TRANSIENT EXPRESSION OF *ELN* GENE IN TOBACCO PLANT

BY

Mona Ahmed Mohamed Abdelghani
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2008.

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APPROVAL SHEET

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SUPERVISION SHEET

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Title of Thesis: Transient expression of *ELN* gene in tobacco plant
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Department: Genetics **Approval:** / /2015

ABSTRACT

Plant molecular farming (PMF) is an important growing prospective approach in plant biotechnology. It includes production of recombinant pharmaceutical and industrial proteins in large quantities from engineered plants. Elastin is a major protein component of elastic fibers. They provide tissues with the required resilience and elasticity. In this work, *Nicotiana tabacum* leaves were used as a bioreactor for the expression and production of the full human tropoelastin protein; the precursor protein of elastin. The agrobacterium-mediated transient expression system into *Nicotiana tabacum* was used *via* syringe agroinfiltration to provide a fast and convenient way to produce recombinant proteins with a greater expression overall the plant leaf. This study aimed to establish an efficient and rapid system for transiently expression and production of human recombinant tropoelastin protein in transgenic *N. tabacum* plants. Modified sequence of the elastin (*ELN)* gene was biosynthesized and cloned into pCambia1390 vector to be transferred into *N. tabacum*. Optimization of codon usage for the human tropoelastin gene, without changing the primary structure of the protein was carried out to ensure high expression in tobacco plants. The obtained data proved that the 5th day post-infiltration is the optimum interval to obtain the maximum production of the recombinant protein. Southern blot analysis was able to detect the 2175 bps fragment representing the *ELN* orf. On the other hand, *ELN* -expression within plant's tissue was visualized by RT-PCR within 3 to 10 days post agroinfiltration. At the protein level, western and ELISA confirmed the expression of recombinant tropoelastin protein. The western blot analysis detected the tropoelastin protein as a parent band at ~70 kDa from freshly extracted protein. While two degraded bands of ~55 and ~45 kDa representing a pattern of tropoelastin were appeared with frozen samples. This study showed that biosynthetic *ELN* gene was successfully expressed into *N. tabacum* leaves using the agroinfiltration technique.

Key words: Molecular farming, transient expression, agroinfiltration, human tropoelastin *hTE, ELN, ELISA*, western blot.

DEDICATION

I dedicate this work to whom my heartfelt thanks: to my mother, aunts, brother, cousins and my friends for their endless support along the period of my post-graduation.

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