J. Agric. Sci. Mansoura Univ., 34 (10):10103 - 10113, 2009

SALT TOLERANCE (STO), A STRESS-RELATED PROTEIN IN ARABIDOPSIS HAS A MAJOR ROLE UNDER OTHER STRESS CONDITIONS

El – Shami , M. I. [1]; M. M. El – Danasory [2]; E. F. El - Hashash[1]; E. I. Zaazaa[1]; M. A. Hager[1]; S. El kafafi[3] and M. Elmalky[4]

(1)Agronomy Department, Faculty of Agriculture, Al-Azhar University, Nasr city, Cairo, Egypt

(2)Biochemistry Department Faculty of Agriculture, Al-Azhar University, Nasr city, Cairo, Egypt

(3)Botany Department Faculty of Agriculture, Al-Azhar University, Nasr city, Cairo, Egypt

(4)Agricultural Research Centre, Rice Research and Training Centre (RRTC), Sakha, Kafr El-Sheikh, Egypt

ABSTRACT

Recently, salt tolerance protein (STO) of *Arabidopsis* and rice were identified and confirmed by STO in yeast cell. Here, we present data of one STO family in *Arabidopsis* which protein was cloned, and the expression levels of the genes were analysed by Reverse transcriptase (RT)-PCR, using gene-specific primers. We examined the expression of *AtSTO* mRNA under different stress conditions. The whole plants in age two weeks were treated by (1mM H_2O_2), NaCl 0, 100, 200, 400 mM of NaCl concentration and 20% for PEG and were incubated for different time. For Brefeldin A (BFA) treatments, were added to the *Arabidopsis* cells culture in three days growth at concentration of 10 mg/ml, and the samples were collected at different times from added BFA. we present data of one STO family in *Arabidopsis* which has Zinc-finger protein, and our result indicted that H_2O_2, BFA were inhibitor of the activity of *AtSTO* mRNA and H_2O_2 decrease the expression level of *AtSTO* mRNA. Generally the expression of STO in presence of different concentrations of NaCl and other stresses factors showed that STO protein play a major role to drought tolerance.

Keywords: Zinc-finger protein /gene structure/ gene expression/ Salt and tolerance/ Arabidopsis/Abbreviations: PEG (polyethylene glycol)

INTRODUCTION

Soil salinity is a major abiotic stress in plant agriculture worldwide, genomic approaches are beginning to revolutionize our understanding of plant tolerance to high salinity and drought. Progress now anticipated through comparative genomics studies of an evolution divers from model organisms and through use of techniques such as high-throughput analysis of expressed sequence tags, large scale parallel analysis of gene expression, targeted or random mutagenesis and gain of function or mutant complementation. The discovery of novel genes determination of their expression patterns in response to abiotic stress and an improved understanding in stress adaptation will provide the basis effective engineering leading to greater stress tolerance (Cushman and Bohnert, 2000). Salt tolerance protein (STO) is a B-box type Zinc-finger protein with sequence similarities to constants, which are required to maintain the folded structure of zinc-finger peptides (Putterill *et al*., 1995; Lagercrantz and Axelsson, 2000; Griffiths *et al*., 2003). Many zinc-finger proteins are known to be involved in transcriptional regulation and developmental control. The first identification of (STO) protein

was through a screening approach using a yeast calcineurin mutant. Thus, yeast null mutants in the catalytic subunit genes (cna1cna2), or in the regulatory subunit gene (cnb1), present a salt sensitive phenotype that can be rescued with STO (Lippuner *et al*., 1996). Surprisingly, in Arabidopsis plants, STO has been shown that overexpression enhances root growth tolerance to high salinity. In addition, STO interacts with CEO1/RCD1, an Arabidopsis protein that complements an oxidative stress-sensitive yeast strain (Belles-Boix *et al*., 2000) and negatively regulates a wide range of stress-related downstream genes (Fujibe *et al*., 2004). CEO1/RCD1 has been recently identified as a new component in the plant salt-stress response, through the interaction with SOS1 (Katiyar-Agarwal *et al*., 2006). However, an interaction of STO with CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1), a negative regulator of photomorphogenesis in the dark, has also been reported (Holm *et al*., 2001and Ma *et al*., 2002).

