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ALLEVIATION OF THE ADVERSE EFFECTS OF SALT STRESS ON WHEAT PLANTS

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ABSTRACT

A pot experiment was conducted to investigate the effect of soaking grains of wheat plants with ascorbic acid in alleviation of the adverse effect of salt stress on wheat growth in parallel with addition of compost rates under three levels of different soil salinity (6, 9 and 12 dS m^{-1}), the grains of wheat divided into two portions, each partition soaked in one only from aqueous solutions, distilled water or 100 mg L^{-1} of ascorbic acid (AsA). Compost manure was mixed with soil at rates of 0, 100 and 200 g pot^{-1}.The results showed a significantly increase in germination percentage up to 46% with used aqueous ascorbic acid solution compared with distilled water. Furthermore, the results proved that AsA partially counteracted the harmful effect of salinity.

Our results indicated that all parameters studied of wheat growth i.e., plant height, number of tillers, number of spikes pot^{-1} and spike length and yield components i.e., wheat thousand grain weight (g), grain yields (g pot^{-1}) and straw yields (g pot^{-1}) as well as dry weight were improved significantly with used aqueous ascorbic acid solution (AsA) as a cultural practice for grains soaking before sowing, this improving gradually reduction with increasing soil salinity but by little degree compared with distilled water. This result evident that ascorbic acid plays a role in the regulation of a number of metabolic processes which were counteracts the harmful effect of salinity, this roll is more effecting with increasing level of organic mater in soil.
Keywords: Soaking, Ascorbic Acid, Wheat, Saline Soil and compost.

INTRODUCTION

Wheat is a staple food in Egypt. Raising wheat production through increasing its cultivated area. Increasing wheat yield per unit area can be achieved by breeding high yielding varieties. Salinity is considered of the major obstruct in North Sinai to increase wheat production. However, we cannot erase salinity but we can accommodate with it using different cultural practices, land smoothing, grading seed bed improvements, irrigation intervals, sowing methods and limited quantity and quality of organic, inorganic and micronutrients cultivation selection.

Recently more of literature studied the agronomic practices as conceptual methods with lowest costs to combat the negative effect of soil salinity stress on plant growth and productivity, among them the use of foliar sprays to increase plant tolerance to salinity. Many works indicated that applying nutrients by foliar application increases tolerance of growing plants to salinity by alleviating Na^+ and Cl^- injury to plants (El-Fouly *et al*., 2002 and El-Fouly *et al*., 2004). Also (Kadria El azab *et al*., 2011) found that application of organic substances such as soaking water extract of compost and humic acid achieve many of the beneficial effects on soil hydrophysical properties and fertility status as well as grown plant parameters under saline soil. Other study by (Amer *et al*., 2011) on applied the raised beds as cultural practice for combating salinity stress Thir results indicated that the soil chemicals properties were improved under Raised beds conditions which have positive effect on the plant health and production compared with traditional system (Furrow row), particularly in the root zone.

Ascorbic acid (AsA) is involved in the regulation of many critical biological processes such as photo-inhibition and cell elongation (Noctor *et al*., 1998). AsA also is involved in cell cycle and many other important enzymatic reactions (biosynthesis of ethylene, for example) (Smirnoff, 2000). The effect of AsA on the content of Proline and soluble sugars can suggest that AsA probably improves growth of stressed plants, further to its antioxidant action, by intensification of their potential for osmotic adjustment and activities of growth (cell division and expansion). Indeed, several studies have shown that AsA plays an important role in improving plant tolerance to abiotic stress (Shalata and Neumann, 2001; Al-Hakimi and Hamada, 2001; Athara *et al*., 2008). One approach for inducing oxidative stress tolerance would be to increase the cellular level of enzyme substrates such as ascorbic acid (vitamin C, AsA). AsA is a small, water-soluble antioxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide (Beltagi, 2008).

