J. Plant Production, Mansoura Univ., Vol. 8 (2): 335 - 345 , 2017

Effect of Auxin Treatment on Growth and Physiological Traits in Two Sunflower Cultivars Under Saline Conditions.

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ABSTRACT

Salinity considered one of abiotic stress affected to crop productivity. The present investigation aimed to give insights about the effect of NaCl saline on two cultivars sunflower plants *(Helianthus annuus. L)* and determine the usefulness of increasing relative salt tolerance by auxin treatment. Different responses of two sunflower cultivars Sakha 53 and China and differently susceptible to salinity stress levels by NaCl treatment in hydroponic culture experiment were investigated. The cultivar more tolerant (Sakha 53) showed less reduction in height , fresh and dry weight of the different plant organs, membrane stability, plastid pigments contents and more expression of antioxidant enzymes under high salinity level (175 mM NaCl) compared with China cultivar. Also the growth parameters were significantly increased due to IAA treatments. The physiological mechanisms of tolerance to salinity are descussed at the cellular, organ was studied. The use of plant hormones in two sunflowers under NaCl stress, improved plants salt tolerance through an improvement of relative water content, membrane stability, enhancement of chlorophyll and carotenoids biosynthesis, some antioxidant enzymes, which reflect an increase the most growth parameters of sunflower. Thus, our tested phytohormones prevent the harmful effects of NaCl in two sunflowers.

Keywords: Sunflower; Salt stress; Antioxidant enzymes; Proline; Photosynthetic pigments; Indole acetic.

INTRODUCTION

Sunflower (*Helianthus annuus*, L.) is an important oil crop all over the world. Sunflower originated in North America, and belongs to the family Asteraceae (Compositae), genus Helianthus (Moreira *et al*., 2010). It is cultivated on all continent, due to its wide adaptability to different stresses. Its productivity is slightly influenced by altitude and photoperiod (Souza *et al*., 2010). As a result of the increase in population there are things to worry about sufficient future global production of food from crop plants because of soil erosion, non-sustainable farming, global climate changes and soil degradation (Lobell *et al*., 2008). A number of abiotic stresses such as drought, salinity, and changes in weather temperature. Suzuki, *et al*., (2005) affects every developmental stage of sunflower (Reddy *et al*., 2003). Sunflower has the ability to extract water from deeper soil layers "when water stress during the early vegetative stage causes stimulation of deeper root system" and a tolerance of short periods of water deficit (Vijay, 2004). Sunflower is moderately sensitive to soil salinity (Ines, *et al*., 2014). The osmotic adjustment in plants can maintain the uptake of water and the turgor of cell, allowing regular physiological metabolisms (Radić *et al*., 2013). The reduction in plant growth under salinization may be also due to the regulation between the endogenous growth substances presented in the plant (El-Nabarawy, 1994). Proline, is an osmoprotectant, contributes to osmotic adjustment, protecting enzymes from oxidative damage under saline condition (Gupta and Huang 2014). Meantime, it was found that salinity stress causes marked decreases in wheat plant growth parameters with significant decreases in photosynthetic pigments and indole acetic acid (IAA) contents (Jasim, *et al.,* 2016). Plant growth and development are regulated by chemically and structurally diverse group of hormones (Marcinska *et al*., 2013). Phytohormones, such as IAA involved in the regulation of plant responses to salt stress and counteract the adverse effect of stress conditions (Javid *et al*., 2011). Akbari *et al*., (2007) showed that application of IAA increased hypocotyls length, seedling fresh and dry weigh and hypocotyls dry weight. Also it was increasing stimulators/inhibitors ratio in the plant tissues and increasing (carbohydrates, proline, organic acids which can used as an indicator in the osmoregulation of tissues under salinity (Upreti and Murti 2010).

The present aimed to study: 1- Evaluating the effect of salinity on growth as well as physiological parameters on the tested two sunflower genotypes and their tolerance to salt stress. 2- Alleviating stress through treatment of plant with growth regulators (IAA with different concentrations) and studying the growth physiological and biochemical parameters. Analysis of the endogenous hormones under salt stress condition was also evaluated.

MATERIALS AND METHODS

Effect of salt stress levels on two sunflower cultivars:

The study was conducted in the Plant Physiology Lab. Faculty of Agriculture - Al-Azhar University, on two cultivars of sunflower *Helianthus annuus L*. (Sakha 53 and China) which were obtained from Sakha Research Station - Oil Crops Department - Kafr El-Sheikh governorate, and the China type from Field Crops Institute - Department of Research germplasm - Bahteem Research Station, Qaliubiya.

For evaluating responses of sunflower to salt stress to Hydroponic experiment were conducted in two years**:**

The first experiment (2014 / 2015): Seeds were germinated in trays float filled with washed sand on a quarter strength Hoagland's nutrient solution used as macronutrient sources [KH_2PO_4(136g/l), KNO_3(101g/l), $Ca(NO_3)_2.4H_2O$(236g/l), and $MgSO_4.7H_2O$(246.5g/l)]. For micronutrient sources $C_4H_4FeO_6$(5g/l), $MnCl_2.4H_2O$ (7.24g/l), $CuSO_4.5H_2O$(0.32g/l), $ZnSO_4.7H_2O$(0.88g/l), BH_3O_3(11.44g/l), $H_2MO_4.H_2O$(.08g/l) and EDTA) (Hoagland and Arnon, 1950) The standard nutrient solution was adjusted on pH 5.8, renewed weekly and aerated continuously. In the first experiment all four

levels (0, 75, 125 and 175) mM NaCl as salt stress. The seeds of both cultivars were planted in trays of cork filled with washed sand and then placed on each tray unit in dishes containing 6 liter of Hogland's solution (pH:5.8), four replicates with eight plants per replicate were planted. After 2 weeks seedlings were exposed to the previous concentrations of NaCl. The plants were harvested after ten days to salt treatment and recorded the growth, physiological and biochemical parameters.