On the other hand, rice has been become a good model plant for genome research of cereals and for isolation of agronomically important genes, owing to its relatively small genome size (430 Mb). In the Rice Genome Research Program (RGP), they have conducted large-scale sequencing of cDNAs randomly selected from various kinds of libraries with the aim of cataloguing all expressed rice genes, they described and isolated seven kinds of cDNAs that show similarities to the Arabidopsis flowering time gene CO and salt-tolerance gene STO (Jianyu SONG *et al*., 1998).

In the present study, we analysed the structure and transcription level of *AtSTO*, an *AtSTO* was analysed has been isolated and characterized and the transcription level of *AtSTO* under different stress condition such as H_2O_2, BFA, NaCl and PEG were analysed by RT-PCR.

MATERIALS AND METHODS

Plant material

Arabidopsis thaliana (L.) Heynh., *Brassicaceae* ecotype Colombia was used for all experiments. Wild type seeds were sown on half MS medium, 0.7% agar plates containing 50 g/ml carbenicillin (Sigma) and grown in 16h light, 8h dark at 18 C. The seeds were sown in pots containing a 3:1 compost: sand mixture and grown in a Sanyo growth chamber at 24 C with 9h light and 15h dark.

Cloning of *Arabidopsis* STO

A homology search BlastP in *Arabidopsis* DNA database using Zinc-binding domain was identified a potential accession number 024174 in BAC number T21E18, locus in chromosome 1. The RT-PCR was carried out using the GATEWAY system(Invitrogene). The construction was affected with RNA from root ofatwo weeks seeding old with two primers; sense primers attB1-STO (5' GGGG ACA AGT TTG TAG TAC AAA AAA GCA GGC T ATG GCT TAC GCA ACA ACA GCA'3) and anti sense primers attB2-STO (5'GGGGACCACTTTGTA CAACAAGAA AGC TGG GTC ATA CAA TAC AGT GCA CAA AC'3). The PCR products flanked by attB1 and attB2 sequences directionally recombined *in vitro* at attP1 and attP2 sites contained in the plasmid (pDoner- 207) when incubated with BP clones enzyme. This

reaction mixture was incubated 1h at 25C and transformed into *E.coli* DH5□. After select gentamycin 12□g/ml, only recombinants can grow because the CCDB gene, contained in the plasmid is lethal, the inserts containing clones were verified by restriction enzymes digestion and by sequencing the full length of STO.

RNA preparation and RT-PCR

Total RNAs during this work was isolated from Arabidopsis as previously described by (Ausubel *et al* 1998). RT-PCR was carried out by use Reverse-iT one step Kit from Abgene following by the manufacture's instructions with equal amounts of RNA from various samples.

Plant treatments with stresses

For H_2O_2 the whole plants at the age of two weeks were treated by spraying *Arabidopsis* plant with (H_2O_2 1mM in 0.95% Nacl, 10mM Tris-Hcl pH 7.5) and incubated for 15, 30, and 60 minutes after application. For BFA treatments, the BFA were added to the *Arabidopsis* cell culture after three days growth at concentration of 10mg/ml, and the samples were collected at: 0, 30, 60, 120, 180, and 240, minutes after addition of BFA. For NaCl and PEG treatments, NaCl or PEG was added to the *Arabidopsis* whole plants in age two weeks in the presence in present of 0, 100,200, 400 mM NaCl concentration and 20% for PEG in half MS medium then incubated for four hours.

