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# Isozyme gene duplication in diploid and tetraploid potatoes

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**Summary.** Isozyme techniques allow the study of gene redundancy in different ploidy levels of potato (*Solanum tuberosum)*. In tetraploid potatoes all isozyme loci are duplicated. No sign of structural or regulatory divergence was found, as is expected due to their tetrasomic inheritance patterns. In addition to this genetic redundancy, produced by a relatively recent polyploidization event, some additional redundancy was found for at least three enzymes even in diploid groups and species. These "older" duplicate genes show structural and regulatory divergence, indicating they appeared by a separate polyploidization event far in the past. Their common origin is still recognizable by both their expression in the same subcellular compartment and by the dimerizing ability of the isozymes they encode. To account for the present chromosome number x = 12 of the *Solanaceae* family, the most frequently found among the species, a hypothetical polyploidization event is proposed.

**Key words:** Gene duplication – Polyploidy – Isozymes – *Solanum* – Potatoes

## Introduction

Gene duplication is a primary mechanism for the acquisition of new genes during evolution (Ohno 1970; Markert et al. 1975).

Isozymes have usually been produced by gene duplication (Markert et al. 1975) and therefore they are very useful tools for studying the evolution of duplicate genes. Many studies have been done on several groups of fishes (Avise and Kitto 1973; Allendorf et al. 1975; Markert et al. 1975; Ferris and Whitt 1977a, b, 1978, 1979; Buth 1979; Stoneking et al. 1981; Whitt 1981) and plants (Hart 1970, 1975; Gottlieb 1973, 1974, 1976, 1977, 1982; Garcia-Olmedo et al. 1978; McMillin and Scandalios 1980; Oliver et al. 1983), providing a great deal of information about the process of evolution by gene duplication.

Duplicated isozyme loci can appear by different kinds of genetic amplification mechanisms (aneuploidy, polyploidy, regional gene duplications, etc.) and, depending on their origin, the fate of duplicated loci can vary markedly (Stoneking et al. 1981; Oliver et al. 1983). For instance, regulatory divergence between duplicates is only found when regulatory sequences have also been duplicated; this is mainly produced by aneuploid and polyploid mechanisms.

Animals usually show a species specific number of isozymes for each enzyme system, whereas in plant species, the number of isozymes reflects the number of subcellular compartments in which the same catalytic reaction is required (Gottlieb 1982). In addition to a basic number of isozymes, usually found for each enzyme system in all plants (see Gotlieb 1982), some species have additional isozymes, probably produced by later duplications.

The cultivated potato *(Solanum tuberosum* L.) belongs to a genus in which different ploidy levels, from diploid to hexaploid, are very often found both among different species and within the same species, always on the same basic chromosome number x = 12 (Hawkes 1978). Several groups and hybrids with ploidy levels ranging from 2x to 5x have been defined for *Solanum tuberosum* by Dodds and Paxman (1962).

We undertook a study on the variability and phylogenetic relationships of the cultivated potatoes and their related wild species (Martinez-Zapater 1983; Oliver and Martinez-Zapater 1984). We were also interested in knowing the gene redundancy shown by the different groups of cultivated potatoes and the possible evolution of its duplicated genes. With this purpose, we analyzed ten different enzyme systems, which showed a total of 25 scorable isozymes, in different groups of *Solanum tuberosum* with different ploidy levels. We also studied two diploid wild species

*of the* same genus: ***Solanum pinnatisectum*** and ***Solanum sparsipilum.*** Several criteria and the genetic analyses carried out in tetraploids (Martinez-Zapater and Oliver 1984a) allowed us to detect a great deal of gene redundancy at the tetraploid level but not in the diploids analyzed. On the other hand, several enzyme systems showed more isozymes than expected, even in diploids, indicating the existence of a duplication event in the origin of all these species.

## Materials and methods

### *Accessions*

We analyzed a total of 35 accessions belonging to ***Solanum tuberosum*** groups Andigena (4x) (15 accessions), Stenotomum (2x) (11 accessions), Goniocalyx (2x) (3 accessions) and the diploid wild species ***Solanum sparsipilum (3*** accessions) and ***Solanum pinnatisectum (3*** accessions). They are all listed in a previous paper (Oliver and Martinez-Zapater 1984). Accessions were provided by the Centro Internacional de la Papa (C.I.P.) (Peru), and the Potato Introduction Station at Wisconsin (U.S.A.). For ***Solanum tuberosum*** group Tuberosum, we analyzed 74 varieties corresponding to the most agronomically important in Europe and the U.S.A. They are also listed in previous papers (Martinez-Zapater and Oliver 1984 b; Oliver and Martinez-Zapater, in preparation). They were provided by the Estacibn de Mejora de la Patata (Vitoria, Spain); Instituto Nacional de Semillas y Plantas de Vivero (Agriculture Ministery, Madrid, Spain), the Potato Introduction Station (Wisconsin, U.S.A.) and the Government Institute for Research on Varieties of Cultivated Plants (Wageningen, Holland). Accessions and varieties were always grown in the greenhouse under uniform conditions.