The second experiment (2015 / 2016):

Seeds of sunflower cultivars were soaked for 12 hours before planting in the previous solutions of hormone Indole - 3 - acetic acid (IAA) used 12.5, 25 and 50 ppm concentrations. Then the seeds were planted in polyethylene bags (5 seeds per bag) and 8 bags for each cultivar. The plants were placed in greenhouse in temperature ranged from 33–35°C during the day and 25–27 °C during the night. Nutrient solutions was added every day, and changed every 3 days. After fourteen days of sowing the seedlings were exposed to NaCl treatment (120mM) added to the nutrient solution. The samples have been taken from the seedlings after 2weeks of NaCl treatment for recorded the growth traits: as following; shoot height and root length (cm), leaf area (cm^2), fresh and dry weight (g) of the root and shoot system. Also certain physiological and biochemical parameters as following;

Physiological studies:

Chlorophyll content:

Chlorophyll levels were estimated in the methanolic extract spectrophotometericaly (63 serious UV/Vis) as described by Lichtenthaler and Wellburn(1985). Chlorophyll was expressed as µg/ml using the following formula:

Chlorophyll a =15.65 A666 - 7.340 A653

Chlorophyll b =27.05 A653 - 11.21 A666

Where A666 and A653 are absorbances at A653 and A666 nm.

Relative water content (RWC):

Leaf relative water content was estimated by incubating leaf samples (100mg) in 20 ml distilled water for 4 h (Weatherley, 1950). The turgid weight of leaf samples was recorded. The leaf samples were oven dried at 65 ºC for 48 h. Dry weights of the samples were taken after confirming that the samples were completely dried out.

RWC=(fresh weight-dry weight)/(turgid weight-dry weight).

Membrane stability index (MSI).

Determination of MSI was done by employing electrical conductivity (EC ; Gen way 4310) of sunflower leaf. at temperatures 40°C and 100°C. Four same sized discs were taken in standard test tubes having 20 ml double distilled water. Four sets of discs were maintained for comparison being kept for 30 min. at 40°C and for 15 min at 100°C in water bath respectively. Electric conductivity's EC_1 and EC_2 were measured by conductivity meter (Sairam and Saxena, 2002).

MSI= [1 – EC1/EC2] x100.

Assay of proline:

Fresh leaf samples (200 mg) were crushed with a pestle in ice-cold mortar liquid nitrogen and extracted with 10 ml of 3-5% sulfosalicylic acid. The homogenate was filtered with a filter paper and the filtrate was used for the analysis. Proline content was determined spectrophotometrically at 520 nm (Bates, 1973).

Proline standard curve:

Proline standard solution was prepared by dissolving 100mg from L- proline in 100 ml of 3% aqueous sulfosalicylic acid. Aliquots of 10µl (20µg) to (100µg) of the proline solution were put into test tubes. Then, total volume up to one ml was reached by using 3% aqueous sulfosalicylic acid. Each tube was treated as previous described (Bates, 1973).

Antioxidant enzymes (catalase and polyphenol oxidase):

Tissue preparation for determining enzymatic antioxidants: Fresh leaves samples (0.2 g) were ground in liquid N_2 and homogenized in an ice-bath in 4 ml homogenizing solution containing 50 mM potassium phosphate buffer and 1% (w/v) polyvinylpyrrolidone (pH 7.8). The homogenate was centrifuged at 14000 rpm at 4°C for 10 min and the resulting supernatant was used for enzyme assays.

a)Assay of catalase (CAT;E.C.1.11.1.6):

CAT action was precise according to Aebi (1984). Concerning catalase action determination 3 ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH = 7.2), 0.5 ml of 75 mM H_2O_2 and 0.03 ml enzyme extraction. The distilled water was used to construct the volume up to 3 ml. Reaction was progressed by the addition of H_2O_2. The absorbance decrease was recorded at 240 nm for 60 seconds. The enzyme activities were accounted by calculating the quantity of decomposed H_2O_2 in sample treatments compared with control.

b) Assay of polyphenol oxidase (PPO;1.10.3.1):

The determination of PPO activity was done according to (Duckworth and Coleman, 1970) method at 420 nm at 25°C. The test solution was prepared by mixing 0.03 ml of enzyme solution with 1.97 ml of 20 mM Catechol solution (which was prepared in 50 mM potassium phosphatebuffer, pH 6.8 at 25°C). The "blank" wasprepared with the same amount of Catechol and 0.03 ml of 50 mM potassium phosphate buffer. The enzyme activities were accounted by calculating in sample treatments compared with control.

Assay of endogenous hormones:

Varian Pro STAR 240 High Performance Liquid Chromatography (made in USA), Milli-Q ultrapure water purification system. Standard substance of ABA, IAA, GA3, ZT were products of Sigma company, methanol was chromatographic pure of Fisher Chemical company, ethanoic acid was analytical pure and water used in the experiment was ultrapure water. Chromatographic column: Hypersil ODS C18 column (150 mm × 4.6 mm, 5 µm), mobile phase: methanol - 0.6% ethanoic acid, gradient elution, column temperature: 35, sample size: 10 µl, flow rate: 1 ml/min, Ultraviolet detection wavelength: 254 nm. Samples of the sunflower leaves were surface dried and cleaned with a paper towel, immediately weighed and frozen in liquid nitrogen and approximately 1 g fresh weight [FW]) were ground in liquid nitrogen, homogenised and

then extracted one week with 30 ml 80% cold aqueous methanol (< 0) in darkness at -20C. The extract was centrifuged at 5000 r/min at 4C for 15 min and the supernatant was collected. Then fresh cold methanol poured into the remnant, extracted three times with aforementioned methods. IAA, ABA, GA3 and ZT were measured by the injection of the extract into a reverse-phase HPLC, with a methanol gradient in 0.6% acetic acid (Chen and Yang, 2005).

