Middle East Journal of Agriculture Research, 3(3): 549-559, 2014
ISSN 2077-4605

Exogenous supply of salicylic acid and IAA on morphology and biochemical characteristics of date palm plantlets exposed to salt stress

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ABSTRACT

Salinity stress is identified the major factor of plant growth deficiency in the plants by affect metabolism process and imbalance hormones which reflected to cell turgor pressure, cell expansion and cell division. This bad effect can modify it by many substances as plant growth regulators. This trial was carried out during two successive seasons 2013-2014 on date palm *Phoenix dactylifera* L. cv. Bartomouda treated with salt stress at 14000 ppm which can be ameliorated by foliar spraying of some plant growth regulators as SA salicylic acid at 400 ppm and IAA at 30 ppm one time/week. Randomized Complete Block Design was used. The obtained results revealed that, under saline stress, the treatments with Salicylic acid and IAA enhanced most of the growth estimations *i.e.* plant height, leaves numbers, fresh and dry weights of leaves. Whereas these parameters were significant reduced under salts 14000 ppm NaCl, chlorophyll a and b was decreased, At saline conditions, increasing of total sugars, proteins, catalase activities (CAT) and peroxidase activities (POD) which act as defense effects in the plants exposed to salinity stress, Na^+, Ca^{2+}, Cl^- and K^+ leaf concentrations were rising under 14000 ppm NaCl. This paper is highlights the significant findings of SA and IAA as foliar application can be increasing growth parameters and tolerant plants under saline conditions.

Key words: *Date palm, salicylic acid, IAA, salts, growth, CAT,POD and proteins*

Introduction

Date palm (*Phoenix dactylifera* L.) which produced *via* tissue culture technique after acclimatization in the green house may be unable to confrontation the abiotic stress conditions when its cultured in the sustainable land i.e. salt stress, drought stress which caused bad effects on the plant growth characteristics as decreasing heights and length, leaves numbers, fresh and dry weights of aerial parts and roots. Na+- specific damage is associated with the accumulation of Na+ in leaf tissues and results in necrosis of older leaves. Growth and yield reductions occur as a result of the shortening of the lifetime of individual leaves, thus reducing net productivity and crop yield (Munns 2002), abiotic stresses remain the greatest constraint to crop production worldwide. Salinity is one major environmental determinant of plant growth and productivity. Salinization is rapidly increasing on a global scale and currently effect more than 10% of arable land, which results in a decline of the average yields of major crops greater than 50 % (Wang *et al.,* 2009), It has been projected that more than 50% of yield reduction is the direct result of abiotic stresses, the major abiotic stresses like drought, high salinity negatively influence the plant growth, survival, biomass production (Sajid and Aftab 2012), leaves numbers/plant and shoot dry weights of Mango cvs (Alphonso, Taimor, Ewaise, Hendy bisinnara and Zebda) decreased with 15, 30 and 45 mM (Kandil *et al.* 2001 on *Beta vulgaris* and Morsy *et al.* 2003 on mango), salinity levels at 0.5, 1.0,2.0, 3.0, 4.5 and 6.0 ds/m reduced heights of seedlings and total biomass (Cavalcante *et al*. 2002 on *Passiflora edulis* F. flavicarpa passion fruit), plant height, number of fronds and leaf length of date palm cvs. Khalas, Khunaizy and Abunarenjeh were decreased significant with increasing Ec from 3 to 18 ds/m (Abdullah and Al-Rasbi 2010), fewer shoots fresh weights of Borage (*Echium amoenum* Fisch & Mey) with increasing salts from 3 – 12 ds/m (Ramezani *et al.* 2011), Leaf number, Collar diameter, Plant height of Jatropha (*Jatropha curcas* L.) under 25 and 50 % water stress (Hedayati *et al*. 2013).

