrate (pH 8.3) gel buffer (Selander et al. 1971). For PGD and MDH enzymes we added EDTA 0.4 M to gel and electrode buffers. Electrophoresis was carried out in 1.0×17.5×20.0 cm gel trays at 200 V and 50 mA. Samples of cv. 'Desirée' were included in all the slab gels as internal markers to determine the electrophoretic mobilities of the different allozyme bands.

Ten consistently scorable enzymes were assayed: esterases (EST) E.C. 3.1.1.1 using alfa-naphtyl acetate in a buffer of PO₄H₂K 0.1 M (pH 6.5) and staining with Fast Blue RR; alcohol dehydrogenase (ADH) E.C. 1.1.1.1 (Pasteur 1973); glutamate oxaloacetate transaminase (GOT) E.C. 2.6.1.1

(Gottlieb 1973); phosphoglucose isomerase (PGI) E.C. 5.3.1.9 (Brewer 1970); phosphoglucomutase (PGM) E.C. 2.7.5.1 (Brewer 1970); peroxidases (POX) E.C. 1.11.1.7 (Shaw and Prasad 1970) with the pH modified at 4.5 according to Rick et al. (1974); malic enzyme (Me) E.C. 1.1.1.40 (Brinkman and Van der Meer 1975) with Tris/ClH 0.2 M (pH 7.0); glutamate dehydrogenase (GDH) E.C. 1.4.1.3 (Fine and Costello 1963); malate dehydrogenase (MDH) E.C. 1.1.1.37 (Philipp et al. 1979); 6-phosphogluconate dehydrogenase (PGD) E.C. 1.1.1.43 (Brewer 1970). The gels were immediately photographed and fixed in methyl alcohol, acetic acid, and water for GOT and EST, glycerol and water for POX and the dehydrogenase enzymes.

Isozymes were designated by a hyphenated capital letter added to the symbol for each enzyme; the isozyme with the most anodal migration was designated A, the next B, and so forth. At each isozyme, the allele with the greatest relative mobility was called a, and then b, c, d, etc. Isozyme and allozyme letter-names were assigned after the electrophoretic survey we have carried out on different diploid and tetraploid groups of *Solanum tuberosum* and other two diploid wild species, *S. sparsipilum* and *S. pinnatisectum* (Oliver and Martínez-Zapater 1984).

Results

Inheritance studies on progenies from different crosses between tetraploid cultivars (Martínez-Zapater and

Oliver 1984 b) and the differential patterns of tissue expression (Table 1) were the criteria we employed in determining the number of isozymes and allozymes at each enzyme. Every isozyme shows the same tissue expression pattern in all groups of cultivated potatoes (Oliver and Martínez-Zapater 1984).

The relative allozyme mobilities, the presence of epigenetic modifications, the subunit number and the subcellular localization of each isozyme are shown in Table 2. Monomeric or dimeric structure was deduced from the absence or presence, respectively, of heterodimeric molecules in the heterozygotes. The subcellular localization was determined as described by Weeden and Gottlieb (1980). In several varieties heterozygous for GOT-B, instead of the normal three-banded phenotype, we found a two-banded one (Fig. 2c, lanes 2, 3, 6, 7, 10). This would be explained if these varieties were heterozygous for a "null" allele. This allele would encode an enzyme subunit capable of forming heterodimeric but not homodimeric active molecules. It was named c' in Table 3, since the heterodimers it forms show the same electrophoretic mobility as those formed by the c allele. The electro-

Table 1. Tissue expression patterns of the different isozymes in potato varieties (*S. tuberosum*)