RESULTS AND DISCUSSION

I- **Effect of (NaCl) salinity on growth:**

The result showed gradual decreases in the values of leaf area, shoot length, fresh and dry weight of shoot and root with increasing the salt (NaCl) concentration in both cultivars when compared with control.

1. **Leaf area:** Salinity tolerance index **(**STI) for leaf area value gradually decreased with increasing the level of NaCl in two cultivars of sunflower plants. The decrease in leaf area value recorded (85.97 %, 70.14% and 25.50 %) with 75mM, 125mM, 175mM of NaCl respectively for Sakha 53 cultivar and (69.71%, 39.64 % and 19.23 %) with the same concentrations of NaCl respectively in China seeds cultivar. This is consistent with the results obtained by (Sadak *et al.,* 2013) who reported that, leaf area values was significantly decreased by increasing of sodium chloride level. Similar results were reported by (Ghoulam *et al*., 2002, Abu-Romman *et al*., 2014 and Hung, 2011).
2. **Shoot length:** The shoot length is one the most important parameter for salt stress because shoot supply water and nutrients from root to the rest of the plant. The results showed that salt stress inhibited the shoot length as showed in Table 1. The highest salinity concentrations 175mM of NaCl recorded the lowest value of shoot length. Shoot length values gradually decreases with increasing salinity levels from 75mM to 175 mM NaCl and recorded (82.80% , 78.52% and 73.71% respectively) in Sakha 53 cultivar, and (76.58, 75.54 and 73.30 respectively) in China seeds cultivar when compared with the control. The obtained results gained in our experiment are in agreement with the results of previous researches. Increased salt concentration caused a significant reduction in the vegetative growth of sunflower (Kumar *et al*., 2014). Higher salinity levels decreased all the growth parameter in this study as reported by several workers in sunflower (Moghanibashi, *et al*., 2012, and safflower (Kaya and Ipex, 2003).

Table 1. Shows S.T.I on growth parameters (leaf area, shoot and root length, fresh and dry weights of shoot and root) in two sunflower cultivars under salt stress of NaCl.

Treatments	S.T.I leaf area		S.T.I Shoot length		S.T.I Root length		S.T.I Shoot F.W		S.T.I Shoot D.W		S.T.I Root F.W		S.T.I Root D.W		S.T.T.I	
Cultivars	Sakha 53	China	Sakha 53	China	Sakha 53	China	Sakha 53	China	Sakha 53	China	Sakha 53	China	Sakha 53	China	Sakha 53	China
NaCl75mM	85.97	69.71	82.8	76.58	160.98	145.44	68.37	86.15	125.97	159.45	74.82	62.27	80.65	72.5	679.56	672.1
NaCl125mM	70.14	39.94	78.52	75.54	116.09	96.94	90.45	56.82	72.2	62.42	66.82	41.62	68.75	55.85	562.97	429.13
NaCl175mM	25.5	19.23	73.71	73.3	93.95	77.41	65.67	55.15	57.27	27.07	29.37	21.35	49.37	41.37	394.84	314.88
LSD 5%	15.57		1.61		13.93		6.57		5.93		9.05		9.49			
LSD 1%	22.51		2.93		21.51		9.08		7.32		11.5		13.12			

Also growth attributes like plant height, shoot and root elongation were severely decreased with salinity (Shila *et al*., 2016). The inhibition of shoot length values by salinity may be due to the ions. It is an established fact that salinity inhibits plant growth (Kandil *et al*., 2016). Unbalanced nutrient uptake by the plants. The lower water potential in saline soil in turn lower cell turgor causing reduction in cell elongation and cell division (Kaya, 2009).

3. **Fresh and dry weight:** It was the lowest values for these parameters at 175 mM concentration for both cultivars subjected in our experiments. These results showed that increasing of salinity rate causes the important decreases on growth characters of both sunflower cultivars. The lowest fresh weight was recorded by NaCl at 175mM for fresh weight value of shoot which showed 65.67%, 55.15 % while fresh weight of root showed the values (29.37 %, 21.35 %) for Sakha53 and for China respectively. Also the lowest value of dry weight for shoot was by NaCl at 175mM which recorded 27.57 %, 27.07 % with Sakha53 and China, but for root dry weight value was 49.37%, 41.37 % for Sakha53 and China respectively. This results accorded with Parida and Das, (2005) who found that the growth parameter were affected by salt stress. Saline stress leads to changes in growth, morphology and physiology of the roots that will in turn change water and ion uptake (Parida and Das, 2005 and Ali, *et al*., 2004). Through total salt tolerance index it has been found that, Sakha53 cultivar gave the highest tolerance indicators than China cultivar for growth parameters with all NaCl concentrations.

II. Effect of NaCl salinity on physiological and biochemical parameters:

Physiological and biochemical parameters significant affected by increased of NaCl concentrations in two tested cultivars of sunflower.

1. **Relative water content (RWC):** One of the early symptoms of salinity stress in plant tissue is the decrease of RWC. The results in Table 2 clearly showed that the gradually decrease in RWC with the increase of NaCl level in the two sunflower treated cultivars compared to those grown in non-saline solution.

The lower RWC values were observed in China cultivar(63.28% , 52.91 % and 48.33 %) when compared toSakha53cultivar (77.32, 69.04 and 61.23) at

NaCl level 75,125,175 mM of NaCl respectively, the maximum reduction was observed at high salinity level (175mM) when compared to the control treatment. The results of present study demonstrate growth reduction of seedlings when subjected to increase in salinity level. This might partially be attributed to the lower leaf water potential and a reduction in relative leaf water content, which resulted in loss of turgor (Qin, *et al*., 2010), which in turn causes stomatal closure and limits of CO2 assimilation and reduced photosynthetic rate (Kamath, 2008). It has been also reported that salinity induced decrease in RWC (Gadallah, 1999). Our results agree also with the findings of (Riaz, *et al*., 2012) and (Amirjani, 2010).

