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Effect of Salycilic Acid on Antioxidant Enzymes and Biochemical Contents of Date Palm Plantlets (*Phoenix dactylifera* L.) under Salt Stress Conditions

Naji Salim Jasim and Muntaha Abd-A. Ati

Date Palm Research Center-Basrah University- Basrah, Iraq
E-mail: ahmidnaji916@gmail.com

Abstract: The study was conducted to observe the effect of Salicylic acid (SA) on physiological and biochemical changes in date palm plantlets. Plantlets were irrigated water with different concentrations of salt stress (10, 15 and 20 ds m^{-1}), while the control treatment was irrigated by tap water only. The salt concentration significantly decreased chloroplast pigments, total chlorophyll and carotenoid, total soluble carbohydrates compared with increased levels of proline contents and antioxidant activities (catalase and peroxidase). The treatments of date palm plantlets with salicylic acid (1.5 mm) under salt conditions led to increase of leaf chemical composition and antioxidant enzymes activity, catalase and peroxidase compared with treatments under salt stress only and control .

Keywords: Date palm plantlets, Salicylic acid, Salinity, Antioxidant enzymes, Chlorophyll, Carotenoid

Date palm (*Phoenix dactylifera* L.) is one of the Arecaceae family of economic importance typically grown in arid and semi-arid parts of the globe. Various environmental stresses effect the development and productivity (Shao et al 2008). One of the most influencing factors is salt stress which affects the plant growth and production of secondary metabolites (Nikolova and Ivancheva 2005). One-third of the areas of land in the world's cultivated are seriously affected by hight salt stress (Kaya et al 2002). The salts stress of 8-10 ds m^{-1} decreased chlorophyll contents total soluble, carbohydrates and increased proline and accumulation of Na and Cl in *Cassia absus* L. (Hussain et al 2009). Salts increased protein content and antioxidant enzyme activity CAT and POD on *Momrdica charantia* (Agrawal and Shahee 2007) and on sunflower(Ebrahimian and Bybordi 2012).

Salicylic acid (SA) is an endogenous regulators of growth with a phenolic nature, it contributes in regulating the physiological processes of the plants and has a role as natural inductor of thermogenesis (Horva´th et al 2007). One of important strategies to alleviated plants against different stress is use of plant growth regulators (PGR) which regulating many of physio-biochemical processes like protein synthesis, antioxidant enzymes, and photosynthesis, which are involved in the stress tolerance mechanism directly or indirectly (Akram and Ashraf 2011). Under salt stress 8-16 ds m^{-1} plant height, leaves numbers, fresh and dry weights of leaves decreased, while treatments of SA 30 mg l and GA3 on Date palm *Phoenix dactylifera* L. increased these estimations and increased proline, superoxide dismutase (POD), peroxidase (POX) and catalase (CAT) (Darwesh and Mohamed 2009, Darwesh 2014). Many trials have shown that the use of salicylic acid in drought trees enhances the activity of antioxidant enzymes such as POD, SOD and CAT (Hayat et al 2008).

The current study was undertaken to find out whether the application of salicylic acid (SA) on date palm plantlet can alleviate the harmful effects of salinity on chemical contents and antioxidant enzymes CAT and POX.

MATERIAL AND METHODS

This study was conducted in greenhouse and laboratories of Date Palm Research Center, the University of Basra during 2017 to 2018. Three levels of salinity stress 10, 15 and 20 ds m^{-1} alone and application of while salicylic acid at 1.5 mM were evaluated along with control (0 ds m^{-1} salt).

Plantlets of the date palm were ten months old. The different versions mentioned above were applied to the plants for 21 days depending on the soil water content of the study soil. Each treatment consists of three replicates (2plantlets pot^{-1}). The following physiological characteristics measurements were recorded after the completion of the experiment.

Total chlorophyll and total carotenoid: The total chlorophyll was estimated by method given by Arnon (1949) from fresh leaves were determined by U.V. spectrophotometer (CECL 2021, ENGLAND) at wave max 663nm , The total Carotenoid contents from fresh leaves were determined by U.V. spectrophotometer (CECL 2021,ENGLAND) at wave max 470nm (Lichtenthaler and Wellburn 1983).

Proline content: Proline was estimated by Bates et al (1973) method the standard curve (mg g^{-1}DW).

