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OXIDATIVE DAMAGE IN WHEAT SEEDLINGS GROWN UNDER SALINITY STRESS CONDITION

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ABSTRACT

The effect of NaCl on seedlings of (*Triticum aestivum* L.) var. Sakha 69 was studied. Obtained results revealed that high salinity treatment (12 dS/m) caused symptoms of oxidative damage such as a decrease in growth rate, superoxide dismutase activity, nitrogen content, protein percentage. On the other hand high salinization led to increase in peroxidase activity, lipid peroxidation, sodium and chloride ions accumulation as well as Na : K ratio.

Additional index words :
Triticum aestivum L. - salt stress – oxidative stress - antioxidant – enzymes - nitrogen – sodium chloride - potassium and protein.

Abbreviations:
SOD:superoxide dismutase MDA=malondialdehyde. RGR : relative growth rate

INTRODUCTION

Salinity in soil and/or water is of increasing important to agriculture as it causes a stress condition to crop plants. Secondary salinization is increasing year after year due to more irrigations given to high water requiring crops and increasing land water table. However, plants differ in their tolerance to salinity, most plants are not capable to tolerate high levels of salinity.

The physiological events associated with salinity stress (decline in photosynthesis, change in cell turgor, metabolite accumulation, disturbance of carbon and nitrogen allocation and change in ion homeostasis) are well documented. Through the last few years, we are beginning to understand the relationships among these physiological events and salt sensitivity or tolerance (Geuta – Dahan *et al*., 1997; Holmstrom *et al*., 1996 and Ishitani *et al*., 1995).

One of the biochemical changes possibly occurring when plants are subjected to harmful stress condition is the production of activated oxygen species. The chloroplasts and mitochondia of plant cells are important intracellular generator of activated oxygen species.

Electrons leaked from electron transport chains can react with oxygen (O_2) during normal aerobic metabolism to produce activated oxygen species such as superperoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH).

These cytotoxic oxygen species are highly reactive and in the absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage to lipids, proteins and nucleic acids (Davies, 1987 and Fridovich, 1986).

Fortunately some plants possess a number of antioxidant enzymes that protect them against the damaging effects of activated oxygen species. The term antioxidant can be considered to describe any compound capable of quenching active oxygen species, without itself under going conversion to a destructive radical (Nishikimi and Yogi, 1996). Hence antioxidants and antioxidant enzymes as ascorbate and glutathione function to interrupt the cascades of uncontrolled oxidation, (Smirnoff and Cumbes 1989).

Evidence suggests that both drought and salinity cause oxidative damage through generation of oxygen radicals or inhibition of antioxidant systems in plants (Jagtap and Bhargava, 1995; Smirnoff, 1993; Zhang and Kirkham, 1994).

The aim of this work, is to estimate the effect of salt stress on antioxidant enzyme activities, lipid peroxidation, rate of plant growth, protein and some ion content in shoot and roots of wheat plant.

MATERIALS AND METHODS

Grains of wheat (*Triticum aestivum* L.) var. Sakha 69 were sown in plastic pots of 5.5 cm in diameter and 6 cm in depth, each with three drainage holes. Each pot was filled with 100 g sandy soil poor in nutrient content (8.19 % clay, 6.4 % silt, 83.3 % sand and 0.38 % organic matter).

Pots were placed in growth chamber under a 16 h photoperiod, 85 % relative humidity and 20/15 ^{o}C (day/night) temperature and a photonflux density of 220 Umol m^{-2} supplied by fluorescent lamps. All pots were, kept well watered with modified Yoshida nutrient solution (Mae, 1993) until seedling stage. Seedling were thinned to three per pot after emergence, then salinization was induced by adding NaCl to one half strength modified Yoshida solution to obtain electrical conductivities of 6 and 12 dS/m, which are equivalent to 60 and 120 mM NaCl, respectively. Nutrient solution without NaCl addition (omM NaCl) served as the control, that is the electrical conductivity was around 0 dS/m. Measurements were taken 7 days after salinity treatments.

Shoot and roots were immediately separated and washed quickly with distilled water to remove any possible salt surface contamination and immediately air dried on absorbing paper for ions and enzyme analyses.

Plants were randomly selected and gently uprooted to estimate growth by shoot dry weight measurements. Dry weight was estimated by drying in an aerated oven at 80 ^{o}C for 48 hours. Relative growth rate (RGR) was calculated from the increase in dry weight of plants at the beginning and at the end of the salt treatment, using the equation :

$$\mathbf{RGR = (l_nW_f - 1l_nW_i) / (t_f - t_i)}$$

where W is the shoot dry weight, t is the time and subscripts denote initial and final sampling, that is 0 and 7 days after salinity treatment.