### *Organs*

In order to determine the tissue expression pattern of each isozyme, we analyzed the following organs in every species and group: tuber, root, shoot, leaf, petal, anther, pollen grains, ovary, calyx, dried seed and seedling.

### *Electrophoresis*

Enzyme extraction, staining and electrophoretic procedures were as described in Martinez-Zapater and Oliver (1984 a).

We analyzed a total of ten enzyme systems, resulting in 25 scorable isozymes: alcohol dehydrogenase [ADH, E.C. 1.1.1.1] (Pasteur 1973); esterases [EST, E.C.3.1.1.1] using alfa-Naphtyl acetate as substrate and staining with Fast Blue RR; glutamate oxaloacetate transaminase [GOT, E.C. 2.6.1.1] (Gottlieb 1973); 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); malic enzyme [Me, E.C. 1.1.1.40] (Brinkman and Van der Meer 1975) with Tris/HCl 0.2 M (pH 7.0); 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); 6-phosphogluconate dehydrogenase [PGD, E.C. 1.1.1.43] (Brewer 1970).

### *Subcellular localization*

Subcellular localization was determined following the technique of Weeden and Gottlieb (1980). This method basically consists of the incubation of pollen grains in Tris/HCl 0.05 M buffer for several hours. Following centrifugation, the supernatant is analyzed by electrophoresis, which allows the identification of the isozymes which are expressed in the cytoplasm. Only cytosol isozymes can be detected in the supernatant after a few hours of incubation. This technique only allows the determination of the cytoplasmic or organelle expression of each isozyme, without providing any additional information for distinguishing between chloroplastic and mitochondrial isozymes.

### *Nomenclature*

Isozymes were called by the corresponding isozyme symbol followed by a letter indicating their anodic mobility, from most to less anodical isozymes. For instance, MDH-A, MDH-B and MDH-C would correspond to malate dehydrogenase isozymes being MDH-A the most anodic one. Isozyme loci are designated in the same way but using lower-case letters.

## Results

The comparative analyses of individual electrophoretic phenotypes, the differential patterns of organ expression and the genetic analyses carried out on tetraploid potatoes (Martinez-Zapater and Oliver 1984 a) were the criteria we employed in determining the genetic control of the different isozymes.

Although the exact demonstration of gene duplication requires the analyses of the nucleotide sequences from both duplicates, the study of the inheritance pattern of isozyme loci allowed us to know their duplicate or single state (Martinez-Zapater and Oliver 1984a). In plants, gene dosage is directly correlated with quantity of gene product (Carlson 1972; DeMaggio and Lambrukos 1974; Roose and Gottlieb 1980). Therefore, the presence of heterozygous individuals exhibiting asymmetrical banding intensities can be interpreted as a probe of gene duplication if both kinds of asymmetrical heterozygotes are found (Allendorf et al. 1975; Ferris and Whitt 1978). The same hypothesis is also supported by the presence of triallelic and tetrallelic heterozygotes. On the other hand, when normal heterozygotes are always found for one isozyme in one species or group, it points to a single isozyme gene state. The above criteria only allows the detection of the duplicate state of variable loci and nothing can be ascertained about the state of non variable ones.

A total of 25 scorable isozymes were detected in the ten enzyme systems analyzed. Their subunit number, deduced from their ability to form hybrid molecules in heterozygotes, and their subcellular localization are shown in Table 1. This table also presents the duplication state deduced for loci encoding these isozymes in tetraploid potato groups (Tuberosum and Andigena) as well as the duplication criteria we used. Diploid groups and species did not usually show dosage effect in the

Table 1. Electromorph relative mobilities, subunit number, subcellular localization, and duplicate state in tetraploid potatoes of each isozyme locus

