

## Growth and Nutrients Status of Wheat as Affected by Ascorbic Acid and Water Salinity

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**Abstract:** A pot experiment was conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt in the winter season of 2009/2010 to study the effect of salinity on growth and yield of wheat. Salinity treatments were: irrigation by diluted red sea water with 3000, 6000 ppm salt concentration and tap water (TW) as a control (300 ppm). Wheat plants were sprayed by ascorbic acid (ASA) in the rate of 100 and 200 ppm twice at 30 and 45 days from sowing. Increasing salt concentration from 3000 to 6000 ppm decreased in the all growth and yield parameters such as plant height, number of leaves, dry weight of stem, leaves and spikes. Spraying ascorbic acid (ASA) improvement the parameters of growth and yield and decreased the salt effect. Increasing the ascorbic acid spraying rate from 0 to 200 ppm increased the uptake of essential nutrients of wheat but decreased the Na and Cl uptake so the ascorbic acid played an important role for decreasing effects of saline water. Meanwhile, the uptake of all determined minerals showed its higher values with spraying 200 ppm ASA. It's interesting that, increasing ascorbic acid rates increased Ca/(Na + K) ratios compared to the another ratios Na/Mg, Na/Ca and Na/K. Therefore, spraying ascorbic acid to wheat plants could be decreased salt the harmful effects of salinity.

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### 1. Introduction:

Soil salinity is a sever problem in agriculture as it results in a noticeable reduction in the productivity of poor drainage in cultivated soils resulted in the accumulation of salts. According to the report of the world's irrigated lands, about 20-27 % may be salt affected (Ghassemi, *et al.* 1995 and Ezz El-Din, *et al.* 2005). In saline environment, NaCl is usually the most injurious and predominant salt but also other salts including Mg<sup>+2</sup>, Ca<sup>+2</sup> and SO<sub>4</sub><sup>-2</sup> may be presented (Yamaguchi and Blumwald, 2005). The biosynthesis of secondary metabolites although controlled genetically, is affected strongly by environmental factors (Ezz El-Din, *et al.* 2005).

Instead of the lack of fresh water, irrigation by saline water adversely affected growth of wheat as mentioned by Munns (2008).

Vitamin C or L-ascorbic acid or L-ascorbate is an essential nutrient for humans and certain other animal species, in which it functions as a vitamin (Padayatty, *et al.* 2003). In living organisms, ascorbate is an anti-oxidant, since it protects the body against oxidative stress. Ascorbate (an anion of ascorbic acid) is required for a range of essential metabolic reactions in all animals and plants. Ascorbic acid is associated with chloroplasts and apparently plays a role in ameliorating the oxidative

stress of photosynthesis. In addition, ASA has a number of other roles in cell division and protein modification. Plants appear to be able to make ascorbate by at least one other biochemical route that is different from the major route in animals, although precise details remain unknown (Smirnoff, 1996).

One approach for inducing oxidative stress tolerance would be to increase the cellular level of enzyme substrates such as ascorbic acid (vitamin C). Ascorbic acid is a small, water-soluble antioxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide (Beltaji, 2008). Improved understanding of ascorbate concentration in plants will lead to the possibility of increasing ascorbate concentration in plants by genetic manipulation. This will have benefits for tolerance of plants to oxidative stresses (Simirnoff, 1996).

Several investigations reported that ASA plays important roles in enhancing the salt tolerance of different plants (Athar, *et al.* 2008 and Paital and Chainy, 2010).

Therefore, the current research work conducted to investigate the effect of ASA in growth and nutrients status of wheat plants grown under saline conditions.

### 2. Material and Methods:

A pot experiment was conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt in the winter season of 2009/2010 to study the effect of salinity and ASA on growth and yield of wheat. The treatments were as follows:

a) - **Salinity:**

Irrigation by diluted Red Sea water 3000, 6000 and tap water (TW) as a control (300 ppm). Chemical composition of the original Red sea water is shown in Table (1).

b) - **Ascorbic acid (ASA):**

Spraying of ascorbic acid in the rate of 100 and 200 ppm twice at 30 and 45 days from sowing.

Experimental pot contained 30 Kg of air dried clay loam soil. The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and metal. A representative soil sample was taken from the experimental units, air dried, ground and analyzed according to the methods of **Chapman and Partt (1961)**. The chemical and physical properties of the soil used are shown in Table (2).