RESULTS AND DISCUSSION

Isolation and characterization of AtSTO

The zinc finger domain of the Arabidopsis flowering time controller protein CONSTANS (CO)was used following Putterill *et al*., (1995), to search by the BLAST in plant database (GeneBank), Three Arabidopsis clones (CO, STO, and COL2), seven rice were identified, (SONG *et al*., 1998) and many other plants clones such as *Glycine max*, *Zea mays* and *Solanum sogarandinum* which have been hypothesized to represent the zinc-finger.To clone *At*STO a reverse transcriptase (RT)-PCR was used. Total RNA was extracted from root in age two weeks and reverse transcriptase was affected to amplify *At*STO with *At*STO gene-specific primers. A comparison of the protein sequence with its homologues in proteins *Oryza sativa* (OSSTO), *Glycine max* (GMSTO), *Solanum sogarandinum* (SSSTO) and *Zea mays* (ZMSTO), showed that *ATSTO* clone encode a 27.6 KDa hydrophilic protein of 249 amino acids (Fig1). The analysis of these predicted protein sequences revealed that two cysteine cores in each finger were interrupted by 23 or 24 amino acid residues with a consensus sequence of "Cys-X2-Cys-X8-Cys-X2-Cys. It has been reported that the zinc-finger domain of GATA-1 transcription factors are in a "Cys-X2-Cys-xi7-Cys-X2-Cys"arrangement. The amino acid sequences of STO also had significant similarity within their C-terminal basic regions. The basic region could be a functional region related to flowering-time and salt tolerance and it is thought to be mediate protein-protein interactions with GATA transcription factors. The GATA factors DNA binding domain is a class IV zinc finger motif in the form CX2CX17–20CX2C followed by a basic region. In plants, GATA DNA motifs have been implicated in light-dependent and nitrate-dependent control of transcription (teakle *et al*., 2002).

Expression profile of the AtSTO gene under H_2O_2

Oxidative stress is arising from an imbalance in the generation and removal of reactive oxygen species (ROS). ROS, especially hydrogen peroxide (H_2O_2), have been associated with a decrease in the activity of different pathways in the plant (Xing *et al*., 2007). Reactive oxygen species (ROS) has long been known to be deleterious to cellular activities, but their transient increase in cells has increasingly been suggested to act as signals and to mediate the regulation of various cellular activities, such as responses to biotic or abiotic stresses, cell death, stomatal movement, and root hair development (Adler *et al*., 1995, 1999; Mount, 1996; Kamata and Hirata, 1999; Rhee, 1999; Guan *et al*., 2000). Among different ROS, only hydrogen peroxide (H_2O_2) can cross plant membranes and it has been increasingly proved to be one of the most important signals in plant cell signalling, especially in elicitor-induced defence responses (Neill *et al*., 2002). The obtained results indicated that H_2O_2 function as a signalling molecule in plants (Desikan *et al*; 2001). Regarding the expression of *AtSTO* mRNA in presence of (1mM H_2O_2), the whole plants were treated by spraying *Arabidopsis* plant and incubated for 0,15, 30, and 60 minutes after spraying. Reverse transcriptase (RT)-PCR was analysed (Fig2). A constant level of loading control (rRNA) was observed in presence of H_2O_2 and (0) control (without H_2O_2). The same expression pattern was observed with short time (15 minutes) of incubation in presence of H_2O_2.Whereas, different expression profiles were observed at long time of incubation in presence of H_2O_2 (30 and 60 minutes). This result indicated that H_2O_2 is an inhibitor of the activity of *AtSTO* mRNA and H_2O_2 decrease the expression level of *AtSTO* mRNA.

Is *AtSTO* induce by brefeldin A (BFA)?

BFA is a vesicle transport inhibitor that specifically blocks cell exocytosis but allows endocytosis, resulting in the internalization and accumulation of recycling plasma membrane proteins in BFA compartments (Nebenfuhr *et al*., 2002). Many plasma membrane proteins, including PIN1, plasma membrane H-ATPase (Geldner *et al*., 2001, 2003), and PIN2 (Geldner *et al*., 2003) have been found in BFA compartments. BFA inhibits not only vesicle trafficking but also root and hypocotyl elongation, lateral root formation, and gravitropism of roots and hypocotyls (Geldner *et al*., 2001, 2003). Regarding the expression of *AtSTO* mRNA in presence of BFA using Arabidopsis cells culture (Figure 3). The cells were treated with Brefeldin A (BFA) and the sample were taken at different periods (0, 30, 60,120,180, and 240 minutes) from adding BFA. Reverse transcriptase (RT)-PCR was analysed (Fig.3), a constant level of loading control (rRNA) was observed in presence of Brefeldin A and (0) control (without BFA). A marked increase of expression level of *AtSTO* mRNA was observed in response for added BFA after 30 and 60 minutes reaching about two to three folds compared with nontreated cells. Whereas, different expression profiles were observed at long time of incubation in presence of BFA (120, 180 and 240 minutes). This result indicated that BFA is an inhibitor of the activity of AtSTO mRNA.

