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RESEARCH ARTICLE

T-DNA insertion in the KUP8 targeted gene improves the survival under salt stress in the *Arabidopsis thaliana*

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Abstract

Salt stress is one of the very harsh abiotic stresses plants face which affects crops growth and productivity. Studying gene regulation in plants under salts attracts scientists worldwide. Using T-DNA strategy has become a crucial approach in studying gene function and regulation. In Arabidopsis, KUP family genes are suggested to be involved in salinity stress in plants. KUP8 gene is classified as a member of one of the KUP family that mainly has a role in K ion transportation. As this gene has not been tested before under salinity stress in Arabidopsis, I interested in understanding the effect of the T-DNA site on the KUP8 regulation. Two KUP8 gene mutants were used comparing to the wild type to study their function in salt stress response. Kup8 mutant 2 'M2' of the KUP8 gene showed higher sensitivity to salinity at 100 mM NaCl compared to the Kup8 mutant 1 'M1' and wild type (Col. 0). This finding is referred to the interruption of the KUP8 gene and preventing the production of the normal transcript. There was no clearer tendency showing the effect of high salt concentration 150mM NaCl on the plant survival among the three tested genotypes. Visible observation indicated the accumulation of anthocyanin on wild type plants followed by *kup*8 mutant 1 'M1' while *kup*8 mutant 2 'M2' showed less darkness. This result indicates that the presence of the insert in the gene promoter may up regulate and enhances the expression the targeted gene under salt stress and may improve the survival ability at plant lavel.

Key words: Salt stress, KUP8, T-DNA, cis-acting elements

Introduction

Plants are subjected to different abiotic stresses as a result of harsh environmental conditions. These unfavorable conditions reduce growth and productivity. Agriculturally, salt stress is not only caused by the effects of irrigation with poor quality water, but also by nature because some lands already contain high amounts of salt. It has been estimated that 20% of all cultivated land and nearly half of irrigated land is salt-affected, leading to reduced yields (Munns 2002; Flowers 2004). Plant susceptibility to salt depends on soil and climate conditions surrounding plants which affect water relations (Osmond *et al.,* 1987). The target plant in this study was Arabidopsis thaliana; the most known and characterized plant in biotechnology. The Arabidopsis genome is composed from more than 27.000 genes encoding proteins from 11.000 families (Bevan and Walsh 2005; Swarbreck *et al.*, 2008). Many of these genes are well known regarding their abiotic stress involvement but a lot of them still not fully understood. The plan of this work was to investigate an insertional mutant line that is involved in the response to salt strees based on some previous QTL analysis in the model plant.

One of the most successful ways to overcome this problem is to engineer crops that tolerate salinity. However, the discovery of the novel genes, determining their function as an expression in response to adverse environment, and improving our understanding regarding stress adaptation will provide effective engineering plans for plants to acquire higher stress resistance (Cushman and Bohnert 2000).

Acclimation to salinity is controlled by different genes as the mechanism of salt tolerance involves a number of processes. Several genes have been identified and isolated, and found to encode proteins that have roles in the antioxidant defense, ion transportation and compartmentalization, and polyamines mechanisms (Sairam and Tyagi 2004).

Arabidopsisis a very susceptible plant to growth inhibition and damage by salinity but the research regarding this kind of stress is still limited. Apart from the sensitivity of this plant to salt, some studies have indicated that glycophytic cells could be adapted to high salt levels. Actually to gain adaptation to salt, plants can be exposed to lower nonlethal levels of salt (Amzallag *et al.,* 1990) and researchers think that salt tolerance character might be preserved in all plants. As mentioned before regarding multigenic control in response to salt, the thought is focused on the quantitative rather than the qualitative pattern of this response. However, the differences in the susceptibility/tolerance to salt may result from the differences in the regulatory circuits or from genes coding for key salt tolerance (Xiong and Zhu 2002).

Potassium (K+) is an essential plant nutrient with key functions in cell growth, enzyme activity, transcription, posttranslational modification, transport and osmotic stress response. The research identifies that many of the KUP family genes are highly expressed under salt stress conditions (Yang *et al.*, 2020a; Yang *et al.*, 2020b) and also found to be drought up regulated (Ou *et al.*, 2018; Yang *et al.*, 2020b). KUP8 is suggested to play an important function as K ion transporter and is up regulated in root tissues under the potassium depletion (Husri and Ong-Abdullah 2018). This gene has not been tested before under salt in Arabidopsis, thus it is suggested to have similar role like the other potassium transporters in plant tissues.

