3190 - Genomics, Molecular Genetics and Biotechnology

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PROTOCOL ADJUSTMENT FOR GENETIC TRANSFORMATION OF WHEAT BY AGROBACTERIUM TUMEFACIENS

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Plant genetic transformation is a powerful tool that can assist the incorporation of desired traits hard to reach by conventional breeding. In Brazil, one of the features targeted for wheat transgenesis is drought tolerance, especially for planting under rainfed conditions in the Cerrado region. The transformation by Agrobacterium tumefaciens is particularly interesting for the good possibility of inserting few copies of the transgene in transcriptionally active sites of the genome. This technique is not yet established for wheat in Brazil. For such reasons, the objective of this study was to evaluate the effect of different factors, below described, in the process of wheat genetic transformation using A. tumefaciens. Two wheat genotypes were used, a Brazilian line developed by Embrapa for rainfed cultivation with good capacity of in vitro regeneration, and ?Bobwhite SH9826', which is widely known for its high regeneration and transformation capacity. The protocol used was based on Wu et al. (2009). Theeffects of pre-incubation of immature seeds collected 9 to 10 days after anthesis at 4 °C for one, two, three and four days before placing the embryos in culture medium were tested. A. tumefaciens AGL1 containing p7UG-AB or pAL154/pAL156 plasmids were used with cell concentration ranging from 1.0 to 2.0 in OD600 for the inoculation step. The effect of rinsing explants with inoculation medium after inoculation step was also tested. Assays consisted of 100-133 transformed explants per treatment, plus 30-35 untransformed control explants. GUS histochemical staining was performed two to three days after the co-cultivation. The most promising treatment was rinsing explants after inoculation, in which 5% and 29% of the Brazilian wheat line explants showed blue spots when inoculated with A. tumefaciens AGL1 containing p7UG-AB and pAL154/pAL156, respectively. The average number of blue spots per explant was 1.0 and 3.1, respectively. This result indicates that the amount of bacteria used in cocultivation is decisive in the transformation efficiency. The fact that the plasmid pAL154 contains additional vir genes was probably responsible for the more efficient transient transformation. Other treatments showed no blue spots. Explants are being conducted in the subsequent culture medium to verify the stable transformation. Mostpromising experiments should be repeated, as well as other factors should be tested in order to improve the transformation efficiency.