Determination of soluble carbohydrate content: Fales (1951) and Schlegel (1956) method was used for estimation of soluble carbohydrate content in aqueous solution

Measurement of catalase (CAT) activity: The assessment of the activity of the catalase (EC: 1.11.1.6) was according to the method given by Chance and Maehly (1955).

Peroxidase (POX) activity: MacAdam et al (1992) procedure was used to assess Peroxidase (EC: 1.11.1.7) activity.

RESULTS AND DISCUSSIONS

Photosynthetic pigments: Results of photosynthetic pigments (Fig. 1 and 2) indicated significant reduction in total chlorophyll and carotenoid contents under salt stress at 15 and 20 ds m^{-1} which were 9.24 and 7.38 mg 100 g^{-1} FW for chlorophyll respectively and 0.029 and 0.0215 mg 100g^{-1} FW for carotenoid respectively. This reduction of pigments contents could be related to the toxic effects of salinity on the pigments biosynthesis elevating their degradation and/or causing damage to the chloroplast thylakoid. Darwesh et al (2006) observed that salinity from 10000-14000 ppm decreased chlorophyll contents in leaves on date palm. Furthermore, Abd El-Samed et al (2011) found that chlorophyll contents decreased under 30-90 mM NaCl on *Zea mays* and broad bean *Vicia faba.* This inhibition of photosynthesis pigments was recovered by the addition of 1.5mM salicylic acid. Exogenous application of SA at 1.5 mM combination with salt concentration 10, 15 and 20 ds m^{-1} caused an increased in total chlorophyll and carotenoid which were 11.721, 13.238 and 9.762 mg 100 g^{-1} FW for chlorophyll and 0.037,0.039 and 0.033 mg 100 g^{-1} FW, respectively. Al-Mayahi (2015) observed that the chlorophyll content reduction in date palm leaves under salt stress. A decline in the level of photosynthesis pigments were due to the formation of protolytic enzymes such as chlorophyllase which is responsible of destruction of chlorophyll under high stress condition. Schutz and Fangmier (2001) observed that the reduction of chlorophyll owing to stress is due to the increase in reactive oxygen species (ROS) production in the cell. SA application causes ROS scavenging and that can enhance chlorophyll biosynthesis and prevent degradation of chlorophyll and enhanced content in date palm plantlets.

Proline content: Both salt stress and salicylic acid affected significantly proline content. In addition, the interaction effect of both salt and salicylic acid on proline content of plantlelts was significant. The salt increased greatly proline content in treated plants. Proline content of the plantlets under salt stresses concentrations 10,15 and 20 ds.m^{-1} were 18.41, 29.24 and 27.49 µg g^{-1} respectively in comparison with control, which was 17.67 µg g^{-1} FW (Fig. 3). The application of SA in combination with salt concentrations increased proline content which were 37.49, 40.66 and 34.24 µg g^{-1}. Dash and Panda (2001) reported that when NaCl concentration and duration of stress increased proline content in *Phaseolus mungo*. Other study founed that NaCl at 50-150 mM increased proline contents in *Salventia natans* L. (Jampeetong and Brix 2009). The application of SA caused enhancement in the synthesis of proline which might be the adaptive mechanism of date palm plantlets under salt stress condition. These results are in agreement with finding of previous studies (El-Beltagi et al 2017, Agamy et al 2013). The increase in proline content in plants allowed water stressed plants to maintain low water potential, the accumulation of proline involved in osmo-regulation seemed to enable additional water to be absorbed from the environment (Gebaly et al 2013).

