ISOLATION OF TWO SALT STRESS RELATED GENES FROM SORGHUM (*Sorghum bicolor*) AND MOLECULAR EVALUATION OF THEIR ROLE IN YEAST

By

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B.Sc. Agric. Sci. (Genetics), Fac. Agric., Assiut Univ., Egypt, 2003.
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APPROVAL SHEET

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ABSTRACT

Two salt stress related genes namely, Glyoxalase II (*Gly II*) and salt overly sensitive (*SOS2*) were isolated by RT-PCR from an Egyptian *Sorghum bicolor* cultivar.The RT-PCR amplified bands for the SbGly II and SbSOS2 genes were 1011 and 1350 bp in size, encoding polypeptide chain of 336 and 449 amino acids with deduced molecular mass 37.0 and 50.3 kDa, respectively. The nucleotide sequences of the *SbGly II* and *SbSOS2* genes were BLASTn searched at NCBI to explore the homologous genes. The sequence comparison of both genes (*SbGly II* and *SbSOS2*) with the published sequences in NCBI database revealed that the *SbSOS2* gene is a unique gene since it showed an amino acid sequence homology ranging from 99-84% with the corresponding sequences in the database. While, the Egyptian *SbGly II* gene revealed 100% homology with the published *S. bicolor*. The full length of the *SbGly II* and *SbSOS2* genes were submitted to the GeneBank database with accession number KP883296 and KY202762, respectively. The phylogenetic analysis for *SbGly II* and *SbSOS2* from Egyptian sorghum showed a high similarity with Gly II and SOS2 from *Zea mays*. While they were distant from those of *Oryza sativa*. Both genes were cloned individually into the pYES2 expression vector for expression in *Saccharomyces cerevisia*. Yeast growth response was examined at OD600 under different NaCl stress concentration, i.e. 0, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2 and 2.3 M.The results revealed that the growth of the transgenic yeasts harboring the SbGlyII-pYES2 or the SbSOS2-pYES2 was significantly higher than the controls under the different concentrations of NaCl. The expression of the two alleles in the transgenic yeast cells was verified by RT-PCR. Moreover, analysis of the proline content revealed a significant increase in the transgenic yeasts harboring the *SbGly II* and the *SbSOS2* genes compared to the control. The present results suggested that the *SbGly II* and *SbSOS2* could be modulated for improving the salt tolerance of different eukaryotic systems.

Key words: Salt stress**,** *glyoxalase II***,** *SOS2***,** *Saccharomyces cerevisiae*, Proline content, *Sorghum bicolor* and RT-PCR.

DEDICATION

I dedicate this work to whom my heart felt thanks: to my father and mother for all the support and encouragement they continually offered along the period of my post-graduation, to my wife (Rehan), to my daughter (Malak), to my brothers (Abd El-Rahman and Taha), to my sister (Asmaa) for their endless support along the period of my post-graduation.

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INTRODUTION

Overcoming food shortage through self-reliance and sustainable agriculture has gained increased attention all over the world. However, abiotic stresses such as water deficit, increased salinity of soil, and extreme temperatures, cause 50 % reduction in crop yield worldwide (Bray *et al.,* 2000; Tuberosa and Salvi, 2006 and Turan *et al.,* 2012).

Drought and salinity are considered the major constraints which affect crop yield and have adverse effects on food security and socioeconomic conditions. Soil salinity is becoming acute problem day by day as it represents a major abiotic stress, limiting growth and productivity of plants in many areas of the world. It was estimated that salt stress adversely affects almost 20 % of cultivated and half of irrigated land (Brini *et al.,* 2007 and Tavakkoli *et al.,* 2011). Salinity stress, in addition of being considered as a hyperosmotic stress that causes various physiological changes, it is also considered as a hyperionic stress (Gupta and Huang, 2014). High concentrations of salts in plants can cause an imbalance between production and scavenging of reactive oxygen species (*ROS*) such as superoxide anion, hydrogen peroxide (H_2O_2) and the hydroxyl radicals (OH^-) particularly in chloroplasts and mitochondria. Thus, causing hyperosmotic stress that can lead to oxidative damage (Zhu, 2001 and Mittler *et al.*, 2004).

The glyoxalase pathway system has been recognized as one of the key detoxification mechanisms for the osmotic aspect of salt