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ROLE OF ANTIOXIDANTS IN SALT STRESS TOLERANT OF DATE PALM OFFSHOOTS (*PHOENIX DACTYLIFERA L.*) FEMALE AND MALE CULTIVARS

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Salinity is one of the most abiotic stress destructive factors which limit growth and production of date palm offshoots considerably. In order to investigate the effects of antioxidant compounds on growth parameters as indicters to salt tolerance at the date palm offshoots cultivars of Kabkab (female) and Ghannami ahmer (male). Experiment was conducted with seven treatments and four replications for each treatment to each cultivar. Offshoots received (Control treatments once without salt water and another with salt water, spraying citric acid at 800 ppm, spraying ascorbic acid at 600 ppm, soil injection by Acetyl salicylic acid at 2000 ppm (aspirin), soil injection by acetyl salicylic acid at 2000 ppm + spraying citric acid at 800 ppm and soil injection by Acetyl salicylic acid at 2000 ppm + spraying ascorbic acid at 600 ppm). The results showed that antioxidant application increased significantly all growth indicators in both cultivars, and the maximum values were recorded in the combined application of acetyl salicylic acid as injection in soil besides spraying of ascorbic acid of kabkab cultivar. Also, antioxidant applications significantly improved RWC as well as carbohydrates content, soluble protein and proline in leaves compared with control treatments in both cultivars. All antioxidant treatments succeeded in decreasing leaf Na+ and Cl- concentrations and increasing leaf K+ concentration and K+/Na+ ratio. The results clear that female cultivar more tolerant to salt stress than male cultivar in offshoot stage.

Key words: Antioxidant, Acetyl salicylic acid, Salinity, Proline, Protein, RWC and K/NA.

INTRODUCTION

Salinity affects many irrigated areas mainly due to the use of salt water. Worldwide, more than 45 million hectares of irrigated land have been damaged by salt, and 1.5 million hectares are taken out of production each year as a result of high salinity levels in the soil (Munns andTester, 2008). High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity (Zhu, 2007). In turn these effects reduce date palm (*Phoenix dactylifera* L.) growth and development. Although the date palm exhibits a high degree of tolerance to salinity FAO (1982), but growth and productivity can be greatly reduced, especially offshoots are more sensitive to salinity than mature plants. Salt stress also generates reactive oxygen species (ROS) such as superoxide ($O_2^{-\circ}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{-\circ}$) causing oxidative stress on plants (Mittler, 2002; Masood *et al.,* 2006). Many compounds as citric acid, Ascorbic acid and salicylic acid act as antioxidant and scavengers of ROS.

Recently, antioxidants are suggested for improving salt tolerance in plants, these compounds as non-enzymatic materials have beneficial effects on scavengers of ROS. Citric acid is considered now as one of non enzymatic antioxidants which act to eliminate free radicals produced in plants under stress in plants (Yan-Lin and Soon, 2001). It is an important substrate in Krebs cycle. In addition, the cycle provides precursors including certain amino acids as well as the reducing agent NADH that is used in numerous biochemical reactions. So, it plays an important role in stimulating biosynthesis processes (Abd El-Al, 2009).

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Ascorbic acid is an important primary metabolite in plants that functions as an antioxidant, an enzyme cofactor and a cell signalling modulator in a wide array of crucial physiological processes, including biosynthesis of the cell wall, secondary metabolites and phytohormones, stress tolerance, photoprotection, cell division and growth (Smirnoff and Wheeler, 2000; Barth *et al.,* 2006). Salicylic acid has been found, among many other functions, to control ion uptake by roots, stomata conductivity and to increase the antioxidant capacity of plants (Raskin, 1992). However, AL- Taey, (2009) found that the Acetyl salicylic acid with (1000, 2000 mg l^{-1}) on orange plants (*Citrus sinensis* L.) increased the plant tolerance of salt water stress significantly in the average of leaf area , the length of vegetative shoots, the fresh and dry weight to the shoots and the amount of total chlorophyll in the leaves. Salicylic acid increased the protein contents inside the plant cells that make the plant have the ability to tolerant the salt stress (Kumer *et al.,* 1999). According to our knowledge, there are no reports on the effects of Acetyl salicylic acid and Ascorbic acid enhancing date palm tolerance to salt stress. Therefore, the objectives of this study were to determine whether the effect of Acetyl salicylic acid, Ascorbic acid and citric acid on the some physiological exchanges in date palm offshoot cultivars Kabkab (female cultivar) and Ghannami ahmer (male cultivar) in improving tolerance of salt stress, and test effect of soil injection by Acetyl salicylic acid in the absorption active root zone as new method.