Soluble carbohydrate contents: The applying 1.5mM SA on the control plants elevated the content of leaf soluble carbohydrate which was 23.99 mg g^{-1} DW compared to control plant (without SA) which was 15.62 mg g^{-1} DW. With salt stress, the soluble carbohydrate content of plantlet leaves was substantially high with increased salt concentrations (Fig. 4). Exposure plantlets were irrigated with growth regulator (SA) at 1.5 mM in combination with salt stress significant more in leaves content of total carbohydrates in plants which were 26.368, 23.226 and 22.307 mg g^{-1} DW for 10, 15 and 20 ds m^{-1} respectively compared with treated plants at the same concentrations which were 22.153, 22.920 and 19.088 mg g^{-1} respectively. Salt stresses, however, resulted in a significant increase in the content of the soluble carbohydrate of date palm leaves. With increasing salt concentrations the rate of rise in soluble carbohydrate content has been reduced, demonstrating a function for soluble carbohydrate in osmotic adjustment. Pe'rez-Lo'pez et al (2010) indicated that osmotic adjustment could involve the accumulation of soluble carbohydrates in crops under stress circumstances. The accumulation of soluble carbohydrates was commonly recorded as a reaction to salinity or drought (Cheeseman 1988). The carbohydrates constitute one of the dry matter's primary organic constituents were influenced by salt stress. The complete carbohydrate content was decreased by growing salinity concentrations. Reducing the content of carbohydrates may be due to reduced photosynthesis and increased photorespiration under salt stress (Namich et al 2007). The application of SA to date palm plantlets under salt conditions increased the contents of total soluble carbohydrates as compared with untreated stressed plants. Photosynthetic pigments were increased in response to salicylic acid treatments thus enhance the polysaccharides biosynthesis

and total carbohydrates significantly which are utilized in the sunflower plants growth (Dawood et al 2012). The application of SA might activate the metabolic consumption of soluble carbohydrates to form new cell constituents as a mechanism to alleviated the growth of date palm plantlets. The high concentration of carbohydrates with its role in reducing water content helps prevent oxidative damages and the preservation of protein structure during water shortage. Carbohydrates also play a molecular role in sugar-responsible genes, which give different physiological responses such as defensive responses and cellular expansion (Simaei et al 2011). Moreover, accumulation of carbohydrate play a key role in alleviating the salinity stress, either via osmotic adjustment, as Ackerson (1985).

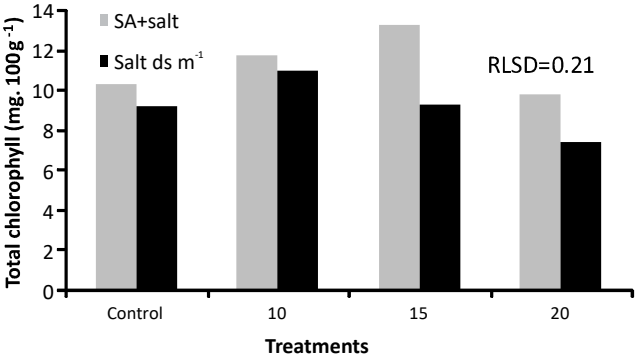

Fig. 1. SA effects on the total chlorophyll content (mg 100 g^{-1}) under salt condition