Many of the physiological processes associated with growth are affected under severe stress, reduction of photosynthesis pigments and accumulation of compatible solutes as proline content, Na, Ca and Cl, and increasing activity of antioxidant enzymes CAT and POD, less chlorophyll contents and highly of accumulation of Na and Ca under salt stress 100-200 mM (Singh *et al.* on *Populus deltoids* and El- Bagoury *et al.* on *Casuarina equisetifolia* L. 1999), salts from 40-80 mM NaCl decreased chlorophyll contents and increased proline and Na and Cl of Citrus reshni *Poncirus trifoliate* x *Citrus sinensis* and Valencia Orange Citrus sinensis (Machacha *et al*. 2000 on rough lemon, Prior *et al.* 2007 and Anjum 2008), total soluble carbohydrates, proline, accumulation of Na and Cl of *Cassia absus* L. under salt stress 8-10 ds/m (Hussain *et al*. 2009), salts increased protein content and antioxidant enzyme activity CAT and POD (Agrawal and Shaheen 2007 on *Momrdica charantia* and Ebrahimian and Bybordi 2012 on Sunflower).

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Though many chemical substances as plant growth regulators (Salicylic acid SA, Indole acetic acid IAA and GA_3 Etc.) were subjected as foliar application or irrigation to overcome the bad effects of salt stress, Growth regulators are involved in altering growth processes in plants, it is possible that they might even reduce the detrimental effects of salinity by stimulating growth (Kaya *et al*. 2010), Indol acetic acid (IAA) is also known to play a significant role in plant tolerance to salt stress (Ribaut and Pilet 1991) In wheat seed germination decreased with increasing salinity level, while the adverse effect of salinity was alleviated by treatment of seeds with IAA or NAA (Gulnaz *et al.,* 1999), Salicylic acid also had been reported which is an essential component of the plant resistance to pathogens and participates in the plant resistance to adverse environmental conditions (Bosch *et al.,* 2007) Salicylic acid is an important secondary signal in plants that plays a major role in the activation of defense genes in response to pathogen attack. Most signalling is buffered by the presence of multiple pathways (Glazebrook *et al*., 2003), optimal concentrations of SA 50 µM caused increasing leaf area, fresh and dry weights of leaves and total soluble sugars contents in tea cuttings *Camellia sinensis* L. (Kaveh *et al*. 2004), salicylic acid (SA) is an endogenous growth regulators of phenolic nature, which participates in the regulation of physiological processes in plants and plays a role as natural inductor of thermogenesis (Horva´th *et al.,* 2007), exogenous application of different plant growth regulators is a well-recognized strategy to alleviate stress-induced adverse effects on different crop plants by regulating a variety of physiobiochemical processes such as photosynthesis, chlorophyll biosynthesis, antioxidant metabolism, and protein synthesis, which are directly or indirectly involved in the mechanism of stress tolerance (Akram and Ashraf 2013), foliar application of SA or tryptophan at 100 µM influenced vegetative growth of geranium *Pelargonium graveolens* (Talaat 2005), foliar application of SA from 5-15 mg/l increased plant height, leaves numbers, fresh and dry weights of leaves, also increased chlorophyll a,b and carotenoides, total proteins and carbohydrates (Hashmi *et al.* 2012 on *Foeniculum vulgaris* Mill), shoot length, fresh and dry weights and pigments were reduced under salt stress at 2000 and 4000 ppm, on the other hand spraying of SA from 0.01 to 0.1 mM increased all vegetative growth, chlorophyll a,b and carotenoides and Na (Delvari *et al.* 2010 on *Ocimum basilicum*), under salt stress 8-16 ds/m plant height, leaves numbers, fresh and dry weights of leaves were decreased, while treatments of IAA and GA_3 increased these estimations and increased proline and POD and CAT (Darwesh and Mohamed 2009 on *Phoenix dactylifera*, and Guru *et al*. 2012 on *Phaseolus mungo*). NaCl at 50 mM depressed plant length, fresh and dry weights and number of runners, whereas spraying of SA 30 mg/l and GA_3 at 50 ppm increased all of these characteristics and Na,Ca and Cl (Hussain *et al* 2011 on *Viola odorata* and Quareshi *et al*. 2013 on strawberry). Keeping this in mind, the present project was undertaken to find out whether the foliar application of SA salicylic acid and indole-3-acetic acid (1AA) on date palm plantlet cv. Bartomouda can alleviate the harmful effects of salinity on plant growth, leaves chemical contents and antioxidant enzymes CAT and POD.