MATERIALS AND METHODS

A field experiment was conducted at the General Authority of Palm station, in Hartha region-Basrah, Iraq (30°36.54'N& 30° 38.60'N to47°44.42'E to 47° 45.18'E), 24 Km from centre of Basrah, during 2014 and 2015 growing seasons on 56 uniform in vigour, 3-4 years-old Kabkab (female cultivar) and Ghannami ahmer (male cultivar) date palm cultivars offshoots effected by salt stress (on Basins Agriculture of 28 offshoot per each cultivar). Texture of Basins is silty clay loam. The selected offshoots are planted at 5x5m. Drip irrigation system was followed. Average of EC of soil was (15.7 dS m^{-1}), as well as average of EC of water (4.7 dS m^{-1}). Spraying foliar and injection at soil by plastic pipe length of 50 cm close to the absorption active root zone were carried out at the first week of March. Acetyl Salicylic acid was initially dissolved in few drops of ethanol and the final volume was reached, using distilled water. Each treatment was replicated four times, with one offshoot for each replicate.

The present experiment included the following treatments to each cultivar:

- T1. Control (irrigated without salinity) (EC of water 0 dSm^{-1}).
- T2. Control (irrigated with salt water).
- T3. Spraying citric acid at 800 ppm.
- T4. Spraying ascorbic acid at 600 ppm.
- T5. Soil injection by Acetyl salicylic acid at 2000 ppm (Aspirin).
- T6. Soil injection by Acetyl salicylic acid at 2000 ppm + Spraying citric acid at 800 ppm.
- T7. Soil injection by Acetyl salicylic acid at 2000 ppm + Spraying ascorbic acid at 600 ppm.
  All treatments from T3 to T7 irrigated with salt water.

After one year from treatments, the following data were recorded

Growth indicators

Plant height (cm): Measured by a measuring tape to leaves was third leaf, from base offshoot to end leaf.

Number of New leaves: by New leaves = Number of leaves when sampling - Number of leaves before treatment.

Roots number: vertical section at the soil of one of the offshoot views were taken from four directions each offshoot (treatment), then were directed stream of water until the roots was emergence, then calculate the number of roots each offshoot and it multiplied by (4), then total replicates divide by the number of offshoots each treatment.

Roots length (cm): root lengths were measured by a ruler from the roots point of contact offshoot base to the top end of root, and then divide the total by the number of roots per offshoot.

Relative water content of leaves (RWC)

Leaf samples were weighed (fresh weight) immediately after harvesting, soaked in distilled water at 25°C for 24 hr to determine the turgid weight , the samples were dried in an oven at 80° C for 48 h and their dry weights were determined. RWC was calculated by the following equation:

RWC = (fresh Weight- dry weight) / (turgid weight- dry weight) x 100.

Analysis of soluble carbohydrates: Samples of fresh leaves were weighed (0.2 g) and homogenized using 70 % ethanol according to Yemm and Willis (1954).

Determination of protein content

The proteins were determined by Bradford's method Bradford, (1976).

Determination of proline content: according to (Irigoyen *et al.,* 1992).

Determination of Potassium and sodium concentration: according to Creser and Parsons (1979). This solution became transparent and used for determinations of K and Na concentrations emission flame photometer.

Determination of Chloride concentration: according to (Kalra, 1998).

Statistical analysis

A completely randomized block design of two date palm cultivars and 14 treatments of antioxidants as well as control treatments replicated four times were used to conduct the experiment. Experimental data on all variables were subjected to analysis of variance (ANOVA) procedures using a statistical package, SPSS version 16.0 (SPSS, Chicago, IL). Significant differences among treatments were considered at the $P \leq 0.05$ levels.

RESULTS

Growth indicators

All growth indicators of height plant, number of leaves, length and number of roots were reduced in both offshoot cultivars due to salt stress significantly as a clear in control treatments to both cultivars compared with control treatment without salt water (EC of water 0 dSm^{-1}) (Table 1). The maximum values were recorded in Kabkab rather than Ghannami ahmer cultivar as compared to control plants. Further, application of antioxidants (spraying citric acid, Ascorbic acid, soil injection by acetyl salicylic acid, soil injection by acetyl salicylic acid + spraying citric acid and soil injection by acetyl salicylic acid + spraying ascorbic acid) increased all growth parameters ascending in both cultivars, and the maximum value due to watering with salt water was recorded in treatment of soil injection by acetyl salicylic acid + spraying ascorbic acid of kabkab cultivar. Kabkab cultivar showed significantly increase in all growth indicators compared with Ghannami ahmer cultivar. These results clear that female cultivar response to antioxidants treatments more than male cultivar in offshoot stage.

