DETERMINATION AND QUANTIFICATION OF SALT STRESS RELATED GENES IN FABA BEAN (*Vicia faba*)

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Salt stress is one of the major environmental factor that affecting plant growth and development. High salt content in the soil causes accumulated stress to the cultivated plants in some areas. Plants may be modified genetically to tolerate these effects that lead to some physiological and morphological modification too (Shanon, 1986; Fisher and Turner, 1978). These interacted changes and modifications are not that simple and a lot of response pathways are required to overcome the unfavorable conditions (Neumann, 1997; Yao, 1998; Hasegewa *et al*., 2000; Munns, 2002). In salty habitat, some plants tolerate these effects and grow in full capacity. These plants have a unique genetic profile tolerating the crucial environment.

Bad drainage and high temperature are affecting the agricultural lands that lead to accumulation of salt in the soil (Zhu *et al*., 2005). These problems are common in most of eastern and southern Mediterranean Sea countries including Syria, Lebanon, Jordan, Egypt, Libya, Tunisia, Algeria and Morocco. In Egypt, bad drainage has bad environmental impacts in the north of Nile delta and the west north coast (FAO, 2005).

Regarding the nutrient uptake in different organs of faba bean seedlings, it is inversely proportional as result the nutrients content decreased by increasing the amount of the salinity. It was stated that the nutrients uptake (N, P, K) were decreased by increasing soil salinity levels. This showed that the excessive content of Na+ and Cl- ions in growth media inhibit the uptake and translocation processes of the most important nutrients (Thalooth *et al*., 2006).

Faba bean (*Vicia faba*) is a major economical crop in Egypt as one of the most important food and feed crop. However, it is one of the most sensitive plants to the abiotic stress especially the soil salinity. On the other hand, there are some wild growing *Vicia* species (*Vicia monantha, V. narbonensis, V. villosa and V. sativa*) that are growing in this region tolerating drought and salt stress. These plants are considered as a good source of abiotic stress genes awaiting their identification and isolation for their relative *V. faba*. Most of the faba bean breeders have a great interest to produce tolerant lines to be cultivated in salinity affected areas.

A lot of physiological analysis studies had been performed to evaluate the

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economic damages of the salinity on important crops to detect the biochemical and molecular regulations that negatively affect the yield and quality of the cultivated crops (Wang *et al*., 2008).

Molecular identification of the stress related genes are helping a lot to understand the physiological regulations of different stress types and levels (Liu and Vance Baird, 2004). On the cellular level, Skirycz and Inze (2010), reported that abiotic stresses inhibit sell growth and reduce enzymes' activities in a group of plant that called reactive oxygen species (ROS). In addition to another plant groups called reactive nitrogen species (RNS), that alters genes actions and exchange the pathways with the ROS to overcome the stress (Molassiotis and Fotopoulos, 2011).

Screening of induced genes during environmental stress showed the expression of some ribosomal genes that may play a role to overcome the abnormal conditions. One of these genes is the ribosomal protein L30e (*RPL30e*) as an essential player of the eukaryotic system. This protein is buffering the directed synthesis of mRNA in the cell during the abiotic stress (Joshi *et al*., 2009). In addition to other proteins that called expansions which cause loosening to plant cell wall, involved in cell enlargement and modification of cell-wall and other developmental processes (Cosgrove, 2000).

The aim of this study was to identify and quantify one of the expansins genes (the beta-expansin *EXPB11*), in addition to the *RPL30e* gene.

MATERIALS AND METHODS

Homogeneous seeds of faba bean seeds (*Vicia faba* L.) cv. Giza 843 was purchased from the Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. The seeds were surface sterilized in 8.0% of commercial bleach and rinsed twice with sterile double distilled water. The seeds were germinated in 35 cm pots that filled of a mixture of peat moss and sand. Three seedlings were maintained in each pot after the emerging of the 2nd true leaf of each seedling. The seedlings were irrigated with Hoagland's solution plus 0.0 concentration (control) and 150 mM of NaCl, respectively, for 10 days. Leaves samples were collected from each pot and subjected to RNA extraction.

Total RNA extraction

Total RNA was extracted and purified from one gram of each sample using SV Total RNA Isolation System® (Promega™) kit. The quality of the RNA was checked on 1.0% agarose formaldehyde gel electrophoresis at 45 volts for 80 minutes.

