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ALLEVIATION THE HARMFUL EFFECT OF SOIL SALT STRESS ON MAIZE PLANT BY USING SOME APPLIED ANTIOXIDANTS.

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ABSTRACT

Two field experiments were performed at Tag El-Ezz Research Station, Agric. Res. Center., Ministry of Agric. Egypt. to investigate the role of selected antioxidants on mitigation or alleviation the harmful effect of soil salt stress on maize plant. Soil salt stress in the first area (A_1) equal 1840 mgl' (2.9 dsm-1); the second salt soil area (A_2) was 6080 mgl' (9.5 dsm-1). Applied antioxidants (Ascorbic, α-Tocopherol, Humic, Seaweed extract and Salicylic) significantly increased all growth characters and yield and its components of maize plant compared with untreated plants in the two soils salt areas (A_1 and A_2) during the two growing seasons. The data also show that applied antioxidant materials were more effective in salt soil area (A_1). The data also show that applied antioxidant materials could alleviate the harmful effect of high soil salt stress levels on growth, yield and its components of maize plant. ASA and SA were more effective in this respect.
Keywords: Antioxidants, Salinity, ASA, SA, SWE, HA, Maize.

INTRODUCTION

Zea maiz is an important crop and its yield is greatly affected by different biotic and abiotic stresses. It could be counteract the harmful effect of these stresses especially salt stress by using some natural applied antioxidants.

Zörb *et al.* (2004) found that corn growth decreased, amounts of proline, Na^+, Na^+/K^+ ratio and the leaf osmolality increased with increasing salinity stress.

Hema *et al.* (2003) stated that hydrogen peroxide and lipid hydroperoxides, reactive oxygen species, are also generated under stress condition. Plants protect themselves from ion toxicity by minimizing toxic ions uptake and transport to the shoots. Potential is accomplished by the accumulation of organic metabolites and/or inorganic ions, which decreased the osmotic potential of the plant (Carvajal *et al.* 2000).

It could alleviate the harmful effect of salinity stress by using some antioxidant materials.

Salicylic acid (SA), a plant phenolic is now considered as a hormone-like endogenous regulator, and its role in the defense mechanisms against biotic and a biotic stresses has been well documented (Szalai *et al.* 2005). Arfan *et al.* (2006) stated that exogenous application of SA promoted growth and yield, and counteracted the salt stress-induced growth inhibition of salt tolerant. The improvement in growth and grain yield of wheat due to SA application was associated with improved photosynthetic capacity and activation of the antioxidative enzymes and accumulation of ionic and non-ionic osmolytes.

Asada (1994) found that ascorbate has been shown to have important functions in photosynthesis, such as in protection of photosynthetic apparatus against the oxygen radicals and H_2O_2 that formed during photosynthetic activity, and against photo inactivation since it is a cofactor of carotenoid de-epoxidation.

Antioxidant Seaweed extracts (SWE) contain not only most of the major and minor nutrients, amino acids, and vitamins B_1, B_2, C, E, but also cytokinins, auxin, GAs, and ABA-like growth substances (Abetz 1980). It could be concluded that biostimulants (SWE) and Humic acid (HA) can alleviate the harmful effect of salinity or drought stress through: (I) Activating root cells (Schmidt 2005). (2) Altering hormonal balances and favor cytokinins and auxins production (Schmidt 2005). (3) Stimulation the biosynthesis of Tocopherol, ascorbic acid and carotenoids in chloroplast that protect photosynthetic apparatus of PSII (Zhang and Schmidt 2000). (4) Protection of plant cells from lipid peroxidation (Smirnoff 1995). (5) Reducing uptake of NaCl (Nabati 1994). (6) Inhibits activity of free radical groups (Fletcher *et al.* 1988). (7) Stimulation the uptake of N, P, K, Mg, Ca, Zn, Fe and Cu by the seedlings. (8) Promoting the accumulation of reducing sugars (O'Donnell 1973).