Isozyme	Tissue ^a											
	YL	ML	R	TU	ST	SB	P	A	PO	O	C	
ADH-A	-	-	+	+	-	-	-	-	-	-	-	
ADH-B	-	-	-	-	-	-	-	+	+	-	-	
EST-A	+	*	-	-	+	*	+	+	-	+	+	
EST-B	*	+	+	-	*	+	-	-	-	-	-	
EST-C	-	-	-	+	-	-	-	-	-	-	-	
GDH-A	+	+	+	+	+	+	+	+	?	+	+	
GOT-A	+	+	+	+	+	+	+	+	+	+	+	
GOT-B	+	+	+	+	+	+	+	+	+	+	+	
MDH-A	+	+	+	+	+	+	+	+	+	+	+	
MDH-C	-	-	-	-	-	-	-	+	+	-	-	
MDH-D	+	+	+	+	+	+	+	+	+	+	+	
Me-A	-	-	-	-	-	-	-	+	+	-	-	
Me-B	+	+	+	+	+	+	+	+	+	+	+	
PGD-A	+	+	+	*	+	+	+	+	?	+	+	
PGD-B	*	+	*	+	*	+	+	+	?	+	+	
PGD-C	+	+	+	^b	+	+	+	+	?	+	+	
PGI-A	+	+	+	+	+	+	+	+	+	+	+	
PGI-B	+	+	+	+	+	+	+	+	+	+	+	
PGI-C	*	*	*	*	*	*	*	*	*	*	*	
PGM-A	+	+	+	+	+	+	*	*	*	*	*	
PGM-B	+	+	+	+	+	+	+	+	+	+	+	
POX-B	*	+	*	^c	+	+	*	*	-	*	+	
POX-C	-	*	+	^c	*	+	*	*	-	*	*	
POX-E	-	*	+	+	-	*	-	-	-	-	-	
POX-F	*	+	-	-	-	+	-	-	-	-	+	

^a YL=young leaf; ML=mature leaf; R=root; TU=tuber; ST=sprout tip; SB=sprout basis; P=petal; A=anther; PO=pollen; O=ovary; C=calyx

^b The activity of this isozyme disappears in old tubers

^c Only expressed in the cortex

* Poor activity

Table 2. Allozyme relative mobilities, subunit number, subcellular localization, and presence/absence of epigenetic modifications at each isozyme in different groups and species of potatoes

Isozymes	Allozymes ^a							Subunit no. ^b	Subcellular localization ^c	Epigenetic modifications
	a	b	c	d	e	f	g			
ADH-A	0.55	0.51	0.47					2	C	no
ADH-B	0.49	0.48	0.43	0.35				2	C	no
EST-A	0.88	0.85						1	—	no
EST-B	0.86	0.84	0.82	0.80	0.77			1	—	no
EST-C	0.68	0.66	0.63	0.61	0.59	0.56	0.53			
GDH-A	0.24	0.17	0.10					2	—	no
GOT-A	0.46	0.40	0.36					2	O	no
GOT-B	0.31	0.23	0.22	0.14	0.06			2	C	no
MDH-A	0.84	0.78	0.72					2	—	no
MDH-C	0.38	0.34						—	—	—
MDH-D	0.15	0.10						—	O	yes
Me-A	0.40							—	—	—
Me-B	0.32							—	—	yes
PGD-A	0.83	0.80						2	—	no
PGD-B	0.72	0.70						2	—	no
PGD-C	0.68	0.64	0.60					2	—	yes
PGI-A	—	—	—					—	O	—
PGI-B	0.41	0.37	0.33	0.27	0.22			2	C	yes
PGI-C	—	—	—					2	C	—
PGM-A	0.52	0.50	0.47					1	O	no
PGM-B	0.44	0.40	0.34					1	C	no
POX-B	0.63	0.60	0.57					1	—	—
POX-C	0.54	0.52	0.50	0.48				1	—	no
POX-E	0.23	0.20						1	—	no
POX-F	0.17	0.08						1	—	no

^a Relative electrophoretic mobilities with respect to the Bromophenol Blue band

^b 1 = monomer; 2 = dimer

^c C = cytosol; O = organelle

phoretic bands always associated with particular electrophoretic morphs, generally with a higher relative mobility, and whose presence cannot be explained through inheritance studies, were considered as epigenetic modifications. Staub et al. (1982) also reported modifications at PGI and Me isozymes of the potato.

The isozymes we used for varietal identification (Figs. 1 and 2) were: ADH-A, EST-C, GOT-A, GOT-B, PGD-C, PGI-B, PGM-B, and POX-C, present in the tuber, and POX-F which can be detected in the sprout (Table 1).