**Table (1) Chemical composition of the original Red sea water**

Salinity (%)	3.5
pH	8.0
<b>Element</b>	<b>Concentration (mg.L<sup>-1</sup>)</b>
Na	10950
Cl	19500
Mg	1300
K	400
Ca	450
N	17
P	Trace
E	Trace
Mn	Trace
Zn	Trace
Cu	Trace
B	4.5

**Table (2) Some physical and chemical properties of the soil used.**

Soil property	Value	Soil property	Value
Particle size distribution (%)		Exchangeable macronutrient (mg.100g <sup>-1</sup> soil)	
Sand	14	P	5.2
Silt	28	K	37.9
Clay	58	Mg	30.7
Texture	Clay loam	Available micronutrients (mg. kg <sup>-1</sup> soil)	
CaCO <sub>3</sub> (%)	1.6	Fe	11.0
Organic matter (%)	0.1	Mn	9.00
pH(1.:2.5soil suspension)	8.3	Zn	3.30
Ec (dSm <sup>-1</sup> ), soil past extract	0.8	Cu	10.2

Grains of wheat (*Triticum aestivum L.*) c.v. Giza 128 sown in the 1st December, 2009. Calcium super phosphate (15.5 % P<sub>2</sub>O<sub>5</sub>) and potassium sulfate (48 % K<sub>2</sub>O) in the rate of 3.0 and 1.50 g/pot were added, respectively, before planting. Ammonium sulfate (20.5 % N) in the rate of 6.86 g/pot was added in two equal doses, the first one was add after two weeks from sowing and the 2<sup>nd</sup> two weeks latter. Irrigation with diluted sea water solution in different concentrations were started 30 days after sowing (one irrigation by salt water and the next was by fresh water alternatively).

Three plants were collected from every treatment at harvest period, cleaned, and dried in electric oven until the dry weight was fixed. Dry samples were ground in a stainless steal mill. Digestion and determination of macro and micronutrients were done according to the method described by **Cottenie et al., (1982)**.

Data collected were subjected to the proper statistical analysis with the methods described by **Sendecor and Cochran (1980)**.

### 3. Results and Discussion

#### 1- Effect of water salinity on growth of wheat plants:

The results in Table (3) indicate that salt stress decreased all growth and yield parameters. Increasing water salinity from 3000 to 6000 ppm depressed plants height, number and surface of leaves, spike length and dry weight of both stem, leaves and spikes and the amount of decline to 19.6, 4.47, 32.1, 4.43, 43.7, 42.4 and 51.0 %, respectively, compared to the unstressed plants. This finding support that results

obtained previously by (Wang, *et al.* 2007; Zheng, *et al.* 2009). This reverse effect may be due to the retarding effect on photosynthesis (Jampeetong and Brix, 2009), protein building (Parida and Das, 2005), mineral disturbances (Grattan and Grieve, 1998), hormonal balance (Shakirova, *et al.* 2003), Water adjustment (Shannon, 1997). This research which the salt stress reflecting on mineral status in shoots and grains which supporting by: Hussein, *et al.* (2008); Shabaan, *et al.* (2008) and Abd El-Baky, *et al.* (2008).

**Table (3): Effect of irrigation by diluted sea water on growth and yield parameters of wheat plants.**

Salinity rates ppm	Plant Height (cm)	No of Leaves/mean stem	Surface area of leaves/mean stem (cm <sup>2</sup> )	Spike length (cm)	Dry weight (g)			
					Stem	Leaves /mean stem	Spikes	Total
300 (TW)	107	3.58	98.0	15.8	3.87	0.66	3.47	8.27
3000	93	3.42	77.4	14.8	2.21	0.56	2.15	4.92
6000	86	3.42	66.5	14.1	2.18	0.38	1.70	4.26
L.S.D. <sub>0.05</sub>	N.S	0.97	9.67	N.S	N.S	N.S	1,75	1.06

#### 2- Interaction effect between ascorbic acid and rates of salinity water on growth and yield parameters.

The results from Table (4) showed that the increasing the rate of spraying of ascorbic acid from 0 to 200 ppm in most cases lead to increase growth and spikes yield of wheat and that under each of the different levels of salinity water or even added to tap water. The rate of spraying of ascorbic acid (200

ppm) in most cases gave the highest value of growth and yield parameters. Ascorbic acid could be used as a potential growth regulator to improve salinity stress resistance in several plant species (Gunes *et al.*, 2005; Khan, 2006 and Sheteawi, 2007). As well as ascorbic acid would inhibit stress-induced increases in the leakage of essential electrolytes following peroxidative damage to plasma membranes (McKersie, *et al.*, 1999).