It could be concluded that some of biological benefits of antioxidant humic acid are: (1). Stimulates plant growth by accelerating cell division (Clapp *et al.* 2002). (2). Increases vitamin content of plants (Ferrini and Nicese 2002). (3). Increases the permeability of plant membranes, promoting the uptake of nutrients (Mackowiak *et al.* 2001). (4). Stimulates plant enzymes.

The aim of this work is treating corn plant by some applied antioxidants to alleviate the harmful effect of salt soil stress on growth and yield of corn plant.

MATERIALS AND METHODS

Two field experiments were performed at Tag El-Ezz Research Station, Dakahlia Governorate, Agric. Res. Center., Ministry of Agric. Egypt. during the successive summer seasons of 2006 and 2007 to investigate the role of selected antioxidants on mitigation or alleviation the harmful effect of soil salt stress on maize plant.

Maize grains (cv Single Cross 122) kindly were supplied by plant breeding section, Field Agric. Res. Center., Ministry of Agric. Giza, Egypt. Two different soil areas differ in their soil salt stress were chosen. Soil salt stress in the first area (A_1) equal 1840 mgl- (2.9 dsm-1); the second salt soil area (A_2) was 6080 mgl- (9.5 dsm-1). Each area was divided into five groups represented by the different applied antioxidants. Uniform grains were presoaked for 6 hours before sowing in any of antioxidants i.e. Ascorbic acid (250 mg/l)., α –Tocopherol (250 mg/l)., Humic acid (1000 mg/l)., Seaweed extract (1000 mg/l) and Salicylic acid (250 mg/l) as well as tap water. Uniform presoaked maize grains were sown on 15th May 2006 and 2007 in the two different soil salt areas.

All the other culture practices of growing maize plants were kept the same as normally practiced in maize fields according to recommendation of Ministry of Agriculture and Land reclamation.

The maize plants were sprayed with the same antioxidant concentrations at 30, 45 and 60 days from sowing. Automatic atomizers were used for spraying the applied antioxidants after adding tween 20 as a wetting agent" (0.05 %). Growth parameters were measured at stage (75 days after sowing) and at harvesting stage, yield and its components were recorded. Each treatment replicated 3 times and arranged in a complete randomized block design.

Statistical analysis:

The dates of all experiments were statistically analyzed as technique of the analysis of variance (ANOVA) according to **Gomez and Gomez (1984).** The treatment means were compared using the least significant differences (LSD).

RESULTS

1- Growth of maize plant:

The data in table (1) show that any of applied antioxidants (Ascorbic, α-Tocopherol, Humic, Seaweed extract and Salicylic) significantly increased all growth characters of maize plant compared with untreated plants in the two soil salt areas (A1 and A2) during the two growing seasons (2006 and 2007). The data also show that applied antioxidant materials were more effective in salt soil area (A1).

It could be concluded that, the different applied antioxidant materials could partially counteract the harmful effect of high soil salt stress levels on growth of maize plant. However Ascorbic acid (ASA) and Salicylic acid (SA) were more effective in this respect. The same results were obtained from pot experiment.

2- Yield and its components of maize plant:

The data in table (2) show that applied antioxidants significantly increased yield and its components (as ear length, ear diameter, number of rows/ ear, number of grains/ row and grain yield) in the two salt soil areas especially (A1) compared with the untreated plants throughout the two growing seasons.

The data also show that applied antioxidant materials could alleviate the harmful effect of high soil salt stress levels on growth, yield and its components of maize plant. ASA and SA were more effective in this respect. It could be observed that this obtained data was taking the same trend of pot experiment.

Table (1): Effect of exogenous applied antioxidants on growth of maize plant under high soil salt stress conditions (1840& 6080 mgl^{-1}) during the two growing seasons (2006 & 2007).

