

Effect of Cold Pretreatment and Period of Preconditioning Inoculation on Transformation Frequency in Rapeseed (*Brassica napus* L.)

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Abstract

Genetic engineering in rapeseed will lead to the generation of plant varieties possessing more agriculturally and economically viable genetic traits. The most gene transformations to rapeseed have been done through *Agrobacterium tumefaciens* method. *Agrobacterium* mediated transformation is depend on many parameters that must be optimized. The purpose of this study was to determine the effect of cold pretreatment (control and 12 h) among a 5 day old-plantlets with preconditioning period (0, 24 and 48 h) and inoculation period of explants in *Agrobacterium* solutions (2, 10, 20 and 40 s) on the *gus* reporter gene transformation frequency in Rapeseed. The experimental design was factorial on basis of completely randomized design (CRD) with four replications. The gene was transferred to a commercial cultivar rapeseed (PF-7045-91) via *A. tumefaciens* (LBA4404 strain) mediated transformation method. Moreover, using PCR technique and *gus* assay, the presence and expression of genes in plants were confirmed. Statistical analysis revealed that there was no significant difference between cold pretreatment and control group. Moreover, the all interaction effects were not significant. Results also demonstrated that there was a significant difference among preconditioning and inoculation period levels for transformation efficiency. The highest effect on transformation efficiency was observed through 24 and 48 h preconditioning periods (with same effects and means 24.21 and 23.55%) and 10, 20 and 40 s inoculation periods (with same effects and means 20.50, 20.63 and 20.54%) respectively.

Keywords: Rape, Cold pretreatment, *Agrobacterium tumefaciens*, Explants preconditioning

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