The promotive effect of AsA on seedling, stem and root lengths may be the result of increasing cell division and cell enlargement due to water uptake caused by a decrease in the osmotic potential by increasing soluble sugars which serve as a substrate for increasing initiation of leaf primordial. Furthermore, the present results indicate that AsA applications counteracted the deleterious effects of salt stress on leaf structure which may be due to a reduction in stomatal opening leading to decrease in the transpiration (Arafa *et al*., 2009), on the other hand, they found that ascorbic acid in combination with salinity increased germination percentage and ascorbic acid was more effective in this respect. Concerning leaf anatomy it was found that low salinity level (1500 ppm NaCl) increased the blade thickness, xylem and phloem tissues thickness and metaxylem vessel diameter as well as main vascular bundle dimensions. At the same time, moderate and high salinity levels (3000 and 6000 ppm NaCl) decreased all these parameters. The great reduction was observed under high salinity level. Application of ascorbic acid through seed soaking enhanced the growth of plants, increased the shoot, root fresh and dry weight. Sodium toxicity was reduced by excluding the sodium from the roots. Chlorophylls contents in *Brassica campestris* s' plants were increased by applying the ascorbic acid as seed soaking. Among all these concentrations (0, 50, 100 mg/L) of ascorbic acid, 100 mg/L was more effective in reducing the salinity stress in *Brassica campestris* s' plants, (Khan *et al*., 2010).

Previous studies showed that the combination of compost with chemical fertilizer further enhanced the biomass and grain yield of crops (Sarwar *et al*., 2008). Moreover, organic manure plays an adaptive role in the tolerance of plant cell to salinity by increasing soil organic matter and hence improve their physical properties which intern improve plant roots growth also the chemical properties, the status of essential nutrients and soil microbial activity (EL-Emam, 1999). In addition to, Mahrous *et al*., (2010), founded that compost can improve the bio- chemicals media soil and at same time increasing wheat yield more than the additional mineral fertilization alone. Matter *et al*., (2007) reported that grain yield of wheat cultivar Sakha-93 increased by using organic fertilizer. The highest wheat grain yield was obtained with treatment consisted of 1.2 : 0.66 : 1.5 : 2.5 ton/fad. from farmyard manure, chicken manure, town refuse and sewage sludge, respectively.

From the earlier mentioned reports it is evident that ascorbic acid plays a role in the regulation of a number of metabolic processes in plants exposed to salt stress. However, information on how ascorbic acid regulates physiological/biochemical processes in wheat plants subjected to salt stress is not much available in the literature. Thus, the main objective of the present study was beneficial from this available information to soaking wheat seed in aqueous AsA solution pre-sowing as a seasonal cultural practice with compost rates addition to alleviate the adverse effects of salt on wheat plant growth and yield under saline soils.

MATERIALS AND METHODS

A pot experiment was conducted to study the effectiveness of the combination treatment between compost and ascorbic acid, for improving the growth and yield of wheat plants under different levels of salinity stress. The soils used for this experiment were sandy loam (5.8, 53.5, 32.6 and 8.1% C.Sand, F.Sand Silt and Clay, respectively), it were collected from three different locations of salinity, at the El Tena plain, North Sina, air dried, sieved (2 mm mesh) and analyzed. Some physical and chemical properties according to the described standard methods after Black *et al*., (1982), Page *et al*., (1982) and Klute (1986), are presented in Table (1).

Table(1): Some physicochemical properties of the experimental soil before sowing.

Locations	pH (1:2.5)	EC (dSm^{-1})	OM (%)	$CaCO_3$ (%)	Available Macronutrients (mg kg^{-1} Soil)			Soluble Ions (m mole/L)						
								Anions				Cations		
					N	P	K	Na^+	K^+	Ca^{++}	Mg^{++}	$HCO_3^=$	Cl^-	$SO_4^=$
1	7.97	6.5	0.72	7.2	44.0	6.2	185.0	21.64	3.63	25.00	24.29	1.66	25.34	47.56
2	8.20	9.3	0.85	7.6	46.5	6.1	191.0	49.47	4.34	33.25	24.60	2.56	61.53	47.58
3	8.12	12.7	0.78	12.2	45.0	6.3	189.0	63.43	4.54	41.00	44.26	2.27	96.72	58.24

plastic pots (30 cm inner diameter) were filled with sieved soil (15 kg pot^{-1}) and divided into three groups according to the levels of salinity; (6, 9 and 12 dS m^{-1}), compost used was analyzed for carbon content, (C/N ratio = 22.5) (Nelson & Sommers, 1996), and macronutrients, 1.83, 0.88 and 2.23 % for NPK respectively, (Ryan *et al*., 2001), as well as mixed with soil before filling at a rates of zero, 100 and 200 g pot^{-1}.