Parameters Treatments mgl^{-1}	Plant height (cm)	Leaves number	Leaves area (cm^2)	Leaves f.wt (gm/plant)	Leaves d.wt (gm/plant)	Stem f.wt (gm/plant)	Stem d.wt (gm/plant)
First season							
A1 + tap water	191	11.3	542	92.3	41.6	217.4	34.5
A1 + ASA (250)	220	13.3	711	115.8	51.6	227.4	40.5
A1 + Tocoph (250)	210	12.7	611	104.0	49.7	260.2	36.3
A1 + HA (1000)	209	12.3	575	102.9	48.0	236.4	35.8
A1 + SWE (1000)	200	12.0	549	98.5	46.8	233.9	34.5
A1 + SA (250)	220	13.3	698	106.9	50.7	263.1	37.1
LSD at 5%	5.90	N.S	15.78	3.78	N.S	N.S	1.71
A2 + tap water	101	8.7	271	43.3	25.8	71.7	17.8
A2 + ASA (250)	129	10.3	418	61.6	31.7	119.8	21.9
A2 + Tocoph (250)	114	10.0	344	53.3	29.9	98.1	20.6
A2 + HA (1000)	110	9.7	337	51.6	28.3	96.1	19.6
A2 + SWE (1000)	107	9.3	310	47.0	27.6	84.4	18.9
A2 + SA (250)	119	10.0	386	57.0	30.8	109.7	21.3
LSD at 5%	8.55	N.S	13.10	3.19	0.9	N.S	N.S
Second season							
A1 + tap water	194	11.7	552	83.7	43.4	231.0	40.3
A1 + ASA (250)	226	13.7	718	112.6	53.4	283.8	54.2
A1 + Tocoph (250)	212	12.7	633	98.9	50.2	265.2	46.5
A1 + HA (1000)	210	12.0	587	94.0	47.9	250.6	42.3
A1 + SWE (1000)	202	12.0	570	89.5	46.2	238.9	41.8
A1 + SA (250)	223	13.3	710	102.7	52.1	271.1	49.8
LSD at 5%	2.65	N.S	N.S	N.S	0.53	3.99	0.98
A2 + tap water	104	8.3	287	44.4	26.8	81.8	18.2
A2 + ASA (250)	133	10.3	420	66.8	32.4	125.3	24.5
A2 + Tocoph (250)	115	9.3	347	56.1	30.2	100.5	21.0
A2 + HA (1000)	113	9.0	340	52.2	28.6	98.1	19.8
A2 + SWE (1000)	108	9.0	313	50.9	27.6	87.7	19.2
A2 + SA (250)	125	10.0	390	62.1	31.4	119.1	22.7
LSD at 5%	2.50	N.S	11.79	3.0	0.46	3.0	0.75

ASA: ascorbic acid. Tocoph: tocopherol. HA: humic acid. SWE: seaweed extract. SA: salicylic acid.
A1: area 1 (Salinity 1840 mgl^{-1} (2.9 dsm^{-1}), A2: area 2 (Salinity 6080 mgl^{-1} (9.5 dsm^{-1}).

Table (2): Effect of exogenous applied antioxidants on yield (after 75 days from sowing) of maize plant under high soil salt stress conditions (1840& 6080 mgl^{-1}) during the two growing seasons (2006 & 2007).

