



Short Communication

Proline accumulation is a general response to abiotic stress in the date palm tree (*Phoenix dactylifera* L.)

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Genet. Mol. Res. 14 (3): 9943-9950 (2015)

Received May 13, 2015

Accepted July 22, 2015

Published August 19, 2015

DOI <http://dx.doi.org/10.4238/2015.August.19.30>

ABSTRACT. Plants exposed to certain abiotic stress conditions tend to produce the amino acid proline, which acts as an active osmolyte, a metal chelator, an antioxidant, and a signaling molecule. There is increasing evidence that proline accumulates in plants due to a wide range of abiotic stress, in particular high soil salinity and drought. Therefore, proline content is often used as a marker-assisted breeding tool aimed at improving drought and salinity tolerance. In this study, it was investigated whether proline accumulation in date palm (*Phoenix dactylifera* L.) seedlings occurs solely due to high salinity and drought stresses or due to other unspecified abiotic stresses, including salinity and salinity shock, drought, extreme temperatures, and abscisic acid. The free proline assays revealed that this amino acid over-accumulated in the roots and leaves of each stress-treated plant, and was remarkably high when leaves were exposed to suboptimum temperatures and salinity stress. These results indicate that the production of proline is a common response to various abiotic stresses and its differential accumulation cannot be used as a molecular marker in date palm breeding programs aimed at improving drought or salinity

tolerance traits in date palms. This conclusion is consistent with the theory that the molecular outcomes of abiotic stresses are often non-specific.

Key words: Proline; Salinity; Abiotic stress; *Phoenix dactylifera* L.

INTRODUCTION

Drought and increases in salinity have recently become a serious worldwide problem in a variety of domains, and play a particularly destructive role in the agricultural sector in countries with a low annual rainfall. This problem causes a significant loss in yield and quality in a variety of plant species, including the date palm (*Phoenix dactylifera* L.), despite the fact that the date palm can survive a wide range of extreme abiotic stresses including drought, high temperatures, and a relatively high level of soil salinity (Yaish and Kumar, 2015; Yaish et al., 2015). Drought and high salinity can cause a significant level of damage to plant tissues. While drought leads to osmotic stress, a reduction in CO₂ assimilation, and an overproduction of destructive free oxygen species, salinity can also trigger toxicity in tissues as it causes an increase in the cellular concentration of Na⁺ (Munns and Tester, 2008).

Despite the fact that the molecular pathways underlying adaptations to high salinity and drought have been partially identified in some plant species, these mechanisms in date palms require thorough investigation at the molecular and physiological levels. Plant cells can tolerate drought and salinity using various strategies, including the synthesis and accumulation of organic metabolites of low molecular weight, known as compatible solutes, such as sugars and sugar alcohols like mannitol, trehalose, and galactinol, as well as through the synthesis of amino acids and amines such as glycine, betaine, and proline (Hayat et al., 2012).

In plants, proline is involved in a variety of developmental processes and is accumulated in remarkable quantities under various abiotic and biotic stresses (Szabados and Savoure, 2010). However, the precise molecular role of this amino acid is not completely understood, to date. Suboptimal abiotic environmental conditions include drought, high or low temperatures, salinity, and nutrient shortage. Moreover, atmospheric pollution and ultraviolet stresses lead to an increase in the accumulated proline in plant tissues at different levels. Additionally, treatment with abscisic acid (ABA), a stress-inducing hormone, caused an increase in proline in plant tissues (Yang et al., 2000).

In addition to its well-known role as an osmoprotectant, acting as a vital factor in enhancing cellular turgor, proline has various crucial roles in plant growth under abiotic stress conditions. These roles include reducing the possible membranous oxidative damage caused by reactive oxygen species, enhancing signal transduction pathways, stabilizing DNA and protein complexes (including membrane proteins), and providing an alternative resource for nitrogen and carbon (Szabados and Savoure, 2010).

An increase in proline content is a positive indicator of salinity and drought tolerance. Thus, proline content in plant tissues was used as a marker-assisted selection molecule in some breeding programs that endeavored to improve salinity and drought tolerance in plants (Flowers and Flowers, 2005; Ma et al., 2007; Ashraf and Foolad, 2013; Wu et al., 2013). In the present study, I determined whether proline accumulation in the date palm tree was specifically correlated to drought, and high salinity conditions, or was a general response to abiotic stresses. For this purpose, date palm seedlings from one fully expanded leaf stage were treated with various abiotic stresses and the proline content was quantified in roots and leaves. The results indicate that all

of the abiotic treatments tested induced accumulation of proline in date palm tissues at significant levels. These results are consistent with the theory that proline accumulation is a general response to stress in plants.