Expression of *AtSTO* in NaCl-treated plants.

Because the *AtSTO* (cDNA) conferred increased salt tolerance in yeast (Lippuner, *et al.*, 1996), it was of interest to determine if the corresponding

gene was induced in plants exposed to elevated levels of salt. Reverse transcriptase (RT)-PCR was analysed with RNA isolated from control and NaCl-treated *Arabidopsis* plants at 100, 200, and 400 mM of NaCl (Fig4 A). A constant level of loading control (rRNA) was observed in presence of NaCl and (0) control (without NaCl). In contrast, the levels of expression of AtSTO were low in zero mM NaCl-treated plants and increased approximately 2-folds in the presence of 100 and 200 mM of NaCl. At the highest NaCl concentration (400 mM) the *AtSTO* mRNA levels were 4-fold higher than in the zero mM NaCl. The results showed a significant increase of the transcript abundance under all of NaCl conditions tested but it could not be named *AtSTO* as a gene induced by NaCl. So an additional experiment was performed to analyse the level of expression of *AtSTO* in presence of NaCl at 0 and 400 mM concentration but with different amounts of total RNA, (0, 50, 100, 200 ng of RNA in order to be sure that the *AtSTO* expression not affected by error of RNA amounts. Total RNA was extracted from plants exposure at NaCl 0, and 400 mM NaCl and *AtSTO* gene expression was obtained by RT–PCR, (Fig4 B). A similar expression level of *AtSTO* was clearly observed under these conditions, measured as being approximately 8-fold higher in plants treated with 400 mM NaCl to plants treated with zero mM NaCl (Fig4 B) especially when compared with 200ng of RNA. These results suggest that *AtSTO* expression is salt regulated and appear to respond to NaCl in the medium and seems to respond differentially at any given salt concentration.

Expression of *AtSTO* in PEG-treated plants.

Drought stress is the most common adverse environmental condition that can seriously reduce crop productivity. Increasing crop resistance to drought stress would be the most economical approach to improve agricultural productivity and to reduce agricultural use of fresh water resources.

The biological basis for drought tolerance is still largely unknown and few drought tolerance determinants have been identified (Ludlow and Muchow, 1990; Bruce *et al*., 2002). Despite the lack of understanding of drought tolerance mechanisms, physiological and molecular biological studies have documented several plant responses to drought stress (Bohnert *et al*., 1995; Blum 1996; Luan, 2002). The products of certain stress-responsive genes could function in alleviating stress damage through still unclear mechanisms (Hasegawa *et al*., 2000). The first identification of *ATSTO* was done by (Lippuner, *et al* 1996), as a salt-responsive gene and (Indorf, *et al* 2007) has identified *ATSTO* as a light signalling gene. Reverse transcriptase (RT)-PCR was analysed with RNA isolated from control and PEG-treated *Arabidopsi* plants at 20% of PEG (Fig5 A). A constant level of loading control (rRNA) was observed in presence of PEG and (0) control without PEG. The levels of expression of *AtSTO* were low in zero mM PEG-treated plants and increased approximately 2 to 3 -fold in the presence of 20% of PEG. In order to confirm the above mentioned data we performed an additional experiment to analyse the level of expression of *AtSTO* in presence of PEG at 0 and 20% PEG but with different amounts of total RNA, (0, 50, 100, 200 ng of RNA in order to be sure that the *AtSTO* expression not effected by error of RNA amounts. Total RNA was extracted from plants exposure at PEG (0, and PEG 20%) and

AtSTO gene expression was obtained by RT–PCR, (Fig4 B). A similar expression level of *AtSTO* was clearly observed under these conditions, measured as being approximately 8 to 10 fold higher in plants treated with PEG 20% as compared to plants treated with zero PEG (Fig4 B) especially when comparing with 200ng of RNA. The obtained data suggest that *AtSTO* is drought response and linked to drought tolerance and may define a novel pathway controlling plant drought tolerance.