Before sowing, Calcium super phosphate (15.5% P_2O_5) and Potassium sulfate (48% K_2O) fertilizers were added at the recommended dose (5 g P_2O_5 and 2 g K_2O pot^{-1}). The grains of wheat divided into two portions, ½ soaked with distilled water and the second ½ soaked with 100 mg L^{-1} of ascorbic acid (AsA) for 6 hour before sowing. Fifteen uniform wheat grains (*Triticum aestivum* L.) (Sakha 93 cultivar) were sown in each pot. After 8 days from sowing the germination percentage (%) was recorded and thinned to 20 plants. Ammonium sulfate (20.5%) was added at a rate of 4 g N pot^{-1}, in two equal portions after 25 and 50 days from planting. Tab water was used for irrigation throughout the experimental period and adjusted regularly near to the field capacity (saturation percentage of 33 %.). The pots were arranged in a split-split design with three replicates. The main treatments were soaked for wheat seeds, sub treatments were levels of soil salinity and the sub sub treatments were the rats of compost.

At the end of the growing seasons, all wheat plants were harvested from each experimental pots for agronomic properties i.e., plant height, number of tillers, number of spikes pot^{-1}, spike length, thousand grain weight (g), dry weight (g pot^{-1}), grain yields (g pot^{-1}) and straw yields (g pot^{-1}), grains sample of wheat plants were dried at 70C$^{\circ}$, crushed and wet digested using mixture of H_2SO_4 + $HClO_4$ acids to determine nutrient contents in aliquots of the digested solutions, *i.e*., N.P.K.(%) (Ryan *et al*., 1996). Soil samples were collected from each experimental pot and prepared for analysis; available nutrients; N which were extracted using K_2SO_4 1% according to the method described by Jackson (1973), and measured according to the modified Kjeldahal method. Also, available P and K were determined by extracting the soil with ammonium bicarbonate-DTPA according to Soltan pour (1985). The obtained data were exposed to proper statistical analysis of variance (ANOVA) by using Minitab computer program and least significant difference (L.S.D) were calculated at level of 5% (Barbara and Brain, 1994).

RESULTS AND DISCUSSION

Data illustrated in Table (2) indicated that wheat agronomic properties were affected significantly with treatments used, sowing seed without soaking was highly decreased the germination percentage up to 0.0% particularly at highest level of salinity (12 dS m^{-1}), this results agreement with Chauban and Sing (1993) who stated that seed germination percentage increased at 6000 ppm for wheat cv Sakha 92. Meanwhile, salinity at 7000 ppm decreased the germination percentage and increased the number of days required for germination but salinity at concentration more than 7000 ppm (8000, 9000, 10000 ppm) completely inhibited seed germination in Sakha 92. Thus, we didn’t need to present these low results which nearing to zero at highest salinity. For this reason, our mains treatments in Table (2) presented only the comparison between solutions of soaking with interactions different levels from salinity and organic matter. The results showed significantly increasing up to 46% in seed germination percentage when soaked in aqueous ascorbic acid solution (AsA) compared with soaking in distilled water for the same time (6 hours).

On the other hand, both distilled water and AsA increased germination percentage under low, moderate and high salinity, but this increasing whereas decreased gradually with increasing soil salinity levels, which salinity stress can affect seed germination through reduction of water uptake leading to moisture stress (osmotic effect), by ion toxicity and/or ionic imbalance, or by the accumulation of Na^{+} and Cl^{-} ions and inhibition of the uptake of several essential nutrients such as K^{+} causing nutritional imbalance in the plants or accumulation of these factors (Taamalli *et al*., 2004) and/or decreasing the activity of certain enzymes by either decreasing the rate of transcription or translation, which lead to decreasing both cell division and cell elongation (Dodd and Donovan, 1999). The recorded values for germination percentage under salinity levels (6, 9 and 12 dS m^{-1}) were 56, 50, 42% and 85, 79, 74% for soaked solutions in distilled water or AsA respectively. Furthermore, AsA was more effective than distilled water in increasing germination percentage of wheat grains. The results proved that AsA partially counteracted the harmful effect of salinity.

