Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate

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Abstract

The interactive effects of salinity stress (40, 80, 120 and 160 mM NaCl) and ascorbic acid (0.6 mM), thiamin (0.3 mM) or sodium salicylate (0.6 mM) were studied in wheat (*Triticum aestivum* L.). The contents of cellulose, lignin of either shoots or roots, pectin of root and soluble sugars of shoots were lowered with the rise of NaCl concentration. On the other hand, the contents of hemicellulose and soluble sugars of roots, starch and soluble proteins of shoots, proline of either shoots or roots, and amino acids of roots were raised. Also, increasing NaCl concentration in the culture media increased Na^+ and Ca^{2+} accumulation and gradually lowered K^+ and Mg^{2+} concentration in different organs of wheat plant. Grain soaking in ascorbic acid, thiamin or sodium salicylate could counteract the adverse effects of NaCl salinity on the seedlings of wheat plant by suppression of salt stress induced accumulation of proline.

Additional key words: cellulose, hemicellulose, lignin, minerals, pectin, proline, soluble proteins, soluble sugars, starch, *Triticum aestivum*.

Introduction

The physiological and biochemical responses of plants to salt stress may be achieved by alteration of certain phases of the metabolic network mediated and directed by certain enzymes, which activate or retard particular metabolic activities. This may divert the perfect balance of the biochemical reactions with the accumulation of some metabolic intermediates or end products.

Considering the specific role of water-soluble vitamins (ascorbic acid, thiamin) and sodium salicylate in plant metabolism, many works estimated the capability of the three treatments (via presowing grain soaking) in ameliorating and modifying the salt-stress induced changes in wheat (*Triticum aestivum* L.) plant via the activation of some enzymatic reactions (Kefeli 1981, Neubaner and Yamamota 1992, Choudhury *et al.* 1993, Hamada 1998, Janda *et al.* 1999, Mishra and Choudhuri 1999). Thus, in this work it seemed necessary to study the effects of various concentrations of NaCl on some metabolic processes and the role of ascorbic acid, thiamin or sodium salicylate in amelioration of these adverse effects of salinity.

Materials and methods

The grains of wheat (*Triticum aestivum* L.) were soaked for 6 h in water, 0.6 mM ascorbic acid, 0.3 mM thiamin, or 0.6 mM sodium salicylate before sowing in plastic pots (11.5 cm in diameter and 10 cm in height, 5 grains in each pot) lined with polyethylene bags and containing soil composed of clay and sand (1:1 by volume). The pots were then irrigated with NaCl solutions to reach the desired concentration (40, 80, 120 and 160 mM NaCl) and then the water content of the soil was adjusted regularly near to the field capacity. Pots with control plants were left untreated. At the end of the experimental period (30 d), fresh shoots and roots were dried in an aerated oven at 70 °C. Cell wall fractionation was conducted essentially according to Dever *et al.* (1968) and Galbraith and Shields (1981). Tissue powder samples were extracted twice in distilled water, twice in 80 %

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ethanol to remove soluble metabolites. The precipitate was then extracted in 2 cm^3 0.5 M NaOH for starch, 0.5 % ammonium oxalate-oxalic acid (90 °C for 24 h) for pectin, 17.5 % NaOH for hemicellulose and in 72 % H_2SO_4 (with 15 min autoclaving) for cellulose extraction. After that, the remaining precipitate was ascribed to the lignin fraction according to Dever *et al.* (1968). Content of soluble sugars, starch and wall polysaccharides were determined by the anthrone sulfuric acid reagent using glucose as a standard (Fales 1951). Free amino acids were determined according to Moore and Stein (1948), free proline according to Bates *et al.* (1973) and amount of protein according to Lowry *et al.* (1951). Sodium and potassium were determined by flame photometer method (Williams and Twin 1960), calcium and magnesium by the versene titration method (Schwarzenbach and Biedermann 1948).

Results and discussion

Cell wall metabolism is an important component in plant growth, not only because it represents a large proportions of the cell biomass, but also because determining wall extensibility for cell enlargement (Zhang and Läuchli 1988). In this investigation, pectin, cellulose, hemicellulose and lignin were determined in stressed wheat plants (Tables 1, 2). The contents of cellulose, lignin of shoots and of roots and pectin of roots were significantly lowered with the rise of NaCl concentration in the rooting medium. On the other side, hemicellulose contents of shoots were raised under all the NaCl concentrations. NaCl up to 160 mM did not induce any significant inhibitory effects on the production of pectin in shoots. NaCl in concentrations 120 and 160 mM stimulated the cellulose production. This could be important for preventing extension (Taiz 1984, Van Volkenburgh and Boyer 1985). Working with tobacco cell cultures in the presence of polyethylene glycol, Iraki *et al.* (1989) recorded a decrease in the percentage of cellulose and an increase in the hemicellulose percentage, whereas the pectic fraction remained more or less unchanged. Similar results were also obtained by Wakabayashi *et al.* (1997). The possible mechanisms for the inhibitory effect of salinity on the incorporation of glucose into cell wall polysaccharides have been discussed (Greenway 1970, Hassan-Porath and Poljakoff-Mayber 1973, Zhang and Läuchli 1988, Al-Hakimi 1995).

