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Alleviation of the Adverse Effects of NaCl on Gas Exchange and Growth of Wheat Plants by Ascorbic Acid, Thiamin and Sodium Salicylate

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Abstract: Interactive effects of salinity stress and ascorbic acid, thiamin and sodium salicylate on gas exchange and some relevant metabolic activities of wheat plants (30-day-old) were studied. The increase in NaCl level, in the range studied, was generally associated with an increase in the net photosynthetic rate and dark respiration. On the other side, the growth rate, leaf area and transpiration rate were lowered in response to salinity stress, while the photosynthetic pigments and the water content of either shoots or roots remained more or less unchanged.

Soaking of wheat grains in 100 ppm ascorbic acid, thiamin or salicylic acid before sowing synergistically enhanced the stimulatory effect of salinity stress on net photosynthesis. On the other hand, the rate of respiration was antagonistically lowered by the application of each of the two vitamins or sodium salicylate. Generally, it can be said that, soaking of grains in vitamins or sodium salicylate before sowing exhibited favorable effects on photosynthetic pigments, growth and transpiration rate counteracting the inhibitory effects of salinity stress.

Key words: Salinity stress, NaCl, synergistic effects, ascorbic acid, thiamin

Introduction

Crops grown in semi-arid zones suffer from lack of rainfall, which causes an increase in the water deficit. This usually results in poor irrigation of crops with water containing a considerable amount of salt. Therefore many trails are now made to help crop plants overcome the noxious effects of salinity using exogenous treatments. Pre-sowing soaking of seeds with certain phytohormones or vitamins was found to be beneficial to plant growth under water stress conditions (Oertli, 1987; Ahmed and Monsaly, 1998; Gilley and Fletcher, 1998; Hamada, 1998). However, the role of vitamins or sodium salicylate in counteracting the inhibitory effects of salinity is poorly investigated no doubt, some vitamins were repeatedly used with unstressed plants to enhance the plant growth. In this respect, exogenous application of vitamins exerted mostly positive effects on plant growth, CO_2 uptake and protein synthesis (Mozafar and Oertli, 1992; Arrigoni *et al.*, 1997). Thus, exogenous addition of vitamins to test organism could lead to growth stimulation through activation of some enzymatic reactions (Kefeli, 1981). However, only some minor works were conducted to follow the role of vitamin treatments in ameliorating the adverse effects of salinity (Shaddad *et al.*, 1990, Ahmed and Monsaly, 1998).

Salicylic acid is known to be a signal molecule in acquired resistance to pathogens in several plant species (Raskin, 1992). It was also reported that salicylic acid accumulates during exposure to ozone or UV light (Yalpini *et al.*, 1994, Sharma *et al.*, 1996), and salicylic acid treatment improved the chilling tolerance of maize (Janda *et al.*, 1999) and the heat-shock tolerance of mustard plants (Dat *et al.*, 1998).

Thus the aim of present work was to follow the interactive effects of salinity and grain soaking with vitamins (ascorbic acid, thiamin) or sodium salicylate on photosynthesis and some related activities of wheat (*Triticum aestivum* L.) plants.

Materials and Methods

Plastic pots (11.5 cm in diameter and 10 cm long) lined with polythene bags and containing soil, composed of clay and sand (1:1 by weight) were used. Before sowing, the grains of wheat (*Triticum aestivum* L.) were soaked for 6 hrs in distilled water (absolute control) or in solutions containing 100 ppm of ascorbic acid, thiamin or sodium salicylate. After sowing (5 grains in each pot), the pots were then irrigated with saline solutions to reach the desired experimental salinization levels (40, 80, 120 and 160 mM NaCl) and then the water content of the soil was adjusted regularly near the field capacity. Some pots were left untreated (00 NaCl and 00 vitamins or sodium salicylate) and were regarded as absolute control plants and some other were salinized (NaCl + 00 vitamins or sodium salicylate) and regarded as reference control plants. At the end of the experimental period (30 days) fresh and dry matter yields were determined. Transpiration rate was measured under 25°C as described by Bozcuk (1975). The contents of chlorophylls a, b and carotenoids were determined spectrophotometrically (Metzner *et al.*, 1965). Net photosynthetic rate (oxygen evolution) and dark respiration (oxygen consumption) were determined manometrically using disks (diameter 16 mm) of leaf tissue exposed at 25°C, irradiance of 12 w m^{-2} (40 w GEF lamps) using the Warburg buffer No. 2961 type VL 85 (Umbreit *et al.*, 1959).

