

Isoenzymatic variation in potato somaclones (*Solanum tuberosum* L.)*

Pedro Canisio Binsfeld¹, José Antônio Peters² and Eliane Augustin³

ABSTRACT

Two enzymatic systems, peroxidase (PER EC 1.11.1.7) and esterase (EST EC 3.1.1.1), were analyzed with polyacrylamide gel electrophoresis using leaves and tubers from first vegetative generation plants obtained from the *in vitro* culture of potato internodes (*Solanum tuberosum* L.). The electrophoretic patterns varied when compared to the control plants. On average 25.6% of the plants had different isoenzymatic patterns in 136 somaclones of the Macaca, 14 of the Santo Amor and six of the Baronesa cultivars; Santo Amor showed the greatest variation (85.7%), followed by Baronesa (33.3%) and Macaca (19.1%). The largest variation was found in the esterase patterns of the Macaca and Baronesa somaclones, while the cultivar Santo Amor showed the greatest number of variants in the peroxidase analyses. The isoenzymatic variability found in the somaclones of the studied cultivars indicates the efficiency of the callus culture technique in the induction of somaclonal variation.

The different responses among the somaclones, obtained from each cultivar, may be attributed mainly to the genotypic differences which are a consequence of vegetative tissue culture.

INTRODUCTION

Agronomic, morphological and/or karyotypic characteristics have been used to assess regenerated plants *in vitro*, but relatively few studies have been carried out using biochemical markers. According to Allicchio *et al.* (1987), isoenzymatic identification shows a series of advantages, compared with the use or

morphological characteristics, mainly because it is a technique of quick application and because the alleles and the enzymatic loci are almost always codominant.

In recent years, the use of enzymatic patterns to identify the somaclonal variants and to monitor the genetic variations which occur during tissue culture has become more frequent. Larkin *et al.* (1984) identified biochemical variants in 142 regenerated wheat plants and showed that the variant characteristics remained stable through two sexual generations. Lipp-João (1987) analyzed somaclones from rice cultivars and found variations in the electrophoretic patterns of three enzymatic systems. Allicchio *et al.* (1987), studying three enzymatic systems in 500 regenerated potato plants, found variation in approximately 28% of the plants.

Isoenzymes have been used as biochemical markers for the identification of intraspecific somatic hybrids (Austin *et al.*, 1985; Hein and Schieder, 1986; Waara *et al.*, 1989) and cultivars (Augustin and Costa,

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¹ Universidade Federal de Pelotas, Caixa Postal 460, 96087-000 Pelotas, RS, Brasil.

² Departamento de Botânica, UFPel, Campus Universitário, Caixa Postal 354, 96010-900 Pelotas, RS, Brasil. Send correspondence to J.A.P.

³ Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa de Fruteiras de Clima Temperado, Caixa Postal 403, 96001-970 Pelotas, RS, Brasil.

1992) of potato. Augustin and Costa (1992) characterized 12 cultivars, using peroxidase, esterase and phosphatase acid patterns, discriminating nine of them. They also found that different plants of the same cultivar, including "Baronesa", show the same enzymatic patterns.

Peroxidases and esterases were found by Deimling (1989) to be the most efficient and universal enzymatic systems for the detection of isoenzymatic variability brought about by somaclonal variation. Berger *et al.* (1985) observed activity and peroxidase polymorphism in apple callus cultures and cell suspensions. De and Roy (1984) found that the isoenzymatic patterns of acid phosphatase altered when cells from calli of *Vigna unguiculata* L. Walp. (Leguminosae) passed from the undifferentiated to the differentiated condition.

The objective of this study was to investigate the isoenzymatic variations of the peroxidases and esterases in potato somaclones obtained by *in vitro* culture, by analyzing the leaves and tubers from the plants of the first vegetative generation.