| Isozyme | Electromorph | Subunit number[a] | Subcellular localization[b] | Duplicate state in 4 x (criteria)[c] |
|---|---|---|---|---|
| ADH-A | 0.55 0.5 1 0.47 | 2 | C | YES (a) |
| ADH-B | 0.49 0.48 0.43 0.35 | 2 | C | YES (a) |
| EST-A | 0.88 0.85 | 1 | ? | Yes (a) |
| EST-B | 0.86 0.84 0.82 0.80 0.77 | 1 | 3 | YES (a) |
| EST-C | 0.68 0.66 0.63 0.6 I 0.59 0.56 0.53 | ? | ? | YES (a) |
| GDH-A | 0.24 0.17 0.10 | 2 | ? | NVL |
| GOT-A | 0.46 0.40 0.36 | 2 | 0 | YES (a, b) |
| GOT-B | 0.31 0.23 0.22 0.14 0.06 | 2 | C | YES (a, b) |
| MDH-A | 0.84 0.78 0.72 | 2 | ? | YES (a) |
| MDH-C | 0.38 0.34 | ? | 3 | ? |
| MDH-D | 0.15 0.10 | ? | O | NVL |
| Me-A | 0.40 | ? | ? | NVL |
| Me-B | 0.32 | ? | ? | NVL |
| PGD-A | 0.83 0.80 | 2 | ? | NVL |
| PGD-B | 0.72 0.70 | 2 | ? | NVL |
| PGD-C | 0.68 0.64 0.60 | 2 | ? | YES (a, b) |
| PGI-A | – – – | ? | O | NVL |
| PGI-B | 0.41 0.37 0.33 0.27 0.22 | 2 | C | YES (a, b) |
| PGI-C | – – | 2 | C | NVL |
| PGM-A | 0.52 0.50 0.47 | 1 | 0 | YES (a, b) |
| PGM-B | 0.44 0.40 0.34 | 1 | C | YES (a,b) |
| POX-B | 0.63 0.60 0.57 | 1 | ? | YES (a) |
| POX-C | 0.54 0.52 0.50 0.48 | 1 | ? | YES (a, b) |
| POX-E | 0.23 0.20 | 1 | ? | YES (a) |
| POX-F | 0.17 0.08 | 1 | ? | Yes (a) |

[a] 1 =Monomer; 2=Dimer
[b] C = Cytosol; 0 = Organelle
[c] YES = Duplicate; NVL = Non variable locus; (a) = Asymmetric heterozygotes; (b) = inheritance analysis

Table 2. Tissue expression patterns of three sets of redundant isozymes in diploid and tetraploid potatoes[a]

| Isozyme | Tissue[b] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | YL | ML | R | TU | ST | SB | P | A | PO | O | C |
| ADH-A | – | + | + | – | – | – | – | – | – | – | |
| ADH-B | | | | | | | + | + | | – | – |
| PGD-A | + | + | + | * | + | + | + | + | ? | + | + |
| PGD-B | * | + | * | + | * | + | + | + | ? | + | + |
| PGI-B | + | + | + | + | + | + | + | + | + | + | + |
| PGI-C | * | * | * | * | * | * | * | * | * | * | * |

[a] + = expressed; – = not expressed; * = poor activity
[b] YL = young leaf; ML = mature leaf; R=root; TU= tuber; ST=sprout tip; SB = sprout basis; P= petal; A= anther; PO = pollen; 0 = ovary; C = calyx

heterozygotes. The only time we found these characteristic patterns was in several plants from the Chaucha accession. The cytogenetic analysis of these individuals showed they were triploids.

The expression of the 25 isozymes was analyzed in 12 organs of the plant. We found the same expression pattern in all species and groups for all (Oliver and Martinez-Zapater, in preparation) but ADH isozymes (unpublished results). In tetraploids, duplicate genes show the same pattern of expression as single genes in diploids. No divergent expression was found between both copies of duplicate genes in tetraploid groups.

On the other hand, when more than one isozyme catalyzes the same reaction in the same cell compartment, they can be considered as initially originating by gene duplication (Gottlieb 1982). For dimeric enzymes, this common origin is easily ascertained since the formation of intergenic heterodimers indicates that both isozymes are expressed in the same cellular compartment and show some structural similarity which allows them to dimerize.

Table 1 shows that three pairs of dimer isozymes are expressed in the same subcellular compartment: ADH-A/ADH-B, PGD-A/PGD-B, and PGI-B/PGI-C (Figs. 1 and 2). These similar isozymes could be the result of gene duplication. There are other isozymes, such as EST-A/EST-B, MDH-AIMDH-B, Me-A/Me-B, which probably originated by the same mechanism, but a lack of data about both their subcellular localization or

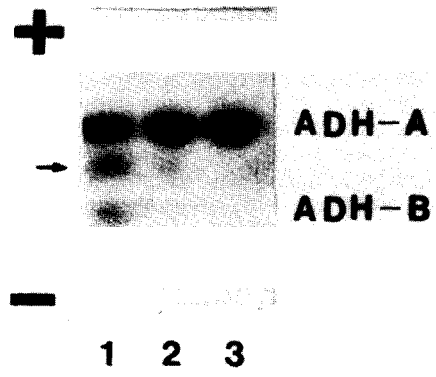


**Fig. 1.** ADH-A and ADH-B isozyme expression in both old (I and 2) and mature (3) tubers of *Solanum tuberosum* group Stenotomum. Note that when ADH-B is expressed (1 and 2) an intergenic heterodimeric band appears *(arrow)*

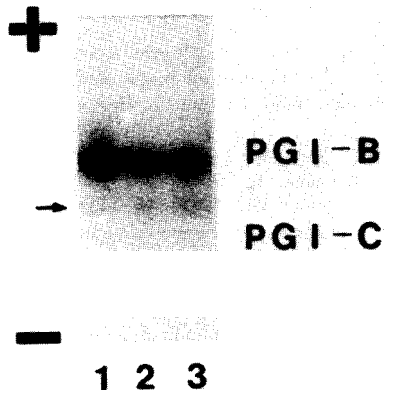


**Fig. 2.** PGI-B and PGI-C isozyme expression in leaves of *Solanum tuberosum* group Stenotomum. PGI-C is poorly expressed but it can be detected by the formation of an intergenic heterodimeric band *(arrow)*

their monomeric nature, unable us to reach any conclusion.