Results and Discussion

In present investigation, the response of wheat plants to high levels of salinity was reflected by decrease in shoot and root growth (Table 1). The presence of applied salt in culture media at a concentration of 80 mM or more considerably attenuated the fresh and dry matter gain in roots and shoots. Moreover, considerable decline in the leaf area of the experimental plants was induced by high levels of NaCl (Table 2). The inhibitory effects of salt stress on the three mentioned parameters add more support to the ubiquitous findings obtained by other investigators using various techniques and plant species (Downton 1977, Robinson *et al.*, 1983, Sànchez-Blanco *et al.*, 1991, Pérez-Alfocea *et al.*, 1993, Hamada, 1996). The reduction in growth could be attributed to the reduction in cell division or in cell enlargement (Nicholls and May, 1963; Terry *et al.*, 1971). Schwarz (1985) also, stated that reduced plant growth under water stress condition results from various factors, the most important of which are physiological drought, induced by the low water potential of the soil solution and osmotic adjustment in plants as a result of increased ionic concentration in their cells, which may result in deformation of macromolecules by disrupting their shell of bound water. The beneficial effects of two applied vitamins (ascorbic acid and thiamin) or sodium salicylate in mitigating partially or completely the adverse effects of salt stress on

Table 1: The action of ascorbic acid, thiamin and sodium salicylate in ameliorating the adverse effects of salt stress on growth (g plant^{-1}) and water content (g g^{-1} dry matter) of wheat plants. *significant (P=0.05) and **highly significant (P=0.01) differences as compared with control.

Treatments	NaCl (mM)	Shoot Fresh matter	Shoot Dry matter	Shoot Water content	Root Fresh matter	Root Dry matter	Root Water content
00 Vitamin or Sodium salicylate							
	00	0.693	0.087	6.966	0.046	0.008	4.750
	40	0.753	0.098	6.684	0.063*	0.011**	4.727
	80	0.512*	0.065*	6.877	0.029*	0.005**	4.800
	120	0.347**	0.044**	6.886	0.024*	0.004**	5.000
	160	0.271**	0.033**	7.212	0.019**	0.003**	5.333
100 ppm Ascorbic acid							
	00	0.815	0.102	6.990	0.056	0.009	5.222
	40	0.723	0.098	6.378	0.050	0.009	4.556
	80	0.633	0.087	6.276	0.047	0.008	4.875
	120	0.663	0.087	6.621	0.036	0.007	4.143
	160	0.542	0.076	6.132	0.029*	0.006*	3.833*
100 ppm Thiamin							
	00	0.812	0.099	7.202	0.550	0.009	5.111
	40	0.829	0.087	8.529**	0.050	0.009	4.556
	80	0.663	0.076	7.724	0.042	0.008	4.250
	120	0.572	0.065*	7.800	0.032	0.006*	4.333
	160	0.407**	0.044**	8.250*	0.026*	0.004**	5.500
100 ppm Sodium salicylate							
	00	0.645	0.076	7.487	0.038	0.007	4.429
	40	0.527	0.076	5.934	0.043	0.007	5.143
	80	0.572	0.076	6.526	0.040	0.006*	5.667*
	120	0.452**	0.054**	7.370	0.026*	0.005**	4.200
	160	0.407**	0.044**	8.250*	0.020**	0.004**	4.000
L.S.D. at 5%		0.171	0.022	1.093	0.017	0.002	0.786
L.S.D. at 1%		0.234	0.033	1.490	0.023	0.003	1.072

Table 2: The action of ascorbic acid, thiamin and sodium salicylate in ameliorating the adverse effects of salt stress on leaf area (dm^2 plant^{-1}) and transpiration rate (mg (H_2O) m^{-2} S^{-1}) of wheat plants. *significant (P=0.05) and **highly significant (P=0.01) differences as compared with control.