MATERIAL AND METHODS

Date palm seeds (*P. dactylifera* L., *Khalas* variety) were germinated in sterilized moist vermiculite and incubated in the dark at 30°C for ten days in a closed container. Subsequently, germinated seeds were carefully cultivated in 2-L pots containing 66% vermiculite and 34% peat moss, and incubated in a growth chamber with a 16-h light cycle, with light intensity of 350 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and temperatures of 35° and 30°C for the light and dark conditions, respectively. During the growth period, humidity was maintained at 60%. Plants then were watered, as needed, to field capacity for six weeks. Consequently, separate sets of six seedlings were either watered regularly (control treatment), watered twice with 300 mM NaCl solution over the course of the entire week (salinity shock), left without irrigation for two additional weeks (drought treatment), incubated overnight in the dark at 50°C (heat treatment) or 4°C (cold treatment), or had the leaves sprayed twice with 10 μM ABA as previously described (Yaish et al., 2010). In the cold and heat treatments, plants were covered with a plastic bag to prevent direct leaf dehydration. The gradual change in salinity treatment was completed and plants were watered daily for six weeks with weekly increasing concentrations of 50, 75, 100, 150, 200, and 300 mM solutions of NaCl.

Root and leaf samples were collected, thoroughly cleaned with tap water, and flash frozen in liquid nitrogen before being grounded by pestle and mortar. Free proline was extracted from 1 g fresh root and leaf tissues, and the proline concentration was colorimetrically quantified according to the previously described protocol (Bates et al., 1973). Water and soluble salt content of the artificial soil were measured using the gravimetric and the electrical conductivity methods following stranded protocols (Rhoades et al., 1989).

RESULTS AND DISCUSSION

Effect of abiotic stress on the phenotype

In this study, a comparative physiological treatment was conducted in order to investigate changes in proline content in response to various abiotic stresses in the roots and leaves of date palm seedlings grown in pots. For the drought treatment, seedlings were unwatered for two weeks, after which point the water content of the soil in the pots was quantified. The average moisture content in the pots before the treatment was 55%. However, after treatment it was reduced to 6%. Since an artificial soil was used in these experiments, it was impossible to measure the soil water potential using the available methods. Additionally, date palm seedlings have spiky, turgid-waxy leaves, and therefore, it was difficult to determine the permanent wilting point which is a symptom under drought conditions that is used as a start point for the proline measurements. For the salinity shock treatment the average electrical conductivity of the soil in the treated and the control pots was 18.13 and 0.68 dS/m, respectively. However, the average electrical conductivity measured for the soil gradually treated with saline solutions was 25.7 dS/m. The seawater salinity ranged from 45 to 50 dS/m, which is an environment only suitable for some halophyte species. Therefore, the salinity levels used in this report were not relatively high, but could still lead to the accumulation of

molecules related to salinity tolerance in date palm tissues. Salinity shock did not have a visible effect on the seedling phenotype because of the short period of the stress treatment. However, the prolonged salinity stress exhibited a pronounced effect on leaf as well as root growth (Figure 1). Due to salinity stress, leaf length was significantly reduced by an average of $63 \pm 2\%$ (SD). In addition, spots of necrosis were observed on the leaves (Figure 1A). The primary root length was also reduced by an average of $34 \pm 7\%$ (SD) due to the long-term exposure to salinity (Figure 1B). This result demonstrated the destructive effect of long-term salinity stress on plant growth rate and health. There are controversial reports regarding the maximum level of salinity that the date palm can withstand. While some research groups reported an insignificant reduction in yield when plants were watered with a NaCl solution up to 24 dS/m (Aljuburi 1992), other reports demonstrated a significant destructive effect on date palm growth and yield when watered with a NaCl solution at concentrations between 4 and 10 dS/m (Sperling et al., 2014). The inconsistency in these results is likely due to the use of different date palm varieties and the application of salt treatments at different growth stages of plant development.