Table 2. Salt tolerance trait indices for physiological and biochemical parameters for two sunflower cultivars under salt (NaCl) stress.

Treatments	S.T.I RWC		S.T.I MSI		S.T.I Chlo.a		S.T.I Chlo.b		S.T.I CX		S.T.I proline		S.T.I catalase		S.T.I polyphinol oxidase		S.T.T.I	
Cultivars	**Sakha 53**	**China**	**Sakha 53**	**China**	**Sakha 53**	**China**	**Sakha 53**	**China**	**Sakha 53**	**China**	**Sakha 53**	**China**	**Sakha 53**	**China**	**Sakha 53**	**China**	**Sakha 53**	**China**
NaCl75mM	77.32	63.28	83.77	75.54	143.06	84.23	152.37	114.03	152.24	91.1	145.98	145.78	148.36	122.08	59.89	32.81	1077.8	814.17
NaCl125mM	69.04	52.91	74.1	61.2	99.7	43.73	106.21	98.96	100	83.44	210.6	215.56	255.11	137.34	70.84	65.58	1078.22	827.44
NaCl175mM	61.23	48.33	30.05	19.46	55.33	66.26	69.86	72.2	56.71	48.41	327.99	323.92	350.64	174.81	110.14	100.82	1115.63	921.63
LSD 5%	1.69		0.24		4.3		3.49		9.37		2.81		18.95		4.45			
LSD 1%	2.4		0.34		6.12		4.96		13.33		3.99		26.95		6.33			

2. **Membrane stability index (MSI)**: Results clearly indicated that, response of MSI to salinity levels was different in both cultivar of sunflower. A decrease in cell membrane stability caused by increasing salt concentration of NaCl Table2. The highest value (83.77% decreased to lowest value 30.05%) in Sakha 53 cultivar and (77.45% to 19.46%) in China cultivar of MSI belonged to 75 mM and 175 mM of NaCl treatments respectively. this is due to genetic variability within a species offers a valuable tool for studying mechanisms of salt tolerance (Azizpour *et al*., 2010). One of these mechanisms depends on the bypass capacity for second oxidative stress that allows growth to continue under saline conditions (Rao *et al*., 2013). Activated oxygen species (AOS) induced lipid peroxidation is a reflection of stress induced damage to cell membranes. Most studies have reported MSI decrease (membrane permeability increase) under salinity stress (Sarwar and Ashraf, 2003). In these studies, MSI exhibited a positive correlation with osmotic potential, K^+ concentration, osmotic adjustment, and/or relative water contents, parameters that are influenced by salt stress (Munns, 2002). MSI has been used as marker of salt injury and salt tolerance in plants (Panda *et al*., 2003). It is suggested that decrease in membrane stability reflects the extent of lipid peroxidation caused by reactive oxygen species (Jamil *et al*., 2012). This is similar to the results obtained by (Bhutta, 2011) and (Chaparzadeh and Mehrnejad, 2013).
3. **Photosynthetic pigments**: The data revealed that different salinity levels and the interaction between salinity and the two cultivars had a significantly affect chlorophyll a, b and carotenoids content. The diminution rate of chlorophyll a, b and carotenoids were the biggest in the Sakha53 followed by China seeds. The reduce under highest salinity level (175mM) it was recorded (55.33, 69.86, 56.71%) for Sakha53 and (66.26, 72.20, 48.41%) for China to Cha, Chb and carotenoid respectively when compared with75mM as showed in Table 2. Reduction in chlorophyll content is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions (Meloni *et al*., 2003). Salinity affects the strength of the forces bringing the complex pigment protein liquid in the chloroplast structure as for the chloroplast in membrane bound its stability is dependent on the membrane stability which under high salinity condition seldom remains intact due to which reduction in pigments (Zhu, 2001) and (Dagar *et al*., 2004). However, other researchers mentioned that reduction in chlorophyll content may be due to variation in its synthesis among the plant species as well as variation in specific enzymes under saline conditions (Keutgen and Pawelzik, 2007).
4. **Proline contents**: Data recorded in Table 2 showed that NaCl application increased in free proline contents. It increased gradually in the two cultivars than control till the last level of salt Table 2. In Sakha 53 cultivar, highly significant increase proline value up to 75mM which showed 14.98 to highest value (327.99) at 175 mM level of NaCl. Similar results were found with China seeds cultivar where they registered the value 145.78 at 75mM and gradually increased until it reached 323.92 at 175 mM which was highly significant value. Similar results were recorded previously by Alam *et al*., 2015 and Wu *et al*., 2015). In many researches in sunflower plant, proline accumulation was observed in all stressed plants of the two varieties compared with control (Patade *et al*., 2008). Proline in plants contributes to osmotic adjustment during stress (Ghasempour and Kianian, 2007) and protects the structure of macromolecules and membranes during extreme dehydration (Prado *et al*., 2000). Melonid *et al*., (2001) Suggested that proline also serves as an important source of nitrogen in plant metabolism, as a readily available source of energy and as a reducing agent thus it is an osmoses protector in plant (Wang *et al*., 2009). Salinity exposure was very effective in proline accumulation in leaves of many crop seedlings (Amirjani, 2010). Similar results have been reached by (Heidari and Sarani, 2012), (Ashrafijou *et al*., 2010) and (Turkyilmaz *et al*., 2014) with other plant species. It was also found that Sakha53 cultivar

was the most tolerant than China cultivar for physiological and biochemical parameters with all NaCl levels (75,125 and175mM).