Materials and Methods

This experiment was done in the green house of Central laboratory of Date palm for Research and Development (ARC), Giza in 2013 - 2014 on the date palm plantlets (*Phoenix dactylifera* L. cv Bartomouda) which sprayed with salicylic acid 400 ppm and 30 ppm of IAA under salinity level 14000 ppm NaCl + $CaCl_2$ 2 : 1 by weight. All plantlets were treated with salinity one time for week and sprayed with salicylic acid and IAA one day before treated with salinity, plantlets were spraying solution was maintained just to cover completely the plant's foliage till drip. All plantlets were ferrtigated with NPK at 2 g/l one time for a week, the control groups were just sprayed with water. Date palm plantlets were cultured in the plastic bags with peat moss + sand 2:1 at Ec 3.06 ds/m. Salicylic acid was dissolved in deionize water and the pH was adjusted at 5.5 ± 0.2 with KOH (1N).

Statistical Analysis:

All analyses were done on a completely randomized design (Snedecor and Cochran 1990), all data obtained was subjected to one- way analyses of variance (ANOVA) and the mean differences were compared by least significant differences (L.S.D test). The experiment was repeated twice with three replicates for each, three plantlets for one replicate and comparisons with p #0.05 were considered significantly different. Plant height (cm), number of leaves were measured, the fresh and dry leaves were weighed for two seasons, all results were taken as the mean values of two seasons tested.

Chlorophyll a and b as described by Lichtentaler and Wellburn (1985), Chl was extracted in the 80% acetone, and the absorbance of supernatant was measured at 663 and 645, Chlorophyll a, chlorophyll b were calculated as follows:

Ch. A = 12.7 (a 663) – 2.69 (a 645) x V/1000x w

Ch. B = 21.426 (a 645) - 4.65 (a 663) x V/ 1000 x w

Indole content: as described by Salim *et al*. (1978)

Total soluble sugar as described by Jayaraman (1981)

Total Soluble Protein: Total soluble protein levels were measured by using BIO-RAD protein assay dye reagent by the method of Bradford (1976).

Na, Ca, Cl and K as described by Chapman and Pratt (1961)

Catalase Activity: as Velikova *et al*., (2000), Catalase (EC 1.11.1.6) activity was assayed by measuring the initial rate of H2O2 disappearance at 240 nm using the extinction coefficient of 40 mM-1 cm-1 for H2O2 The 3 ml reaction solution consisted of 50 mM potassium phosphate buffer (pH= 7.0), 15 mM H2O2 and 100 µl of enzyme extract. Addition of H2O2 started the reaction and the decrease in absorbance was recorded after 30s.

Peroxidase: as Plewa *et al.* (1991)The peroxidase (EC1.11.1.7) activity was determined using the method of following the formation of tetraguaiacol by measuring the absorbance at 470 nm and using an extinction coefficient of 25.5 mM-1 cm-1. Reaction mixture (3 ml) contained 25 µl of enzyme extract, 2.77 ml of 50 mM phosphate buffer (pH 7.0), 0.1 ml of 1% H2O2 and 0.1 ml of 4% guaiacol. The increase in absorbance at 470 nm due to the guaiacol oxidation was recorded for 3 min.

Results and Discussion

Growth parameters:

Growth performance of date palm (*Phoenix dactylifera* L.) plantlets under 14000 ppm NaCl + $CaCl_2$ as irrigation and sprayed with some of growth regulator treatments as salicylic acid (SA) at 400 ppm and IAA at 30 ppm was estimated by measuring growth parameters as plant height cm, leaves numbers and fresh and dry weights of leaves (g). A negative relationship was detected between salt concentration in irrigation water and plant height cm Table (1) from 87.4 to 91.0 cm with control treatment in 1st and 2nd seasons respectively, meanwhile, 14000 ppm NaCl + $CaCl_2$ produced 72.7 to 74.3cm respectively for 1st and 2nd seasons, foliar application of salicylic acid SA at 400 ppm and IAA at 30 ppm attain the significant promoting effects for mitigated the bad effects of salts, longest height 80.5cm in the 1st season and 86.2cm in the 2nd season attribute to foliar spraying of 400 ppm salicylic acid followed by 73.7 and 77.5cm in 1st and 2nd seasons respectively associated with IAA at 30 ppm. In relation to leaves numbers, data in Table (1) clearly indicated that salt stress 14000 ppm led to marked reduction in the two seasons for leaves numbers of plantlets 9.0 in the 1st season and 12.7 leaves/plantlet in 2nd season. On the other hand foliar applications with salicylic acid (SA) at 400 ppm and 30 ppm of IAA enhancing leaves numbers, this enhancing and increment in leaves numbers was appeared to be larger at SA at 400 ppm which scored 12.1 and 14.5 in 1st and 2nd seasons respectively, followed by 10.7 and 13.1 leaves respectively in 1st and 2nd seasons under spraying of 30 ppm IAA with significant differences between them. Significant marked retarded was to be found on fresh weights of leaves under NaCl + $CaCl_2$ at 14000 ppm (15.8 and 16.6g respectively in 1st and 2nd seasons for leaves fresh weight) and (7.6 and 8.0g for dry weight in 1st and 2nd seasons respectively, More pronounced positive effects were found of the growth regulator treatments SA at 400 ppm and IAA at 30 ppm which was spraying at the time of salinization at 14000 ppm on the fresh and dry weights of leaves, However, Application of foliar SA at 400 ppm and 30 ppm IAA was found to be more effective in accelerate the fresh and dry weights of leaves, significant increasing seasons for fresh and dry weights of leaves 16.9 and 18.4g for leaves fresh weight and 8.1 and 8.8g for leaves dry weight respectively in 1st and 2nd seasons related to application of SA at 400 ppm graduated by foliar spraying of 30 ppm IAA,

Table 1: Effect of salt stress and exogenous supply of SA and IAA on the plant growth parameters of date pam (*Phoenix dactylifera* L.)

Treatments	Plant growth parameters							
	Plant height cm		Leaves numbers		Fresh weights (g)		Dry weights (g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Con.	87.4	94.6	14.5	15.6	18.2	22.3	8.7	9.1
14000 ppm	72.7	74.3	9.0	12.7	15.8	16.6	7.6	8.0
14000 ppm+ 400 mg/l SA	80.5	86.2	12.1	14.5	16.9	18.4	8.1	8.8
14000 ppm+ 30 mg/l IAA	73.7	77.5	10.7	13.1	14.8	16.2	7.0	7.3
L.S.D.	= 5.9	= 7.9	0.2	0.9	1.3	1.2	0.5	0.6

The reduction of different growth parameters i.e. plant height, leaves numbers and fresh and dry weights of leaves were affected by salts stress may be attributed to decrease in turgor pressure which is essential and needed for cell expansion and cell division, also this reduction due to hormonal imbalance which associated to disturbance in cell wall extensibility (Hoad *et al.,* 2001 and Hussain *et al*., 2008), Salinity is one of the most common environmental stress factors. Salinity adversely affects plant growth and development, hindering seed germination (Dash and Panda 2001), sea water 20-60% decreased trunk growth of *Nicotiana tabacum* and *Pistacia terebinthus* (Germana *et al.* 2000), length and fresh and dry weights of leaves decreased under high level of salts 7.22 and 26.5 ds/m (Al- Rokibah *et al.* 1998 on date palm and Alencar *et al*. 2003 on yellow melon), salts from 0.7 to 4.0 ds/m reduced canopy area and trunk growth of *Prunus armeniaca* cv. palsteyn

(Volschenk *et al*. 2000), A common adverse effect of salt stress on crop plants is the reduction in fresh and dry biomass production (Kusvuran 2010), Ec from 3-18 ds/m reduced plant height, number of leaves of *Ricinus communis* L (Sun *et al.* 2013.), on the other hand exogenous application of SA and IAA can be modify this bad effects of salts, IAA at 150 and 200 mg/l reduced the adverse effect of salt stress 16-26 ds/m and increased leaf numbers/seedling and leaf matter of date palm cv lulu (Al-Juburi and Al- Masry 2000), growth regulators are involved in altering growth processes in plants, it is possible that they might even reduce the detrimental effects of salinity by stimulating growth (Nasser *et al*. 2001), pre-sowing wheat seeds with plant growth regulators like IAA alleviated the growth inhibiting effect of salt stress (Sastry and Shekhawa, 2001 and Afzal *et al*., 2005 on wheat), spraying of 20-80 mg/l ALA and GA_3 at 200 mg/l increased shoot length and fresh and dry weights of leaves and improve all growth parameters under 150 mM NaCl (Akbari *et al*. on *Vigna radiata* 2008), exogenous application of salicylic acid significantly increased overall growth; shoot length, leaf area, fresh and dry weights of root, shoot of *Coriandrum sativum* L (Niakan *et al.,* 2010). foliar application of SA from 10-3 to 10-5 increased plant height and dry weights of *Zingiber officinale* Roscoe (Borsani *et al*. 2001 on *Arabidopsis* and Ali and Hawa 2013), IAA produced by *Klebsiella oxytoca* at 100 ppm can obviously promote plant height, dry weights under salt stress (Liu *et al*. on cotton seedlings and Majid *et al*. on *Triticum aestivum* L. 2013 and recently Paravaiz 2014 on *Zea maize*).

