Ain Shams University
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IMPROVEMENT OF TRANSGENE EXPRESSION IN WHEAT

A Thesis

Submitted for Partial Fulfillment of Master Degree of Science in Botany

(Genetics)

By

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2010

ABSTRACT

Heba Hamdy Sobhy Mahmoud Abou Seada. Improvement of transgene expression in wheat. Unpublished Master of Science in Botany (Genetics), Department of Botany, Faculty of Science, Ain Shams Univ., 2010.

The major aim of this study was to improve the expression of the drought tolerance transgene *HVA1* in wheat. The HVA1 transgene was transferred from the transgenic line 111 to two high yielding Egyptian cultivars, Giza 168 and Sakha 92, using crosses/backcrosses approach. Five backcrosses were done using the Egyptian cultivars as a recurrent parents, then the plants were self-pollinated to achieve the homozygoisty of the introduced gene. SSR and AFLP techniques were performed to insure the recovery of the genomic background of the recurrent parents. The BCF1 plants were subjected to drought stress at field. The statistical analysis revealed that the improved genotypes (G168x111 and Sk92x111) were insignificantly surpassed their parental cultivars. Accumulation of two transgene copy was as an approach to enhance the *HVA1* transgene expression in wheat. The homozygous-one-copy transgenic lines (357, 344, 111, 84 and 1.6) were crossed with each other. The obtained genotypes are either one-copy or two-copy in hemizygous state. Drought experiment at field was conducted to compare between the different copies. Statistical analysis showed that the genotype

111x1.6 (two-copies, hemizygous) possessed the highest values in five traits out of seven. But this increase is non-significant compared to the transgenic line 111. As a general view, the *HVA1* transgene copy number is not positively or negatively correlated to its expression level. But the increase in *HVA1* expression, as reflected by plant tolerance to drought stress, is related to the nature and expression of the copy involved

Key words: *Triticum aestivum*, *HVA1*, AFLP, SSR, transgene copy number, backcross, recurrent parent, drought tolerance

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Acknowledgment

First and foremost, I am indebted to ALLAH almighty forever, the most merciful and compassionate, for his support, help and generosity.

I like to express my deep gratitude and sincere appreciation to **Prof. Dr. Soheir El-Saeed Elkhodary**, *Professor of Cytology and Genetics, Botany Department, Faculty of Science, Ain Shams University for her help, revising the manuscript, continuous guidance and for her keen supervision.*

Special thanks and sincere appreciation to **Prof. Dr. Ahmed Bahieldin** *head of the Agricultural Genetic Engineering Research Institute (AGERI), ARC, for providing all facilities and supplies to carry out this work and for his constructive ideas to solve the problems that I faced in this study.*

I would like also to express my deepest appreciation, grateful thanks and love to **Dr. Hala Fawzi Fissa,** *Senior scientist, Agricultural Genetic Engineering Research Institute (AGERI), ARC, for her unlimited help, continuous follow up, faithful guidance and too much patience throughout this work and finally numerously revising the manuscript.*

Thanks from all my heart to **Dr. Raeefa A. Hassanin**, *Prof. of Plant Physiology, Botany Department, Faculty of Science, Ain Shams University, my Spiritual mother for her motherly continuous advice, encouragement, understanding, love and help.*

Thanks also to **Dr. Amira A. Hassanin**, *Prof. of Plant Physiology and Head of Botany Department, Faculty of Science, Ain Shams University, for her faithful help.*

Thanks also to all members of the Environmental Stress Lab. (ESL) at AGERI: **Dr. Osama Meselhy, Dr. Ahmad Ramadan, Dr. Sameh, Omnia, Lamiaa mostafa, hanan, Abd El-Rahman, Adel, Randa, Lamia, Rasha, Nelly and Dalia** *for their continous and generous help.*

Sincerely, I express my deepest thanks and loyal appreciation to my colleagues **Dr. Nour Eldin Omar** *at the Genetic Engineering and Biotechnology, National Research Center,* **Hussein Fotouh, Marwa Hanafy, Mohammed Abd-Elsattar and Yasser** *at AGERI that a convential word of acknowledgment cannot pay for their standing by my side as well as their great and valuable efforts, endless support, kind advice and faithful care throughout the hard times I faced in this work.*

