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# Isozyme gene expression in potato tumors incited by *Agrobacterium*

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**Summary.** Two plant tumors (crown galls and hairy roots) were experimentally provoked on potato cv. 'Désirée' by oncogenic strains of *Agrobacterium tumefaciens* and *A. rhizogenes*. A marked shift in the expression of some organ-specific genes occurred in crown galls derived from the central zone of tubers: two novel isozyme genes (*Est-B* and *Pox-E*) were expressed, two others (*Est-C* and *Pox-F*) were suppressed and the remaining ones were maintained in the original state. When the starting tissue was the stem segment, a smaller shift occurred, namely the activation of *Adh-A* and the suppression of *Pox-F*. In all cases, the isozyme profiles characterizing all crown galls, whatever their origin, were identical. Under normal aeration conditions, *Adh-A* was not expressed in either tumoral or non-tumoral roots. However, under the relative anaerobic conditions of in vitro cultures, a difference existed between both types of roots: *Adh-A* was expressed in normal but not in tumoral roots. This means that hairy roots can tolerate higher levels of anaerobiosis without giving rise to an anaerobic response. For the remaining isozymes, no alteration occurred in either organized (hairy root) or unorganized (crown gall) tumors, as compared to the corresponding non-tumoral tissues (normal root and callus, respectively).

**Key words:** Crown gall tumors – Hairy root disease – Potato – Isozyme gene expression – *Agrobacterium* – *Solanum tuberosum*

## Introduction

Crown gall and hairy root disease are plant tumors resulting from a set of genes with morphogenic effects which alter normal cell development and differentiation being introduced into the nuclear plant genome. Both tumors are induced in many dicotyledonous plants by oncogenic strains of *Agrobacterium tumefaciens* and *A. rhizogenes*, respectively (Schell et al. 1984; Nester et al. 1984). Virulent bacteria harbor a large plasmid (Ti or Ri), a specific segment of which, known as T-DNA, covalently integrates into plant chromosomes, thus initiating the neoplastic transformation. Once tumoral growth has been initiated, plasmids are not necessary to maintain it, thus allowing the axenic culture of tumor tissue. This enables the analysis of the genetic and biochemical changes associated with the conversion of normal plant cells to tumor cells.

An electrophoretic survey on several enzymes in potatoes has been previously carried out (Oliver and Martínez-Zapater 1984). The genetic basis for a total of 25 potato isozymes was established through the determination of the number and relative mobilities of the allozymes, subunit numbers, subcellular localizations and normal expression patterns in ten tissues and organs of entire plants (Oliver and Martínez-Zapater 1985), the study of several well-known pedigrees of cultivated varieties (Martínez-Zapater 1983), and the genetic analysis carried out on progenies derived from appropriate crosses (Martínez-Zapater and Oliver 1984; Staub et al. 1984; Quirós and McHale (personal communication)). Most of the potato isozymes show a generalized tissue expression pattern, being expressed in all the investigated cellular types; however, esterases (EST) and peroxidases (POX) are organ-specific, being differentially expressed during development. In plants, peroxidases are known to be related to cell differentiation and organogenesis (Mäder et al. 1975; Gaspard et al. 1979), possibly through their established role in plant hormone metabolism (Thorpe 1980). In addition,

it is known that plant alcohol dehydrogenase (ADH) isozymes are induced in response to different environmental factors, such as anaerobic (Hageman and Flesher 1960; Martínez-Zapater 1983) and auxin (Freeling 1973) treatments. Since isozymes represent the end-products of specific gene functions, ADH, EST and POX isozymes were selected to investigate gene expression changes associated with tumoral growth in plants. Here, the isozyme patterns found in experimentally induced tumors (crown gall and hairy root disease) are reported, comparing them with those found in non-tumoral calli as well as in other normal tissues and organs of the common potato.