The ground plant material was digested using perchloric acid for estimation of Na and Cl^{-} ions using spectrophotometry absorption. K was determined photometrically using a flame photometer. Nitrogen was

determined using microkjeldahl as described by Van Schouwenburg and Walinga (1978) Crude protein percentage was calculated by multiplying the nitrogen percentage by the factor of 6.25. For enzyme assays and estimation of lipid peroxidation frozen shoot and root samples were ground to a fine powder with liquid nitrogen in a cold mortar and extracted using 50 mM phosphate buffer (pH 7.0). The extracts were centrifuged at 4 ^{o}C for 30 min at 20000 x g and the resulting supernatants were used as the crude extracts. SOD activity was estimated by Stewart and Bewley (1980). Peroxide activity was determined using the guaiacol oxidation method (Chance and Maehly, 1955).

Lipid peroxidation was measured by measuring the amount of malondialdehyde (MDA) formation using the thiobarbituric acid method described by Stewart and Bewley (1980). Most of the chemical procedures were carried out in the central labs of the national Research Center and Faculty of Agriculture, Cairo, University.

Data were analyzed according to Snedecor and Cochran (1980). Treatment means were compared by Least significant difference test (L.S.D) ate 5 % level of probability.

RESULTS

Effect of salt stress on :

One. Relative growth rate (RGR)

Relative growth rate based on shoot dry weight of wheat plants subjected to a week salinity treatments are presented in Fig. (1). At high salinity level (12 dS/m), wheat seedlings showed growth retardation compared to non-salt treated plants, while at moderate salinity level (6 dS/m), plants showed slight increase in growth rate than the control.

Morphologically, the most typical symptom of saline injury to a plant is retarded growth due to inhibition of cell elongation (Neiman 1965), resulting in a stunted plant.

Two. Chemical composition :

It was clear in table (1), that salinity increased the accumulation of Na^+ and Cl^- ions in shoots and roots, but higher contents of Na^+ and Cl^- ions were found in roots than in shoots. The different concentrations of Na^+ and Cl^- ions in roots and shoots, suggest a different pattern of their uptake and translocation (Ciscato et al., 1997).

Table (1): Effect of salinity on Na^+ and Cl^- content in shoots and roots of wheat seedlings.

	NaCl (dS/m)	Shoots		Roots	
		m.g.Dw^{-1}	L.S.D at 5%	m.g.Dw^{-1}	L.S.D at 5%
Na^+	0	0.49	n.s	0.61	n.s.
	6	24.07	7.1	28.1	9.6
	12	29.4	8.5	32.7	14.1
Cl^-	0	1.0	n.s	0.63	n.s
	6	9.86	0.5	10.55	0.7
	12	12.15	1.1	13.75	1.5

Table (2): The effect of salinity on Na : K ratio in shoots and roots of wheat .

NaCl dS/m	Na : k	
	Roots	Shoots
0	0.05	0.05
6	0.06	0.06
12	0.10	0.07

Table (3): Effect of salt stress on nitrogen content mg/g dw. and protein percentage in shoots and roots of wheat seedlings.

Salinity level dS/m	Nitrogen mg/g d.w.		Protein %	
	root	shoot	root	Shoot
0	13.6	20.4	8.50	12.75
6	10.80	19.6	6.75	12.25
12	10.40	18.8	6.50	11.75

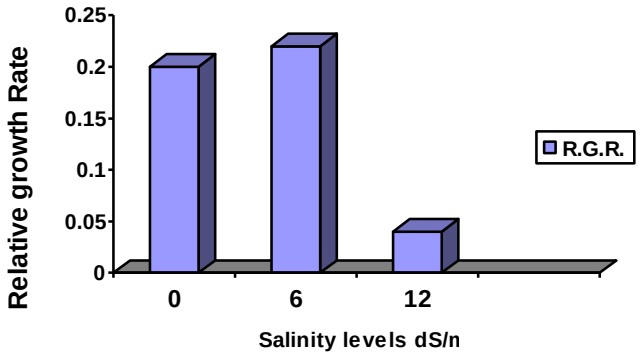

Fig. (1) : Effect of salinity on relative growth rate (RGR) in terms of shoot dry weights.