Table 1. Effect of wheat grains soaking in 0.6 mM ascorbic acid, 0.3 mM thiamin, or 0.6 mM sodium salicylate on the content of cell wall polysaccharides [mg g^{-1}(d.m.)] of shoots of wheat plants treated with NaCl (*, ** - significant at $P = 0.05$ and 0.01, respectively, as compared with reference controls).

Soaking	NaCl [mM]	Pectin	Hemicellulose	Cellulose	Lignin
Water	0	24.03 ± 0.859	22.05 ± 0.472	60.80 ± 0.909	172.30 ± 3.823
	40	22.33 ± 0.219	23.70 ± 0.580	43.80 ± 1.178**	137.61 ± 3.543**
	80	22.05 ± 0.266	23.99 ± 0.475	39.12 ± 1.619**	129.17 ± 2.099**
	120	24.09 ± 0.585	25.13 ± 0.928*	29.72 ± 0.820**	119.59 ± 1.207**
	160	23.19 ± 0.877	25.59 ± 0.626**	20.76 ± 1.122**	107.55 ± 1.359**
Ascorbic acid	0	24.84 ± 0.411	20.35 ± 0.171	51.09 ± 0.502**	181.78 ± 2.877**
	40	23.33 ± 0.200	24.32 ± 0.400	56.51 ± 1.238**	157.04 ± 3.252**
	80	22.05 ± 0.266	23.50 ± 1.098	50.78 ± 0.936**	162.01 ± 2.371**
	120	24.58 ± 0.200	22.91 ± 1.023	50.05 ± 0.318**	166.40 ± 1.015**
	160	25.61 ± 0.399	23.17 ± 0.370	48.12 ± 0.384**	172.11 ± 1.789**
Thiamin	0	27.96 ± 0.340**	25.59 ± 0.198**	53.03 ± 0.888**	177.51 ± 1.465**
	40	26.31 ± 0.556	21.54 ± 0.206	58.02 ± 0.376**	150.00 ± 0.330**
	80	24.92 ± 0.995	20.30 ± 0.478	54.14 ± 1.418**	156.55 ± 1.497**
	120	22.52 ± 0.989	20.20 ± 0.941	56.28 ± 1.293**	158.83 ± 1.103**
	160	22.26 ± 0.400	19.96 ± 0.399	51.07 ± 0.500**	147.51 ± 1.240**
Sodium salicylate	0	20.83 ± 0.276*	21.64 ± 0.583	54.98 ± 1.308**	184.05 ± 0.279**
	40	23.63 ± 0.824	23.17 ± 0.585	59.08 ± 1.469**	161.29 ± 1.903**
	80	22.09 ± 0.175	21.97 ± 0.470	56.41 ± 1.039**	169.18 ± 0.522
	120	27.65 ± 0.940**	23.96 ± 0.851	58.58 ± 1.486**	172.02 ± 1.566
	160	26.79 ± 0.400*	23.75 ± 0.240	55.13 ± 1.612**	179.78 ± 1.135**

The adverse effects of salt treatments on cellulose and lignin contents in shoots and roots and pectin in roots were partially or completely alleviated by soaking grains in ascorbic acid, thiamin or sodium salicylate. In shoots the applied vitamins did not exert any effect on the pectin content, while the sodium salicylate stimulated pectin production. The cellulose contents in shoots and roots remained mostly unchanged. Vitamins or sodium salicylate could perhaps alleviate the inhibitory effects of salinity on glucose incorporation into cell wall polysacchariedes. Similarly, cellulose biosynthesis was particularly enhanced (Zhang and Läuchli 1988). These authors suggested that high Na^+ concentrations reduced cellulose synthesis in cotton roots via disturbance of plasma membrane integrity. In this work treatments with vitamins or sodium salicylate could, however, counteract this adverse effect (Tables 1, 2).

Table 2. Effect of wheat grains soaking in 0.6 mM ascorbic acid, 0.3 mM thiamin, or 0.6 mM sodium salicylate on the content of cell wall polysaccharides [mg g^{-1}(d.m.)] of roots of wheat plants treated with NaCl (*, ** - significant at $P = 0.05$ and 0.01, respectively, as compared with reference controls).