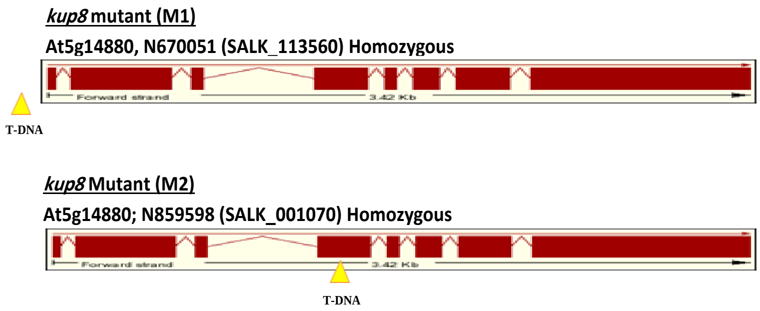
Materials and methods

The experiments were conducted in a controlled growth and cold room in the Horticulture Department, Faculty of Agriculture at the University of Tripoli, Libya. Plants of Arabidopsis thaliana homozygous lines of KUP8 gene (At5g14880) and Columbia 0 wild type were grown in separate pots. T3 seeds provided by Nottingham Arabidopsis Stock Centre (NASC) ((http://arabidopsis.info/) were used for this study. Seeds of two kup8 Salk lines; SALK_113560 (N670051); T-DNA is inserted in the promoter (named as mutant 1'M1), and SALK_001070 (N859598); T- DNA is inserted in the exon four (named as mutant 2; M2) of the KUP8 gene (Figure1), and Columbia 0 (N70000) were sown on peat moss produced by Floragard. After sowing, pots were covered with cling film and put in a cold room (±4Cº) and continuous light for 4-5 days. Pots were, then, kept at normal growth conditions; room temperature 20± 1 Cº and Short-day conditions (10hrs day - 14hrs night, with light density about 170 µmol m^{-2} s^{-1} (400-700nm), and 50-65% relative humidity. Generally, plants used for this study were grown for a period recognized between stages 3-6 according to (Boyes *et al.*, 2001). The experiment was designed to include three treatments (salt concentrations; 0.0, 100 and 150 mM NaCl) with three replicates, each of which include nine plants with eighteen plants for each treatment.

Imposition of the salt stress

Before exposing to salt stress, plants were left to grow for 4 weeks under standard conditions. Then, adaptation to salt was done by exposing plants (wild type and kup8 mutants) to lower salt concentration (20 mM NaCl) by immersing pots (lower feeding) in the salt solution for 20 minutes for 3 days. Control plants were watered with distilled water; EC 2.5ppm (Elhaj 2009). Liquid fertilizer was added to the salt solution for all pots including those for control plants. Then, plants were left in the growth room for four days before exposing higher salt concentrations; 100 and 150 mM NaCl for 20 minutes for 5 days. Five millilitre of mineral solution was added to the salt and control solutions. Few days later after stopping salt treatments, surface irrigation using tap water with liquid fertilizer added, was done whenever needed to prevent soil dryness. After 10 days, plants were recovered by washing salt twice and left for grow for time-course survival counting.

Fig.1: Exonic, intonic and T-DNA insertion site of the KUP8



Results and discussion

Interesting in understanding the effect of the T-DNA site on the gene regulation may help in engineering plant genome to enhance abiotic stress tolerance. In this experiment, KUP8 mutant (M2) which carry T-DNA in the exon 4 (SALK_001070, 'N859598') of the KUP8 gene showed higher sensitivity to salinity at 100 mM NaCl compared to the KUP8 mutant (M1) which carry the T-DNA in the gene promoter (SALK_113560 'N670051') and wild type (Col. 0 'N70000') (figures 2 and 3). The possible clear evidence for that is the interruption of the gene by the foreign DNA inserted within the gene coding sequence. This leads to the absence of the transcript from the KUP8 gene which ends up with a specific protein related to salt stress response. Therefore, these results confirm the involvement of the KUP8 gene in the response to salt stress. Growth observation indicated the development of dark color on wild type plants which indicates the anthocyanin accumulation, followed by *kup*8 mutant 'M1' while *kup*8 mutant 'M2' showed less dark color. On the other hand, there was no clearer tendency showing the effect of high salt concentration at 150mM NaCl on the plant survival among the three tested genotypes. Also, the obtained results indicate that locating the insert in the gene promoter may contribute to up regulate the targeted gene under salt stress. Changing the promoter sequence by the left border of the insert indicates the presence of some cis-acting elements that are involved in the salt stress response to the region of gene promoter. A search using PLACE database (Higo *et al.*, 1999), was done to identify the response elements in the promoter of KUP8 and the T-DNA left border junction. Research results show that the T-DNA left border may contain certain cis-acting elements that may increase the gene up regulation to when the T-DNA being inserted in the gene promoter.