Chemical contents:

Photosynthetic pigments mg/g f.w.:

The spectrophotometric estimation of chlorophyll pigments Fig. 1 indicated progressive reduction of chlorophyll a and b content under NaCl + $CaCl_2$ at 14000 ppm which had 0.7 and 0.6mg/g f.w. for chlorophyll a respectively for two tested seasons and 0.22 and 0.19 mg/g f.w. for chlorophyll b in 1st and 2nd seasons respectively. Pigments which identified as the green substances in the higher plants is responsible to the photosynthesis process converts light energy to chemical energy from the sun into food for the plants Photosystem and produced all ingredients and accumulation of plant biomass. This depression of pigments contents as many scientists found, The severe reduction in the photosynthetic pigments might be attributed to the toxic action of NaCl on the biosynthesis of pigments, increasing their degradation and/or maintaining damage of the chloroplast thylakoid (Rao and Rao 1981), salinity from 10000-14000 ppm decreased chlorophyll contents in leaves of date palm (Darwesh *et al*. 2006 on date palm and Hassanein *et al*. 2009 on Wheat), chlorophyll contents of *Rough lemon* and *Bitter sweet orange* decreased under 30-90 mM NaCl (Abd El-Samad *et al.* 2011 on *Zea mays* and broad bean *Vicia faba* and Shahid *et al*. 2013 on Pea (*Pisum sativum* L.)), This inhibition was recovered by the addition of 400 ppm salicylic acid. Exogenous application of SA at 400 ppm and IAA 30 ppm caused an increase in chlorophyll a and b, the increasing of pigments a and b was to be associated with foliar application of SA at 400ppm 1.1 and 1.2 mg/g f.w. for chlorophyll a and 0.75 and 0.86 mg/g f.w. for chlorophyll b respectively for two tested seasons which is developed than IAA at 30 ppm. SA provides a pool of compatible osmolyts in the presence of salinity. The increase in production of photosynthetic pigments in SA treated plants was concomitant with the accumulation of saccharides and growth yield of wheat plant under salinity levels as compared with control plants (Tari *et al*. 2002), salicylic acid at 50-1000 µM caused significant increased in chlorophyll content in leaves under 100 µM NaCl, this accumulation of photosynthetic pigments as a result of exogenous application of SA may be due to increase in photosynthetic efficiency as reflected by increasing in both chl a, chl. b and carotenoids (El-Mergawi and Abdel-Wahed 2007 on *Zea mays* and Misra and Misra 2012 on *Rauwolfia serpentina*), foliar application of IAA 1-2 mM increased total chlorophyll contents of *Zea mays* with 100 mM NaCl (Kaya *et al.*2010),

Indoles mg/g f.w.:

Decline in indoles amounts Fig. 2. were observed at salinity stress 14000 ppm Na Cl + Ca Cl_2 (10.9 and 9.8 mg/g f.w. respectively for two seasons) compared to the contents of indoles with untreated plantlets which produced higher significant contents of indoles contents for two seasons, in concern the exogenous spraying of two growth regulators which produced significant performed in total indoles contents in combination of 14000 ppm salts, maximum total contents of indoles were belonging to exogenous spraying of 30 ppm IAA (19.1 and 22.7 mg/g f.w. respectively for 1st and 2nd seasons graduated by treatment of SA at 400 ppm. NaCl caused a significant reduction in IAA concentrations in rice leaves (Prakash and Prathapasenan 1990), Salinity caused 75% reduction in IAA levels of tomato (Dunlap and Binzel 1996), salinity 16000 – 18000 ppm causes a progressive decline in the level of IAA in the root and leaves system of plants, meanwhile under yeast and amino acids increased indoles contents (Pervaiz *et al.* 2003 and Darwesh 2013 on date palm)