Table (2): Effect of applied treatments on wheat growth parameters and Yield Components. (Mean Values)

Treatments			Parameters of wheat growth				Yield Components		
Solution of soaking	Salinity of soil used (dS m^{-1})	Compost rates (g pot^{-1})	Germenation %	Number of tillers pot^{-1}	Plant height (cm)	Number of spikes pot^{-1}	Grain yields (g pot-1)	Straw yields (g pot-1)	Thousand grain weight (g)
Soaking by water	6	0	47.3	25.0	50.2	20.0	26.41	78.59	31.43
		100	56.0	37.0	60.4	29.6	42.33	126.00	34.07
		200	63.3	48.7	62.5	38.9	47.65	142.02	37.20
	9	0	40.7	19.7	46.7	15.9	22.62	75.38	30.83
		100	52.7	27.7	56.0	22.1	33.63	101.37	33.03
		200	56.7	34.0	59.0	27.2	37.13	108.21	34.10
	12	0	26.7	14.0	34.0	11.7	13.18	43.49	26.17
		100	46.7	25.0	51.0	20.0	27.01	81.99	31.40
		200	52.0	28.7	54.7	22.9	26.80	87.54	32.50
Soaking by AsA	6	0	61.3	37.3	53.7	29.9	32.30	97.70	32.67
		100	83.3	48.7	65.6	38.9	47.74	145.26	40.90
		200	92.0	56.7	70.3	45.3	54.54	164.79	44.63
	9	0	51.3	30.0	48.3	24.0	24.52	82.15	31.00
		100	79.3	42.3	59.0	33.9	41.60	136.40	39.63
		200	84.7	47.3	63.7	37.9	49.58	146.42	40.93
	12	0	36.7	23.0	36.7	18.4	21.61	61.59	27.33
		100	76.0	31.3	56.0	25.1	35.97	91.53	37.80
		200	80.7	33.0	57.4	26.4	35.00	107.66	38.97
Statistical Analysis									
LSD at (5%)	Soaking (A)		**1.3**	**1.6**	**0.9**	**1.2**	**1.55**	**4.44**	**0.73**
	Salinity (B)		**1.6**	**1.9**	**1.1**	**1.5**	**1.90**	**5.44**	**0.90**
	Compost (C)		**1.6**	**1.9**	**1.1**	**1.5**	**1.90**	**5.44**	**0.90**
	A*B		**ns**	**2.7**	**1.6**	**2.2**	**ns**	**ns**	**ns**
	A*C		**2.3**	**ns**	**ns**	**ns**	**ns**	**ns**	**1.3**
	B*C		**2.8**	**3.3**	**1.9**	**2.6**	**3.30**	**9.42**	**1.56**
	A*B*C		**ns**	**ns**	**ns**	**ns**	**ns**	**13.32**	**ns**

For all the above discussions, the results obtained in Table (2) indicated that all wheat growth parameters and yield components were significantly improved with used aqueous ascorbic acid solution (AsA) as a cultural practice for grains soaking before sowing, this improving gradually reduction with increasing salinity soil but by little degree compared with using distilled water for soaking. This result evident that ascorbic acid plays a role in the regulation of a number of metabolic processes which are alleviate of the adverse effects of salt stress on plant growth (Khan *et al*., 2010). On the other hand, this role is more effecting with increasing level of organic mater in soil. For example, the results of dry weight at harvest appeared the same trend, which the figure (1_a) appear the positive effect of AsA on dry weight compared with distilled water as a soaked solutions although gradually decreased with increasing soil salinity.

