Abstract

Khaled Abd El-Fattah Mahmoud El-Mangoury, Biotechnological Studies on Some Wheat Cultivars. Unpublished Ph. D. thesis, Ain Shams University, Faculty of Agriculture, Department of Agronomy, 2005.

This study was carried out in the laboratory of Plant Cellular and Molecular Genetics (PCMG), Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation, Giza, during the period from 2001 till 2004.

The aim of the present investigation was two main goals, the first, was to optimize high efficient regeneration system and the second was to optimize reporter gene expression in callus using the biolistic method, causing highest gene expression and minimal tissues damage.

To efficiently complement traditional wheat breeding with genetic transformation technology, it will be desirable to introduce transgenes into the ideal genetic background. Poor tissue culture performance is limiting the number of wheat cultivars that can be stably transformed. Therefore, tissue culture response of three current Egyptian wheat cultivars cv. Sids 1, Sakha 69 and Giza 168 were statistically analyzed in this research. Regenerable callus cultures were initiated from immature embryos of the three wheat cultivars and regeneration characteristics differed highly significantly among the mentioned cultivars.

On the other hand, this research announced a rapid and facile method to investigate both osmotic conditioning treatments of the target tissues (mixture as 0.2 M mannitol and 0.2 M sorbitol or 0.4 M mannitol), and bombardment

pressure (1100 psi or 1350 psi) affecting beta-glucuronidase (GUS) expression in the three Egyptian wheat cultivars. Immature embryo derived calli were bombarded with gold particles coated with *pBA6* plasmid, harboring *gus* and *bar* gene. GUS activity was observed after two and seven days from bombardment. At 1100 psi, number of blue spots and clusters per bombarded callus (GUS activity) was higher compared with 1350 psi across the three tested cultivars and reached 5% level of significance. Mixture osmotic treatment had resulted high levels of *gus* gene expression in both Sakha 69 and Sids 1 cultivars, while, mannitol was favorable for Giza 168 cultivar. Applying biological and physical parameters (osmotic treatment and bombardment pressure; respectively), high levels of *gus* gene expression were obtained.

In this study, we report one procedure that drastically reduce bombardment damage by optimizing the osmotic treatments and bombardment pressure protocol and the combination of these procedures with improving regeneration protocol for each cultivar, causes high frequency in regeneration capabilities of the bombarded calli.

Transgenic plants were recovered from the cultured immature embryo-derived callus of the three wheat cultivars after biolistic transfer and bialaphos selection. Transgene integration and expression were confirmed by herbicide application and polymerase chain reaction. The introduction of transgenes into the ideal genetic background will allow an evaluation of their crop improvement potential

Key Words: biolistic, osmotic, bombardment pressure, regeneration, transformation, *gus* gene, *bar* gene.

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