Parameters Treatments mgl^{-1}	Ear length	Ear diameter	No of rows/ear	No of grains/row	Grain yield (ardb/fad)
First season					
A1 + tap water	13.1	4.0	11.7	36.2	12.8
A1 + ASA (250)	17.7	4.8	13.6	42.4	20.1
A1 + Tocoph (250)	16.0	4.3	12.5	39.2	16.9
A1 + HA (1000)	15.4	4.2	12.2	38.9	15.1
A1 + SWE (1000)	13.9	4.1	12.1	37.7	14.6
A1 + SA (250)	16.8	4.6	13.3	40.6	18.3
LSD at 5%	0.44	N.S	0.34	1.15	0.70
A2 + tap water	7.4	2.6	7.7	19.9	7.7
A2 + ASA (250)	9.9	3.2	10.0	29.5	10.0
A2 + Tocoph (250)	8.8	2.9	9.2	24.3	8.8
A2 + HA (1000)	8.3	2.8	8.7	23.4	8.8
A2 + SWE (1000)	7.7	2.7	8.2	22.0	8.2
A2 + SA (250)	9.5	3.0	10.0	24.7	9.7
LSD at 5%	0.56	N.S	0.38	1.11	0.68
Second season					
A1 + tap water	13.2	4.0	12.5	37.3	13.4
A1 + ASA (250)	17.0	4.9	13.9	34.8	20.5
A1 + Tocoph (250)	16.4	4.4	13.0	40.9	17.8
A1 + HA (1000)	15.1	4.3	12.7	39.8	15.8
A1 + SWE (1000)	14.3	4.1	12.6	38.8	14.9
A1 + SA (250)	17.1	4.7	13.2	41.9	19.8
LSD at 5%	0.23	N.S	0.16	0.58	0.37
A2 + tap water	7.5	2.8	7.9	21.7	8.1
A2 + ASA (250)	10.2	3.4	10.3	30.7	10.3
A2 + Tocoph (250)	8.9	3.0	9.5	25.9	9.3
A2 + HA (1000)	8.4	2.8	8.7	24.1	8.9
A2 + SWE (1000)	7.8	2.7	8.4	23.0	8.2
A2 + SA (250)	9.7	3.2	10.1	28.4	10.0
LSD at 5%	0.27	N.S	0.20	0.52	0.41

ASA: ascorbic acid. Tocoph: tocopherol. HA: humic acid. SWE: seaweed extract. SA: salicylic acid. A1: area 1 (Salinity 1840 mgl^{-1} (2.9 dsm^{-1}), A2: area 2 (Salinity 6080 mgl^{-1}(9.5 dsm^{-1}).

DISCUSSION

The inhibitory effect of salt soil stress areas (A1 and A2) on corn growth in the present investigation may be due to a decrease in water absorption, metabolic processes, merestematic activity and/or cell enlargement (Khadr *et al.*, 1994 and Sakr *et al.*, 2010) or by damaging growth cells so that they can not perform their functions (Chen and Murata 2002). Moreover, the decrease in growth due to salinity may be attributed to an increase in respiration rate resulting from higher energy requirements. Yang *et al.* (1990) reported that there are two ways that salinity could retard growth (a) by damaging growth cells so that they cannot perform their functions or (b) by limiting their supply of essential metabolites.

The reduction in grain yield caused by salt soil stress in the two areas (A1 and A2) is largely due to (1) reduction in pollen viability has been related to decreased calcium mobilization from plant leaves treated with sodium chloride, which is important in pollen germination and pollen tube growth. (2) abscission of flowers or young fruit due to ethylene induction by salinity. (3) moreover, decreasing production pollen grain, mean number of perfect flowers, and fruit set. (4) decreasing the leaf area and number per plant, resulting reduction in the supply of carbon assimilate due to decreasing the net photosynthetic rate and biomass accumulation (Sakr *et al.,* 2007).

As for SA it could be concluded that this antioxidant can alleviate the harmful effect of ROS caused by soil salt stress through (1) accumulation of inorganic or organic osmolytes makes the surplus of water uptake possible as it can also be seen from the increased relative water contents of tissues (Szepesi *et al.*, 2005). (2) decreased the Na^+/K^+ ratio in the roots and increased it significantly in the leaves. (3) improved the photosynthetic performance of plants under stress conditions (Ananieva *et al.,* 2004). (4) accumulated different compatible osmolytes, such as sugars, sugar alcohol, proline, superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) (Sakhabutdinova *et al.* 2003). (5) increased the level of reduced glutathione (GSH) with an increase in the ratio of reduced to oxidised glutathione (GSSG) indicating higher antioxidant potential [Srivastava and Dwivedi 1998]. (6) it prevented any decrease in IAA and cytokinin contents and thus reduced stress-induced inhibition of plant growth. (7) induced activation of the division of root meristem cells, which contributes to an SA-induced growth of the maize (Kuznetsov and Shevyakova 1999). (8) accumulation of this amino acid under stress through maintaining an enhanced level of ABA in the seedlings (Ervin 2005).