## Materials and methods

Three *Agrobacterium* strains were used for tumor induction: LBA 958 (with nopaline Ti plasmid pTiC58) and LBA 1010 (with octopine Ti plasmid pTiB6) of *A. tumefaciens* and ATCC 15834 of *A. rhizogenes.*

Healthy tubers from the potato cultivar 'Désirée' (*Solanum tuberosum* group Tuberosum), not pretreated with sprout inhibitors, were kindly supplied by the Instituto Nacional de Semillas y Plantas de Vivero (Ministerio de Agricultura, Pesca y Alimentación, Madrid, Spain). To obtain non-tumoral callus tissue, surface sterilized (70% ethanol and 10% commercial bleach) stems of *S. tuberosum* cv. 'Désirée' were cut into 0.5 cm segments and placed in Petri dishes containing Murashige and Skoog medium (MS; Murashige and Skoog 1962) supplemented with 1-Naphthaleneacetic acid (1.8 mg/l) and kinetin (2.1 mg/l). Developed calli were directly used for electrophoretic assays. The induction of unorganized crown gall tumors, using fresh cultures of the bacterial strains mentioned above, was performed on potato tuber discs and stem segments following in detail the bioassay developed by Anand and Heberlein (1977). Hairy root disease was only provoked on tuber discs. After tumor induction, tumoral tissues were transferred to MS medium containing Carbenicillin (1 mg/ml) to decontaminate it from bacteria. Plates with calli or tumors were incubated under a 16-h light and 8-h dark cycle.

Small pieces (about 50 mg) from the actively growing surface of crown galls or calli, as well as from the hairy root tips, were used for electrophoretic assays. Horizontal starch gel electrophoresis, with LiOH/Borate (pH 8.1) electrode buffer and Tris/Citrate (pH 8.3) gel buffer (Selander et al. 1971), was carried out in 9×9×0.5 cm gel trays, using a Sartophor® electrophoresis chamber at 200 V and 25 mA. Samples of the central zone of fresh tubers of cv. 'Désirée' were included in all the slab gels as internal markers to control the electrophoretic mobilities of the different electromorphs. Three enzymes were extracted and assayed as described elsewhere (Martínez-Zapater and Oliver 1985): Alcohol dehydrogenase {ADH, E.C. 1.1.1.1}, Esterases {EST, E.C. 3.1.1.1}, and Peroxidases {POX, E.C. 1.11.1.7}.

## Results and discussion

The expression patterns for nine isozyme genes (Table 1) were determined in at least three independent gels using samples of two tumors (crown gall and hairy root) experimentally provoked on both tuber discs and stem segments by the infection with *Agrobacteria.* Non-inoculated tuber discs and stem segments were used as controls. For comparison, isozyme patterns were also determined in a proliferative, but non-tumoral, callus tissue, derived from stem segments. No differences in the isozyme patterns were found between nopaline (incited by strain C58) and octopine (incited by strain B6) crown gall tumors; so, they were grouped in the same column (CG) of Table 1.

**Table 1.** Isozyme gene expression patterns[a] in different tissues[b] of potato

| Isozyme gene | Source tissues | | In vitro cultures | | | Control tuber discs | Other tissues | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Non-tumoral | Tumoral | | | | | | | |
| | CZT | SB | NTC | CG | HR | CTD | PT | ST | YL | ML | R |
| *Adh-A*[c] | + | – | + | + | – | + | – | – | – | – | – |
| *Est-A* | * | * | * | * | – | – | * | + | + | * | – |
| *Est-B* | – | + | + | + | + | – | – | * | * | + | + |
| *Est-C* | + | – | – | – | – | + | + | – | – | – | – |
| *Pox-B* | * | + | + | + | * | + | + | + | * | + | * |
| *Pox-C* | * | + | + | + | + | + | + | * | – | * | + |
| *Pox-D* | – | – | – | – | + | + | + | – | – | – | + |
| *Pox-E* | – | * | + | + | + | + | + | – | – | * | + |
| *Pox-F* | * | + | – | – | – | – | + | – | * | + | – |