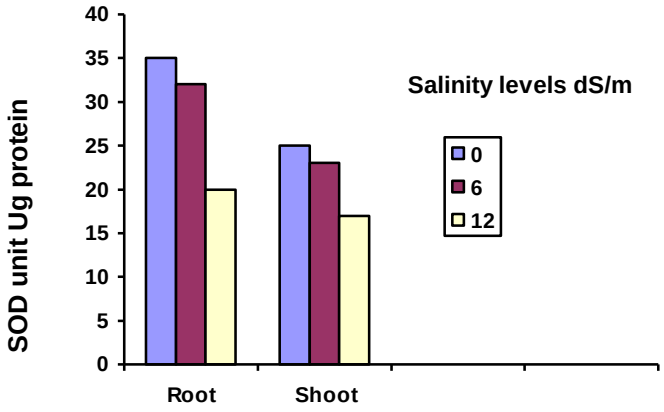

Fig. (2) : Effect of salinity on SOD activity.

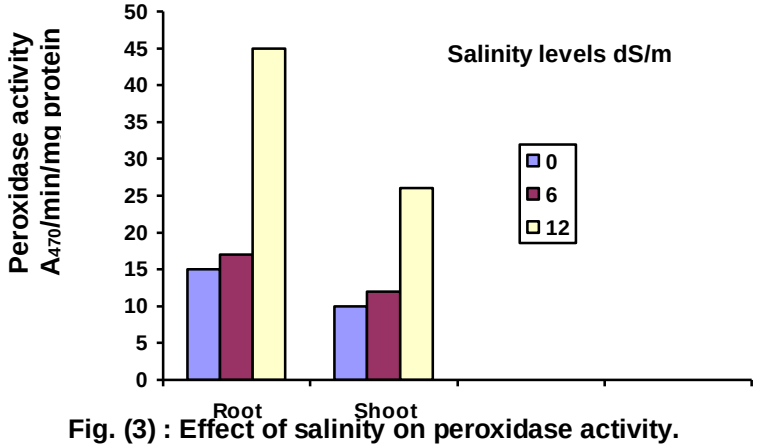

Fig. (3) : Effect of salinity on peroxidase activity.

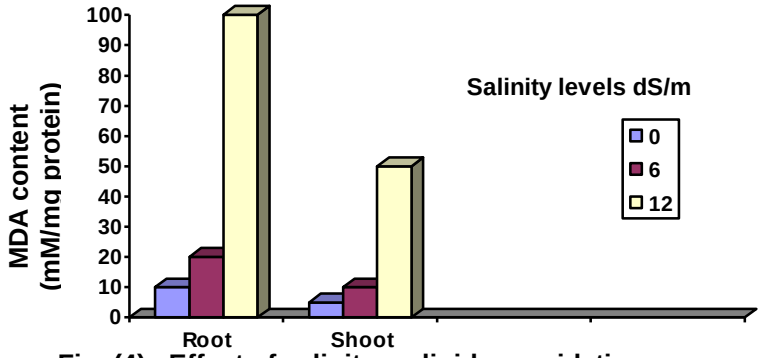

Fig. (4) : Effect of salinity on lipid peroxidation.

The Na : K ratio as presented in table (2), remained almost constant in roots and shoots at 0 and 6dS/m, whereas the ratio increased at high salinization of 12 dS/m, this was approved by (Sharma, 1996).

It was evident in table (3) that nitrogen content in roots decreased by increasing salinity levels by 20.5 % in roots and 4 % in shoots at 6 dS/m and 23.5 % in roots and 8 % in shoots at 12 dS/m salinity level. The same trend was observed in protein percentage, as it reduced by 20.5 % in roots and 2 % in shoots at 6 dS/m and 23.6 % in roots and 7.5 in shoots of seedling wheat plants at 12 dS/m, previous results were supported by (Meneguzzo et al., 1999).

C. Superoxide dismutase (SOD) and peroxidase activities :

Fig. (2) shows effect of the increasing level of NaCl on SOD activities in shoots and roots of wheat seedlings. It was clear that plants exhibited a clear decline in SOD activity with increasing the magnitude of salinity stress. The SOD reduced by 43 % in roots and by 32 % in shoots at 12 dS/m.