Treatments	NaCl (mM)	Leaf area	Transpiration rate
00 Vitamin or Sodium salicylate			
	00	0.810	8.479
	40	0.700	8.559
	80	0.590*	6.519*
	120	0.593*	5.369**
	160	0.455**	3.289**
100 ppm Ascorbic acid			
	00	0.938	8.785
	40	0.876	8.657
	80	0.756	8.020
	120	0.635	6.828*
	160	0.577*	4.296**
100 ppm Thiamin			
	00	1.084**	9.720
	40	1.014*	9.006
	80	0.802	8.152
	120	0.765	7.535
	160	0.699	5.457**
100 ppm Sodium salicylate			
	00	0.943	8.720
	40	0.829	8.426
	80	0.731	7.817
	120	0.710	6.780*
	160	0.606*	4.343**
L.S.D. at 5%		0.176	1.461
L.S.D. at 1%		0.240	1.992

plant growth were clearly exhibited by the test plants (Tables 1 and 2). This means that two vitamins or sodium salicylate may act as growth stimulants which can play a role in reversing the effect of NaCl on metabolic activities relevant to growth, like cell division and cell enlargement. Such promoting effects of vitamins were, also, obtained by some other authors (Ansari and Khan, 1986; Mozafar and Oertli, 1992; Ahmed and Monsaly, 1998).

The water content of shoots and roots remained more or less unchanged up to the high level of salinity (Table 1). Tolerance of the experimental plants was closely associated with a relatively stable water content (Hamada, 1996).

Transpiration rate was markedly affected by the salinization level and it decreased gradually as salinity increased (Table 2). Furthermore, the data obtained herein clearly demonstrate the effectiveness of ascorbic acid, thiamin or sodium salicylate in alleviating partially or completely the depressive effects of salinizing the growth media on transpiration rate of the test plants. Salt stress in the root medium strongly retards the availability of water (Hayward and Spurr, 1943) in addition to the water movements in the roots (O'Leary, 1969) and consequently alters the transpiration of plants (Gale *et al.*, 1967; Kaplan and Gale, 1972).

The effect of NaCl supply on chlorophyll a, chlorophyll b and carotenoids in the leaves of salt stressed wheat plants, in addition to interactive effects of salinity and each of the ascorbic acid, thiamin or sodium salicylate are shown in Table 3. The data clearly indicate that the pigment contents were slightly affected by different salinization levels and their interaction with each of the two applied vitamins or sodium salicylate. The observation that chlorophyll content was not affected by the water stress, is in accordance with the findings of Kulshrehtha *et al.* (1987).

Special emphasis was laid on the influence of salinity stress and its interaction with ascorbic acid, thiamin or sodium salicylate on net photosynthesis and dark respiration of the test wheat plants. The results presented in Table 3 reveal that all of the investigated salinity levels induced stimulatory

Table 3: The action of ascorbic acid, thiamin and sodium salicylate in ameliorating the adverse effects of salt stress on pigments (Chl. a, Chl. b and Carot.) contents [mg g^{-1} (d.m.)], net photosynthesis, P_N [μmol (O_2) g^{-1} (d.m.) S^{-1}] and dark respiration rate, R_D [μmol (O_2) g^{-1} (d.m.) S^{-1}] of wheat plants. *significant (P=0.05) and **highly significant (P=0.01) differences as compared with control.