Soaking	NaCl [mM]	Pectin	Hemicellulose	Cellulose	Lignin
Water	0	65.56 ± 1.832	47.01 ± 0.801	51.34 ± 0.871	189.66 ± 2.341
	40	68.65 ± 2.176	55.94 ± 0.343**	44.32 ± 1.792**	152.39 ± 3.037**
	80	41.80 ± 1.434**	61.38 ± 1.664**	41.01 ± 1.759**	145.35 ± 1.938**
	120	33.09 ± 0.517**	64.41 ± 2.008**	34.78 ± 0.898**	129.39 ± 1.982**
	160	30.63 ± 1.717**	68.42 ± 0.572**	19.72 ± 0.382**	120.97 ± 1.733**
Ascorbic acid	0	39.33 ± 1.259**	42.88 ± 1.031*	56.17 ± 1.480**	196.65 ± 3.468**
	40	40.82 ± 1.777**	47.29 ± 0.748	56.21 ± 1.783**	171.00 ± 1.648**
	80	37.73 ± 0.465**	49.24 ± 0.988	55.46 ± 2.122**	169.11 ± 2.024**
	120	37.16 ± 1.320**	45.52 ± 1.718	51.42 ± 2.205	166.82 ± 0.819**
	160	30.06 ± 0.573**	47.18 ± 0.915	51.00 ± 0.816	157.85 ± 2.136**
Thiamin	0	24.91 ± 0.344**	77.01 ± 0.861**	53.83 ± 1.056*	192.10 ± 2.225
	40	27.20 ± 0.572**	47.86 ± 2.060	55.11 ± 1.617**	167.89 ± 0.897**
	80	35.96 ± 0.915**	46.38 ± 0.458	51.92 ± 0.829	164.07 ± 4.494**
	120	39.45 ± 1.030**	44.49 ± 1.258	54.80 ± 1.416**	158.24 ± 1.776**
	160	58.68 ± 1.596**	57.25 ± 2.122**	54.13 ± 0.382**	151.68 ± 3.086**
Sodium salicylate	0	47.63 ± 0.915**	54.62 ± 1.333**	58.02 ± 0.628**	203.02 ± 2.190**
	40	27.42 ± 0.345**	49.07 ± 0.176	57.15 ± 1.014**	184.09 ± 1.262**
	80	28.11 ± 0.571**	44.57 ± 0.230	58.41 ± 1.482**	179.77 ± 0.775**
	120	37.33 ± 0.799**	48.01 ± 0.341	55.98 ± 1.002**	177.17 ± 1.289**
	160	42.83 ± 1.604**	47.63 ± 0.915	55.40 ± 1.099**	165.76 ± 1.125**

Salinity stress stimulated soluble sugars accumulation in roots and retarded their biosynthesis in shoots (Table 3). The applied vitamins or sodium salicylate enhanced the stimulatory role of water stress on the production of soluble sugars in roots in NaCl stressed wheat plants. However, treatments with sodium salicylate at high NaCl concentrations (120 and 160 mM) lowered the contents of soluble sugars. Also in this context, the applied vitamins or sodium salicylate retarded the accumulation of soluble sugars in shoots (Table 3). The starch was accumulated at all investigated NaCl concentrations in shoots, whereas in roots starch contents were more or less unchanged whatever salinity used. The applied vitamins or sodium salicylate were generally effective in antagonizing partially or completely the stimulatory effect of salt stress on starch accumulation in shoots of test plants. Starch accumulation in roots was stimulated by the application of ascorbic acid at all salinity concentrations. Thiamin induced nonsignificant effects. With application of sodium salicylate, starch accumulation in roots was significantly reduced at high levels of NaCl. Gordon *et al.* (1986) showed that re-growth of defoliated white clover was associated with a decrease in starch and other sugars of shoots and roots. It is accepted that with the demand for sugars starch degradation increased, but that was not strictly associated with low concentrations of sucrose, glucose and fructose (Baur-Hoch *et al.* 1990). Salt stress was found to affect the incorporation of internally available sugars into various fractions of cell wall in excised root segments or cell suspension cultures (Iraki and Carpita 1986, Solomon *et al.* 1987). Imamul-Huq and Larher (1983) observed that leaves of plants subjected to water stress often show a decrease in starch, which was generally accompanied by an increase in reducing sugar content.

