STUDIES ON FACTORS EFFECTING GENETIC TRANSFORMATION OF BARLEY (*Hordeum vulgare* L.) USING MATURE EMBRYO

By

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B. Sc. Agric. Sc. (Biotechnology), Cairo University, 2004

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ABSTRACT

Shimaa El-Sayed Rashad El-Sayed: Studies on Factors Effecting Genetic Transformation of Barley (*Hordeum vulgare* L.) Using Mature Embryo. Unpublished M.Sc. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2012.

The effects of types of medium and methods of sterilization on mature embryo culture of barley (*Hordeum Vulgare* L.) were tested. Mature seeds were surface sterilized in Sodium hypochlorite followed by 70% ethyl alcohol, and then washed several times in sterile distilled water. Plant regeneration from direct and indirect embryogenic callus of eight barley genotypes Giza 123, Giza 124, Giza 125, Giza 126, Giza 130, Giza 2000 and two genotypes wild types from Marssa Matrouh (El kasr and Awlad Ali) was tested on seven different media were tested using maltose as the carbon source.

Out to these cultivars two commercially important cultivars (Giza 130, Giza 126) and one wild type Elkasr, were tested on three different of hormones only and stability of all component media of medium M1. Revealed the highest frequencies of direct regeneration of embryogenic calli for M1 (60.42%), shoots (51.67%) and roots (48.75%) and frequencies of indirect regeneration of embryogenic calli for M1 (91.11%), shoots (51.11%) and roots (38.89%). Regenerated plants were obtained from this callus and genotypes. These genotypes were examined for somaclonal variation. Somaclonal variation has been tested intensively also in the three barley (El Kasr, Giza 130 and Giza 126) genotypes using molecular genetic analysis (protein and isozymes) and RAPD PCR. The Transformation of barley based on the infection of mature embryos with *Agrobacterium*

tumefactions system used for transformation of explants Elkasr village using the strain harbors the pITB-AFP plasmid vector which contains defensin (*AFP*) gene, hygromycin phosphor transferase (*hpt*) and β-glucuronidase (*GUS*) genes, as selectable and marker genes. The Transient expression of *AFP* gene was investigated as a preliminary test of the transformation efficiency. Transformation experiments were carried out with different parameters and the one barley genotype Elkasr. The tested transformation parameters included: the type of explants mature embryos based on the results of GUS transient expression. Selection was initiated on (M1) Media containing 60 mg L-1 hygromycin. PCR analysis using primers specific for the *AFP* and *GUS* genes using specific probe.

Key words: Barley, *Hordeum vulgare*, mature embryos, plant regeneration, somaclonal variation, protein, Isozymes, RAPD, transformation Agrobacterium, defensin, *AFP* gene, Gus assay.

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