Figures:-

Figure (1): Alignment of Amino acid sequences of STO cDNAs, clones encoding putative zinc finger proteins of Arabidopsis (ATSTO) AC 024174, Oryza sativa (OSSTO) AC NM_001063369, *Glycine max* (GMSTO) AC ABB29467, *Solanum sogarandinum* (SSSTO) AC ABC25454 and *Zea mays* (ZMSTO) AC ACG41790. The Structure of STO sequences protein of crops revealed that two cysteine cores in each finger were interrupted by 23 or 24 amino acid residues with a consensus sequence of "Cys-X2-Cys-X8-Cys-X6-Cys-X2- Cys.

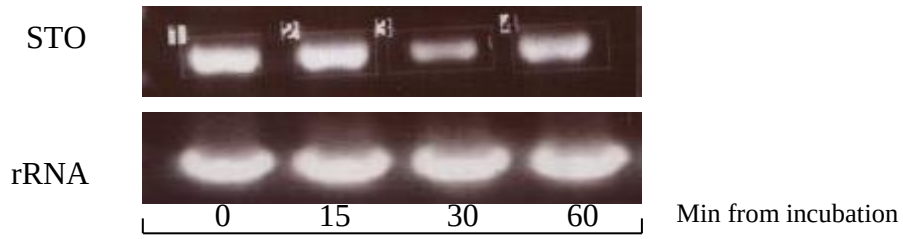


Figure (2):H_2O_2 affects *At*STO gene expression. RT–PCR was obtained from (0,15,30 and 60 minutes from incubated *Arabidopsis* plants in presence of 1 mM H_2O_2.

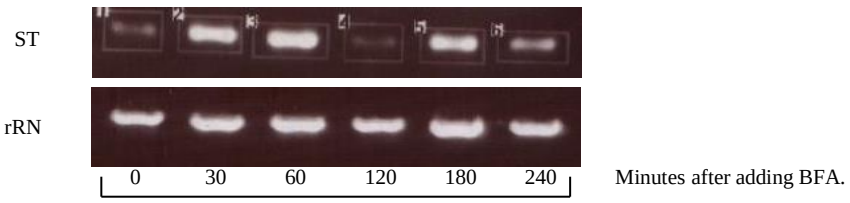
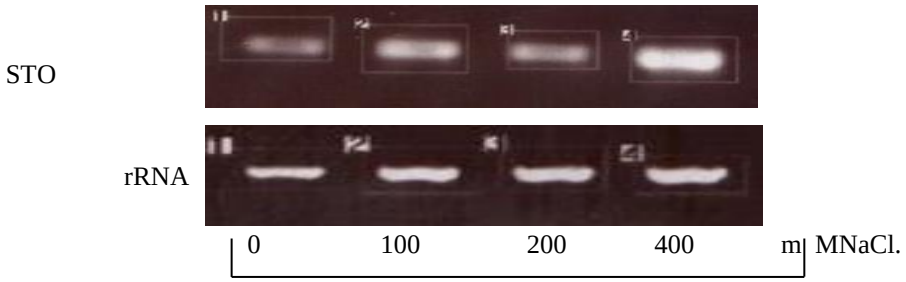


Figure (3):Expression in presence of BFA at 1mM concentration for 0, 30,60,120,180 and 240 minutes from incubation

(A)



(B

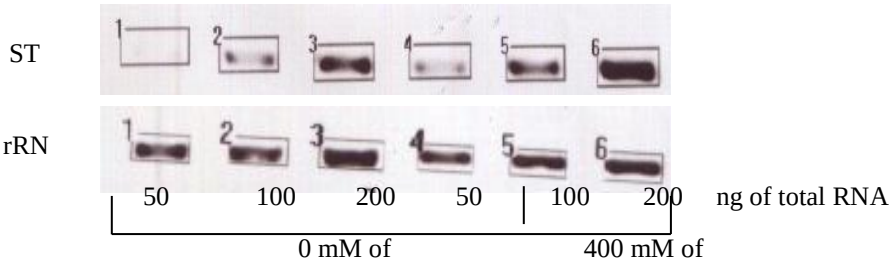


Figure (4): Expression of *AtSTO* in NaCl-treated plants.