MATERIAL AND METHODS

Meristems from the potato plant cultivars Macaca, Santo Amor and Baronesa, cultivated in the MS medium (Murashige and Skoog, 1962) without hormones and with 3% sugar were used to obtain somaclones. The plants were grown for eight weeks, until they were sufficiently large to have the internodes removed. These were put in MS medium with the addition of (in mg/l) 4.5 thiamine, 500 inositol, 0.1 kinetin (K) and 5.0 acetic naphthalene acid. The callus formed after four weeks was then transferred to the MS plant regeneration medium with the addition of (in mg/l) 5.0 benzilaminopurine (BAP), 2.0 of K, 0.1 indoleacetic acid (IAA) and 5.0 gibberellic acid (AG3). The regenerated plants, called somaclones, were micropropagated in MS medium without growth regulators, at $25 \pm 1^\circ\text{C}$, 3,500 lux luminosity and a photoperiod of 16 h. Approximately 220 somaclones were regenerated and transplanted to soil. Of these, 156 completed the cycle and formed small tubers which were used for field planting. This planting used six plants of each somaclone and six control plants of each cultivar (the control plants did not pass through tissue culture).

To analyze the peroxidase electrophoretic patterns (PER EC 1.11.1.7) the main leaflet of the fifth leaf from the apex of the flowering plants was used. The electrophoretic patterns of esterase (EST EC 3.1.1.1)

were obtained from the tubers, eight days after harvesting.

Approximately 20 mg of fresh vegetative tissue was squashed at 4°C in 0.02 ml of buffer solution used in the gel preparation (Scandalios, 1969). Homogenization was carried out by a glass rod on porcelain plates, over an ice cube, and the samples absorbed on rectangles of Whatman 3 MM, 4×1.5 mm paper.

The horizontal electrophoresis technique was used in 6% polyacrylamide gel, using the buffers described by Scandalios (1969). The samples were applied 12 cm from the cathodic electrode. The cubes were maintained at a temperature of 4 to 6°C . The isoenzymatic migration was carried out at a field intensity of 10 V/cm, and was stopped when the bromophenol blue band used as a marker reached a distance of 9 cm from the point of application.

The bands with peroxidase activity were visualized by the immersion of the gel, at room temperature, in a 1:1 solution of benzidine (0.5 g, added to 9 ml of acetic acid and 36 ml of water) and H_2O_2 (0.075%). They were left in a 1:1 (v:v) solution of ethanol and water for 30 min to fix.

The technique described by Scandalios (1969) was used to reveal the esterase isoenzymes.

RESULTS AND DISCUSSION

Variation in the electrophoretic patterns was found in both enzymatic systems, detected by the difference in the staining intensity or presence/absence of some bands. Of a total of 156 somaclones examined from the three cultivars (Macaca, Santo Amor and Baroneza), an average of 25.6% of the plants presented enzymatic patterns different from those of their controls (Table I). The greatest percentage of variants was found in the Santo Amor cultivar. These showed differences in the number and position of the bands. Variability was also observed by Heinz and Mee (1971) who obtained 80.9 and 31.0% variation in four enzymatic systems analyzed in two groups of sugar cane somaclones. Allicchio *et al.* (1987) obtained 28% of the somaclones from the *in vitro* culture showing isoenzymatic variations.

The control plants from the three cultivars showed different peroxidase electrophoretic patterns (Figure 1). In the somaclones, 16 patterns differed from the controls (Table II, Figure 1). The frequencies were low but indicated genetic or epigenetic alterations.

The presence or absence of electromorphs relative to the control plants was detected by the separate analyses of the different enzymatic patterns.

Table I - Number of somaclones with isoenzymatic variations originating from the callus *in vitro* culture from three potato cultivars.

Cultivar plants	Total of examined plants	Number of somaclones different from the controls		
		Esterase (tuber)	Peroxidase (leaf)	Total
Macaca	136	16	14	26
Santo Amor	14	7	10	12
Baronesa	6	2	0	2

*Plants with more than one difference were counted only once.

