IMPROVEMENT OF Agrobacterium-MEDIATED TRANSFORMATION OF POTATO Solanum tuberosum L USING ANTI-OXIDANT COMPOUNDS

Gil A Enríquez,[1] Nadia Ramírez,[1] María A López,[1] Fernando Bravo,[2] Marlén Pérez,[1] Pedro Oramas[1] y Guillermo Selman-Housein[1]

[1]Centro de Ingeniería Genética y Biotecnología, Habana, Cuba. [2]INGBI, Buenos Aires, Argentina.

Introduction

Agrobacterium mediated transformation of several potato cultivars (*Solanum tuberosum L.)* has been possible by using different kind of explants. Nevertheless, the transformation frequencies are often low, variable and very genotype dependent (1).

For practical commercial use it is important to have fast and efficient means of transferring gene to any cultivars.

We have to establish an efficient method for leaves and stem segments.

This procedure is based on the addition of anti-oxidising compounds and silver nitrate an inhibitor of ethylene synthesis.

Materials and Methods

The plants were grown as *in vitro* shoot cultures in glass tubes on MS (2) medium containing 3 % sucrose, and 0.6 % agar (SIGMA). All cultures were maintained in a 25 °C tissue culture room under controlled illumination and humidity.

For transformation the leaves and stem were taken from plantlets at 4-5 weeks after subculturing. The leaf explants were floated overnight on MS supplemented with 10 mg/L BAP and ANA, 1 mg/L silver nitrate, 40 mg/L cysteine and 15 mg/L ascorbic acid. The explants were then transferred to callus induction on MS with 0.1 mg/L AIA and 2.25 mg/L BAP. After 7 days the leaves were transferred to regeneration medium containing BAP and 3.5 mg/L GA_3.

On the other hand, the stem were cut across the nodal discarding both buds and then transferred on MS medium with 3 mg/L BAP and 0.01 mg/L ANA (about 10 days) and after this time transferred to shoot induction medium containing 0.3 mg/L GA_3 removing ANA and BAP.

Agrobacterium tumefaciens strains containing pCibΩ5ncCP plasmid were grown at 28 °C in YEB mediun.For transformation it was preinduced or not by acetosyringone before co-cultivation with plant tissue.

Results and Discussion

We found that this procedure allowed us to increase two fold (leaves on Wenzler [3]) and four fold (stem on Newell [4]) in the transformation efficient, through 100 mg/L kanamycin. Although, in our method, the stem segments allowed major resistant shoots per explant than the leaves (Table 1).

Table 1. *Comparison between different transformation methods for shoot regeneration.*

Source	Total (•#)	Total shoots	Ave/exp.	Kan^r (%)
Wenz. meth. (leaf)	100	250	2,5	20 (8 %)
Our meth. (leaf)	100	500	5,0	220 (44 %)
Newel meth. (stem)	100	50	0,5	20 (40 %)
Our meth. (stem)	100	200	2,0	150 (75 %)

The addition of this compounds was noxious when they remained for long time in the medium. At the same time we observed, by Evans Blue test, that the use of these compounds allowed the tissue survival more than the controls. Perhaps with the use of these treatments it is possible to decrease the production of a variety of chemicals, many of which are of a phenolic nature and interact with plant cells provoking disturbances.

1. Hulme J, Elaine SH and Shields R Plant Cell, Tissue and Organ. Culture 1992;31:161-167.

2. Murashige T and Skoog F Physiol. Plant 1962;15:473-497.

3. Wenzler H, Mignery G, May G and Park W Plant Science 1989;63:79-85.

4. Newel CA, Rozman R, Hinchee MA, Lawson EC, Haley L, Sanders P, Kaniewski W, Tumer NE, Horsch RB and Fraley RT Plant Cell Reports 1991; 10:30-34.