



EFFECTS OF AGROBACTERIUM TUMEFACIENS STRAINS AND PLASMID VECTORS ON TRANSIENT TRANSFORMATION OF WHEAT IMMATURE EMBRYOS

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Genetic transformation mediated by Agrobacterium tumefaciens has some advantages over biolistic approaches, and the insertion of small copy numbers of the transgene into transcriptionally active site in the genome is one of them. In wheat, this technique has been established in some public and private institutions around the world, but its implementation is still a challenge for many other institutions. Several factors can affect this interaction, such as plant and bacteria genotype, culture medium, co-cultivation conditions, etc. This study aimed to evaluate which strains of A. tumefaciens and plasmid vectors are able to promote transient expression of the GUS gene in the Brazilian wheat genotype PF020037, which has high in vitro regeneration capacity. The basic protocol was according to Wu et al. (2009). Immature embryos with 10 to 13 days post anthesis (0.8 to 1.5 mm) were collected one day prior to processing and maintained at 4°C until ready to use. They were inoculated during 15 minutes in a suspension containing either one of three strains of A. tumefaciens (AGL1, EHA105 or LBA4404) combined with different vectors (pAL154/ pAL156 or p7UG-AB or pAL154/p7UG-AB). Excess of bacterial suspension was removed, explants were transferred to new inoculation medium and co-cultivation was performed during two days at 22°C. Each treatment had 70-100 explants inoculated and 35 not inoculated controls. GUS histochemical assay was performed two days after embryos were transferred to the induction medium in two replications containing 18 explants. Analyzed variables were the percentage of embryos with blue spots and the number of blue spots per embryo. The frequency of embryos with blue spots ranged from 0 to 50% among treatments. Best results were obtained when explants were inoculated with AGL1 pAL154/pAL156, with 50% of embryos showing blue spots and an average of 2.3 spots per embryo, and EHA105 pAL154/p7UG-AB with 38.9% of embryos with blue spots and blue spots average of 1.5 per embryo. These results indicate that the helper plasmid pAL154, which contains extra copies of the vir genes, increases significantly the transient transformation efficiency of PF020037 immature embryos. The remaining explants are being cultured in vitro to verify the efficiency of production of transgenic plants.