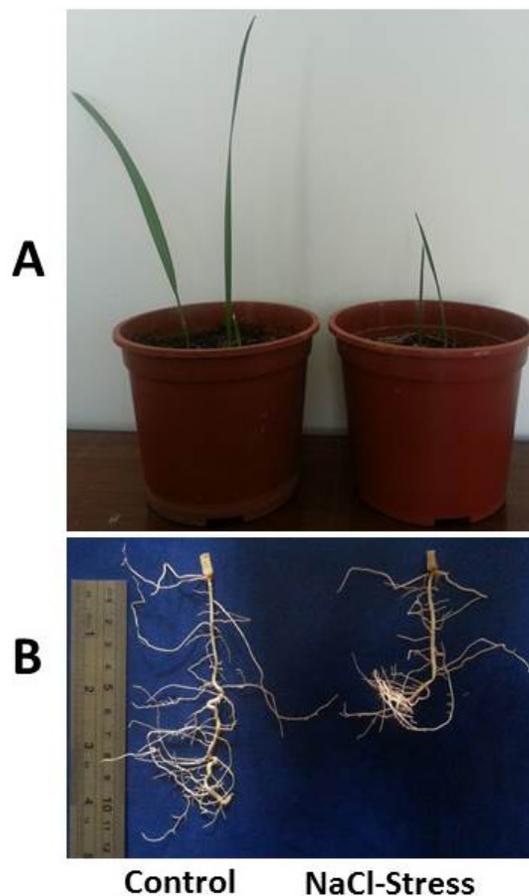


Figure 1. A. Date palm seedlings grown in soil under standard (control) and high salinity stress conditions (NaCl-stress). B. Influence of prolonged salinity treatment on root system compared to the control.

Proline differentially accumulated in seedling roots and leaves under abiotic stress

Biochemical analysis of the roots and leaves showed that there was a variation in proline accumulation in response to abiotic stresses in date palm seedlings. This content also varied between the roots and leaves of seedlings that experienced the same stress conditions (Figure 2). It was noticed that the level of proline in leaves was slightly higher than that in roots of the untreated seedlings, but was significantly higher in the leaves when seedlings were exposed to heat or cold stresses. Among the other abiotic stress treatments, the highest level of proline accumulation was observed in roots when seedlings were either slowly (gradual salinity stress) or swiftly (salinity shock) treated with saline solutions and then the roots were exposed to cold temperatures. Surprisingly, drought and ABA had the least effect on the accumulation of proline in both roots and leaves (Figure 2A). In this context, differential proline accumulation analysis showed that proline levels increased by 9.3-, 6.3-, and 6.0-fold than the control treatment in salinity stress, salinity shock, and cold treatment of roots, respectively. Drought, heat, and ABA treatments also showed a significant increase in root proline content by 2.5- to 3.5-fold than the control (Figure 2B).

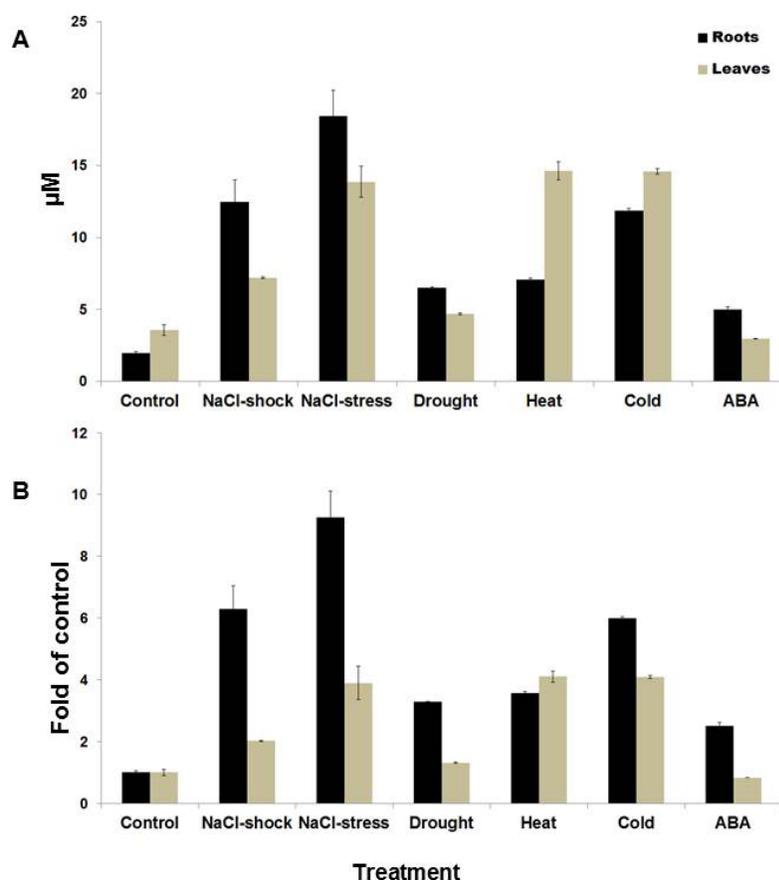


Figure 2. A. Proline accumulation in the roots and leaves of date palm seedlings grown under different abiotic stresses. **B.** Relative proline accumulation (fold of control) in roots and leaves.

The effect of salinity treatments on the proline content in roots was remarkable. However, the content was induced to a lower level in the corresponding leaves (Figure 2). These data may confirm the main conventional role of proline in salt tolerance as a cellular osmoprotectant. Due to the direct contact between salts and the roots in the soil, the effect of salts on root metabolism, including proline synthesis and accumulation, is evident. It is unknown, however, whether Na^+ or Cl^- is stored in the roots, taken up by the roots, and accumulated in leaves, or is extruded by the roots. According to the findings of the present study, roots accumulated higher concentrations of proline in response to salinity stress. This could be due to the direct osmotic pressure or a different signaling pathway induced by excessive *in planta* NaCl concentrations. A previous study showed that the level of proline is directly correlated with the NaCl concentration in the date palm callus as well as in seedlings exhibiting early germination (Djibril et al., 2005).