[a] + = expressed; – = not expressed; * = poor activity

[b] CZT = central zone of tuber; SB = shoot basis; NTC = non-tumoral callus; CG = nopaline and octopine crown gall; HR = hairy root; CTD = Control tuber disc non-infected with Agrobacteria; PT = periderm of tuber; ST = shoot tip; YL = young leaf; ML = mature leaf; R = normal root

[c] The expression pattern scored for this gene is that it showed under normal aeration. However, under anaerobic conditions *Adh-A* was inducible in all but HR tissues (see text)

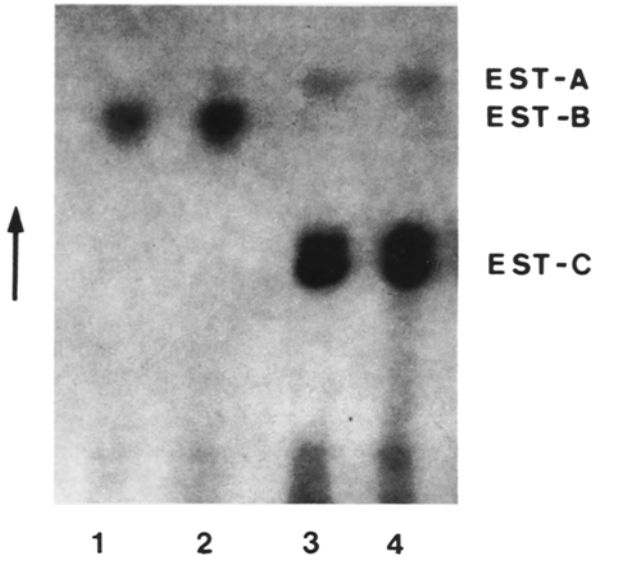


**Fig. 1.** Shift in the expression of esterase isozyme genes in crown galls derived from the central zone of tubers. *Lanes 1 and 2* correspond to samples of crown galls induced on potato tubers by the strain C58 of *A. tumefaciens. Lanes 3* and *4* show the normal esterase pattern of the central zone of tubers

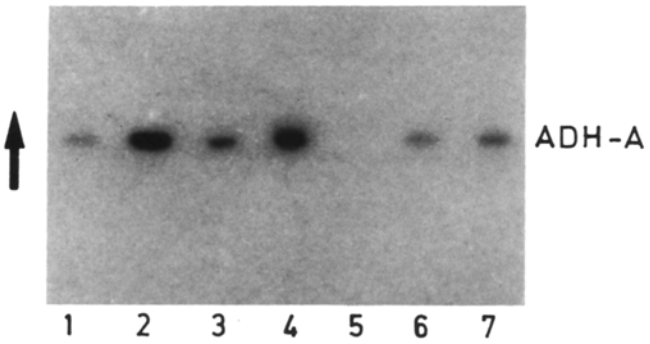


**Fig. 2.** ADH-A isozyme expression in different tissues of potato cv. 'Désirée'. *1* and *7*=non-tumoral callus; *2*=crown gall induced on stem segments by the strain C58 of *A. tumefaciens;* *3*=non-inoculated tuber control discs; *4*=central zone of tubers; *5*=hairy roots induced on tuber discs by the strain ATCC 15834 of *A. rhizogenes;* *6*=normal roots cultivated in vitro

Changes in the isozyme profiles can be observed when different proliferative tissues are compared with those from entire plants, which could be attributed to epigenetic modifications or gene lost. However, since the isozyme profiles in proliferative tissues are almost the same as those seen in some well known tissues of entire plants (shoots and leaves, see Table 1), gene loss is not the probable explanation. Furthermore, since the different isozymes are genetically well-characterized (Martínez-Zapater and Oliver 1984) and since their changes in tissue specificity in entire plants are well-known (Oliver and Martínez-Zapater 1985), epigenetic modifications can be ruled out and an interpretation in terms of changes in gene expression seems to be more adequate.