When NaCl was provided in low concentrations

(40 and 80 mM) more soluble proteins were synthesized in shoots and roots of wheat plants than when the plants were treated with higher concentration (160 mM) of NaCl. Soaking of grains in ascorbic acid, thiamin or sodium salicylate exhibited a favourable effect on the accumulation of soluble proteins and ameliorated the inhibitory effects of high salinity concentrations on soluble protein accumulation in shoots. In roots thiamin application exerted no significant effect, whereas ascorbic acid and sodium salicylate inhibited production of soluble proteins. The present results and those obtained by others (Ziska *et al.* 1990, Lopez *et al.* 1994, Perez-Alfocea *et al.* 1995, Ashraf and O'Leary 1999) are indicative of the extent to which sugar and nitrogen metabolism in plant cells is affected by salinity stress. The remarkable responsiveness in the biosynthesis of sugars and proteins to the presence of ascorbic acid, thiamin or sodium salicylate may be taken as a further evidence of the role played by the two vitamins or sodium salicylate in plant adaptation mechanisms. It is generally assumed that stress-induced proteins play a role in stress tolerance, which may be essential for the survival of plants under extreme stress conditions (Skriver and Mundy 1990, Chandler and Robertson 1994). Munns *et al.* (1979) recorded that soluble proteins could play an important role in osmotic adjustment in water-stressed plants. Therefore, in salt resistant plants more proteins were accumulated than in susceptible ones (Thakur and Rai 1985).

Table 3. Effect of wheat grains soaking in 0.6 mM ascorbic acid, 0.3 mM thiamin, or 0.6 mM sodium salicylate on the content of soluble sugars and starch [mg g^{-1}(d.m.)] of shoots and roots of wheat plants treated with NaCl (*, ** - significant at $P = 0.05$ and 0.01, respectively, as compared with reference controls).

Soaking	NaCl [mM]	Shoots soluble sugars	Shoots starch	Roots soluble sugars	Roots starch
Water	0	14.47 ± 0.550	36.65 ± 0.925	2.73 ± 0.300	51.76 ± 1.894
	40	12.99 ± 0.643	39.50 ± 0.756	5.63 ± 0.191**	52.50 ± 1.092
	80	12.13 ± 0.497*	41.81 ± 0.852**	4.53 ± 0.400**	56.05 ± 1.847
	120	12.26 ± 0.550*	42.97 ± 0.822**	4.91 ± 0.285**	51.93 ± 1.611
	160	11.72 ± 0.674**	45.16 ± 0.901**	5.44 ± 0.386**	51.93 ± 2.060
Ascorbic acid	0	10.84 ± 0.424**	38.44 ± 0.619	2.39 ± 0.190	51.93 ± 2.196
	40	11.72 ± 0.540**	39.10 ± 0.947	4.25 ± 0.477**	55.06 ± 2.189
	80	9.21 ± 0.577**	34.97 ± 0.549	4.91 ± 0.079**	60.97 ± 1.029**
	120	8.94 ± 0.521**	28.96 ± 0.837**	4.10 ± 0.246**	60.76 ± 1.206**
	160	9.20 ± 0.644**	36.07 ± 0.521	4.82 ± 0.095**	61.60 ± 2.183**
Thiamin	0	6.18 ± 0.477**	30.38 ± 0.526**	6.44 ± 0.096**	66.53 ± 0.871**
	40	6.36 ± 0.239**	34.27 ± 0.631	6.54 ± 0.477**	66.53 ± 1.374**
	80	6.27 ± 0.515**	35.14 ± 0.819	5.58 ± 0.095**	52.44 ± 1.622
	120	9.68 ± 0.573**	36.36 ± 0.775	5.92 ± 0.246**	51.41 ± 1.099
	160	7.79 ± 0.387**	35.45 ± 0.628	4.96 ± 0.190**	46.09 ± 1.718
Sodium salicylate	0	5.60 ± 0.386**	31.50 ± 0.572**	4.44 ± 0.201**	60.35 ± 2.519*
	40	6.59 ± 0.558**	29.94 ± 0.458**	3.01 ± 0.285	46.13 ± 2.175
	80	6.76 ± 0.631**	32.46 ± 0.915**	2.62 ± 0.096	47.46 ± 1.548
	120	6.70 ± 0.400**	32.38 ± 0.920**	1.58 ± 0.095**	45.34 ± 1.663*
	160	9.17 ± 0.643**	31.31 ± 0.516**	1.29 ± 0.095**	42.94 ± 1.145**

Proline accumulation is a well-known response to water stress (Cusido *et al.* 1987, Yadav *et al.* 1997). The increase in NaCl concentration stimulated the accumulation of proline in the different organs of wheat plants (Table 4) and the highest proline content was found in plants, which grow under 160 mM NaCl. Proline is important osmotic agent, however, in some cases sugars and sugar alcohols might be more important (Bolarin *et al.* 1995). Vitamins or sodium salicylate treatments reduced the water stress-induced proline accumulation (Table 4). Since proline accumulation could be regarded as an indicator of stress severity (Hanson *et al.* 1979, Stewart and Larher 1980), it can be concluded that vitamins or sodium salicylate could ameliorate salinity stress.

