

## Transformation of different developmental stage maize immature embryo by *Agrobacterium tumefaciens*

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**Keywords:** *Zea mays* L., genetic engineering, pCAMBIA3302, HEA105 *Agrobacterium*, gene *gus*.

Plant genetic engineering opens new perspectives for the genetic improvement of maize (*Zea mays* L.), allowing gene transfer for superior genotypes development. The transformation by *Agrobacterium tumefaciens* has been the preferred method for this purpose. However, maize is considered the most recalcitrant crop to genetic transformation. The critical points to use this technology are related to the use of *in vitro* responsive genotype, type and development stage of the explants, strains of *Agrobacterium*, conditions of infection and co-cultivation, as well as the culture media for embryogenic callus induction, selection and plant regeneration. This study aimed to evaluate the possibility of transferring genes at different developmental stage of immature embryos (1-2mm, 2-3mm and 3-4mm) of BR451 maize variety (made available by the Embrapa Milho e Sorgo). The experiment was conducted at the Laboratory of Plant Biotechnology, Bacteriology and Virology at FAMV/UPF. The explants were infected with the strain EHA 105 of *Agrobacterium tumefaciens* containing the plasmid pCAMBIA 3301 carrying the *gus* reporter and the *bar* marker genes controlled by the CaMV35S promoter. The protocol used was adapted from Vega et al. (2008). To evaluate the transfer of T-DNA to the immature embryos, it was used the GUS histochemical assay (JEFFERSON et al., 1987) 3 days after infection, just after the co-cultivation step. The controls explants (not infected with *Agrobacterium*) did not present *gus* staining. Six percent of immature embryos of 1-2mm showed the *gus* staining, being the best size for T-DNA transference. All blue spots were localized at the scutelum, tissue that produces the embryogenic callus for plant regeneration in maize (Ishida et al., 2007). The percentage of 2-3 mm immature embryos showing *gus* staining was lower than all stages (only 2%). Immature embryos of 3-4mm presents 4% of *gus* staining, however the spots were observed only in the embryo axis, structure that does not give rise to a embryogenic callus.