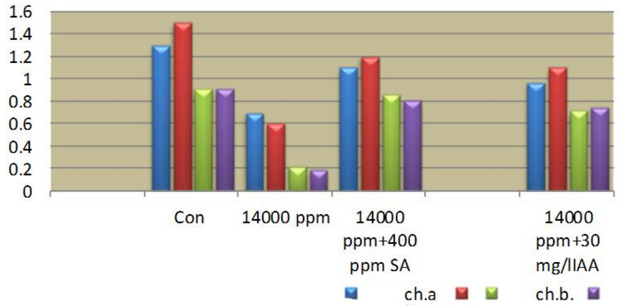

Fig. 1: Effect of exogenous supply of SA and IAA under salt stress on chlorophyll a and b mg/g f.w. at 1st and 2nd seasons of date palm (*Phoenix dactylifera* L.)

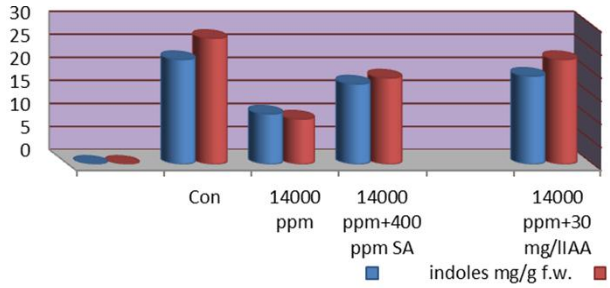

Fig. 2: Effect of exogenous supply of SA and IAA under salt stress on indoles contents mg/g f.w. at 1st and 2nd seasons of date palm (*Phoenix dactylifera* L.)

Total sugar mg/g d.w.:

Data obtained in Fig 3. show that application of salts 14000 ppm NaCl+ $CaCl_2$,strongly rising of total soluble sugars under salt stress for two tested seasons, moreover, exposure plantlets with spraying of growth regulators 400 ppm SA and 30 ppm IAA in combined with salt stress significant exceeding in leaves contents of total soluble sugars without any differs in between. Total sugars were closely related to compatible solutes which required in the case of tolerance plant to stress Sugars contribute up to 50% of the total osmotic potential in glycophytes subject to saline conditions. The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity or drought (Murakeozy *et al.*2003),Osmotic adjustment, defined as lowering of osmotic potential due to net solute accumulation in water stress, has been considered to be a beneficial drought tolerant mechanism for some crop species (Hamdia, 2002), 200 mmol/l NaCl increased soluble sugars of *Aloevera* seedlings (Jianfeng 2005), Many plants accumulate compatible osmolytes, such as proline (Pro), glycine (Gly) betaine, manitol or sugars, under osmotic stress. Pro biosynthesis from glutamine (Glu) appears to be the predominant pathway under stress conditions (Delauney *et al.,* 1993 and Yamada *et al*., 2005 and Nasser 2011 on Wheat), 1088 ppm soil salinity with 100 ppm SA increased total sugars of date palm (El-Khawaga 2013),

Proteins:

Results obtained regarding leaves proteins contents of date palm Fig 3. impact of salt stress 14000 ppm was highly significant accumulations 2.7 and 2.8 mg/g d.w. in 1st and 2nd respectively, compared to control treatment, exogenous application of SA 400 ppm and IAA 30 ppm had the same trend for significant progressive contents of proteins without significant variance in between for two tested seasons. Proteins accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is re-utilized when stress is over and may play a role in osmotic adjustment (Pareek *et al.* 1997), compatible solutes in plant stress responses is not limited to conventional osmotic adjustment, but also includes some other regulatory or osmoprotective functions. One such function is in mainting cytosolic K+ homeostasis by preventing NaCl induced K+ leakage from the cell (Cuin and Shabala 2005), NaCl at 50-150 mM increased proline contents in *Salventia natans* L (Jampeetong and Brix 2009 and Patel *et al.* 2010 on *Ceriops tagal*), salt stress 18000 and 20000 ppm increased amino acids contents (Darwesh *et al.*2011 on *Phoenix canariensis*), The accumulation of compatible solutes is often regarded as a basic strategy for the protection and survival of plants under abiotic stress conditions, including both salinity and oxidative stress (Zhonghua *et al.* 2014),SA 50-75 mM with NaCl at 60 mM increased proteins of *Solanum Tuberosum* L (Sajid and Aftab 2012),total soluble proteins of sunflower increased under NaCl 150mM and L-argenine (Havva *et al.* 2014),