***(A) AtSTO* expression in presence of NaCl at 0,100, 200, and 400 mM concentration. Total RNA was extracted from plants after 24 h of exposure to NaCl and *At*STO gene expression was obtained by RT–PCR and equal loading was confirmed by rRNA in all lanes.**

***(B) AtSTO* expression in presence of NaCl at 0 and 400 mM concentration. Total RNA was extracted from plants after 24 h of exposure to NaCl and *AtSTO* gene expression was obtained by RT–PCR with different amounts of total RNA, (0, 50, 100, 200 ng of RNA.**

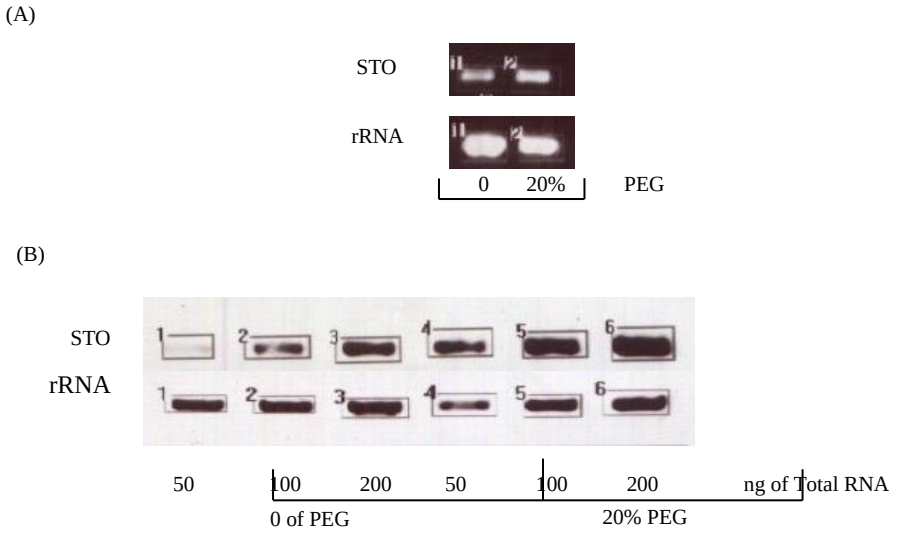


Figure (5): Expression of *AtSTO* in PEG 20% treated plants.

(A) *AtSTO* expression in presence of PEG 20%concentration. Total RNA was extracted from plants after 24 h of exposure to PEG at 20% and STO gene expression was obtained by RT–PCR.

(B) *AtSTO* expression in presence of PEG at0 and 20% . Total RNA was extracted from plants after 24 h of exposure to PEG at 20% and *STO* gene expression was obtained by RT–PCR with different amounts of total RNA, (0, 50, 100, 200 ng of RNA.

REFERENCES

Adler, V.; Schaffer, A.; Kim, J.; Dolan, L. and Ronai, Z. 1995. UV irradiation and heat shock mediate JNK activation via alternate pathways. Journal of Biological Chemistry. 270, 27071–26077.

Adler, V.; Yin, Z.; Tew. K.D. and Ronai Z. 1999. Role of redox potential and reactive oxygen species in stress signaling. Oncogene, 18, 6104–6111.

Ausubel, E.M; Breent, R.; Kingston, R.; Moor,D; Seidman, J.G; Smith, J.A and Strihl, K. 1998. New york Greene Publishing/Wiley Interscience,.

Belles-Boix, E., Babiychuk, E., Van Montagu, M., Inze, D. and Kushnir, S. 2000. CEO1, a new protein from *Arabidopsis thaliana*, protects yeast against oxidative damage. FEBS Lett., 482, 19–24.

Blum, A. 1996. Crop responses to drought and the interpretation of adaptation. J. Plant Growth Regul., 20: 135–148

Bohnert, H. J.; Nelson, D.E. and Jensen, R.G. 1995. Adaptations to environmental stresses. Plant Cell, 7: 1099–1111

Bruce, W.B.; Edmeades, G.O. and Barker, TC. 2002.Molecular and physiological approaches to maize improvement for drought tolerance. J. Exp. Bot., 53: 13– 22

Cushman, JC; Bohnert, HJ. 2000. Genomic approaches to plant stress tolerance. Curr Opin Plant Biol. 3(2):117-24.