Table 1. Effect of antioxidants application on plant height (cm), leaves number (leaf $plant^{-1}$) and roots length (cm), roots number (root $plant^{-1}$)

Cultivars	Treatments	Height plant (cm)	Leaves number (leaf $plant^{-1}$)	Root length (cm)	Roots number (root $plant^{-1}$)
Kabkab	T1	142.6	9.3	22.6	52.0
	T2	109.0	3.0	14.3	37.6
	T3	114.0	4.0	15.3	42.0
	T4	120.6	5.0	17.3	43.3
	T5	128.0	6.0	18.0	44.0
	T6	134.6	7.0	20.6	47.0
	T7	138.0	8.3	21.3	50.3
Ghannami ahmer	T1	104.3	5.6	20.6	45.6
	T2	69.0	2.3	13.6	31.3
	T3	78.6	2.6	15.0	34.0
	T4	83.3	3.3	16.6	35.3
	T5	87.3	4.0	18.0	37.0
	T6	92.0	5.0	18.6	39.3
	T7	99.3	5.0	20.0	43.6
	R.L.S.D.	4.2	1.9	1.7	1.6

Table 2. Effect of antioxidants application on RWC (%), carbohydrates (mg g^{-1}), soluble protein (mg g^{-1}) and proline (mg g^{-1})

Cultivars	Treatments	RWC (%)	Total soluble carbohydrates (mg g^{-1})	Protein (mg g^{-1})	Proline (mg g^{-1})
Kabkab	T1	83.5	49.5	7.2	8.3
	T2	64.5	32.4	4.6	10.5
	T3	67.0	34.8	5.4	10.8
	T4	70.7	37.4	5.8	11.4
	T5	73.6	41.2	6.4	11.7
	T6	75.6	44.0	6.7	11.9
	T7	80.2	47.6	7.1	12.4
Ghannami ahmer	T1	79.1	49.0	5.0	7.3
	T2	60.3	29.6	3.7	8.6
	T3	62.3	33.5	4.1	8.6
	T4	64.7	36.3	4.3	8.8
	T5	65.0	40.0	4.6	9.3
	T6	65.9	44.3	4.7	9.6
	T7	71.7	47.9	4.9	9.9
	R.L.S.D.	2.1	2.4	1.4	0.91

Biochemical characteristics

It is clear from the data in Table 2 that antioxidant applications significantly improved RWC, carbohydrates content, soluble protein and proline in leaves compared with control treatments in both cultivars. The combined application of acetyl salicylic acid as injection in soil + spraying of ascorbic acid or citric acid superior than using either acetyl salicylic acid as injection in soil or spraying of ascorbic acid or citric acid each one alone. Also, kabkab cultivar (female cultivar) significantly responded in this respect rather than Ghannami ahmer cultivar (male cultivar).

Table 3. Effect of antioxidants application on Na, Cl, K concentration (mg g^{-1}) and K/Na ratio

Cultivars	Treatments	Na (mg g^{-1})	K (mg g^{-1})	Cl (mg g^{-1})	K/Na
Kabkab	T1	4.5	12.6	6.5	2.7
	T2	9.6	12.3	11.6	1.2
	T3	9.2	12.4	11.2	1.3
	T4	9.0	12.9	11.0	1.4
	T5	7.7	13.0	9.7	1.6
	T6	7.2	13.8	9.2	1.9
	T7	6.8	15.9	8.8	2.3
Ghannami ahmer	T1	6.1	14.7	8.1	2.4
	T2	11.9	12.7	13.9	1.0
	T3	11.2	12.8	13.2	1.1
	T4	10.7	12.3	12.7	1.1
	T5	10.4	12.5	12.4	1.2
	T6	10.0	13.1	12.0	1.3
	T7	9.1	13.6	11.1	1.5
	R.L.S.D.	1.4	1.2	0.4	0.2

Leaf Na, K, Cl concentration and K /Na ratio

Data in Table 3 clear that all antioxidant treatments significantly decreased leaf Na^+ and Cl^- concentration and increasing leaf K^+ concentration in leaves of offshoots, thus increase K^+/Na^+ ratio. Application of acetyl salicylic acid as injection in soil combined with ascorbic acid or citric acid was in significant increase K^+/Na^+ ratio as compared with control treatment in both cultivars under salt stress. Also, female cultivar of kabkab significantly response in this respect rather than male cultivar of Ghannami ahmer.

Application of acetyl salicylic acid as injection in soil combined with ascorbic acid or citric acid was in significant increase K^+/Na^+ ratio as compared with control treatment in both cultivars under salt stress. Also, female cultivar of kabkab significantly response in this respect rather than male cultivar of Ghannami ahmer.