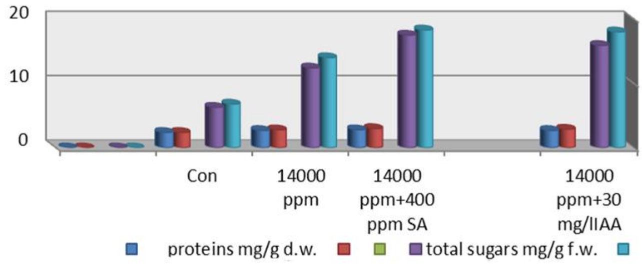

Fig. 3: Effect of exogenous supply of SA and IAA on proteins mg/g d.w. and total sugars mg/g f.w. at 1st and 2nd of date palm (*Phoenix dactylifera* L.)

Ions concentrations Na,Ca, Cl and K:

In concerning of ions concentration in aerial parts of date palm *Phoenix dactylifera* L. affected by salt stress are given in Table Figs 4 and 5. exhibited that, salt stress at 14000 ppm alone or in combination of exogenous application of growth regulators SA 400 ppm and IAA 30 ppm caused a significant excess of ions Na,Ca and Cl in the two tested seasons, while in the contrary, under 14000 ppm NaCl+ $CaCl_2$ the leaves contents of K^+ was significantly reduced than untreated plantlets which had biggest leaves contents of K^+, on the other hand under exogenous application of 400 ppm SA and 30 mg/l IAA significant increased contents of K^+

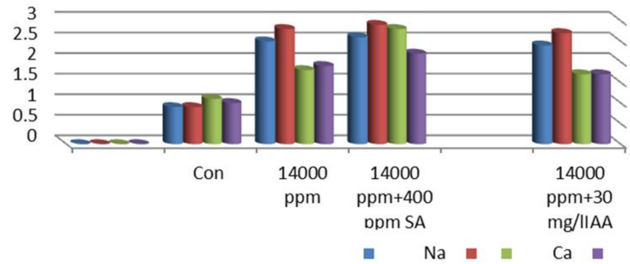

Fig. 4: Effect of exogenous supply of SA and IAA under salt stress on Na ppm and Ca % at 1st and 2nd seasons of date palm (*Phoenix dactylifera* L.).

In a saline environment plants take up excessive amounts of Na at the cost of K and Ca, high Na/Ca and Na/K ratios in a saline growth medium may impair the selectivity of the root membrane and greater accumulation of Na in plant roots. In a saline environment plants take up excessive amounts of Na at the cost of K and Ca. High Na/Ca, The greater accumulation of Na in plant roots (Boursier and Lauchi 1990), salinity decreased K+ concentration in date palm seedlings, and citrus leaves (Grattan and Grieve 1994), Chloride is a more sensitive indicator of salt damage than Na, since it is stored by plants, whereas Na is absorbed in smaller quantities despite high Na concentrations in the soil (Alam 1994), Leaf Na+, Cl- and Fe+ concentrations of date palm seedlings were increased, when irrigated with salt application alone or in combination with IAA (Aljuburi and Al-Masry 2000), Na+-specific damage is associated with the accumulation of Na+ in leaf tissues and results in necrosis of older leaves. Growth and yield reductions occur as a result of the shortening of the lifetime of individual leaves, thus reducing net productivity and crop yield (Munns 2002), Na and proteins were accumulated in the leaves of *Acacia ampliceps* under NaCl from 5-15 ds/m (Roomi *et al.* 2002), the application of growth regulators could assist the accumulation of mineral elements in leaves and roots of treated plants, increasing plant growth and its resistance to salt stress, spraying of GA_3 at 20 ppm increased K+ in Citrus sinensis L. old Washington navel orange trees (Eman *et al.* 2007), SA at 1.0 mM with 50 mM NaCl decreased Na and Cl and increased K+ of *Vigna radiata* L. (Khan *et al.* 2010), salinity from 14000 to 20000 ppm increased Na,Ca, Cl (Darwesh and El-Banna 2011 on *Phoenix dactylifera* L.)