The production and distribution of free amino acids other than proline in shoots and roots were also substantially affected by the various NaCl concentrations with or without vitamins or sodium salicylate treatments. NaCl stimulated the production of the free amino acids in roots. On the other hand, the production of other free amino acids in only salinized wheat shoots was mostly unchanged. However, treatments with ascorbic acid, thiamin or sodium salicylate exerted a stimulatory effect

on the production of free amino acids in salinized wheat shoots. Boggess *et al.* (1976) concluded that water stress induced a promotion of proline accumulation at the expense of other free amino acids. On the other hand, Stewart and Larher (1980) and Yadav *et al.* (1997) showed that, free amino acids other than proline were accumulated under water stress. In some cases, the pattern of changes in amino acids was opposite to that of soluble proteins, indicating that the increase in soluble proteins could be at the expense of other free amino acids.

Table 4. Effect of wheat grains soaking in 0.6 mM ascorbic acid, 0.3 mM thiamin, or 0.6 mM sodium salicylate on the content of soluble proteins, proline and amino acids [mg g^{-1}(d.m.)] of shoots and roots of wheat plants treated with NaCl (*, ** - significant at $P = 0.05$ and 0.01, respectively, as compared with reference controls).

Soaking	NaCl [mM]	Shoots			Roots		
		soluble sugars	starch		soluble sugars		starch
Water	0	70.44 ± 1.534	0.17 ± 0.008	5.00 ± 0.523	48.53 ± 1.050	0.08 ± 0.005	34.95 ± 0.422
	40	77.43 ± 1.468*	0.22 ± 0.009	5.00 ± 0.801	61.50 ± 1.154**	0.18 ± 0.010**	48.87 ± 0.475**
	80	92.67 ± 0.947**	0.44 ± 0.060**	5.84 ± 0.353	54.67 ± 0.804**	0.28 ± 0.020**	54.99 ± 0.988**
	120	70.91 ± 1.259	0.62 ± 0.064**	5.19 ± 0.084	51.34 ± 1.050	0.44 ± 0.013**	55.70 ± 0.873**
	160	61.71 ± 1.574**	0.87 ± 0.038**	5.25 ± 0.969	41.18 ± 1.050**	0.63 ± 0.009**	59.99 ± 0.983**
Ascorbic acid	0	77.85 ± 0.837*	0.16 ± 0.004	5.17 ± 0.548	26.28 ± 1.504**	0.11 ± 0.009**	53.52 ± 1.129**
	40	91.93 ± 1.363**	0.18 ± 0.009	5.80 ± 0.210	36.44 ± 1.195**	0.09 ± 0.008	36.22 ± 0.701
	80	79.37 ± 1.052**	0.20 ± 0.003	6.06 ± 0.041	36.98 ± 0.701**	0.13 ± 0.005**	54.85 ± 0.562**
	120	90.30 ± 3.148**	0.37 ± 0.002**	7.47 ± 0.675**	36.27 ± 1.050**	0.14 ± 0.004**	66.39 ± 1.406**
	160	87.05 ± 1.256**	0.59 ± 0.017**	6.98 ± 0.296**	41.88 ± 1.751**	0.17 ± 0.003**	71.52 ± 1.828**
Thiamin	0	66.97 ± 0.521	0.20 ± 0.009	5.95 ± 0.674	28.21 ± 1.052**	0.12 ± 0.009**	53.59 ± 1.125**
	40	87.47 ± 1.784**	0.20 ± 0.004	8.67 ± 0.210**	46.26 ± 1.401	0.08 ± 0.008	48.66 ± 1.124**
	80	89.73 ± 1.940**	0.31 ± 0.007**	6.16 ± 0.025*	45.88 ± 1.051	0.12 ± 0.006**	49.02 ± 0.422**
	120	87.20 ± 2.054**	0.31 ± 0.069**	6.31 ± 0.379*	49.76 ± 1.402	0.16 ± 0.008**	63.29 ± 0.561**
	160	80.53 ± 2.045**	0.32 ± 0.008**	7.68 ± 0.843**	45.74 ± 0.700	0.18 ± 0.003**	41.77 ± 0.844**
Sodium salicylate	0	85.21 ± 1.844**	0.19 ± 0.004	5.30 ± 0.886	26.81 ± 0.350**	0.11 ± 0.002**	37.34 ± 0.563**
	40	82.10 ± 1.153**	0.18 ± 0.002	8.02 ± 1.011**	44.85 ± 0.700*	0.18 ± 0.003**	37.94 ± 0.141**
	80	77.06 ± 1.151*	0.24 ± 0.010**	8.31 ± 0.299**	38.90 ± 0.904**	0.16 ± 0.004**	36.19 ± 0.846
	120	78.06 ± 2.940*	0.25 ± 0.036**	8.10 ± 0.169**	28.98 ± 0.103**	0.16 ± 0.005**	28.69 ± 0.848**
	160	84.10 ± 1.471**	0.31 ± 0.069**	8.38 ± 0.043**	30.66 ± 1.752**	0.17 ± 0.008**	26.37 ± 0.703**