Desikan R, Mackerness A-H S, Hancock JT, Neill SJ. 2001. Regulation of the Arabidopsis transcriptome by oxidative stress. Plant Physiol. 127(1):159-72.

Fujibe, T., Saji, H., Arakawa, K., Yabe, N., Takeuchi, Y. and Yamamoto, K.T. 2004. A methyl viologen-resistant mutant of Arabidopsis, which is allelic to ozone-sensitive rcd1, is tolerant to supplemental ultraviolet-B irradiation. Plant Physiol, 134, 275–285.

Geldner, N., Anders, N., Wolters, H., Keicher, J., Kornberger, W., Muller, P., Delbarre, A., Ueda, T., Nakano, A., and Jurgens, G. 2003. The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. Plant Cell, 112: 219–230.

Geldner, N., Friml, J., Stierhof, Y.D., Jurgens, G., and Palme, K. 2001. Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature. 413: 425–428.

Griffiths, S., Dunford, R.P., Coupland, G. and Laurie, D.A. .2003. The evolution of CONSTANS-like gene families in barley, rice, and Arabidopsis. Plant Physiol,131, 1855–1867.

Guan, L.; Zhao, J. and Scandalios, J.G. 2000. Cis-elements and transfactors that regulate expression of the maize CAT1 antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for the response. The Plant Journal, 22, 87–95.

Hasegawa, PM.; Bressan, RA.; Zhu, JK and Bohnert, HJ. 2000. Plant cellular and molecular responses to high salinity. Ann. Rev. plant physiol. 51: 463–499

Holm, M., Hardtke, C.S., Gaudet, R. and Deng, X.W. 2001. Identification of a structural motif that confers specific interaction with the WD40 repeat domain of Arabidopsis COP1. EMBO J., 20, 118–127.

Indorf Martin, Julio Cordero, Gunther Neuhaus, and Marta Rodrı´guez-Franco.2007. Salt tolerance (STO), a stress-related protein, has a major role in light signalling.The Plant Journal. 51, 563–574

Jose´ C. Reyes, M. Isabel Muro-Pastor, and Francisco J. Florencio. 2004. The GATA Family of Transcription Factors in Arabidopsis and Rice1. Plant Physiol. Vol. 134, 1718-32

Jianyu SONG, Kimiko YAMAMOTO, Ayahiko SHOMURA, Hiraku TADANI, Hui Sun ZHONG, Masahiro YANO, and Takuji SASAKI.1998. Isolation and Mapping of a Family of Putative Zinc-finger Protein cDNAs from Rice. DNA RESEARCH 5, 95-101

Kamata H, and Hirata H. 1999. Redox regulation of cellular signalling. Cellular Signalling 11, 1–14

Katiyar-Agarwal, S., Zhu, J., Kim, K., Agarwal, M., Fu, X., Huang, A. and Zhu, J. K. 2006. The plasma membrane Na+/H+ antiporter SOS1 interacts with RCD1 and functions in oxidative stress tolerance in Arabidopsis. Proc. Natl Acad. Sci. 103, 18816–18821.

Lagercrantz, U. and Axelsson, T. 2000. Rapid evolution of the family of CONSTANS LIKE genes in plants. Mol. Biol. Evol. 17, 1499–1507.

Lippuner, V., Cyert, M.S. and Gasser, C.S.1996. Two classes of plant cDNA clones differentially complement yeast calcineurin mutants and increase salt tolerance of wild-type yeast. J. Biol. Chem. 271, 12859–12866.

Luan S. 2002. Signaling drought in guard cells. Plant Cell Environ. 25: 229–237

Ludlow MM, Muchow RC.1990. A critical evaluation of trait improving crop yields under water-limited environments. Adv Agron 43: 107–153.