## Discussion

In the potato species and groups analyzed here, we can distinguish two different duplication events. Firstly, since all groups, even the diploid ones (Stenotomum and Goniocalyx), and species (S. *sparsipilum* and S. *pinnatisectum),* showed apparently redundant isozymes (ADH-A/ADH-B, PGD-A/PGD-B, andPGI-B/PGI-C), these isozymes have probably originated by the same duplication event. For these three isozymes, both duplicated genes have diverged both structurally, since they do not share any allele (Table 1), as well as in their organ expression pattern (Table 2). Using the divergent expression categories defined by Ferris and Whitt (1979) for *Catostomid* fishes, we can classify the regulatory divergence of these three duplicate genes. Thus, PGI-B and PGI-C would show unidirectional divergence. Both isozymes are present in all tissues, being the more anodical migrating isozyme (PGI-B) predominantly expressed. The lower PGI-C activity in all tissues could be produced by mutations at regulatory or structural sequences. For ADH and PGD isozymes, both duplicate genes show bidirectional divergence, which means differential gene expression, but no consistent predominance of one locus product over another among the different organs was observed. The divergence between ADH-A and ADH-B seems to be larger than the divergence between PGD-A and PGD-B. For ADH, both isozymes are expressed in different organs, their expression being only coincident in some developmental steps (Table 2). However, PGD-A and PGD-B are simultaneously expressed in all the organs analyzed, differing only in the relative activity they show in each organ (Table 2).

Bidirectional divergence in the expression of duplicate genes is probably due to the divergence of regulatory sequences (Ferris and Whitt 1979). This usually occurs when the amplification mechanism has been aneuploidy or polyploidy (Oliver et al. 1983), allowing the duplication of both structural and regulatory sequences. The fact that regulatory divergence has been found for several redundant isozymes in all groups and species, points to a polyploid origin of the gene redundancy in potatoes.

If so, a polyploidization event can be hypothesized accounting for the origin of the genus *Solanum* with its basic chromosome number of x = 12. A similar hypothesis has also been proposed for the origin of *Nicotiana* genus where many species share this basic chromosome number x = 12 (Goodspeed 1954; see Swaminathan and Magoon 1961 for revision). Therefore, we could postulate that the basic number x = 12 of the *Solanaceae* is secondary and derived through polyploidy from x=6, the ancestral basic number of this family. This hypothesis would also be supported by the great deal of *Solanaceae* species with chromosome numbers fewer than 12; for example, there are several *Petunia* species *(P. hybrida, P. axillaris, P. integrifolia, P. parodii)* with x = 7 (Darlington and Wylie 1945). This hypothesis is also in agreement with that of Stebbins (1950) who considers that all *Fanerogama* genera with basic chromosome number x = 12 or higher have originated by polyploidy.

The time elapsed from this first event would have been so long as to allow diploidization to occur for nearly all duplicated genes. In Catostomid fishes, where this process has been widely analyzed, over half of the duplicated genes have been silenced within the 50 million years after the polyploidization event (Ferris and Whitt 1977 b).

The second amplification event has been produced among diploid species and groups by different polyploidization mechanisms (Oliver and Martinez-Zapater 1984), thus originating the tetraploid potatoes. This event probably took place recently less than 10,000 years ago. This probably dates the origin of the potato culture (Ugent et al. 1983), and would be the source of the isozyme gene duplication observed in tetraploid groups. Table 1 shows that all testable isozyme loci (the variable ones) show evidence of duplication in tetraploids, suggesting that this would be the general pattern for all loci.

We have found no structural or regulatory divergence nor diploidization signs in these recently duplicated genes. This is what can be expected since they show tetrasomic inheritance (Martinez-Zapater and Oliver 1984a). The divergence is not possible until the disomic inheritance pattern has been reached (Allendorf et al. 1982).

We have shown here that at least two gene amplification events have taken part in the origin and evolution of potato species, providing a great deal of gene redundancy at the molecular level. This could explain the high variability levels found in this culture as far as isozyme (Oliver and Martinez-Zapater 1984), morphological and agronomical traits (Landeo and Hanneman 1982) is concerned.

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