Agrobacterium *MEDIATED TRANSFORMATION FOR THE PRODUCTION OF TRANSGENIC CABBAGE* (Brassica oleracea) cv. *HÉRCULES PLANTS*

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Introduction

The genus *Brassica* includes a wide range of crop species with a great economic importance as a source of oil, condiments and vegetable. Regeneration of plants from green explants culture is very important for genetic improvement, recent advances in cell and molecular biology have facilitated the transfer of foreing gene into plant, which is the first step for the genetic improvement of crops using the new biotechnological approaches. *Agrobacterium*- mediated transformation has been the most widely used in this types of works, it has been known for many years that some species including this genus are susceptible to infection by *Agrobacterium tumefaciens* strains. Althought *Brassica oleracea* cv. Hércules have been omitted from this studies.

In this report, the transformation of a *Brassica oleracea* cv. Hércules with *Agrobacterium tumefaciens* strain containing the binary vector pCIB- BtK, the Cry IA (b) gene from *B. turingiensis* var. kurtaki under the control of CaMV 35S promoter version and the NPT II gene (conferring resistance to kanamycin) as a selection marker for transgenic plants.

Determination of Cry IA (b) protein expressed in transgenic plants was done by ELISA The problem for the regeneration of transgenic plants was relatied with the hypersensitive response of the explants to the *A. tumefaciens* strain but this methodology show the conditions for selections, the morphology of the transgenic plants that were indistinguishable from controls were recovered.

Materials and Methods

Bacterial strain: Bacterial strains *E. coli* MC1061 and *A. tumefaciens* C58C1 Rif r, containing plasmid pGV2260 lacking the total T-region were used in this work.

Plant material: Seeds from *Brassica oleraceae* cv. Hércules were surface-sterilized in ethanol 70 % for 30 s, followed by continuous agitation for 30 min. in 15 % sodium hypochlorite solution (v/v), then rinsed thoroughly with sterile distilled water and grown aseptically on autoclaved medium (Basal Murashige and Skoogs medium (1962), 20 g/L sucrose, 6 g/L phyto-agar, pH= 5,8) and incubated at room temperature in the darkness for 48 h, at this time, the container was placed in a tissue culture room under a 16 h. light/ 8 h. dark cycle for 7 days.

Hypocotyls excised from just below the shoot apex of germinated seedlings were cut in segments, 7-8 mm long. each.

The effect of growth regulators on shoot regeneration was investigated by transferring the hypocotyl segments in diferent combinations of BA, AG3, Kin and NAA. Explants were evaluated for shoot regeneration after 45 days in culture. For rooting, regenerated shoots were transferred to MS mediun regulator free. The rooted shoots were transplanted into pots containing soil under green houses conditions.

Co-cultivation of explants with *Agrobacterium*: The procedures for co-cultivation and culture of explants were therefore modified to miniminize tissue necrosis. The overnight grown bacteria were suspended in liquid shoot induction medium consisting of MS salts and vitamins, 30 g/L sucrose suplemented with 1 mg/L AG3, 0,1 mg/L NAA and 1 mg/L Kin.

The density of the *Agrobacterium* was adjusted to A600= 0,1. Hypocotyls excised were pre-cultured in this medium at 28 °C in the dark. After 12 h. of co-cultivation, explants were washed with sterile water and finally transferred to the regeneration media suplemented with 500 mg/mL cefotaxime and 20 mg/mL kanamycin.

Determination of the recombinant Cry IA (b): A DAS-ELISA system, based on anti-Cry IA immunopurified policlonal IgG was performed to quantify the recombinant Cry IA (b) protein in transgenic cabbage. The sensitivity of the assay is 5-10 ng per mg of soluble protein, using 50 mg of total protein per ELISA microtiter dish well. Molecular Analisys of transgenic plants: Transgenic plants were tested by PCR and Southern blot analysis.

Results

In first experiments, the stems, leaves, hypocotyls and cotyledonns of *B. oleracea* cv. Hércules were inoculated with *A. tumefaciens* but compline plants developed only from the hypocotyls and ocassionally from the cotyledons. The differentiation of roots and shoots always occurs at the proximal cut end of the petiole or hypocotyl that is in contact with the medium.

After about six weeks on initiation medium supplemented with 500 mg/mL cefotaxime and 20 mg/mL Km, a characteristic gel-like callus was for-

med at the site of tissue contact with the medium. The upper part of the gelatinous tissue contained small vectors of poutative embriogenic tissue. When the shoots appears, they were transferred to hormone -free MS medium supplemented with 20 mg/mL Km. In contrast, for control plants (non transgenic) root development was inhibited in a medium suplemented with Kanamicyn.

The clones grown in Kanamycin were tested by PCR and Southem blot.

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