Table II - Frequency of the peroxidase enzymatic patterns in leaves from somaclones of the potato cultivars Macaca, Santo Amor and Baronesa.

Isoenzymatic patterns	Cultivars		
	Macaca	Santo Amor	Baronesa
A	122*	-	-
B	1	-	-
C	1	-	-
D	2	-	-
E	1	-	-
F	1	-	-
G	6	-	-
H	2	-	-
I	-	-	6*
J	-	3*	-
L	-	2	-
M	-	1	-
N	-	1	-
O	-	2	-
P	-	1	-
Q	-	1	-
R	-	1	-
S	-	1	-
T	-	1	-
Total	136	14	6

*Isoenzymatic patterns similar to those of the controls for specified cultivars.

Seven patterns of peroxidase were found in the somaclones of the Macaca cultivar (Figure 1: IB to IH).

Figure 1 and Table II show that the greatest variation occurred in the isoperoxidases of the cultivar Santo Amor somaclones (11/14). There was no pattern different from the control plants in the cultivar Baronesa.

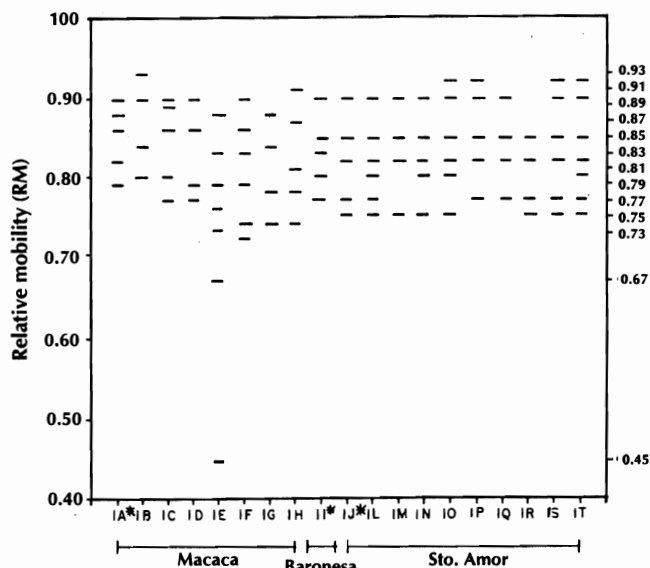


Figure 1 - Diagram of the peroxidase isoenzymatic patterns in leaves from potato somaclones obtained from *in vitro* culture. Control patterns IA, II and IJ are for the Macaca, Baronesa and Santo Amor cultivars, respectively. EMBRAPA-CNPFT, UFPel, Pelotas, 1992.

The isoenzymatic analysis of esterase was carried out on samples of tubers obtained shortly after harvest, which is the ideal period for such assessments, according to Allicchio *et al.* (1987). Similarly to the peroxidases, the esterases were shown to be an effective system for detecting somaclonal variants. Figure 2 and Table III show, for the three cultivars, 16 patterns different from those of the control plants. This is due to the presence of several polymorphic loci controlling the enzymatic system, which involves various different isoenzymes.

Although the frequency of the pattern variation among the somaclones was considered low, it was highly representative, as in the cultivar Santo Amor, where (8/14) of the somaclones varied in their esterase enzymatic patterns.

Nine out of the 136 somaclones studied showed enzymatic alteration in both the systems. Potato somaclone esterase, peroxidase, acid phosphatase, alfa and beta amylase and superoxide dismutase enzymatic systems, analyzed by Deimling (1989), showed alteration in the patterns of up to five systems. These results showed stable alterations via *in vitro* culture as they were not observed in only one enzymatic system.

For Allicchio *et al.* (1987), the enzymatic variations observed in the potato somaclones determined a new sequence and rearrangement, involving the loss and appearance of new gene functions. Thus, it may be supposed that the variation obtained represents alteration in the cells during the *in vitro* culture. It cannot

Table III - Frequency of the esterase enzymatic patterns in tubers from somaclones of the potato cultivars Macaca, Santo Amor and Baronesa.