5. **Antioxidant enzymes:** It was found that the activities of antioxidant enzymes were increased in leaves under NaCl stress. The responses of polyphenol oxidase (PPO) and catalase (CAT) were particularly significant in the leaves indicating a high defence capability of antioxidant enzymes to salt stress at the early growth stage (Xiaoli *et al*., 2009). Significantly increased CAT activity was also observed with higher levels of sodium chloride, 175 mM compared to the lowest level which recorded 152.24% at 75 mM and the highest level was 350.64% of Sakha53, for China cultivar the highest value was observed at 175mM NaCl which recorded 174.81% and the lowest level (122.08%) at 75mM . Phenol accumulation could be cellular adaptive mechanisms for scavenging oxygen free radicals during stress. The highest rate of PPO activity was measured in 175 mM NaCl about 110.14% for Sakha53 and 100.82% for China cultivar increases as compared with 75 mM NaCl which recorded (59.89%, 32.81%) for Sakha53 and China cultivars respectively. Tolerance of salinity stress in higher plants correlates with the levels of antioxidant systems and substrates (Turkyilmaz *et al*., 2011). These changes in the levels of antioxidant enzymes molecules are signals of plant tolerance/adaptation to stress conditions (Koca, 2007). The activity of these enzymes are correlated with oxidative stress tolerance of plants (Lee, *et al*., 2001). Oxidative stress is initiated by reactive oxygen species ROS (are chemically reactive molecules containing oxygen) such as superoxide radical (O^{-2}), hydrogen peroxide (H_2O_2) (Athar, *et al*., 2008). CAT is an important antioxidant enzyme that converts H_2O_2 to water in the peroxysomes. Higher activity of CAT and PPO decreased H_2O_2 level in cell and increased the stability of membranes and CO_2 fixation because several enzymes of the Calvin cycle within chloroplasts are extremely sensitive to H_2O_2. A high level of H_2O_2 directly inhibits CO_2 fixation (Yamazaki *et al*., 2003). ROS scavenge by various antioxidant enzymes such as CAT and PPO, which increased during salt stressed conditions (Nawaz and Ashraf, 2007). The increased activities of the antioxidant enzymes upon salt stress are often related to the enhanced tolerance to salt stress (Mohammadkhani and Heidari, 2007). Our results were consistent with the results of previous studies in other crops such as (Emamverdian *et al*., 2015) and (Loukehaich *et al*., 2011).

III. **The effect of auxin (IAA) on induction salt tolerance:**

Auxins play a major role on regulating plant growth (cell elongation, and apical dominance) to salinity in plants. The data indicated that, we found that salt stress caused deleterious morphological and cellular effects as presented below:

A- The effect of auxin on growth.

The effects of IAA on leaf area, plant height, and other vegetative growth studied of two sunflower cultivars treated (Sakha 53 and China) grown under salinity stress (120 mM NaCl) were showed in Table 3.

Table 3. The effects of (IAA hormones) on S.T.I of the growth parameters of sunflower under salinity stress.

Treatments	S.T.I leaf area		S.T.I shoot length		S.T.I root length		S.T.I shoot fresh weight		S.T.I shoot dry weight		S.T.I root fresh weight		S.T.I root dry weight		S.T.T.I	
Cultivars	sakha 53	China	sakha 53	China	sakha 53	China	sakha 53	China	sakha 53	China	sakha 53	China	sakha 53	China	sakha 53	China
NaCl120mM,12.5ppmIAA	119.71	127.65	93.46	102.58	108.35	114.31	111.58	86.69	95.69	74.83	101.57	69.69	101.25	117.5	731.61	693.25
NaCl120mM,25ppmIAA	167.1	152.84	136.82	162.74	168.08	169.7	161.96	188.62	229.52	201.66	202.48	167.3	292.5	370	1358.45	1412.86
NaCl120mM,50ppmIAA	82.64	69.6	85.58	93.18	109.52	104.47	98.58	74.03	93.62	74.06	118.22	67.42	173.75	111.25	761.91	594.01
LSD 5%	30.82		29.64		20.72		8.51		7.84		21.26		47.16			
LSD 1%	40.25		39.67		36.95		11.75		10.84		33.55		65.64			

1. Leaf area: Inboth sunflower cultivars, a significant reduction in leaf area was observed under conditions of salt stress. Pre-treatment with auxin (IAA) concentrations (12.5 ppm, 25 ppm and 50 ppm) showed effective increase in leaf area when compared with non treated plants` under salt stress (NaCl) 120 mM. All seed soaking treatments with IAA were alleviated the growth inhibiting effect of salt stress. Highest STI value for leaf area recorded (167.97 %, 152.84%) in Sakha53 and China cultivars respectively which were treated with 25 ppm IAA accompanied with 120 mM NaCl when compared with other concentrations of IAA. Under saline condition it was found that new cell starts its elongation process and the excess of Na^+, Cl^- and other ions modifies the metabolic activities of cell wall, which causes deposition of several materials on cell wall and limits the cell wall elasticity (Yasar *et al*., 2006). Cell walls become rigid and turgor pressure efficiency in cell enlargement was decreased with application of elevated salt treatment. The other anticipated cause of reduction in leaf area and dry matter accumulation could be the reduced development and differentiation of tissues (Ali, *et al*., 2004). These results were compatible with Bahrani, *et al*., (2010) who concluded that the effects of stress are often manifested at morphophysiological, biochemical and molecular levels, such as inhibition of growth and changes in endogenous contents of phytohormones and their effect on plant growth (Perales, *et al*., 2005) and (Dobra, *et al*., 2010). But the Indole acetic acid (IAA) stimulates cell enlargement that is a necessary step in cell growth. The application of IAA to plants promotes vegetative and reproductive growth (Hussain *et al*., 2011). Also (Kusvuran, 2010) and (Zhu, 2001) reported that pre-sowing wheat seeds with plant growth regulators like IAA alleviated the growth inhibiting effect of salt stress and increased in seedling growth parameters under different salinity levels.