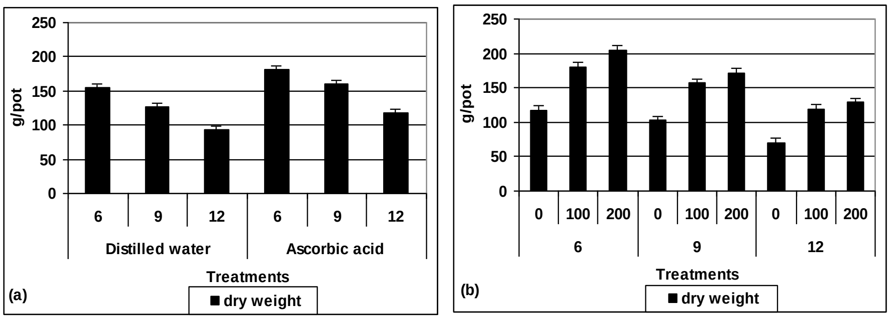

Figure (1): Effect of applied treatments on dry weight of wheat (Mean Values)

On the other hand, the figure (1_b) appear the positive effect of compost addition (OM) on dry weight under all salinity levels used which it can improve the bio-chemicals soil media and soil structure that lead to better root development due to more nutrient uptake, compost not only slowly releases nutrients but also prevents the losses of chemical fertilizers through denitrification and volatilization. However, it was found that the values of dry weight with all compost level used were decreased at high salinity soil to 12 dS m^{-1} may be due to increasing NaCl in soil which inhibited growth and nutrient uptake by plants, these results are in agreement with those reported with (El-Fouly *et al*., 2010).

Data presented in Table (3) showed that macronutrient N and P contents as well as crude protein percentage in wheat grains not only significantly increased as a general trend with soaking grains in solution AsA before sowing compared with distilled water, but also with increasing the rates of compost addition to soil in case individual effect or interaction together, which the content of NPK and crude protein in grains were increased to 41.3, 31.7, 27.8 and 41.9 % respectively. With the highest rates of OM addition to soil compared with non addition of OM, theses results may be due to increasing the biological nutrients uptake by wheat plant in presence of OM. Also, data appeared the same trend but by little degree with used AsA as a soaking solution which increased to 7.5, 5.5 and 7.1 % for N, P and crude protein in grains respectively, these results may be indicated that the role principal for AsA through soaking process is decreases in the osmotic potential as soon as its antioxidant action which encourage some biological process while plays an important role in improving plant tolerance to salinity stress particularly in primary age (germination) (Beltagi, 2008 and Arfat *et al*., 2009). In contrast, the content (%) NPK and crude protein were significantly decreasing with increasing level of salinity soil.

Table (3): Individual effects of applied treatments on N, P and K contents (%) and crude protein percentage of wheat grains yield. (Mean Values of Individual Factors).

Treatments		Grains			
		N%	proteine %	P%	K%
Solution of soking (A)	Distilled water	1.86	10.67	0.18	0.49
	Ascorbic acid	1.99	11.43	0.19	0.50
	LSD at 5%	**0.043**	**0.251**	**0.007**	**ns**
Salinity of soil used (dSm^{-1}) (B)	6.0	2.08	11.98	0.20	0.49
	9.0	1.90	10.95	0.18	0.50
	12.0	1.78	10.24	0.18	0.50
	LSD at 5%	**0.053**	**0.307**	**0.008**	**ns**
Compost rates (g pot^{-1}) (C)	0.0	1.55	8.88	0.16	0.42
	100.0	2.03	11.69	0.19	0.54
	200.0	2.19	12.60	0.21	0.53
	LSD at 5%	**0.053**	**0.307**	**0.008**	**0.017**
LSD at (5%)	A*B	**ns**	**ns**	**ns**	**ns**
	A*C	**0.074**	**0.435**	**ns**	**ns**
	B*C	**0.091**	**0.532**	**0.015**	**ns**
	A*B*C	**ns**	**ns**	**ns**	**ns**

Data in Table (4) showed an obvious clear response for soil available macronutrient contents to the OM addition, which the available macronutrients were significantly increased with increasing the levels of compost addition, this is due to rich the compost by nutrients. Also, data appeared significantly increasing in soil available macronutrients with increasing soil salinity may be due to reduce plant growth thus decrease nutrient uptake as soon as the residual effect of compost addition non consumption. On the other hand, there is no positive change in soil content of macronutrient with soaking processes, but it was showed decreasing relatively in macronutrients particularly at used AsA which enhance the plant growth and uptake these elements.

Table (4): Individual effects of the different applied treatments on available Macronutrients (mgkg^{-1}Soil) of soil after wheat harvesting.

