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Genetic transformation of immature maize embryos mediated by *Agrobacterium rhizogenes*

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The *Agrobacterium rhizogenes* is a natural plant pathogen responsible for the phenotype named root hairy, which is the excessive proliferation of roots at the site of infection in dicots. Monocots plants are not natural host of this bacterium. The ability of *A. rhizogenes* to form these roots is determined by the presence of a high molecular weight plasmid called Ri (root inducing). In last decades the process of transformation mediated by *Agrobacterium* has been broadly studied and new protocols for transformation of recalcitrant species, such as maize, have been established. The transformation of maize via *A. rhizogenes* has the following advantages: (1) formation of transgenic adventitious roots in a short period of time; (2) easy manipulation of genetically modified roots in tissue culture; (3) high rate of roots growth in tissue culture; (4) possibility of roots produced via *A. rhizogenes* being colonized by endophytic and symbiotic microorganisms, assisting in studies of these interactions. This study aimed to evaluate the virulence of four strains of *A. rhizogenes*, A4, 8196, R1601 and 2659 to zygotic immature embryos of the Hi-ll maize. The strains used for infection carried the binary plasmid pTF102 which contains the gene cassettes *35SP*::*gus*::*35ST* and *35SP*::*bar*::*vspT*. For infection of a single colony of *A. rhizogenes* was initially grown in medium YEP supplemented with 50 mg.L^{-1} spectinomycin during 72h at 19°C. Then, transferred to activation medium containing salts and vitamins of N6 and supplemented with 100 µM acetosyringone, for four to five hours, 23°C. *A. rhizogenes* and immature maize embryos were placed in contact for five minutes. After infection the embryos were transferred to co-cultivation medium, in the dark, at 20°C during five days. Transient β-glucuronidase expression was evaluated by GUS histochemical test, in order to determine the efficiency of infection of different strains tested. After the period of co-cultivation the embryos were transferred to resting medium for fifteen days and then to selection medium until the appearance of roots. The *bar* and *rol* genes were detected by PCR in some of generated roots. The results confirm that the *A. rhizogenes* was able to infect immature maize embryos of Hi-II and the efficiency was higher for the R1601 and A4 strains.

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