**Table (4) Effect of ascorbic acid and irrigation by diluted seawater on growth of wheat plants**

Salinity rates ppm	Ascorbic acid ppm	Plant height	No. of leaves/mean stem	Area of leaves/mean stem	Spike length	Dry weight (g):			
						Stem	Leaves /mean stem	Spikes	Total
300 (TW)	0 <sup>a</sup>	102	4.75	87.2	15.5	3.16	0.69	2.27	6.12
	100	105	5.00	90.2	16.3	4.21	0.75	3.34	8.30
	200	114	5.00	116.6	15.7	4.21	0.74	4.79	9.55
3000	0 <sup>a</sup>	96	4.75	73.2	16.0	2,07	0.55	1.58	4.64
	100	98	4.75	78.8	16.7	2.21	0.74	2.44	3.39
	200	96	3.75	90.3	16.7	2.81	0.79	2.43	5.65
6000	0 <sup>a</sup>	83	3.25	58.6	15.4	2.17	0.76	1.35	3.98
	100	83	3.25	58.7	16.3	2.34	0.41	2.11	4.89
	200	92	3.75	83.2	17.3	2.03	0.28	1.61	3.98
LSD <sub>0.05</sub>		N.S	N.S	16.45	N.S	N.S	N.S	N.S	N.S

<sup>a</sup> Sprayed by the same quantity of distilled water.

#### 3- Effect of water salinity on nutrients uptake in shoot of wheat plants

The results in Table (5) indicate that the uptake of N, P, K, Ca and Mg in shoot of wheat plants

decreased by increasing irrigation water salinity from 3000 to 6000 ppm compared to tap water (control), but increasing irrigation water salinity increased the Na and Cl uptake of wheat shoot. Poustini and

**Siosemardeh (2004)** showed that increasing water salinity rate led to decrease and increase of K and Na, respectively, in shoot of wheat. **Asik *et al.*, (2009)**

reported that the uptake of N, P and K of wheat plants decreased when the water salinity increased.

**Table (5) Effect of irrigation by diluted seawater on nutrients uptake in shoot of wheat plants**

Salinity rates ppm	Nutrients uptake ( mg /plant)						
	N	P	K	Na	Ca	Mg	Cl
<b>300(T.W)</b>	24.55	2.128	6.494	0.394	3.499	8.406	7.159
<b>3000</b>	14.70	1.519	4.864	0.552	2.554	5.564	8.523
<b>6000</b>	13.15	1.463	3.450	0.680	1.753	4.758	13.04
<b>L.S.D.<sub>0.05</sub></b>	0.419	0.171	0.119	0.168	0.110	0.267	0.180

#### 4- Interaction effect between ascorbic acid and rates of salinity water on nutrients uptake

The interactive effect of salinity and ascorbic acid application act significantly on K, Ca, Na, Mg and Cl uptake (Table 6). Under a good water irrigation, the uptake of all nutrients uptake increased when the rates of ascorbic acid increased. This increasing up to the high rate of ascorbic acid (200 ppm) increased N, P, K, Na, Ca and Cl uptake, unless the second rate of ascorbic acid (100 ppm) was enough to increasing Mg uptake. Under water salinity irrigation, increasing the ascorbic acid spraying rate from 0 to 200 ppm increased the uptake of essential nutrients of wheat but decreased the Na and Cl uptake so the ascorbic acid played an important role of decreasing effects of salinity of water irrigation.

**Grattan and Grieve (1998)** stated that despite a large number of studies that demonstrate that salinity reduces nutrient uptake or affects nutrient partitioning within the plant, little evidence exists that adding nutrients at levels above those considered optimal in non-saline environments and improves crop yield, this effect called “salt effect”. The authors added that antioxidant such as ascorbic acid generally affected the mineral uptake of different plants and decreased the effects of salinity.

**Gunes, *et al.* (2005)** revealed that ascorbic acid strongly inhibited  $\text{Na}^+$  and  $\text{Cl}^-$  accumulation, but stimulated N, Mg, Fe, Mn and Cu concentrations of salt stressed maize plants. These results suggest that ascorbic acid could be used as a potential growth regulator to improve plant salinity stress resistance.