A marked shift in the expression of some organ-specific genes occurs in crown galls derived from the central zone of tubers (compare CZT and CG columns of Table 1). The shift occurs through the expression of novel isozyme genes (*Est-B* (Fig. 1) and *Pox-E*) and the suppression of some of the previously expressed ones (*Est-C* and *Pox-F*); some other genes (*Adh-A* (Fig. 2), *Est-A* (Fig. 1), *Pox-B, Pox-C* and *Pox-D*) were maintained in the previous state of expression or non-expression. A partial shift also occurs in the non-inoculated control discs (see Table 1), the increase in number and activity of all but *Pox-F* peroxidases being the most significative change. The shift in isozyme gene expression observed in crown galls of tuber origin does not seem to be, however, the general rule for all types of crown galls, since when the starting tissue was the stem segment (SB), a smaller shift occurred, the activation of *Adh-A* and the suppression of *Pox-F* being then observed. It is interesting to note that the isozyme differentiation reached by all crown galls, whatever their origin, was identical (column CG of Table 1).

Note, furthermore, that the isozyme gene expression patterns of crown galls were not specific of tumoral but simply of proliferative cell growth, since they were also observed in non-tumoral calli derived from stem segments (compare NTC and CG columns of Table 1). The expression patterns of all the monitored isozyme genes were therefore identical in both crown galls and non-tumoral callus tissues. This means that the factor/s determining isozyme differentiation in tumoral and non-tumoral proliferative tissues seem to be very similar. Since both actively growing tissues should have similar auxin-cytokinin balances in common (endogenous in crown gall, exogenous in non-tumoral callus), one can hypothesize that these phytohormone levels seem to be sufficient to achieve and maintain the specific isozyme gene expression patterns observed in both types of proliferative cells.

The expression of *Adh-A* deserves an additional comment. This isozyme gene is not organ-specific in the potato, being normally expressed in those tissues where relative anaerobic conditions exist, such as, for example, the central zone of tubers (Martínez-Zapater 1983; see Fig. 2). Relative anaerobiosis may also exist when tissues are grown for several days in Petri dishes, and this would explain the expression of *Adh-A* in crown galls, non-tumoral calli and control discs (see Table 1 and Fig. 2). The situation for *Adh-A* was different in root tissues; under normal aeration conditions, this isozyme gene was not expressed in either tumoral (HR) or non-tumoral (R) roots (Table 1). However,

under the relative anerobic conditions of in vitro cultures, a difference exists between both types of roots: *Adh-A* was expressed in normal but not in tumoral roots. This means that hairy roots can tolerate higher levels of anaerobiosis without giving rise to an anaerobic response, a conclusion connected with that pointed by Tepfer (1984), who shows that tobacco plants transformed by Ri T-DNA, but not normal plants, are able to grow in sealed Petri dishes.

When normal aeration conditions exist, both tumoral (HR) and non-tumoral (R) roots showed identical expression patterns for all the monitored isozyme genes (Table 1). In hairy roots, derived from the central zone of tubers, a typical expression pattern of normal roots was established through the activation (*Est-B, Pox-D* and *Pox-E*), the suppression (*Adh-A, Est-A, Est-C* and *Pox-F*) or the maintenance in the original state (*Pox-B* and *Pox-C*) of the different isozyme genes. Through these changes, the isozyme differentiation achieved by root tumoral cells was identical to that reached by normal, non-tumoral root cells. This result paralleled the close morphological and functional similarities between tumoral and non-tumoral roots.

In conclusion, the only alteration found in the isozyme gene expression of plant tumors incited by *Agrobacteria* was the non-induction of *Adh-A* in hairy roots under anerobic conditions. For the remaining isozyme genes, no alteration occurred in either organized (hairy root) or unorganized (crown gall) tumors, as compared to the corresponding non-tumoral tissues (normal root and callus, respectively). In this way, plant tumors showed few perturbations in isozyme gene expression, thus differing from animal tumors (Lindahl et al. 1982; Stigbrand et al. 1982). The maintenance of plant tumoral growth through the modulation of balances of naturally occurring hormones might explain these results.

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