Treatments			Soil available Macronutrients (mg kg^{-1}Soil)		
Solution of soaking	Salinity of soil used (dS m^{-1})	Compost rates (g pot^{-1})	N	P	K
soaking by water	6	0	65.3	6.9	183.0
		100	58.0	6.5	184.0
		200	64.3	7.1	190.0
	9	0	66.4	7.0	184.0
		100	61.3	6.8	186.3
		200	67.4	7.3	191.3
	12	0	68.7	7.2	185.4
		100	65.0	7.1	186.7
		200	75.0	7.5	193.0
soaking by AsA	6	0	65.3	6.7	176.3
		100	58.4	6.5	183.4
		200	63.4	7.0	191.0
	9	0	64.7	6.7	181.7
		100	60.3	6.6	185.4
		200	66.3	7.2	190.0
	12	0	68.7	7.3	183.0
		100	64.0	7.0	185.0
		200	73.7	7.4	191.3
Statistical Analysis					
LSD at (5%)	Soaking (A)		ns	0.149	1.546
	Salinity (B)		1.369	0.183	1.893
	Compost (C)		1.369	0.183	1.893
	A*B		ns	0.259	ns
	A*C		ns	ns	ns
	B*C		2.371	ns	ns
	A*B*C		ns	ns	ns

CONCLUSION AND PERSPECTIVE

Using of aqueaus ascorbic acid solution for soaking wheat seed appeared segnificantly increasing in all parameters of wheat growth and yield components under salinity soils conditions, which plays an important role in improving plant tolerance to salinity stress particularly in primary age (germination), but may be there are other effects for used ascorbic acid as a foliar. Indeed, we need to several studies cooperating with anatomy or physiology plant department to compare the two systems addition of AsA, its concentration, time of soaking and its effect on plants growth and productivity under saline soils conditions.

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التخفيف من الآثار السلبية للإجهاد الملحى على نباتات القمح

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أجريت تجربة أصص للتحقق من أثراستخدام حمض الأسكوربيك للتخفيف من الآثار السلبية للإجهاد الملحى على نمو القمح من خلال نقع حبوب القمح بالتوازي مع اضافة السماد العضوى إلى أراضى مختلفة فى مستوى الملوحة (6 ، 9 ، 12 ديسيمينز/م) تقريبا ، تم نقع جزء من حبوب القمح في الماء المقطروالجزء الأخر فى محلول مائى يحتوى على 100ملجم/لتر من حمض الأسكوربيك (AsA) لمدة 6 ساعات قبل الزراعه. تم خلط السمادالعضوى مع التربة المستخدمة قبل ملء الأصص بمعدل بدون و100 و200 جم/كجم تربه. اظهرت النتائج زيادة كبيرة في نسبة الإنبات تصل إلى 46٪ عند نقع حبوب القمح فى المحلول المائي لحمض الاسكوربيك مقارنة بالمنقوع فى الماء المقطر. وبذلك، أثبتت النتائج تصدى جزئي لحمض الاسكوربيك للأثر الضار للملوحة.

أشارت النتائج أن جميع الصفات المدروسة لنمو القمح (ارتفاع النبات، عدد الأشطاء، عدد السنابل لكل وعاء و طول السنبلة) ومكوناته (وزن الألف حبة (جم) محصول الحبوب (جم / وعاء) و محصول القش (جم/ وعاء) بالأضافه الى الوزن الجاف لنبات القمح عند الحصاد جم / وعاء) قد ازدادت بشكل ملحوظ فى معامله النقع بالمحلول المائي لحمض الاسكوربيك (AsA) قبل الزراعه ، هذا التحسن الملحوظ فى تلك الصفات يتناقص تدريجيا مع زيادة ملوحة التربة ولكن بدرجة اقل مقارنة بالماء المقطركمحلول للنقع. هذه النتيجة توضح أن حمض الاسكوربيك يلعب دورا في تنظيم عدد من عمليات التمثيل الغذائي التي تقاوم التأثير الضار للملوحة، وهذا الدور يكون أكثر وضوحا مع تزايد مستوى المواد العضوية المضافه الى التربة.

قام بتحكيم البحث

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