We only considered the presence or absence of each allozyme irrespective of their staining intensity. Thus, we obtained for each potato variety an array of characters indicating the presence/absence of each allele. The different isozymes were hierarchically ordered according to the numbers of classes they divide the collection of 74 potato cultivars. POX-F was considered the latest due to its expression in the sprout but not in the tuber. Isozyme data were then sorted in a computer, obtaining a matrix with the ordered phenotype arrays of the 74 potato varieties. A dichotomous classification table was then derived from this matrix (Table 3).

Discussion

The dichotomous classification of Table 3 shows that 68 out of 74 potato varieties analyzed were entirely identified by their electrophoretic phenotype arrays at nine isozymes. Only three pairs of varieties ('Cardinal'–'Diamant', 'Katahdin'–'Sebago', and 'Onda'–'Belda') show an identical phenotype in all the isozymes studied; nevertheless, the first two varieties are easily distinguishable by their skin colour: 'Cardinal' skin is red whereas 'Diamant' has a yellow one. Morphological distinction of tubers is more difficult in the other two pairs of varieties; increasing the number of isozymes studied will probably permit their separation.

With the exception of both EST-C and POX-F, we have used electromorph variants from isozymes whose genetic control was known (Martínez-Zapater and Oliver 1984b). May et al. (1982) have recently pointed out the usefulness of genetic studies on isozymes for potato variety identification. Genetic markers have several advantages with respect to the use of both storage protein profiles (Stegemann and Loeschke 1976; Stegemann 1979) and isozyme bands without any consideration about their genetic control (Desborough and Peloquin 1968). Mainly, it avoids the variation in patterns with the physiological conditions of the tubers, since although these conditions also affect the expression of some multilocus iso-

Table 3 (continued)

EST C	POX C	PGI B	GOT B	GOT A	ADH A	PGM B	PGD C	POX F	Variety										
bcefg -	bd	a	c	de	c'd	cd	[a	ab	'Marijke'										
									'Roja Riñon'										
									'Urgenta'										
									'Libertas'										
									'Pimpernel'										
									'Record'										
									'Sientje'										
									'Heida'										
									'Flora'										
									'Alda'										
									'Green Mountain'										
									'Cardinal'										
									'& Diamant'										
'Palogan'																			
'Buesa'																			
'Fenix'																			
bcdefg -	ad	c	d	cd	[b	bc	[ab	bc	'Batoche'										
									'Furore'										
									'President'										
									'Draga'										
									'Belle Isle'										
									'Ackersegen'										
									'Turia'										
									'Alava'										
									'Ari'										
									'Netted Gem'										
									abde	ac	c	cd	bc	[c	d	cd	[b	bc	
																			'Batoche'
																			'Furore'
'President'																			
'Draga'																			
'Belle Isle'																			
'Ackersegen'																			
'Turia'																			
'Alava'																			
'Ari'																			
'Netted Gem'																			
'Royal Kidney'																			