As for biostimulants (SWE), it could be concluded that SWE can alleviate the harmful effect of soil salt stress through: I)- activate root cells at the same time stimulate biosynthesis of endogenous cytokinins from roots (Schmidt 2005). II)- enhancing leaf water status, some plant nutrients uptake, shoot growth and root pull strength (Demir *et al.,* 2004). III)- altering hormonal balances and favor cytokinins and auxins production (Schmidt 2005). IV)- enhancement of antioxidant enzymes (SOD, GR, ASP) for protection against adverse environmental conditions (Schmidt, 2005). V)- stimulation the biosynthesis of Tocopherol, ascorbic acid and carotenoids in chloroplast which protect photosynthetic apparatus of PSII (Zhang and Schmidt 2000). VI)- protection of plant cells from lipid peroxidation and inactivation of enzymes that occur under stress (Smirnoff 1995). VII)- stimulation stem elongation and exhibits auxin-like activity (Crouch and Van-Staden 1993). VIII)- reduced uptake of NaCl (Nabati 1994) while increased K and Ca content in the leaves (Demir *et al.*, 2004). IX)- stimulation of chlorophyll biosynthesis (Garbay and Churin 1996) and regulation cell membrane components under drought stress (Yan 1993). X)- inhibits activity of free radical groups which are major elements for chlorophyll degradation (Fletcher *et al.* 1988). XI)- stimulation the uptake of N, P, K, Mg, Ca, Zn, Fe and Cu by the plants that alleviate the inhibitory effect of Na toxicity and restored growth

(Nelson and Van-Staden 1984). XII)- promoted the accumulation of reducing sugars which increased wilting resistance through enhancing osmotic pressure inside plant. In addition nucleic acids metabolism were stimulated (O'Donnell 1973). XIII)- Stimulation of chloroplast development and enhancing phloem loading and delay senescence (Demir *et al.*, 2004).

The enhancing effect of Humic acid on alleviation salinity or drought stress may be through (1). Stimulates plant growth by accelerating cell division, increasing the rate of development in root systems, and increasing the yield of dry matter (Clapp *et al.*, 2002). (2). Increases germination of seed and viability (Clapp *et al.*, 2006). (3). Increases vitamin content of plants (Ferrini and Nicese 2002). (4). Increases the permeability of plant membranes; promoting the uptake of nutrients N, P, K, Ca and Mg (Mackowiak *et al.*, 2001). (5). Stimulates root growth, especially lengthwise (Tan and Nopamornbod 2005). (6). Increases root respiration and formation (Hopkins and Stark 2003). (7). Stimulates growth and proliferation of desirable soil micro-organisms as well as algae and yeast (*Neri et al.*, 2006). (8). Aids in photosynthesis. (9). Stimulates plant enzymes. (10). Acts as an organic catalyst. (11). One reason is that humic acids permanently tie up the sodium ion. This helps plants tolerate the higher sodium concentrations, avoiding toxicity and osmotic related problems. (12). Chelates nutrients (Super-Grow 2006). (13). Enhances root development (Vaughan and Macdonald 2005). (14). Improves plant vigor and appearance (Obatolu 2006). (15). Promotes thatch decomposition (Ozdoba 2006). (16). Reduces chemical fertilizer use (Vladimir Vaslenko 2002). (17). Better resistance to stress (Clapp *et al.*, 2006).