The applied NaCl induced Na^+ accumulation in shoots and roots of the tested plants; the highest Na^+ accumulation was consistently displayed in plants subjected to the highest salinity (Tables 5, 6). Simultaneously, the accumulation of K^+ and Mg^{2+} decreased gradually with the rise of salinity and this trend was generally accompanied by reciprocal variations in the concentration of calcium. The alterations in distribution and accumulation of mono- and divalent cations in the different organs of salt stressed plants may be an indication of the role of these cations in regulating the physiological activities of these plants (Benzioni *et al.* 1992). The positive correlation between increased NaCl concentration in the medium and the increase in Na^+ content in tissues is well documented (He and Cramer 1992, Perez-Alfocea *et al.* 1994, Kinraide 1999, Santos and Caldeira 1999). Na^+ and Cl^- are highly water soluble and are readily taken up by plants and transported into the shoots, most likely they are acting as osmotica, but only moderate concentrations can be tolerated before growth and photosynthesis are reduced (McCree 1986). Serrano and Gaxiola (1994) reported that the high concentrations of Na^+ negatively affected the intercellular K^+ accumulation, presumably either by competing for sites through which influx of both cations occurs (Jeschke and Wolf 1988) or affecting membrane stability causing leakage of K^+ (Watad *et al.* 1991). K^+ efflux has been used as an indicator of cellular damage for a range of toxic compounds, and losses of K^+ have been also documented during salinity stress (Graifenberg *et al.* 1995, Kennedy and De Filippis 1999). The significantly lower levels of Mg^{2+} due to salinity are probably related to the lower levels of chlorophylls present in NaCl treated shoots (Kennedy and De Filippis 1999). Variations in the concentration of K^+ and Mg^{2+} in wheat plants due to increased NaCl concentrations were generally accompanied by reciprocal variations in the concentration other mono (Na^+) and divalent (Ca^{2+}) cations, *i.e.* Na^+/K^+ and Ca^{2+}/Mg^{2+} ratios increase along with NaCl concentration; a trend which is in accordance with some

Table 5. Effect of wheat grains soaking in 0.6 mM ascorbic acid, 0.3 mM thiamin, or 0.6 mM sodium salicylate on the content of Na^+, K^+, Ca^{2+}, Mg^{2+} [mg g^{-1}(d.m.)] and Na^+/K^+ and Ca^{2+}/Mg^{2+} ratios in shoots of wheat plants treated with NaCl (*, ** - significant at $P = 0.05$ and 0.01, respectively, as compared with reference controls).