2. Shoot and Root length: It has been observed that IAA increased shoot and root length (Table 3). The

best treatment (25 ppm) of IAA recorded a higher of shoot length value (136.82 for Sakha 53 and 162.74 for China) and (168.08 and 169.70) for root length value in both cultivars respectively, followed by (12.5 ppm IAA) which recorded (93.46 and 102.58) for shoot of Sakha53 and China also (108.35 and 114.31 for STI roots lingth in both cultivars respectively). Saline stress may reduce plant growth by water deficit, ion toxicity, ion imbalance or combination of these factors. With increasing salinity reduction of shoot and root length. It was mentioned that the exogenous IAA application at high concentrations (50 ppm) may be harmful and cause retardation of plant height, as reported by Nejadalimorad *et al*., (2014) on different plant species. The application of IAA hormone increased shoot length under salinity level Sadak *et al*., (2013) and Abdelhamid *et al*., (2013). Exogenous application of IAA at cell division stage of seeds growth increased cell elongation (Majid, 2013) and therefore increase shoot and root length. The adverse effect of salinity was alleviated by IAA (Afzal *et al*., 2005).

3. **Shoot and root fresh and dry weight**: The seed treatments with IAA positively affected significantly fresh and dry biomass value of shoot and root in plant stressed by NaCl (Table 3). Maximum STI of shoot fresh biomass were with seeds treated at 25 ppm IAA under saline conditions 120 mM NaCl that was recorded (161.96 and 188.62) for both Sakha53 and China cultivars respectively) followed by the 12.5 ppm treatment which recorded (111.58, 86.69). The maximum shoot dry biomass value recorded (229.52) for Sakha53 and (201.66) for China cultivar at 25 ppm of IAA under saline conditions (120mM NaCl). Our results were consistent with the results of previous researches in other plants (Mostafavi and Heidarian, 2012 and Hussain *et al*., 2011). It was also reported by Kaya *et al*., (2010), in the case of biomass attributes, the IAA has appreciably increased the shoot and root fresh and dry biomass.

Also the application of IAA increased STI value for root fresh and dry biomass in plants stressed by NaCl. The higher root fresh biomass was recorded at 25 ppm IAA (202.48, 167.30) and (292.50, 370.00) for root dry biomass in the two cultivars Sakha53 and China respectively when compared with other concentrations of hormone. The exogenous application of IAA increased the shoot length, root length, shoots fresh biomass, root fresh biomass and leaf area. Similar results were reported. The overall vegetative growth was measured in terms of root, shoot fresh and dry weight (Hoad, 2001). Also Pop and Pamfil, (2011) reported that the maximum increase was achieved at the concentration of (12.5 ppm) which caused significant improvement in root and shoot length, root and shoot fresh, dry biomass and leaf area. The effect of IAA on cell division and elongation of cells might have resulted in the increased plant height. Akbari *et al*., (2007) showed that the application of IAA increased germination percentage, plant height and dry biomass in wheat plant. For total salt tolerance index (S.T.I) 25ppm of IAA gave the highest value for growth parameters for both cultivars.

Effects of IAA on physiological traits:

The adverse effect of salinity was alleviated by treatment of seeds with IAA. Auxin (indole-3-acetic acid) have central roles in plant growth regulation and plant defense against abiotic stresses e.g. salinity. To studies the response of two cultivars sunflower for auxin pre-sowing treatments on some physiological traits such as Relative water content, Membrane stability index, Photosynthetic pigments, Proline content and activity of some antioxidant enzymes under saline conditions.

1. **Relative water content (RWC):** RWC decreased with increased NaCl concentration, but IAA application showed an increased RWC table 3. The highest value of relative water content (106.19% and 116.00%) was recorded in Sakha53 and China cultivars respectively with (25 ppm IAA) compared with the lowest value of RWC was recorded at 50 ppm IAA (97, 89% and 94.54%) for Sakha53 and China cultivars respectively.

Table 4. S.T.I for physiological parameters of two sunflower genotypes treated with (IAA) different concentrations under salt stress conditions.

Treatments	S.T.I RWC		S.T.I MSI		S.T.I Chlo.a		S.T.I Chlo.b		S.T.I CX		S.T.I proline		S.T.I catalase		S.T.I polyphinol oxidase	
Cultivars	Sakha 53	China	Sakha 53	China	Sakha 53	China	Sakha 53	China	Sakha 53	China	Sakha 53	China	Sakha 53	China	Sakha 53	China
NaCl120mM,12.5ppmIAA	99.93	102.16	111.26	229.43	136.37	117.79	108.91	133.47	130.60	121.40	89.04	99.90	136.73	48.08	90.06	103.51
NaCl120mM,25ppmIAA	106.19	116.00	125.89	126.70	154.33	105.81	120.63	112.96	131.29	131.41	86.84	96.90	154.33	130.64	161.63	108.44
NaCl120mM,50ppmIAA	97.89	94.54	119.3	114.82	117.90	77.45	89.13	82.06	83.52	73.31	130.66	104.65	117.90	124.82	160.13	105.72
LSD 5%	0.40		1.95		16.99		20.29		23.27		15.63		4.58		0.29	
LSD 1%	0.57		2.79		24.15		25.36		34.37		22.23		6.51		0.41	

This reduction in salt treated plants was caused by an increase in of evapotranspiration in plants (Santos *et al*., 2002). It has been proved that IAA triggers some metabolic processes in plants as well as affects plant water relations (Hayat *et al*., 2010). Decrease in RWC indicated a loss of turgor that resulted in limited water availability for the cell extension process in sugar beet and other plants (Katerji *et al*., 1997). Also, Nazar, *et al*., (2011) reported that, this decrease in RWC could be because of lower water availability under stress conditions or root systems which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface. But with IAA application showed an increased RWC for plant stressed by NaCl (Shaharoona *et al*., 2006).

