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Thesis Submitted in Partial Fulfillment of the Requirements for PhD degree in the field of agricultural engineering, the trend of Biotechnology

Title
Expression of *Hva1* LEA in transgenic *Solanum tubersum* in order to enhances tolerance to cold stress

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September 2021

ABSTRACT

After wheat, maize, and rice, potato is not only an important food crop but also a substantial source of income throughout the world. Agria cultivar is native to Iran and is widely cultivated. Developing a practical and effective transformation method for cultivars (such as Agria cultivar) that are recalcitrant in tissue culture is vital. *Hva1* encodes the protein of the LEA III superfamily that involves in reactions to abiotic stresses such as low temperatures. Here, a protocol has been designed for an *Agrobacterium*-mediated transient transformation in tissue culture-independent conditions *in-planta*. The protocol establishes for *hva1* and *EPSPS* transformations by direct injection of the bacterial suspension into the potato tuber sprout to encode resistance to cold and against glyphosate herbicide. A two-stage selection was involved using 1% and 2% Glyphosate to eliminate the chimeric and non-transformed plants. Ultimately, the protocol enabled confirmation of gene integration into the plant, transgene expression of the gene and transgene expression, which was made possible by competitive PCR reaction, RT-PCR, and ELISA, respectively. In this research, the transformation efficiencies acquired in potatoes (up to 46%) were higher than those reported using conventional *Agrobacterium*-mediated approaches in previous studies. Apart from a slower growth rate, the vegetative potentials of the transgenic lines are similar to non-transgenic lines. Thus, the constitutive expression of the integrated T-DNA neither slowed down the growth rate nor affected potato tuberization significantly. The *Hva1* gene was expressed successfully leading to the accumulation of the hva1 protein in transgene-generated tubers. This study is the first report on a successful transformation of potato in-planta whereby Agrobacterium can be directed at potato seed sprouts through injection. Herein, It is made a comprehensive phylogenetic and evolutionary analysis for LEA proteins in Poaceae.The expansion of the gene number in the Poaceae was approved by the duplication events in the preexisting genes rather than by the appearance of the altered LEA gene. Our data will provide novel insights for further studies of the Late Embryogenesis Abundant protein family in Poaceae.

Keywords: Potato, *Hva1*, *EPSPS*, *In-planta*, phylogenetic analysis, evolutionary analysis, Poaceae.