**Table (6) Effect of ascorbic acid and irrigation by diluted sea water on uptake of nutrients in shoots of wheat plants**

Salinity rates ppm	Ascorbic acid ppm	mg / plant						
		N	P	K	Na	Ca	Mg	Cl
<b>300</b>	<b>0<sup>a</sup></b>	14.26	1.840	5.343	0.550	3.223	6.750	9.790
	<b>100</b>	26.14	1.737	5.670	0.693	3.487	9.933	14.417
	<b>200</b>	33.23	2.800	8.470	0.737	3.787	8.533	18.917
<b>3000</b>	<b>0<sup>a</sup></b>	11.55	0.973	3.947	0.540	1.787	4.827	9.733
	<b>100</b>	13.35	1.263	4.427	0.383	3.263	4.917	9.930
	<b>200</b>	17.22	2.153	6.220	0.333	5.613	6.950	9.907
<b>6000</b>	<b>0<sup>a</sup></b>	12.34	1.243	3.820	0.737	1.243	4.500	6.857
	<b>100</b>	11.73	1.347	4.080	0.407	1.973	4.780	7.070
	<b>200</b>	15.39	1.967	4.450	0.403	2.040	4.933	8.250
<b>LSD<sub>0.05</sub></b>		0.725	0.297	0.029	0.282	0.367	0.463	0.311

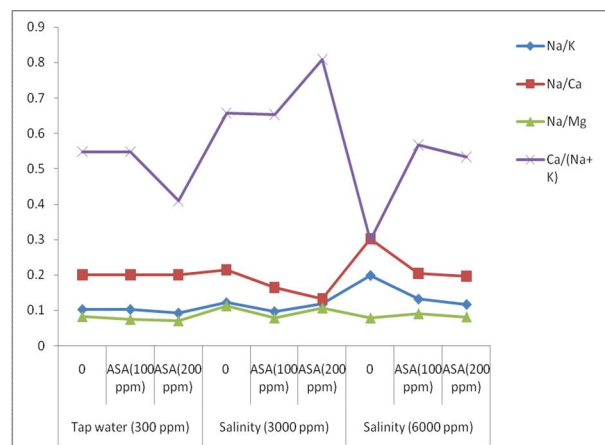
<sup>a</sup> Sprayed by the same quantity of distilled water

#### 4. Relationship between nutrients ratio and salinity.

As shown in Fig (1) increasing ascorbic acid rates increased Ca/(Na + K) ratios compared to the another ratios ( Na/Mg, Na/Ca and Na/K) so that spraying ascorbic acid to wheat plants decreased salt effects. Under water tap irrigation, increasing ascorbic acid rates depressed Na/K ratio from 0.103

to 0.093 as well as Na/Mg ratio from 0.082 to 0.070 but ascorbic acid didn't effect of Na/Ca ratios. Under the first rate of water salinity (3000 ppm), the second rate of ascorbic acid (100 ppm) enough to decrease Na/K and Na/Mg ratios but the third rate of ascorbic acid (200 ppm) decreased and increased Na/Mg and Ca/(Na+K) ratios, respectively. Under the second rate of water irrigation, ascorbic acid addition led to

decrease Na/K, Na/Ca and Na/Mg ratios while increasing Ca (Na+K) ratio.



**Fig (1) effect ascorbic acid rates on nutrients ratios under water salinity irrigation.**

Increasing numbers of salt-tolerant transgenic plants have been generated with over expression of vacuolar  $\text{Na}^+/\text{H}^+$  antiporter proteins mediating lower concentrations of Na and higher ratios of K/Na in cytosol (Zhang and Blumwald, 2001; He *et al.*, 2005; Yamaguchi and Blumwald, 2005). These results indicate that both the absolute concentrations of K, Na and Ca in plants and the magnitude of the imbalance between these ions in the critical cell organelles such as cytosol and vacuoles are important in the differential expression of salt tolerance. In many research groups, investigations dealing with the development of salt-tolerant, and accumulation of K, Na and Ca in plants (Colmer *et al.*, 2006 Munns *et al.*, 2006). The concentrations of these nutrients and their ratios (e.g., K/Na and Ca/Na) are widely used as screening varieties for their tolerance to salt toxicity. These parameters are reliable and useful in screening varieties for salt-stress tolerance.

#### Conclusion:

From the above mentioned data it could be concluded that salinity adversely affected of growth and nutrients uptake, however, ASA application as antioxidant and improved growth and nutritional status of wheat plant through its enhancing oxidant defense and decreasing the salt stress damages.

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