Isoenzymatic patterns	Cultivars		
	Macaca	Santo Amor	Baronesa
A	120*	-	-
B	1	-	-
C	3	-	-
D	1	-	-
E	2	-	-
F	2	-	-
G	3	-	-
H	1	-	-
I	3	-	-
J	-	-	4*
L	-	-	2
M	-	6*	-
N	-	1	-
O	-	1	-
P	-	1	-
Q	-	1	-
R	-	2	-
S	-	1	-
T	-	1	-
Total	136	14	6

*Isoenzymatic patterns similar to those of the controls for specified cultivars.

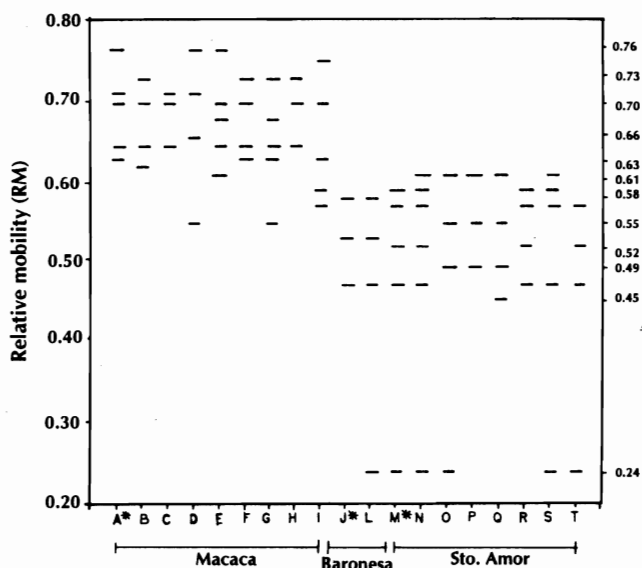


Figure 2 - Diagram of the esterase isoenzymatic patterns in tubers from potato somaclones obtained from *in vitro* culture. Control patterns A, J and M are for the Macaca, Baronesa and Santo Amor cultivars, respectively. EMBRAPA-CNPFT, UFPel, Pelotas, 1992.

be said that such alterations are of genetic or epigenetic origin. Lassner and Orton (1983), however, speculated that the loss or appearance of bands may result from mutation of the mitotic recombination type, deletion, duplication, modification in the nitrogenized bases or transposition of mobile elements. Quantitative alterations, besides resulting from chromosome deletion or duplication, may originate from modification of the regulating system of genic expression.

The enzymatic system used allowed the identification of variants in the three cultivars studied, indicating that the *in vitro* passage of cells and/or tissues from calli and later plant regeneration induces genetic variability (Larkin and Scowcroft, 1981; Larkin et al., 1984; Lipp-João, 1987) that can be detected by the use of enzyme studies.

RESUMO

Dois sistemas enzimáticos, peroxidase (PER EC 1.11.1.7) e esterase (EST EC 3.1.1.1), foram analisados através de eletroforese em gel de poliacrilamida em folhas e tubérculos das plantas de primeira geração vegetativa, obtidas através da cultura *in vitro* de entrenós de batata (*Solanum tuberosum* L.). Os padrões eletroforéticos revelaram o aparecimento de algumas isoenzimas bem como a supressão de outras, quando comparados aos das plantas controles. Em 136 somaclones da cv. Macaca, 14 de Santo Amor e seis de Baronesa, foi observada uma média de 25,6% de plantas com padrões isoenzimáticos diferentes, tendo ocorrido a maior variação na cultivar Santo Amor (85,7%), seguida pela Baronesa (33,3%) e Macaca (19,1%). A maior variação foi encontrada nos padrões esterásicos dos somaclones de Macaca e Baronesa, enquanto que a cultivar Santo Amor apresentou maior número de variantes nas análises das peroxidases.

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