Applications of HA improves plant cell permeability. This means that the plant cell absorbs more nutrients so fulvic acid is a great additive to fertilizers. (Tattini *et al.*, 1991).

As for α-Tocopherol, ascorbic acid and glutathione, it could be concluded that, these plant antioxidants can alleviate the harmful effect of reactive oxygen species (ROS) caused by salt soil stress (1840 and 6080 mgl^{-1}) through several ways such as :(1) inhibiting the lipid photoperoxidation (Thomas *et al.*, 1992) (2) involving in both electron transport of PSII and antioxidizing system of chloroplasts. (3) as membrane stabilizers and multifaceted antioxidants, that scavenge oxygen free radicals, lipid peroxy radicals and singlet oxygen (Diplock *et al.*, 1989). (4) reacting with peroxyl radicals formed in the bilayer as they diffuse to the aqueous phase . (5) scavenging cytotoxic H_2O_2 and reacts non-enzymatically with other ROS: singlet oxygen, superoxide radical and hydroxyl radical (Blokhina *et al.*, 2002). (6) regenerating another powerful water-soluble antioxidant, ascorbic acid, via the ascorbate–glutathione cycle. (7) stabilize membrane structures (Blokhina *et al.*, 2002). (8) modulating membrane fluidity in a similar manner to cholesterol, and also membrane permeability to small ions and molecules (Foyer 1992). (9) decreasing the permeability of digalactosyldiacylglycerol vesicles for glucose and protons (Berglund *et al.*, 1999).

Generally, it could be concluded that tocopherol, ascorbic, SA, SWE and HA can help to alleviate the harmful effect of ROS may be through several ways such as: (1) inhibits the lipid photoperoxidation (Thomas *et al.*, 1992). (2) involves in both electron transport of PSII and antioxidizing system of chloroplasts (Thomas *et al.* 1992). (3) membrane stabilizers (Thomas *et al.*, 1992). (4) can react with peroxyl radicals (Sairam and Servastava 2002). (5) It scavenges cytotoxic H_2O_2 and reacts non-enzymatically with other ROS (Sairam and Servastava 2002). (6) regenerate another powerful water-soluble antioxidant, ascorbic acid (Blokhina *et al.*, 2002). (7) stabilize membrane structures (Blokhina *et al.*, 2002). (8) modulates membrane fluidity in a similar manner to cholesterol, and also membrane permeability to small ions and molecules (Foyer 1992). (9) decrease the permeability of digalactosyldiacylglycerol vesicles for glucose and protons (Berglund *et al.* 1999).

From the above mentioned results it could be noticed that the applied antioxidants could alleviate or minimize the harmful effect of Nacl salinity on Zea maize plant growth and ascorbic proved to be more effective in this respect.

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التغلب على الآثار الضارة لاجهاد ملوحة التربة على نبات الذرة باستخدام المواد المصادة للاكسدة

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أجريت تجربتان حقليتان فى محطة بحوث تاج العز التابعة لمركز البحوث الزراعية خلال موسمى ٢٠٠٦، ٢٠٠٧ لدراسة دور عدة مواد من مضادات الاكسدة فى التغلب على الاثار الضارة لمستويات الاجهاد الملحى على نبات الذرة الشامية هجين فردى ١٢٢.

أظهرت النتائج أن اضافة المواد المضادة للاكسدة (حمض الاسكوربيك – التوكوفيرول – حمض الهيوميك – مستخلص أعشاب البحر– حمض السالسيليك) أدت الى زيادة معنوية لجميع صفات النمو والمحصول ومكوناته بالمقارنة بالنباتات الغير معاملة فى مستويات الاجهاد الملحى ١، ٢ .

أوضحت النتائج أن المواد المضادة للاكسدة المضافة كانت أكثر تأثيرا فى مستوى الملوحة الاول و كانت معاملات الاسكوربيك والسالسيليك هى الاكثر تأثيرا و فاعلية فى هذا الشأن.

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