DISCUSSION

Although salinity stress reduced growth parameters of the two date palm offshoot cultivars, there were major differences in their reduction. Kabkab (female cultivar) seems to be high salt tolerant compared with Ghannami ahmer (male cultivar).

This was judged with the ability of kabkab cultivar to enhance its tissue water contents (RWC%) and K^+/Na^+ ratio more than Ghannami ahmer cultivar (Table 2, 3). Accordingly, plant salt tolerance is determined by cultivars and biochemical pathways that facilitate retention of water and synthesis of osmotic active metabolites (Sadak *et al.,* 2013).

This reverse effect may be due to the retarding effect on photosynthesis (Jampeetong, 2009), protein building (Parida, 2005), mineral disturbances (Grattan, 1998) hormonal balance (Shakirova *et al.,* 2003) and water adjustment (Shannon, 1997). The electrolytes in saline solution first induce an imbalance in water potential between the symplast and apoplast and this causes decreased turgor, which could led to growth reduction (Bohnert and Jensen, 1996). Reduction in the growth rate of the leaf can represent an adaptation to salt stress, since increased levels of salts in the soil impedes the uptake of water by the plant and the reduction in leaf area limits transpiration (Queiroz *et al.,* 2012).

Lower leaf RWC may be due to lower water availability under saline conditions or plant roots could lose their ability to reabsorb water lost through transpiration (Gadallah, 2000), whereas the antioxidants with all treatments increased all growth indicators. The ameliorative effect of antioxidants on growth improvement comes from the fact that they act as an antioxidant under salinity. Our explanation is consistent with previous reports that different antioxidants such that ascorbic acid, citric acid and salicylic acid mitigated salinity effects and thus enhanced salt tolerance on various plants. Many authors proposed that antioxidants ameliorate the damaging effect of salinity through interaction of antioxidant response and protection of membranes (Ekmekçi and Karaman, 2012).

Regarding to carbohydrate constituents of the two cultivars, data clearly showed that antioxidants treatments significantly increased total carbohydrates in leaves of the two cultivars concomitantly with the increased growth rate which led to the conclusion that the photosynthetic efficiency was increased in response to antioxidant treatments and thus led to enhance biosynthesis of carbohydrates which are utilized in growth of offshoots. Also, the treatment of acetyl salicylic acid by injection in soil increased growth rate due to stimulatory roles of them as antioxidant on two offshoots cultivars by increased length and number of roots and protein and proline content in leaves. These results could be explained by the stimulatory effect of the used antioxidant on metabolic activities of the two cultivars. As well as the antioxidants treatments decreased Na^+ and Cl^- concentration and increasing K^+ concentration in leaves of both cultivars which might be attributed to enhance the selective absorption in roots. Szepesi *et al.,* (2009) suggested that the SA–induced fine-tuning of the net ion uptake by the roots, xylem transport for long-distant distribution, and vacuolar compartmentalization of Na^+ along the plant axis are required to facilitate osmotic adjustment and plant growth in a saline environment. However, the contribution and interaction among antioxidants compounds eventually improved roots and leaves growth at the offshoots, which may be to alleviated salinity damages in the roots by Acetyl salicylic acid. Also, Salicylic acid provided protection against salinity in plants, probably due to the increased activation of aldose reductase and APx enzymes and to the accumulation of osmolytes, such as sugars, sugar alcohol or proline (Szepesi *et al.,* 2005), and offshoots may be treated with Acetyle salicylic acid exhibited less Na^+ and more K^+ accumulation and soluble sugars in roots and leaves. The application of exogenous salicylic acid appeared to induce a pre-adaptive response to salt stress, leading to the promotion of protective reactions to the photosynthetic pigments and the maintenance of membrane integrity in plants, which was reflected in an improvement in plant growth (El Tayeb, 2005). Furthermore, Ascorbic acid acts as scavengers ROS. Also, ascorbate enhanced α-tocopherol synthesis, which protects the plant from programmed cell death induced by ROS (Conklin and Barth, 2004).

Conclusion

It can be concluded that antioxidants application helped improving water status of offshoots exposed to salt stress conditions and subsequently improved salinity tolerance. Based on the the respond and the ability of the experimental date palm offshoots cultivars to cope with salinity stress, cultivar kabkab could be regarded as more salt-tolerant than cultivar Ghannami ahmer. Also, antioxidant treatments were not only alleviated the inhibitory effect of salt stress on growth criteria in the two used cultivars, but also in some cases induced a stimulatory effect greater than that estimated in control offshoots (with salinity). As well as the combined application of acetyl salicylic acid as injection in soil besides spraying of ascorbic acid or citric acid as new method which was accompanied by marked increases growth parameters.

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