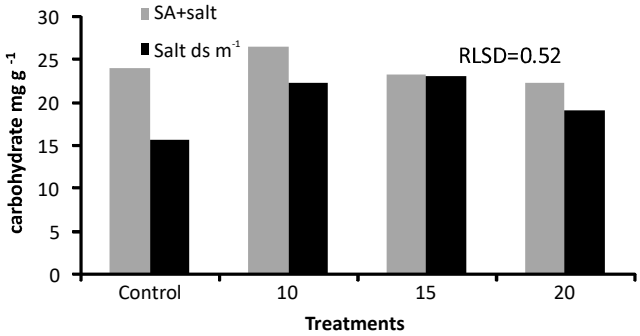

Fig. 4. SA effect on the carbohydrat content (mg g^{-1}) under salt condition

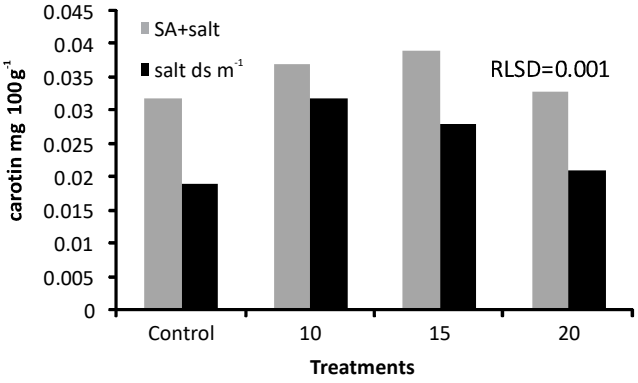

Fig. 2. SA effects on the Carotenoid content (mg 100g^{-1}) under salt condition

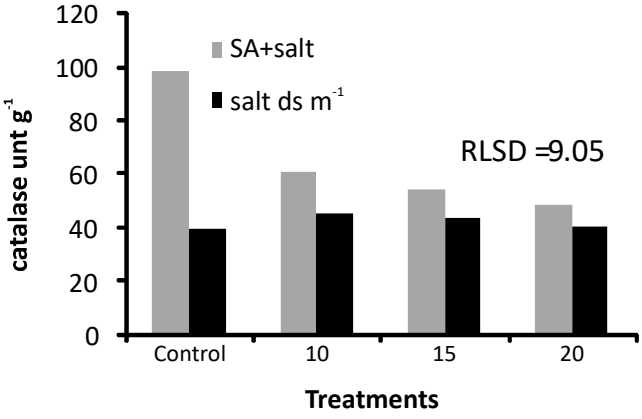

Fig. 5. SA effect on the catalase (unit g^{-1}) activity under salt condition

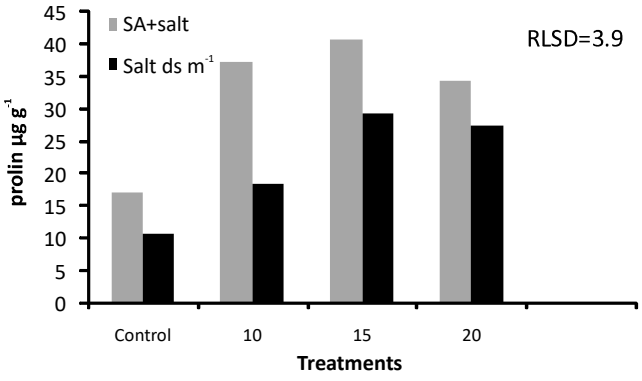

Fig. 3. SA effect on the proline content (µg g^{-1}) under salt condition

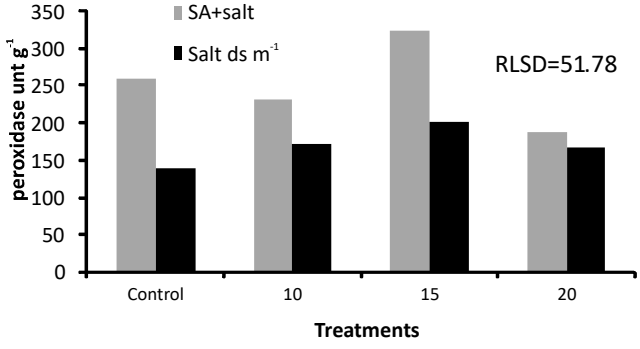

Fig. 6. SA effect on the peroxidase (unit g^{-1}) activity under salt condition

Catalase (CAT) and Peroxidase (POD): The CAT and POD activity increased during salt stress and this increase was positively related to 10 and 15 ds.m^{-1} salt concentrations. Additionally, the results showed that high levels of antioxidant enzymes CAT and POD activity occurred in plants at 10 and 15 ds m^{-1} salt concentrations with SA combination at 1.5mM. Control crops revealed the smallest concentrations of CAT and POD operations. The enzymes of antioxidants include catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) (Zhu 2001, Ashraf 2002). The study of Mittler (2002), showed that salinity induces and increases the activity of these antioxidant enzymes. CAT involving degradation of hydrogen peroxide and prevention of oxidative damage. The resistance to environmental stress may therefore depend at least partially on the production by enhancing the antioxidant defense system (Azevedo et al 2006). CAT and POD markedly increased under salt stress 40-200 mM (Abdulwahid 2012 on date palm). Increasing CAT and POD activities with SA application at 1.5mM was the highest under salt pressure, exogenous application of SA can control the activity of intracellular antioxidant enzymes such as POX, SOD and boost plant sensitivity to environmental stress (Sakhabutdinova et al 2004). Study of Saruhan et al (2012) discovered that the application of SA increased the activity of antioxidant enzymes such as CAT, POD and APX in plants.

CONCLUSIONS

Salt stress was significantly reduced chemical compositions chloroplast pigments (total chlorophyll and carotenoid), total soluble carbohydrate, antioxidant activity (CAT and POD) and increased proline content. The adverse effect of salinity can be alleviated by applying SA, thereby raising all above physiological characteristics.

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