Catalse CAT and Peroxidase POD:

Under salt stress plant cells have evolved intricate defense systems including enzymatic (superoxide dismutase (SOD), POD and catalase (CAT) etc, antioxidant resistance mechanisms may provide a strategy to enhance plant stress tolerance. Data presented in this study Fig. 6. showed that POD and CAT activity increased during salt stress and this increase was positively related to 14000 ppm NaCl + $CaCl_2$ concentration. Plants possess an impressive array of defense mechanisms against oxidative stress including the enzymatic and nonenzymatic antioxidant systems. Plants possess an impressive array of defense mechanisms against oxidative stress including the enzymatic and nonenzymatic antioxidant syste, the antioxidant enzymes include superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), The latter of antioxidant enzymes includes catalase (CAT), superoxide dismutases (SOD), peroxidase (POD) and glutathione reductase (GR) (Zhu 2001 and Ashraf 2002), NaCl salinity generates and increase the activity of this antioxidant enzymes. CAT, which is involved in the degradation of hydrogen peroxide and preventing oxidative damage (Mittler 2002). 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 and Mehmet *et al.* 2013 on *Ctenanthe setosa*).

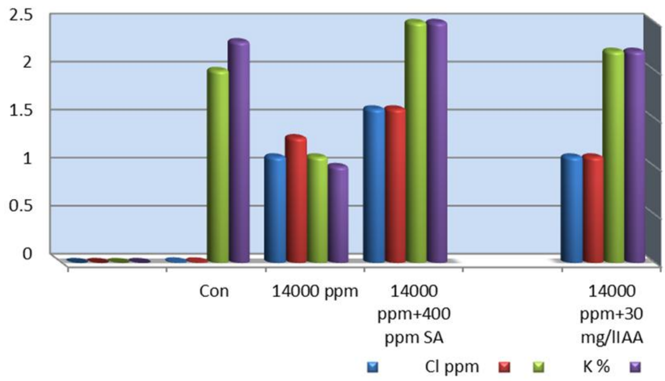

Fig. 5: Effect of exogenous supply of SA and IAA under salt stress on Cl ppm and K% at 1st and 2nd seasons of date palm (*Phoenix dactylifera* L.)

The increasing activities of CAT and POD with spraying of SA at 400 ppm and IAA at 30 ppm were highest under stress of NaCl + $CaCl_2$ without significant differences in between, exogenous SA can regulate the activities of intracellular antioxidant enzymes such as SOD, POX and increase plant tolerance to environmental stresses (Sakhabutdinova *et al*., 2004),foliar application of SA under NaCl 120 mM rising CATPOD and SOD of sunflower (Sibgha *et al*. 2009 on *Helianthus annuus* L., Mutlu *et al*. 2009 on wheat and War *et al*. 2013 on *Cicer arietinum*), salinity 7.25 mmohs/cm with spermidine from 50-100 ppm increased CAT and POD of *Helianthus tuberosus* (Sofy and Fouda 2013). These results explained the obvious increasing of antioxidant enzymes accomplished a defense system for protecting oxidative damage in the plants were impressed to higher salts condition.

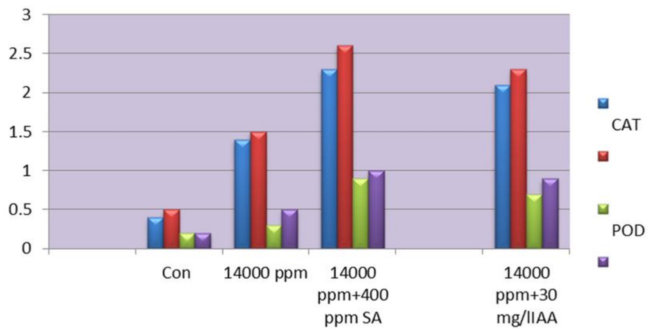

Fig. 6: Effect of exogenous supply of SA and IAA under salt stress at 1st and 2nd of date palm (*Phoenix dactylifera* L.)

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