RT-PCR analysis

Approximately 5 ug of total RNA were reverse transcribed using Access RT-PCR System® (Promega™). Specific primers for both genes were designed by the online software of Primer3 program (http://bioinfo.ut.ee/primer3-0.4.0/). The primers were (5'-GGGCAAGTACACGCTAGGAT-3' as a forward primer and 5'-

AGGGTCTGTGATGGACAAGG-3' as reverse primer for the *RPL30e* gene) and (5'- CAGTGCAGAGTTGCGGTAAA-3' as a forward primer and 5'-ACAAATCGACGACGACACAG-3' as a reverse primer for the beta-expansin *EXPB11*) followed by the thermocycler program of 94°C for 3 min, followed by 30 cycles of 95°C for 45 s and 54°C for 1 min and 72°C for 1 min and a final extension at 72°C for 7 minutes (Joshi *et al*., 2009). The amplified fragments were displayed on 1.0% agraose gel electrophoresis.

Analysis of target genes expression using the quantitative real-time PCR

The quantification of genes expression had been done by the indirect measurement method using SYBR green technology. The quantification was performed using the ABI PRISM 7900. The calculation and the comparison were done using the arithmetic equations to reach the same output for relative quantification. The quantity of the expression of the target gene was normalized to an internal control (β-actin gene) and finally given by $2^{-\Delta\Delta C}{}_{T}$. The used β-actin primers were (5'-CGTCTTCCCCTCCATCG-3' as a forward primer and 5'-CTCGTTAATGTCACGCAC-3' as a reverse primer).

The amount of target, normalized to an endogenous reference and relative to a calibrator (fold differences), is given by $2^{-\Delta\Delta C}{}_{T}$. Using the expression level of target genes in the control tissue as a base line, the expression folds have been detected in the treated ones assuming that both standard and target have same efficiencies (Molestina and Sinai, 2005).

Threshold cycle values (Ct) were used, as Ct indicates the PCR cycle number at which the amount of amplified target reaches a fixed threshold. In order to convert threshold cycles in copy numbers. The used formula for copy number detection was:

“"Y molecules μL^{-1} = (Xg μL^{-1} DNA/ [Length of PCR product in base pairs x 660]) x 6.022 x 10^{23}) which is a simple method for determining the variable values in experimental biology" (Reed and Muench, 1938).

RESULTS

cDNA and the RT-PCR was applied in order to determine expression of the genes under investigation. The RT-PCR products were displayed on 1.0% agaorse gel electrophoresis (Fig. 1a and b.). Figure (1a) was the amplified cDNA fragment of the *RPL30e* gene and figure 1 b. was the amplified fragment of the expansin *EXPb11*. As shown in the Fig. (1), Lanes "C" which were the control 0.0 NaCl and lanes T which were 150 mM NaCl treatment whereas lanes "M" in Fig. (1a) was for the 100 bp DNA-Low ladder (Core Bio Service. Cat# 19-109) and "M" in figure 1.b. was (TrackIt™ 100 bp DNA ladder Cat# 10488058). The amplification of the fragments was accurate, distinguishable, and in the expected size of the designed primers (285 bp for the *RPL30e*

and 198 bp for the expansin, respectively) according to the used DNA markers.

Gene expression quantification

Real-time PCR was performed to determine the C_T value of the gene under investigation in the treated samples and to be compared to the untreated sample's C_T value to quantify the expression folds in both. Some differences were observed between the C_T of both samples, treated sample was observed to have lower C_T value that means that the expression of these genes are high in the presence of the salt in the soil (Table 1).

The expression folds of the target gene were calculated and represented in Fig. (2). It is clear that the expression folds of both genes were elevated due to the treatment of the samples of 150 mM NaCl.

DISCUSSION

Due to the contentious abusing to the environment and global worming consequences, abiotic stresses became more for crops production over the whole world. Salt stress is one of the common abiotic stresses that limit the yield of growing plants. Faba bean is a salinity sensitive plant that may be dramatically damaged when being exposed to salty conditions. Most of the salts' ions are toxic to the plants because it changes the osmotic potential around the plants which leads the plant to go through what is called "osmotic stress" (Cramer, 2010).

Previous researches investigated the salinity effects on plant's growth (length, leaf number, fresh weight, dry weight, photosynthesis rate, the osmotic potential, and protein content (Ha *et al*., 2008; Yilmaz and Kina, 2008; Rui *et al*., 2009; Taffouo *et al*., 2009 & 2010; Abdul Qados, 2011; Kapoor and Srivastava, 2010; Memon *et al*. 2010).