Prolonged salinity treatment had a greater effect than salinity shock in terms of final proline accumulation in date palm tissues. While the long-term salinity treatment increased the proline content by 9.3- and 4.0-fold than the control in roots and leaves, respectively, the salinity shock increased the proline content by only 6.3- and 2.0-fold in roots and leaves, respectively (Figure 2B). Plants react differently to salinity shock and salinity stress (Shavrukov, 2013). However, date palm seedlings accumulated proline in similar trends when NaCl was either gradually or suddenly applied at a high concentration to date palm seedlings. This suggests there is a common functional response leading to tolerance in both situations.

Unlike salinity, drought stress showed a remarkably lower effect on the concentration of proline accumulated in date palm seedlings. While the amount of the accumulated proline in the drought treated roots was approximately 3-fold higher than that in the control treatment, drought only slightly increased the proline level in the leaves. This result may imply that either the date palm has the ability to tolerate and adapt to drought in roots and leaves with a relatively low amount of accumulated proline, or that proline is not a major player in the mechanism leading to drought tolerance in date palms. Unlike the other treatments, other than salinity stress, the seedlings were gradually exposed to drought conditions in soil. Perhaps the seedlings had enough time to adapt to drought stress and therefore produced a minimum amount of proline in response. In addition to proline, plants tend to accumulate other compatible sugars and amino acids in order to adapt to and withstand drought, however, they were not quantified in this study.

Cold and heat treatments showed a significant increase in proline content in roots and leaves. However, cold conditions caused a higher final level in proline content than heat, especially in roots (Figure 2). Plants tend to accumulate proline in response to high temperatures and it was suggested that proline may buffer the cellular redox potential in plants that are exposed to heat (Wahid and Close, 2007). Alternatively, plants possess numerous mechanisms to survive cold and freezing temperatures (Griffith and Yaish, 2004), including the accumulation of proline, which has been linked to plant resistance to damage from frost in some plant species (Chu et al., 1978). When date palms were exposed to a cold temperature (4°C) for 16 h, a significant increase in proline accumulation in both root and leaf tissues was observed (Figure 2). The proline content in the leaves is almost the same when seedlings were exposed to either hot or cold conditions (Figure 2); this observation may indicate that at this growth stage there is a higher sensitivity to extreme temperatures in the leaves, despite the fact that adult date palm trees can adapt to mildly cold and warm temperatures when grown in open fields or oases.

Exogenous foliar application of ABA led to a slight increase in the amount of proline accumulated in roots, but surprisingly this increase was not observed in leaves (Figure 1). The over

accumulation of proline is probably due to the ABA transduction signal received from the leaves, which induces ABA and proline synthesis, even if proline synthesis in the location of ABA application (leaves) is transient. This may suggest that ABA was catabolized shortly after application on the leaves, and consequently led to instability in proline synthesis. At the molecular level, ABA induces proline synthesis in some plant species, and thereby enhanced drought and salt tolerance (Yang et al., 2000). Recent studies proposed that phytohormones play a role in regulating and controlling proline metabolism during salinity tolerance (Iqbal et al., 2014). In *Arabidopsis*, ABA is required to activate the two *P5CS* genes, which control the level of proline synthesized in the cell (Strizhov et al., 1997). There is crosstalk between the mechanisms that respond to drought and high salinity conditions in plants, where ABA plays a central role (Knight and Knight, 2001). Thus, *de novo* production of ABA enhances both drought and salinity tolerance in some plant species. It is worth noting that in this regard, an overall increase of ABA did not necessarily always lead to improved drought tolerance since this increase is not consistent in all plant tissues and cell types, this includes the guard cells of the stomata that play a crucial role in plant drought tolerance (Yaish et al., 2010).

In conclusion, in an attempt to understand mechanisms of stress adaptation in date palm trees, the role of proline accumulation was assessed as a possible unique response leading to the tolerance of different abiotic stresses. Proline accumulated in date palms when grown under a wide range of abiotic stresses; in particular, when grown under high salinity and suboptimum temperature conditions. Therefore, proline in date palms may function as an extra player leading to resistance or may represent a common tolerance mechanism of each tested abiotic stress. Since abiotic stress tolerance mechanisms can overlap, proline is an unsuitable biochemical selective marker for drought and salinity screening in conventional plant breeding programs.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by a generous grant from the Research Council of Oman (TRC) (#151) to M.W. Yaish.

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