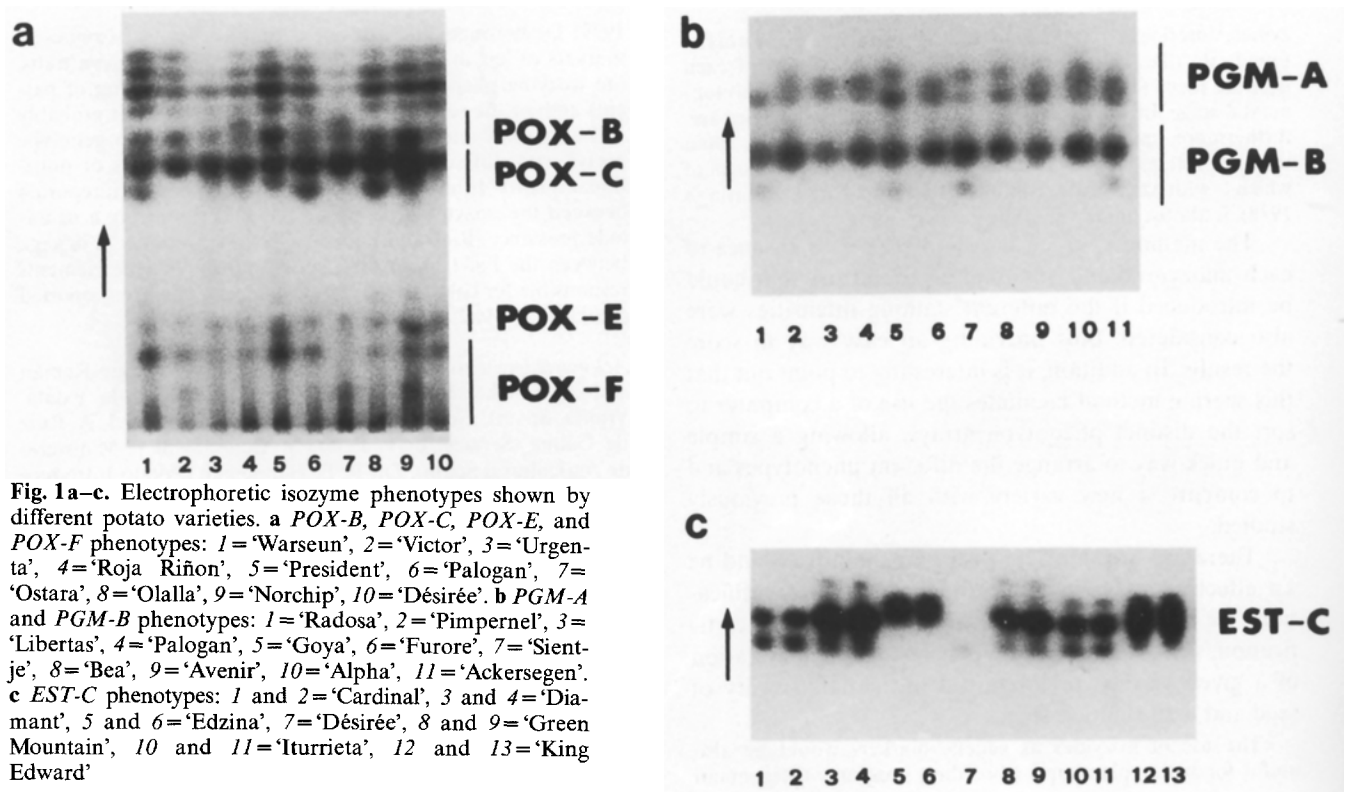


Fig. 1a-c. Electrophoretic isozyme phenotypes shown by different potato varieties. **a** *POX-B*, *POX-C*, *POX-E*, and *POX-F* phenotypes: 1='Warseun', 2='Victor', 3='Urgenta', 4='Roja Riñon', 5='President', 6='Palogan', 7='Ostara', 8='Olalla', 9='Norchip', 10='Désirée'. **b** *PGM-A* and *PGM-B* phenotypes: 1='Radosa', 2='Pimpernel', 3='Libertas', 4='Palogan', 5='Goya', 6='Furore', 7='Sientje', 8='Bea', 9='Avenir', 10='Alpha', 11='Ackersegen'. **c** *EST-C* phenotypes: 1 and 2='Cardinal', 3 and 4='Diamant', 5 and 6='Edzina', 7='Désirée', 8 and 9='Green Mountain', 10 and 11='Iturrieta', 12 and 13='King Edward'

