DEVELOPMENT OF GENETICALLY MODIFIED STRAINS OF WHEAT EXPRESSING THE *HAL2*-LIKE GENE

By

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B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., Egypt, 1999
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APPROVAL SHEET

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SUPERVISION SHEET

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ABSTRACT

Wheat yield is severely affected by soil salinity. This study was conducted in an attempt to improving the salt stress tolerance of two wheat (*Triticum aestivum* L.) cultivars, i.e., Giza 164, an Egyptian wheat cultivar and Bobwhite 56. Immature embryo derived calli of the wheat genotypes were bombarded with a plasmid containing the rice halotolerance gene (*HAL2*-like gene) for salt stress tolerance and the *bar* gene for herbicide resistance. Three putatively transgenic plants from Bobwhite 56 cultivar namely B56-10, B56-20 and B56-24, and three putatively transgenic plants from Giza164 cultivar namely G164-5, G164-16 and G164-26 were detected by herbicide application through leaf painting. The transformation efficiency was 0.22 and 0.13 for Bobwhite 56 and G164, respectively. For molecular confirmation of putative transgenic plants, PCR analysis has been carried out and revealed the presence of both of the *HAL2*-like and the *bar* genes in the DNA of the putatively transgenic plants. Southern blot hybridization confirmed the integration of the gene of interest (*HAL2*-like) into the genome of the wheat transgenic plants. Moreover, RT-PCR analysis confirmed the expression of both of the *HAL2*-like and the *bar* genes. Sand culture experiment was conducted to investigate the effect of the introduced *HAL2*-like gene on wheat salt tolerance. T_2 transgenic plants and wild type plants were subjected to salt stress of 6000 ppm and 8000 ppm NaCl solution. The data showed that the transgenic lines expressed different levels of salt tolerance as expressed by the performance of the yield related traits. Transgenic lines G164-5/2 and B56-20/2 manifested the lowest reduction in yield traits performance. The results confirmed that the *bar* and the *HAL2*-like genes was successfully integrated into the genome of wheat, transmitted and expressed in the transgenic progeny.

Key words: wheat, immature embryos, *HAL2*-like gene, *bar* gene, salt stress, transformation, biolistic gun.

DEDICATION

I dedicate this work to whom my heart felt thanks: to soul of my Dear father, the most influential person in my life, who showed me what the attitude towards life's responsibilities should be., to my mother who brought me into this world and made me who I am with constant dedication and unconditional support, to my brothers and specially to my dear husband Taye and my dear children for their patience, support and encouragement they continually offered along the period of my post graduation.

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INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is a hexaploid species (2*n* = 6*x* = 42, AABBDD) belonging to the family Poaceae.. Almost 95% of the wheat grown today is of the hexaploid type, while nearly all of the remaining 5% is durum wheat (tetraploid) (Patnaik and Khurana, 2001). It has a large genome of approximately 17000 Mb, a~8-fold larger than that of maize and a 40-fold larger than that of rice (Gill *et al*., 2004). Wheat is a major staple food for more than one third of the world population, with an annual yield production of 616.8 million tons in 2006 (http:// faostat.fao.org).

Abiotic stresses are the major environmental challenges for crops in most of the countries, they are considered as limiting factors of plant growth and crop yield. About 7% of the world's total land area is affected by salinity (Flowers *et al*., 1997). Thus, salinity is one of the important factors responsible for low yield and restricted economic utilization of land resources both in arid and semi arid regions of the world. The 21 century is marked by global scarcity of water resources, environment pollution and increased salinization of soils and water (Tester and Bacic, 2005). Egypt is one of the countries that suffer severe salinity problems, as 33% of the cultivated land which represents only 3% of the total land area in Egypt is already salinized (Ghassemi *et al*., 1995).

Wheat is known as moderately tolerant crop to salt. Nevertheless, the possibilities for increasing tolerance to abiotic stresses are enormous and genetic transformation is a powerful tool to develop salinity-tolerant genotypes. However, there are different

methods of transformation and different selection schemes. The most common approach for wheat transformation is the bombardment of tissue derived from immature embryos followed by selection based on resistance to the *bar* gene.

Many genes have been used for wheat transformation to improve salinity-stress tolerance such as: *HAL1* (Gaxiola *et al*., 1992), *AKT1* (Sentenae *et al*., 1992), *HAL2* (Glaser *et al*., 1993), P5CSF129A (Zhang *et al.*, 1995), chimeric accumulating gene (*cpy/sacB*) (Eissa, 2001; Bahieldin *et al*., 2003), *mtlD* (Abebe *et al*., 2003; Ramadan, 2005), *HVA1*(Patnaik and Khurana, 2003; Bahieldin *et al*., 2005), *LEA* (Brini *et al*., 2007), antiporter *hvnhx2* (Miroshnichenko *et al*., 2007), *ATHK1* (Chen *et al*., 2009) .

Even with all these successful reports, hurdles still exist for this recalcitrant crop. Of these hurdles, low transformation rates, tools for transgene expression, and transgene silencing in subsequent generations are probably the most critical (Weeks *et al*., 1993; Becker *et al*., 1994; Janakiraman *et al*., 2002; Sahrawat *et al*., 2003 ; Huw *et al*., 2005).

The rice halotolerance gene (*HAL2*-like gene) or RHL gene belongs to the inositol monophosphatase family. This gene encodes a cation-sensitive nucleotidase required for sulfate assimilation and RNA processing. The *HAL2* gene was identified in yeast by functional assay for supporting the growth of cells under high salinity stress (Glaser *et al*., 1993) and over expression of this gene in yeast improved salt tolerance. Peng and Verma (1995) isolated and characterized rice *HAL*-like (RHL) cDNA. The RHL cDNA complemented both cysQ

and met 22 mutants in bacteria and yeast, respectively. Thus, they have demonstrated that the proteins encoded by *cysQ*, *HAL2* and *RHL* genes have the same function in sulfur assimilation pathway in *E.coli*, yeast and plants. They have further shown that the enzyme encoded by the RHL gene is 3'(2'), 5'-diphosphonucleoside and uses both adenosine 3'-phosphate 5'-phosphosulfate (PAPS) and 3'(2')-phosphoadenosine 5'-phosphate (PAP) as substrates. Availability of active sulfur seems essential to overcome oxidative stress imposed by salinity and drought stresses. Over expression of this gene in plants resulted in accumulation of glutathione which also reduces oxidative stress.

Thus, the present investigation was carried out to address the following objectives:

1. To transform an Egyptian wheat cultivar (Giza 164) and an American wheat cultivar (Bobwhite 56) using the biolistic particle delivery system and the plasmid pHAL harboring the *HAL2*-like gene for salt tolerance beside the *bar* gene as a selectable marker.
2. To produce putative transgenic wheat plants tolerant to salt stress.
3. To confirm the expression of the *bar* gene by leaf painting and herbicide spraying.
4. To confirm the integration of the introduced genes in the putative transgenic plants using PCR and Southern blot analyses and the expression using RT-PCR.
5. To evaluate the performance of transgenic wheat lines constitutively expressing the *HAL2*-like gene based on yield and yield-related traits under saline as well as non saline conditions.