Membrane stability index (MSI): Maintaining integrity of cellular membranes under stress conditions is considered an integral part of salinity tolerance

mechanisms. In the present result, MSI decreased significantly in both sunflower cultivars under salinity stress. All concentrations of IAA treatment increased MSI significantly in plants treated in both sunflower cultivars. The best concentration of IAA was (25ppm) for Sakha53 cultivar which recorded (125.89%), but for China seeds which recorded (229. 43%) at 12.5 ppm of IAA. The lowest MSI value was recorded (111.26 and 114.82) at 50 ppm IAA for Sakha53 and China cultivars respectively. Salinity impairs membrane stability by increasing electrolyte leakage (Wael *et al*., 2014). IAA treatment increased MSI significantly in plants treated with hormone concentrations in both sunflower cultivars (Singh *et al*., 2011).

3- **Photosynthetic pigments:** Salt treatment decreased the concentration of photosynthetic pigments of two sunflower cultivars Table 3. However, IAA treatment reduced the extent of salinity-induced decline in concentration of these pigments; best improvement with 25 ppm for Sakha53 which recorded (Chlorophyll a 154.33 and Chlorophyll b 120.63%), followed by 12.5ppm of IAA this recorded (Chlorophyll a 136.37 and Chlorophyll b 108.91%). But with China cultivar (12.5ppm) was the best concentration which recorded (Chlorophyll a 117.79 and Chlorophyll b 133.47%) followed by 25ppm which recorded (Chlorophyll a 105.81 and Chlorophyll b112.96%). Photosynthetic pigments are directly related to growth and productivity of plants. The highest level of carotenoids showed with 25ppm IAA concentration in Sakha53 and China cultivar where it recorded (131.29, 131.41 %) improvement percent compared with salt treatment without IAA. At high concentrations value of auxin deterioration occurs on growth and physiological parameters of plants. The decrease in chlorophyll content and carotenoids under salt stress might be due to chlorophyll synthesis, which in turn depends on adequate ion balance (Radha *et al*., 2015). Auxin, such as (IAA), has been demonstrated to regulate plant responses to salinity, its photosynthetic apparatus, and the chloroplast structure (Tognetti *et al*., 2012). IAA and GA_3 which may be involved in protecting the photosynthetic apparatus and consequently increasing the photosynthetic pigments (Saeidi-Sar *et al*., 2013). IAA presumably acts as a coenzyme in the metabolism of higher plants, thus it plays an important role in the formation of the photosynthetic pigments (Husen *et al*., 2016). These increases in pigments content may be attributed to the promotion of pigments synthesis and/or retardation of pigments degradation (Taslima, *et al*., 2011). This is supported by the results of (Majid *et al*., 2013) on other plants.

4. Proline contents: Under salt stress, osmolyte such as proline maintains cellular homeostasis through osmotic regulation and induces physiological processes favorably. In our experiment, application of IAA at three concentrations combined with salt stress 120 mM NaCl resulted changes in the proline content in two sunflower cultivars. It has been observed that the less accumulation of proline which recorded (86.84 for Sakha 53 and 96.90 for China) at 25ppm of IAA treatment following by 12.5 ppm that is recorded (89.04 and 99.9%). Based on our results, proline can be considered a stress sign in both sunflower cultivars plant. Therefore, the treatment with IAA determines the concentration of proline in presence of salt. Also our data suggest that proline plays a fundamental role in IAA mediated defensive processes in the two sunflower against salt, reducing the detrimental influence of stress. The results in agreement with the findings by Sakhabutdinova *et al.* (2003), (Hamdia, 2004) and (Kaya *et al*., 2013). Since proline is one of the important components of defense reactions of plants against salinity (Kuznetsov and Shevyakova, 1999). It has also been previously demonstrated that the exogenous treatment with plant growth regulators such as IAA elevated proline content in plants (Ding *et al*., 2010). These data suggests that proline is an important component in the spectra of IAA mediated protective reactions of plants in response to salinity (Vardhini and Rao, 2003) which contribute to a reduction of injurious effects of stress factors and acceleration of restoration processes during the period after action of stress, which might be a manifestation of the protective action of IAA on plants (Filippou *et al*., 2014).

5. Antioxidant enzymes: IAA play an effective role by protecting the fluidity and integrity of plant cell membranes. Table 4 showed that salinity and their interactions with IAA significantly affected the studied antioxidant enzymes. The maximum expression of CAT and polyphenol oxidase activity in two cultivars of sunflower plants with IAA at (25ppm) concentration which recorded (154.33 and 130.64 %) for CAT activity and (161.63, 108.44%) for PPO activity in Sakha53 and China cultivars respectively. For PPO the lowest activity at 12.5ppm IAA which recorded (90.06 and 103.51%). It has been observed that, when increasing the concentration of the hormone (IAA 50ppm), the activity of antioxidant enzymes which recorded (117.90 and 160.13%) for CAT and PPO respectively in Sakha53 cultivar and (124.82 and 105.72%) for CAT and PPO respectively in China cultivar. They properly mediate enzymatic PPO, CAT and nonenzymatic machinery with the result of preventing cell membrane damage by ROS (Jungklang and Songklanakarin, 2012). Tolerance of salt-stress in higher plants correlates to the levels of antioxidant systems and substrates (Jahnke and White, 2003). It has also been demonstrated that cellular induction of the antioxidant machinery is important for the protection of plants against stress (Azzedine *et al*., 2011). The treatments with any of our tested phytohormones like auxin, alleviated the negative effects of salt on growth and physiological traits and metabolic processes by diminishing the build-up of ROS at high salinity levels, and thus enhancing the tolerance to salinity (Hassanein *et al*., 2009). Additionally, applied of plant growth regulators reduce moisture loss through the transpiration rate and protect the plants from stress (Azzedine *et al*., 2011).