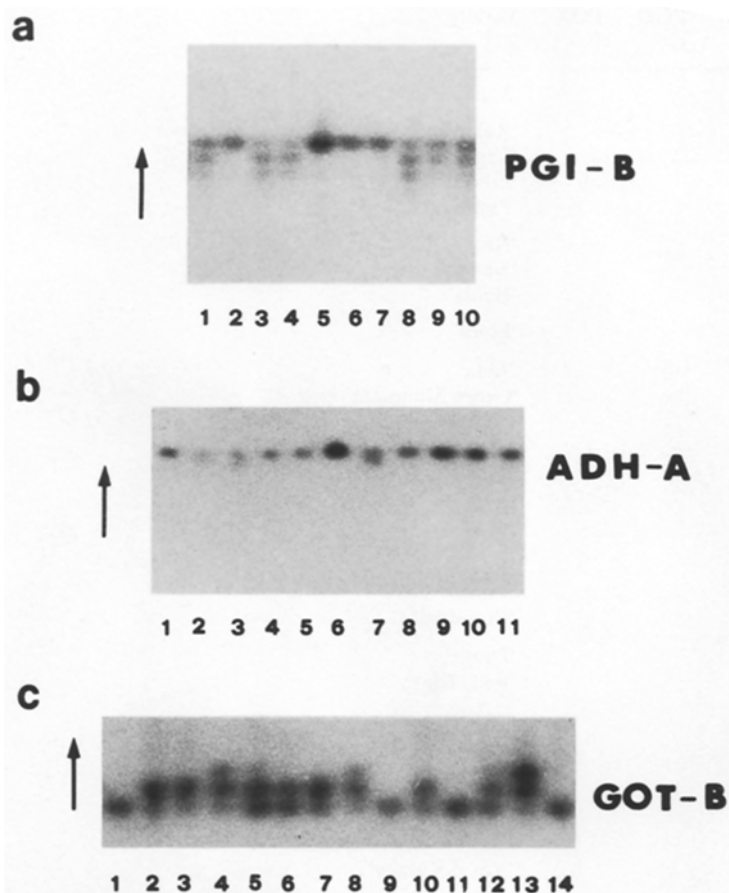


Fig. 2a-c. Electrophoretic isozyme phenotypes shown by different potato varieties. **a** *PGI-B* phenotypes: 1='Mirka', 2='Pimpernel', 3='Norchip', 4='Claustar', 5='Désirée', 6='Diamant', 7='Radosa', 8='Heida', 9='Ackersegen', 10='Sientje'. **b** *ADH-A* phenotypes: 1='Kennebec', 2 and 3='King Edward', 4 and 5='Libertas', 6='Désirée', 7='Edzina', 8='Lora', 9 and 10='Marijke', 11='Netted Gem'. **c** *GOT-B* phenotypes: 1='Gauna', 2='Red Pontiac', 3='Lora', 4='Kennebec', 5='Spunta', 6='Flora', 7='Onda', 8='Fenix', 9='Désirée', 10='Nipigon', 11='Cardinal', 12='Edzina', 13='Norchip', 14='Diamant'

zymes, interlocus but not interallelic changes are normally found. By determining the tissue expression pattern of each isozyme locus (Table 1), one can choose the optimal developmental stage for the analysis of these isozymes. Allozymes are, furthermore, more variable among potato cultivars than storage proteins (Kuhns and Fretz 1978), the genetic control of which, with very few exceptions (Rickman and Desborough 1978), is almost unknown in the potato.

The method is based on the presence or absence of each allozyme band, eliminating the errors that could be introduced if the different staining intensities were also considered, thus providing an easy way to score the results. In addition, it is interesting to point out that this scoring method facilitates the use of a computer to sort the distinct phenotype arrays, allowing a simple and quick way to arrange the different phenotypes and to compare a new variety with all those previously studied.

Therefore, we think that isozyme analysis would be an effective and reliable method of potato identification; we recommended it particularly in varietal certification, since it allows to quickly test the denomination of a given variety, or to control the varietal purity of seed and tissue culture stocks.

The use of isozymes as genetic markers would be also useful for better planning in breeding programs (Stegemann

1979; Desborough 1983). If one can utilize allelic isozymes as markers of loci in close association with genes for given traits, the isozyme phenotype would facilitate the choosing of parents and/or the selecting of progeny (Whitt 1983). It probably will also allow one to relate individual genotypes or genotype arrays with different disease resistances, yield traits or nutritional quality. In the tomato, a close linkage has been reported between the isozyme gene *Aps-1* and that conferring a nematode resistance (Rick and Fobes 1974). More recently, a linkage between the *Pgi-1* and *Adh-2* genes and the genetic elements responsible for cold tolerance in this species has been reported (Zamir et al. 1982; Vallejos and Tanksley 1983).

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