Ma, L., Gao, Y., Qu, L., Chen, Z., Li, J., Zhao, H. and Deng, X.D. 2002. Genomic evidence for COP1 as a repressor of light-regulated gene expression and development in Arabidopsis. Plant Cell, 14, 2383–2398.

Mount, DW. 1996. Reprogramming transcription. Nature. 383, 763–764.

Nebenfuhr, A., Ritzenthaler, C., and Robinson, D.G. 2002. Brefeldin A: Deciphering an enigmatic inhibitor of secretion. Plant Physiol. 130:1102–1108.

Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT. 2002. Hydrogen peroxide and nitric oxide as signaling molecules in plants. Journal of Experimental Botany. 53, 1237–1247.

Putterill, J., Robson, F., Lee, K., Simon, R. and Coupland, G. 1995. The CONSTANS gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell, 80, 847–857.

Rhee SG. 1999. Redox signaling: hydrogen peroxide as intracellular messenger. Experimental and Molecular Medicine 31, 53–59.

Teakle GR, Manfield IW, Graham JF, Gilmartin PM. 2002.*Arabidopsis thaliana* GATA factors: organisation, expression and DNA-binding characteristics. Plant Mol Biol. Sep; 50 (1):43-57.

Yu Xing.I, Wensuo Jia, and Jianhua Zhang. 2007. AtMEK1 mediates stress-induced gene expression of CAT1 catalase by triggering H2O2 production in *Arabidopsis*. Journal of Experimental Botany, Vol. 58, No. 11, pp. 2969–2981,

بـروتين (STO)وعلاقتـة بتحمـل النباتـات للملوحـة ودوره الكبيـر تحـت ظـروف الإجهاد الأخرى.

محمود إبراهيم الشامى[١] , محمد مبروك الدناصوري[٢]، عصام فتحي الحشاش[١], عزالدين إبراهيم زعزع[١], محمد أحمد هاجر[١] , السيد الكفافى[٣] و محمد المالكى[٤]

[١] قسم المحاصيل، كليّة الزراعة، جامعة الأزهر

[٢]قسم الكيمياء الحيوية، كليّة الزراعة، جامعة الأزهر

[٣] قسم النبات، كليّة الزراعة، جامعة الأزهر

[٤]مركز بحوث الأرز والتدريب – مركز البحوث الزراعية - سخا – كفر الشيخ

في السنوات الأخيرة ، تم التعرف على بروتين (STO) لتحمل الملوحة فى نبات الأرابيدوبسيس والأرز ومؤكدا ذلك بدراسته في الخميرة لتحملها للملوحة . وفى دراستنا هنا نقدم النتائج لبروتين من أحد عائلات (STO) نبات الأرابيدوبسيس وقد تم عمل استنساخ (كولنة)لهذاالبروتين ،وأيضا دراسة مستويات التعبير لهذا الجين بواسطة طريقة النسخ العكسي لتفاعل البلمرة المتسلسل Reverse transcriptase (RT)-PCR ،وذلك باستخدام بوادئ مخصصة لهذا الجين تحت ظروف إجهاد مختلفة.وقد تمت معاملة النباتات في عمر أسبوعين بواسطة H_2O_2 (1mM) وكلوريد صوديوم NaCl بتركيز 400, 200,100, 0,ملى مول وبولى إثيلين جليكول PEG 20% وقد تم تحضينها أثناء المعاملة لفترات مختلفة. وعوملت معلق خلايا الأرابيدوبسيس المنماه لمدة ثلاثة أيام بواسطة Brefeldin A (BFA) بتركيز 10mg/ml وجمعت العينات على فترات مختلفة من المعاملة . وقد أوضحت النتائج في دراستنا أن هذا البروتين يمتلك بصمة الزنك.كما أوضحت أن المعاملة نبات الأرابيدوبسيس ب H_2O_2, BFA قد أدت إلى تثبيط نشاط mRNA *AtSTO* وقللت من مستوى تعبيره.وعموما تأثر تعبير ***AtSTO*** في وجود تركيزات مختلفة من كلوريد الصوديوم وأيضا عوامل الإجهاد الأخرى مما يوضح أن البروتين (**STO**)يلعب دورا كبيرا في تحمل النباتات للملوحة