Endogenous hormones: At non stressed plants it was observed that, higher concentrations of endogenous hormones (IAA, $GA_3$and Kin) compared to plants

exposed to salinity stress, which recorded (9.91 IAA, 61.31 GA_3 and 153.48 Kin) for Sakha53 cultivar and (81.56 IAA, 78.76 GA_3 and 0.74 µg Kin) for China cultivar. Under saline condition recorded (4.16 IAA, 39.59 GA_3 and 124.59 Kin) for Sakha53 cultivar and (20.27 IAA, 29.85 GA_3 and 0.01Kin) for China cultivar.

Table 5. Effect of IAA treatment on endogenous hormones of two sunflower cultivars under salt stress condition.

Treatments	Cultivars	IAA µg/g FW	GA_3 µg/g FW	KIN µg/g FW	ABA µg/g FW
Controle	Sakha53	9.91	61.31	153.48	41.60
	China	81.56	78.76	0.74	16.05
NaCl 120mM	Sakha53	4.16	39.56	124.59	70.91
	China	20.27	29.85	0.01	32.58
NaCl 120mM, IAA 25ppm	Sakha53	8.98	50.48	193.4	60.41
	China	35.75	99.34	0.80	24.52
LSD 5%		0.76	0.33	0.39	0.23
LSD 1%		1.08	0.47	0.55	0.34

It is clear that IAA at 25 ppm and NaCl at 120mM was the most effective treatment as it caused the highest contents of endogenous hormones (IAA, GA_3 and Kin) which recorded (8.98 IAA, 50.48 GA3 and 193.4 µg Kin) for Sakha53 cultivar and (35.75 IAA, 99.34 GA3 and 0.80 µg Kin) for China cultivar. For endogenous ABA the lower concentration observed in unstressed plants which recorded (41.60µg and 16.05 µg) for Sakha53 and China cultivars respectively but it increased significantly under saline condition which recorded (70.91 µg and 32.68 µg) for Sakha53 and China cultivars respectively. It is well known that the increase in the endogenous content of auxin, cytokinin and gibberellins promote cell division and cell enlargement and hence increased plant growth and total carbohydrate as well as crude protein contents and affect positively on plant growth and reflects the negative effect of salinity (El-Saeid *et al*., 2010). On the other hand, high levels of auxins and gibberellins together were found to enhance leaf production (Bing *et al*., 2010). IAA at 25 ppm and NaCl at 120mM, ABA recorded (60.41 µg and 24.52 µg) for Sakha53 and China cultivars respectively. The increases in the content of the endogenous growth promoters might be attributed to the increase in their biosynthesis and/or decrease in their degradation and conjugation (Barakat, 2011). It's evident that IAA treatment increased endogenous GA, IAA and CK stressed plants when plants treated with (25ppm IAA). The high levels of GA and IAA which produced from plants treated by IAA may explain the increase in plant height (Sen and Maharana, 1999). These results are consistent with El-Saeid *et al*., (2010) on cowpea. S.T.T.I for physiological and biochemical parameters 25ppm of IAA was the best concentration which gave the maximum values for Sakha53 cultivar but 12.5ppm concentration was the best for China cultivar.

In conclusion, different responses of two sunflower cultivars Sakha 53 and China and differently susceptible to salt stress levels by NaCl treatment in hydroponic culture experiment were investigated. The cultivar more tolerant (Sakha 53) showed less reduction in growth and more expression of antioxidant enzymes under high salinity level (175 mM NaCl) compared with China cultivar. The use of auxin in two sunflowers under salt stress conditions, improved the plants tolerance through an improvement of relative water content, stability of membrane, enhancement of PP biosynthesis, some antioxidant enzymes, which reflect an increase the most growth parameters of sunflower.

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تاثير المعاملة بالأوكسين على النمو والقياسات الفسيولوجية لصنفين من عباد الشمس تحت ظروف الاجهاد الملحى

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تعتبر الملوحة من المعوقات البيئية الرئيسية على إنتاجية المحاصيل في جميع أنحاء العالم. وقد أجريت هذه الدراسة فى محاولة لفهم الآليات الفسيولوجية لتحمل الملوحة وتحديد جدوى زيادة تحمل الاجهاد الملحي على صنفين من نبات عباد الشمس (سخا٥٣ والصنف الصينى) عن طريق المعاملة بهرمون الأوكسين. كانت هناك استجابات مختلفة لكلا الصنفين للاجهاد الملحى عند المعاملة بتركيزات مختلفة منه فى تجربة المزارع المائية. الصنف سخا٥٣ كان الاكثر تحملا حيث كان معدل النقص اقل فى قياسات (طول الساق والجذر كذلك الوزن الجاف والرطب لكلا من الساق والجذر, معدل ثبات الاغشية, المحتوى من الكلوروفيل, الكاروتينويد و تعبير اعلى لمضادات الاكسدة الانزيمية تحت مستوى عال من الملوحة (175mM) مقارنة بالصنف الصينى. ايضا قياسات النمو (طول الساق والجذر كذلك الوزن الجاف والرطب لكلا من الساق والجذر) ارتفعت عند المعاملة بالأكسين. المعاملة بالأوكسين لكلا الصنفين تحت ظروف الاجهاد الملحى حسنت من تحمل النبات لظروف الاجهاد من خلال تحسين المحتوى المائى النسبى, مستوى ثبات الاغشية و تحفيز تخليق الكلوروفيل والكاروتينويد و بعض مضادات الاكسدة الانزيمية والذى انعكس على زيادة جميع قياسات النمو للنبات وكذلك ادائة الفسيولوجى. وهكذا فان الهرمون موضع الاختبارقلل من الآثار الضارة للملوحة على صنفين من عباد الشمس, والتي يمكن اعتمادها كمنظمات للنمو أو المواد المضادة للاكسدة والتى يمكن استعمالها فى تحسين نمو النبات تحت ظروف الاجهاد الملحى.