I feel deeply grateful and sincere to **Dr. Ahmed Shokry** *for his keen advice, following up the stage of the laboratory work, and his precious help during the computing analysis,* **Dr. Sherif Edrees,** *Lecture at the Molecular Cytogenetics Laboratory, Faculty of Agriculture, Ain Shams University for his help and advice in the molecular work.*

Many thanks are also to all the members and colleagues of the botany department, science faculty, ain shams university especially **Dr. Seham M. Ali** *Prof. of Plant Physiology,*

Botany Department, Faculty of Science, Ain Shams University, **Dr. Azza M. Saber** *Prof. of Plant Physiology, Botany Department, Faculty of Science, Ain Shams University*, **Dr.Al-safa Hassan, Zahar Ibrahim, Dina Mohammed, Abd El-Aziz** *and* **Haitham Zalat** *for their help and support.*

Last but not least, from my whole heart I wish to express my deepest thanks to **my great parents** *that offered me and still offering whatever I need throughout my life not only to complete this work starting from their prayers ending with too much effort that words can never describe,* **my husband Mohammed El-Helaly** *for his unlimited love, patience and encouragement and* **all my family members**, *for their help, love and care.*

Heba Hamdy

I. INTRODUCTION

Wheat (*Triticum aestivum* L 2n=6x=42 chromosomes, AABBDD) is a member of the family Poaceae which includes major cereal crops of the world such as maize and rice. Wheat is the major food grain crop consumed by humans, and is the most widely grown crop in the world over an area of 240 million hectar **(FAO statistics, 2006)**. Ninety-five percent of wheat grown is the hexaploid type, used for the preparation of bread and other baked products (**Patnaik and Khurana, 2001**).

Drought is an important environmental factor limiting the productivity of wheat and other crops world-wide. As irrigation water sources have become scarcer, engineering genes that protect and maintain the function and structure of cellular components can enhance tolerance to the stress. Genetically engineered wheat plants that constitutively over-express certain plant proteins, such as late embryogenic abundant (LEA) proteins encoded by the *HVA1* gene, have shown great progress, better vegetative growth and water use efficiency under water deficit conditions **(Sivamani *et al*., 2000)**. Under field experiments the *HVA1* T4 transgenic plants showed tolerance to drought stress (**Bahieldin *et al*., 2005**)

Many problems are associated with genetic manipulation of elite wheat genotypes, such as poor regenerability and low transformation frequency. Many laboratories optimized the transformation protocol for good tissue culture laboratory which is usually poorly adapted and lower yielding genotype (**Patnaik and Khurana, 2001**). Once the transgene integration and expression is confirmed in one genotype, crossing the elite genotypes is more efficient than transforming new genotypes. In conventional breeding programmes backcross method was used for transferring qualitative characters such as disease resistance. Using backcrossing approach (BC), expressed transgene could be moved from transgenic line (donor parent) to elite cultivar(s) (recurrent parent(s)) with recovery of almost 98% after the fifth backcross (BC5) (**Library of crop technology**).

In order to ensure the recovery of almost all the genomic background of the recurrent genotype, molecular-marker-based techniques could be used. Molecular markers were used by plant breeders to assess genetic diversity in wheat **(Stachel *et al*., 2000)**. Simple sequence repeats (SSRs) are highly variable, showing co-dominant inheritance **(Morgante and Olivieri, 1993)**. For each microsatellite amplified with specific primers, a PCR

product for a defined molecular size is diagnostic for a given allele **(Macaulay *et al*., 2001)**. SSR markers for wheat have become available in increasing numbers **(Prasad *et al*., 2000)**. Amplified fragment length polymorphism (AFLP) method developed by **Vos *et al*. (1995)** is a highly polymorphic and reproducible system. Alleles are generally scored in dominant manner. AFLPs have been used for cultivar identification in sugar beet **(De Riek *et al*., 2001)**.

Scientists working in a range of plant species have previously noted direct correlations between transgene copy numbers and transcript accumulation (**Hobbs *et al*., 1993; Schubert *et al*., 2004; Stockhaus *et al*., 1987**). There are contradictory reports about the relationship between copy number and expression level. **Hobbs *et al*. (1993)** concluded that increased copy numbers of the *gus* gene may either elevate or decrease the level of expression in tobacco transgenics, whereas **Bhattacharyya *et al*. (1994)** found a clear positive correlation in the same species. Conversely, the analysis of transgenic citrus plants revealed a significant negative effect of transgene multicopies on *GUS* activity levels (**Cervera *et al*., 2000**).