Treatments	NaCl (mM)	Pigments Chl. a	Chl. b	Carot.	P_N	R_D
00 Vitamin or Sodium salicylate						
	00	1.080	0.698	0.702	1014.70	386.90
	40	1.117	0.789	0.731	1373.60**	630.49**
	80	1.191*	0.820*	0.733	1443.61**	777.32**
	120	1.113	0.773	0.721	1402.82**	861.36**
	160	1.056	0.697	0.693	1169.34**	1001.61**
100 ppm Ascorbic acid						
	00	1.062	0.676	0.694	1637.08**	3005.54
	40	1.086	0.686	0.714	1700.33**	448.92
	80	1.197**	0.819*	0.788	1606.07**	523.86*
	120	1.179*	0.767	0.763	1435.09**	659.86**
	160	1.138	0.773	0.755	1327.43**	670.58**
100 ppm Thiamin						
	00	0.989*	0.596	0.631	1353.94**	357.36
	40	1.034	0.831*	0.760	1447.98**	376.53
	80	1.149	0.769	0.760	1503.28**	559.57**
	120	1.170*	0.848*	0.751	1439.35**	591.18**
	160	1.209**	0.876**	0.773	1343.26**	575.82**
100 ppm Sodium salicylate						
	00	0.962**	0.636	0.613	1547.36**	336.30
	40	0.967**	0.644	0.607	1642.29**	390.58
	80	1.045	0.723	0.666	1575.19**	398.66
	120	0.974*	0.612	0.609	1536.36**	461.57
	160	0.988*	0.626	0.624	1463.20**	545.37**
L.S.D. at 5%		0.083	0.116	0.099	139.410	106.359
L.S.D. at 1%		0.113	0.158	0.135	190.135	145.058

effects on the photosynthetic activity of wheat plants. Furthermore, the data demonstrated the capability of vitamins or sodium salicylate in enhancing the stimulatory role of salinity stress on the activation of net photosynthetic rate in leaves of the stressed wheat plants. The increase in net photosynthetic rate at low salinity levels can also be attributed to an increase in chlorophyll concentrations per unit leaf area (Plaut *et al.*, 1990). For wheat leaves, Rawson (1986) has shown a larger increase in net photosynthetic rate for a given rise in stomatal conductance under salinity as compared with non-saline conditions, also suggesting that net photosynthetic rate was less sensitive to salinity than transpiration. Evidence to support this suggestion can be obtained from the data presented herein which indicated that the water content of shoots or roots remained unchanged up to the high level of salinity, in the range studied. There is a considerable amount of evidence (Huq and Palmer, 1978), that water stress can generate the superoxide anion radical (O^{-2}) in plant tissues which is converted to hydrogen peroxide (H_2O_2). H_2O_2 strongly inhibits CO_2 fixation, possibly by inactivating transketolase (Kaiser 1976) or by inactivating several Calvin cycle enzymes. Neubauer and Yamamota (1992) and Choudhury *et al.* (1993) attributed positive effects to vitamins for stabilizing and protecting the photosynthetic pigments and the photosynthetic apparatus from being oxidized. To a certain extent, the action of salicylic acid is similar to the effects of the other regulatory molecules i.e. jasmonic acid and abscisic acid, on the processes of germination, growth and aging, and also similar in the manner of effect on the stomata (Popova *et al.*, 1987, 1988). The established effects of salicylic acid on stomatal function, chlorophyll content, transpiration rate and respiratory pathways rise the assumption that salicylic acid might possess another physiological function, most probably involved in regulation of some photosynthetic reactions.

The results presented in Table 3 reveal that dark respiration was stimulated at all investigated salinity levels. The magnitude of this increase was more pronounced with further rise of salinity level. Treatment with ascorbic acid, thiamin or sodium salicylate significantly retarded the dark respiration rate in comparison with reference controls. The reduction of growth under salinity conditions could be related to the increase of maintenance respiration (Schwarz,1985). The applied vitamins or sodium salicylate was generally effective in antagonizing partially or completely the stimulatory effects of salt stress on dark respiration. The improvement in growth criteria of the treated wheat plants was found to be in concomitance with the marked and progressive retardation in the rate of respiration.

From the proceeding results and discussion, it can be concluded that treatment of wheat grains with ascorbic acid, thiamin or sodium salicylate could alleviate the inhibitory effects of salt stress and also stimulate the growth *via* enhancement of the photosynthetic rate and retardation of dark respiration.

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