Soaking	NaCl [mM]	Na^+	K^+	Ca^{2+}	Mg^{2+}	Na^+/K^+	Ca^{2+}/Mg^{2+}
Water	0	14.84 ± 0.500	39.22 ± 1.980	15.46 ± 1.040	7.24 ± 0522	0.38 ± 0.163	2.14 ± 0.269
	40	22.99 ± 1.360**	35.55 ± 1.300*	16.10 ± 1.492	6.50 ± 0.799	0.65 ± 0.182**	2.48 ± 0.153**
	80	38.25 ± 1.980**	28.36 ± 1.099**	18.36 ± 1.177**	6.71 ± 0.820	1.35 ± 0.224**	2.74 ± 0.213**
	120	60.45 ± 2.000**	21.99 ± 0.719**	19.27 ± 1.247**	4.92 ± 0.571**	2.75 ± 0.221**	3.92 ± 0.372**
	160	72.21 ± 2.000**	17.65 ± 0.439**	23.58 ± 1.592**	3.77 ± 0.672**	4.09 ± 0.317**	6.26 ± 0.399**
Ascorbic acid	0	12.98 ± 0.340	40.04 ± 2.600	17.84 ± 1.025*	8.33 ± 0.660*	0.32 ± 0.057	2.14 ± 0.284
	40	16.56 ± 0.800	38.60 ± 2.029	19.66 ± 1.050**	8.18 ± 0.359	0.43 ± 0.076	2.40 ± 0.314**
	80	29.17 ± 0.899**	33.17 ± 2.059**	22.02 ± 1.124**	7.64 ± 0.509	0.88 ± 0.075**	2.88 ± 0.334**
	120	38.07 ± 2.000**	29.97 ± 1.939**	20.35 ± 1.099**	7.05 ± 0.378	1.27 ± 0.257**	2.89 ± 0.222**
	160	51.13 ± 2.221**	25.08 ± 1.048**	24.80 ± 1.409**	5.89 ± 0.307*	2.04 ± 0.153**	4.21 ± 0.363**
Thiamin	0	14.02 ± 0.560	38.99 ± 1.659	18.24 ± 0.762**	7.96 ± 0.812	0.36 ± 0.061	2.29 ± 0.258
	40	17.28 ± 0.780	37.83 ± 1.519	18.75 ± 0.764**	8.22 ± 0.874	0.46 ± 0.072**	2.28 ± 0.150
	80	27.97 ± 1.106**	35.56 ± 1.493*	20.81 ± 0.913**	6.98 ± 0.984	0.79 ± 0.300**	2.98 ± 0.161**
	120	40.46 ± 1.399**	31.48 ± 0.880**	21.27 ± 1.060**	7.26 ± 0.630	1.29 ± 0.439**	2.93 ± 0.261**
	160	53.85 ± 0.200**	27.76 ± 0.520**	22.53 ± 1.199**	6.51 ± 0.494	1.94 ± 0.805**	3.46 ± 0.201**
Sodium salicylate	0	13.98 ± 0.400	28.30 ± 0.882**	17.99 ± 0.832*	7.19 ± 0.567	0.49 ± 0.019**	2.50 ± 0.111**
	40	19.67 ± 0.780**	32.84 ± 1.599**	19.46 ± 1.126**	7.65 ± 0.661	0.60 ± 0.039**	2.54 ± 0.063**
	80	29.39 ± 1.200**	30.18 ± 0.784**	22.36 ± 1.127**	7.27 ± 0.659	0.97 ± 0.047**	3.08 ± 0.152**
	120	43.52 ± 2.800**	34.21 ± 1.502**	21.44 ± 1.147**	6.32 ± 0.789	1.27 ± 0.239**	3.39 ± 0.207**
	160	55.77 ± 3.399**	27.46 ± 0.920**	28.68 ± 1.985**	6.06 ± 0.631*	2.03 ± 0.269**	4.73 ± 0.221**

Table 6. Effect of wheat grains soaking in 0.6 mM ascorbic acid, 0.3 mM thiamin, or 0.6 mM sodium salicylate on the content of Na^+, K^+, Ca^{2+}, Mg^{2+} [mg g^{-1}(d.m.)] and Na^+/K^+ and Ca^{2+}/Mg^{2+} in roots of wheat plants treated with NaCl (*, ** - significant at $P = 0.05$ and 0.01, respectively, as compared with reference controls).