In this research, two genes were examined for their importance in salt tolerance in faba bean. The first one was the expansin (*EXPB11*) which showed high induced expression in the salt treated faba plants. It recorded 20 folds more than the control (Fig. 2). Our data support the role of the expanisin in salt tolerance of previous studies; the *EXPB11* showed higher expression formost of plants including rice plant in a high salt soil. Four families of the expansin plants are recognized on the basis of phylogenetic sequence (Kende *et al.*, 2004). These protein families are designated from largest to smallest α-expansin (*EXPA*), β-expansin (*EXPB*), expansin-like A (*EXLA*) and expansin-like B (*EXLB*). α-Expansin and β-expansin proteins have demonstrated by experimental means to cause loosening in cell-wall (McQueen-Mason *et al*., 1992).

The second part of this research was the quantification of the expression of the ribosomal protein coding gene *RPL30e*. The expression of this gene scored 15 folds over the control (Fig. 2). That supports the role of these kinds of genes that lead to changes in protein metabolism processes. Plant could do the

balance by tolerating the aggressiveness of the environment by production of novel genes and sometimes to break down some other proteins. In addition to, a lot of changes of the accumulation of ribosomal proteins are happening in both eukaryotic and prokaryotic systems (Sarhadi *et al*., 2010; St-Sauveur, 2013).

Increasing in ribosomal genes activity is going in parallel with the elevation of the stress (either biotic or abiotic stress). In either ways, by producing new sets of proteins to heel the damage or to degrade other existing proteins to avoid extra damages (Vincent *et al*., 2007; Bandehagh *et al*., 2011).

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Table (1): C_T values and standard deviation of the untreated and salt treated faba bean samples.

NaCl Concentration mM	Ct Value	
	RPL30e	*EXPb11*
0	35.76± 0.21	29.22 ± 0.56
150	10.53± 0.07	8.87 ± 0.21

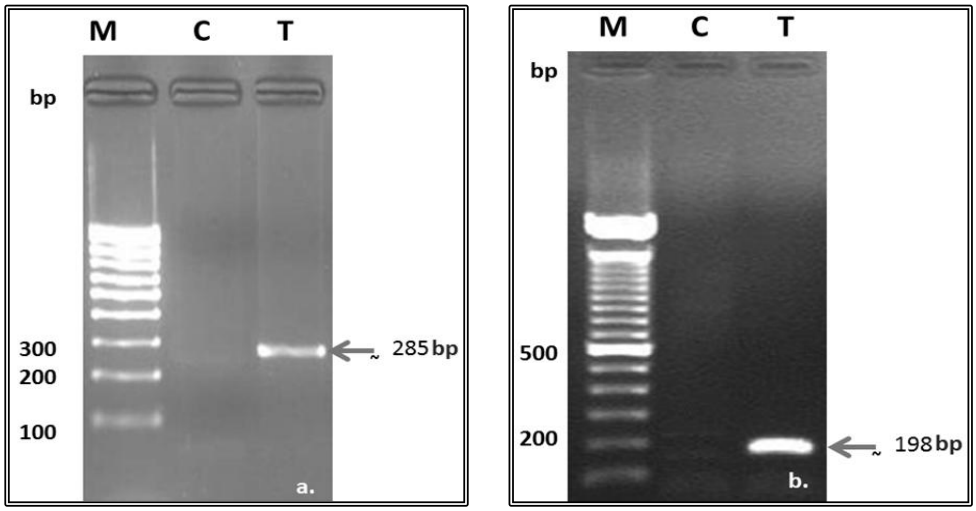

Fig. (1): Agarose gel electrophoresis of the RT-PCR for both *EXPB* and *RPL30e*.

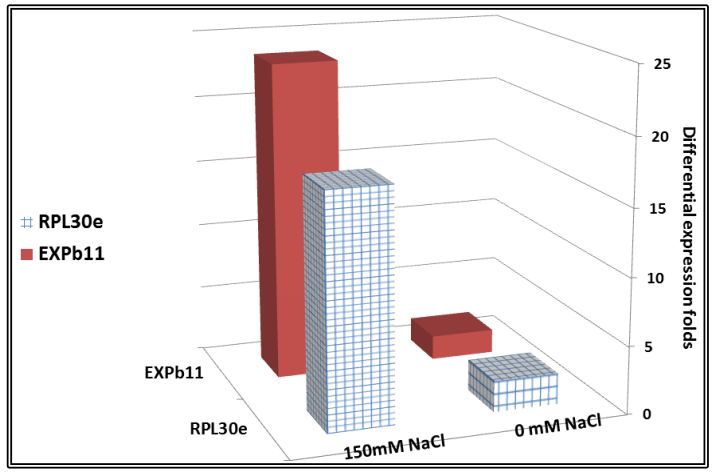

Fig. (2): Differential expression folds of the untreated (0 mM Nacl) and salt treated (150 mM) faba bean samples.