These transcription elements act as transcriptions activators to enhance gene expression in response stress (Elhaj 2009). The results indicated that there are many drought, ABA, potassium and calcium signalling response elements in the mutant. These are known to activate different dehydration stress and ABA signalling involved genes which may affect survival under salt stress (Elhaj 2009). It has been confirmed that Fifteen putative cis-acting regulatory elements, including the stress response, hormone response, circadian regulation, and nutrition and development were identified in the promoter of some K^+ transporters under stress condition that contribute to enhance the gene expression (Liang *et al.*, 2020).

Potassium ion is required for the activity of many enzymes in the plant cell and has a crucial role in maintaining turgor. Usually, plant roots uptake potassium from soil and have developed a mechanism that can operate at low external potassium ions with higher capacity. The process of K+ uptake takes place through a biphasic (high- and low-affinity) pattern of potassium uptake and transportation confirming two mechanisms that work together at the plasma membrane (Epstein *et al.*, 1963). Under stress conditions, abscisic acid (ABA) plays a role in responses to abiotic different stresses (Osakabe *et al.*, 2013). It triggers signaling cascades that essentially lead to activities modulation in the K+ channels and transporters (Chérel and Gaillard 2019).

To conclude, locating of T-DNA within the coding sequence usually interrupts the gene and prevents the normal transcription, thus no protein is produced, while inserting a peace of forging DNA in the promoter of a gene may alter the gene expression leading to up- or dowan-reglation. This results in increased sensitivity or raising stress tolerance depending on the type of regulatory elements that the T-DNA brings to the original promoter.

Fig.2: The effect of salt concentration NaCL (mM) on plant part survival (%) of Arbidiopsis wild type (w) Columbia 0 and two *kup8* mutants (M1: SALK_113560 "T-DNA in the promoter", and M2: SALK_001070 "T-DNA in the exon 4", after 9 days of finishing salt treatment

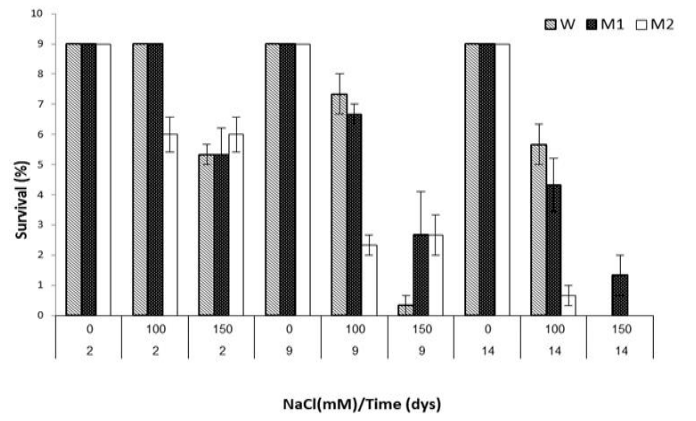
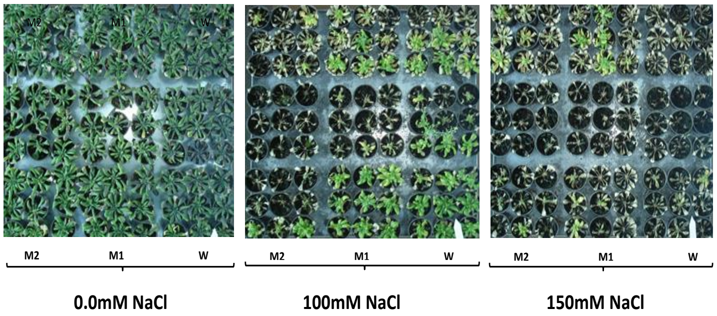


Fig.3: The effect of salt concentration NaCL (mM) on plant part survival (%) of Arbidiopsis wild type (w) Columbia 0 and two *kup8* mutants (M1: SALK_113560 "T-DNA in the promoter", and M2: SALK_001070 "T-DNA in the exon 4", after 2, 9 and 14 days of imposition of the salt treatment



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