ATTEMPTING GENETIC TRANSFORMATION OF BEAN BY HIGH VELOCITY MICROPROJECTILES

A. Allavena and G. Bernacchia
Istituto Sperimentale per l'Orticoltura
Via Paullese 28, 20075 Montanaso Lombardo (MI), Italy

Due to the lack of highly efficient protocols for plant regeneration from somatic tissues and protoplasts, genetic transformation of bean is not feasible to date by conventional methods such as leaf disk cocultivation with Agrobacterium spp or DNA uptake by protoplasts. in vivo and in vitro infection of germinating seeds with Agrobacterium spp. and bombardment of meristems with DNA coated microprojectiles appear to be, at the moment, the most promising methods for genetic transformation of species recalcitrant to regeneration.

The principle of bombardment with microprojectiles is based on the acceleration of tungsten or gold particles (1 to 3 μm diameters), coated with DNA, toward target cells. Due to the high velocity and high density, the microprojectiles penetrate membranes and deliver the DNA into cells. Alien DNA can be transiently expressed and also stably integrated. It has been demonstrated (Christou, 1990) that when foreign DNA is delivered into meristematic cells chimeric or fully transformed tissues can rise from transformed cells and the introduced genes can be inherited in the progenies.

A home-made, easy to assemble and inexpensive microprojectiles accelerator (gene gun) has been built up and set up at our laboratory during 1990. Data published by Klein et al. (1987) and the design kindly provided by Dr. S. Hughes (Nuovo C.R.A.I., Caserta, Italy) were helpful for our purpose. Information regarding the home made gene gun was reported by Genga et al. (1991).

Two plant organs were used as targets in the experiments: a) immature cotyledons of P. coccineus Streamline 770 which are able to regenerate full plants via organogenesis and somatic embryogenesis (Allavena, unpublished); b) embryo axis from immature seeds of both P. vulgaris and P. coccineus species from which plants can be recovered by micropropagation.

The plasmid (pBI 221) carrying the β-glucuronidase gene fused to the CaMV 35S promoter (Jefferson, 1987) and a construct carrying the same GUS gene together with the NOS-neomycin phosphotransferase (NPT II) gene conferring resistance to kanamycin were used to coat tungsten microprojectiles.

Six cotyledons with the adaxial face exposed to the flight path of the microprojectiles were laid on a circular area of 570 mm². An average number of 400 + 178 (mean + confident limit) GUS expressing units were observed per shot. A maximum of 350 units per cotyledon and 1200 units per shot were counted. When 20 embryos were laid on the same area 274 + 112 units/shot were found with a maximum of 150 unit/embryo. This data was comparable considering that the target area of embryos was less expanded. Embryo axis with the primary leaves removed were also exposed to bombardment in the upright
position. On average, 60% of the apical meristems showed at least one GUS expressing unit after three shots on the same plant material.

Transformed sectors positive to GUS histochemical assay were observed in plantlets grown by micropropagation from shooted embryos.

References