On the other hand, the peroxidase activity as in Fig. (3) showed an opposite trend with regard to salinization, peroxidase activity increased with

increasing salinity levels by 13.3 % at 6 dS/m and 200 % in roots at 12 dS/m, while in shoots increased by 20 % at 6 dS/m and by 61 % at 12 dS/m. Meneguzzo et al., (1999) found that the peroxidase activities increased under saline condition in the organ that firstly suffer stress.

d. Lipid peroxidation :

With increasing salinity levels, the MDA content increased, thus indicating an increase in lipid peroxidation as presented in Fig. (4). It increased by one fold at 6dS/m and 9 folds at 12 dS/m in roots, while in shoots it increased by half a fold at 6dS/m and approximately 4 folds at 12 dS/m.

DISCUSSION

The observed high Na^+ accumulation in seedlings of wheat in response to salinity, resulted in symptoms of oxidative damage such as decrease in SOD activity, increase in lipid peroxidation and peroxidase activity and decrease in growth rate.

In this respect Hernandez *et al* (1993), reported that the catalytic activity of SOD isozymes from cowpea plants decreased as a function of salt concentration in vitro.

Also, Singha et al., (1990), found that salinity decrease SOD activity in leaves, chloroplasts and mitochondria of pea plants and stated that inhibition of SOD activity under salt stress is a consequence of an altered synthesis and accumulation of less active enzymes in salt treated plants cannot be entirely ruled out.

The observed decrease in SoD activity, could diminish the ability of seedlings to scavenge O_2^- radicals favoring an accumulation of oxygen radical species, which could cause membrane damage. The extent of damage to the membrane was monitored by measuring of (MDA) content. Peroxidase in plants are involved in the biosynthesis of cell wall Negrel *et al* (1987), including lignification and suberization (Polle et al 1994 and Espelie *et al* 1986).

Kalir et al, (1984) reported an increase in peroxidase activity under salt stress. It could be due to increased activity of peroxidase encoding genes or increased activation of already existing enzymes.

Also, high peroxidase is correlated with the reduction of plant growth (Mc Adam *et al* 1992 and Zheng *et al.,* 1992). This may be attributed to peroxidase catalysis of ferulic acid conversion to diferulic acid on polysaccharides, the feruloylation of hemicelluloses of the insolubilization of hydroxyproline – rich glycoprotein causing cell wall stiffening (Fry *et al.*, 1986).

In conclusion plant grown under salt stress, with low Na+ accumulation and relatively unchanged SOD and peroxidase activity by bringing about an unchanged capacity for oxygen radical scavenging and maintenance of cellular membranes as well as cell wall functions, could explain the NaCl tolerance over sensitive ones.

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الضرر الناتج عن الأكسدة فى بادرات القمح النامية تحت ظروف الإجهاد الملحى

مايبل سعد جاب الله ، بدور أبو ليلة

قسم النبات – المركز القومى للبحوث – الدقى - القاهرة - مصر

أجريت تجربة أصص تحت ظروف محكمة (غرفة التنمية) لدراسة تأثير إضافة ملح كلوريد الصوديوم بتركيزات مختلفة، حيث كان التوصيل الكهربى لتركيزات المحلول المستخدم صفر ، 6 ، 12 dS/m على نمو بادرات القمح صنف (سخا 69) وقد أظهرت النتائج المتحصل عليها أن التركيز العالى 12 dS/m سبب نقص فى معدل النمو ونشاط انزيم السوبر أكسيد – ديسموتيز ومحتوى النيتروجين والبروتين بالنسبة لمعاملة المقارنة، كما أدى تركيز الملوحة العالى الى زيادة نشاط انزيم البيرواكسيديز واكسدة الليبيد بالإضافة الى تراكم كل من أيون الصوديوم والكلوريد فى الجذور التى كانت أعلى منها فى السيقان.

لقد دلت النتائج على ان محتوى كل من النيتروجين والبروتين قد نقص فى الجذور بدرجة أكثر مما فى السيقان بنسبة 23.6 % فى الجذور، 7.5 % فى السيقان. وفيما يتعلق بنسبة الصوديوم الى البوتاسيوم فكانت ثابتة بالنسبة للتركيز المتوسط واختلفت فى التركيز الملحى العالى (12 dS/m). كما انخفض انزيم السوبر أكسيد ديسموتيز بنسبة 43 % فى الجذور و 32 % فى السيقان مقارنة بمعاملة المقارنة.

وبالنسبة لنشاط انزيم البيراوكسيديز فقد زاد فى الجذور بنسبة 20 %، 61 % فى السيقان. أماذ بالنسبة لأكسدة الليبيدات فقد زادت بنسبة الضعف فى حالة الملوحة المتوسطة وتسعة أضعاف فى الملوحة المرتفعة (12 dS/m).