Soaking	NaCl [mM]	Na^+	K^+	Ca^{2+}	Mg^{2+}	Na^+/K^+	Ca^{2+}/Mg^{2+}
Water	0	26.83 ± 1.068	10.82 ± 0.937	12.60 ± 0.917	6.79 ± 0.731	2.48 ± 0.419	1.86 ± 0.084
	40	30.75 ± 1.660*	10.00 ± 0.799	17.22 ± 0.722**	6.02 ± 0.531	3.08 ± 0.340*	2.86 ± 0.155
	80	46.96 ± 2.420**	8.03 ± 0.595**	16.96 ± 0.937**	5.17 ± 0.622**	5.85 ± 0.422**	3.28 ± 0.300*
	120	68.18 ± 1.920**	6.47 ± 0.657**	19.34 ± 0.962**	4.44 ± 0.541**	10.54 ± 0.824**	4.36 ± 0.078**
	160	81.52 ± 2.312**	5.02 ± 0.300**	21.55 ± 1.100**	3.18 ± 0.501**	16.24 ± 0.877**	6.78 ± 0.537**
Ascorbic acid	0	20.48 ± 0.760**	11.62 ± 1.098	13.36 ± 0.661	7.82 ± 0.450	1.76 ± 0.167	1.71 ± 0.080
	40	26.80 ± 0.959	10.95 ± 1.108	14.52 ± 0.859**	7.17 ± 0.471	2.45 ± 0.245	2.03 ± 0.103
	80	36.72 ± 1.439**	10.08 ± 0.890	18.72 ± 0.874**	6.84 ± 0.561	3.64 ± 0.564*	2.74 ± 0.317
	120	48.17 ± 1.600**	9.26 ± 0.802**	21.15 ± 0.724**	6.03 ± 0.777	5.20 ± 0.388**	3.51 ± 0.418**
	160	62.38 ± 2.140**	8.65 ± 0.880**	23.62 ± 1.198**	5.18 ± 0.479**	7.21 ± 0.438**	4.56 ± 0.321**
Thiamin	0	24.76 ± 1.379	12.43 ± 0.859**	12.99 ± 0.660	6.98 ± 0.559	1.99 ± 0.046	1.86 ± 0.045
	40	25.91 ± 1.620	11.22 ± 0.581	16.87 ± 0.609**	7.57 ± 0.857	2.31 ± 0.181	2.23 ± 0.081
	80	33.69 ± 1.820**	11.04 ± 0.559	16.29 ± 0.721**	6.18 ± 0.502	3.05 ± 0.132**	2.64 ± 0.313
	120	50.08 ± 2.159**	9.88 ± 0.832	19.57 ± 0.857**	5.27 ± 0.539**	5.07 ± 0.157**	3.71 ± 0.228**
	160	58.57 ± 2.340**	9.28 ± 0.601**	22.18 ± 0.925**	4.95 ± 0.524**	6.31 ± 0.367**	4.48 ± 0.354**
Sodium salicylate	0	22.46 ± 0.699**	8.39 ± 0.780**	11.42 ± 0.820*	6.84 ± 0.479	2.68 ± 0.149	1.67 ± 0.012
	40	28.35 ± 0.920	9.87 ± 0.957	15.94 ± 0.892**	6.82 ± 0.619	2.87 ± 0.182	2.34 ± 0.058
	80	40.02 ± 1.119**	9.00 ± 0.741**	18.80 ± 1.034**	6.77 ± 0.481	4.45 ± 0.380**	2.78 ± 0.083
	120	52.19 ± 2.040**	8.35 ± 0.699**	20.37 ± 1.164**	5.09 ± 0.810**	6.25 ± 0.687**	4.00 ± 0.117**
	160	64.28 ± 2.379**	7.33 ± 0.801**	24.06 ± 1.307**	4.76 ± 0.472**	8.77 ± 0.482**	5.06 ± 0.335**

of the results of Garcia-Reina *et al.* (1988) and Torres-Schumann *et al.* (1989) using other plant tissues. During stress, the increase in Na^+ content and decrease in K^+/Na^+ ratio in leaves resulted in rapid increase in electrolyte leakage, H_2O_2 and O_2^- content (Chen *et al.* 1994, 1998). Finally, membrane lipid, protein and integrity of membrane were damaged, which brought about a decrease in membrane-binding protein activity (such as H^+-ATPase). This result suggests that the decrease in H^+-ATPase activity may be related with accumulation of active oxygen causing lipid peroxidation (Chen *et al.* 1999). The activity of tonoplast H^+-PPase also decreased under salt stress in *Hordeum vulgare* (Matsumoto and Chung 1988, Chen *et al.* 1999).

Grain soaking in ascorbic acid, thiamin or sodium salicylate had an inhibitory effect on the accumulation of sodium in the different organs under various concentrations of NaCl. Furthermore, their application ameliorated the inhibitory effects of NaCl on K^+ and Mg^{2+} accumulation in the different organs of the test plants. Also, these treatment enhanced the stimulatory effect of salt stress on the accumulation of Ca^{2+} in wheat organs.

In general, effects of ascorbic acid or thiamin in mitigating partially or completely the adverse effects of salt stress may be one aspect of the role of these vitamins in the activation of some enzymatic reactions (Kefeli 1981). Also, Neubauer and Yamamota (1992), Choudhury *et al.* (1993) and Hamada (1998) attributed such positive effects of vitamins in counteraction of the adverse effects of salt stress to stabilizing and protecting the photosynthetic pigments and the photosynthetic apparatus from being oxidized. The salicylic acid, an ubiquitous plant phenolic compound, was recognized as an endogenous regulator in many plant physiological processes (Enyedi *et al.* 1992, Yalpini and Raskin 1993). In this context, Janda *et al.* (1999) observed that salicylic acid pre-treatment at normal growth temperature induced protection against low-temperature stress in young maize plants, probably due to increased antioxidant activity. Also, Mishra and Choudhuri (1999) found that deterioration at heavy metal stress was partially alleviated by the exogenous application of salicylic acid in *Oryza sativa*.

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