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Effect of salinity on water relations, sodium accumulation, chlorophyll content and proteolytic enzymes in a wild wheat

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Abstract

The effect of 50 to 200 mM NaCl on two lines (CP with solid stem and CV with hollow stem) of ×*Haynaldoticum sardoum* was studied. NaCl significantly reduced root and shoot fresh and dry masses, root length and less markedly shoot length of CP and CV plants. The sodium accumulated in the leaves in relation to the concentration of NaCl and length of the treatment; CP leaves contained twice as much sodium as CV leaves. The leaf chlorophyll *a/b* ratio was not affected by NaCl. NaCl decreased the leaf water and osmotic potentials. The pressure potential increased due to the increased concentration of dissolved solutes in the leaf, particularly sodium. The proteinase and exopeptidase activities increased during NaCl treatment.

Additional key words: aminopeptidase, carboxypeptidase, growth, ×*Haynaldoticum sardoum*, proteinase.

Introduction

Negative effects of salinity depend on environmental conditions and plant genotype. Some crops, such as sorghum, maize and soybean, respond to salinity by accumulation of soluble organic compounds leading to osmotic adjustment.

×*Haynaldoticum sardoum* is a potential crop species for less favourable environmental conditions, such as dry and saline soils, or when irrigation water of

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Abbreviations: CBZ-Phe-Ala - carbobenzoxy-phenylalanyl-alanine; Chl - chlorophyll; CP - Culmo Pieno; CV - Culmo Vuoto; d.m. - dry mass; f.m. - fresh mass; Leu-p-NA - leucine *p*-nitroanilide; ψ_p - pressure potential; ψ_π - osmotic potential; ψ_w - water potential.

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low quality is used (Cremonini *et al.* 1992). This wild wheat, growing exclusively among crops of durum wheat in Sardinia, Sicily, central and southern Italy, is characterized by two lines, one with a solid stem (CP) and the other with a hollow stem (CV; Meletti and Onnis 1975). The main aims of this work were to study the influence of increasing NaCl concentration on growth and water relations, and to measure the accumulation of sodium, the chlorophyll content, and the proteolytic enzyme activities in leaves of salt stressed plants to find some possible mechanism of tolerance in two wild lines of ×*Haynaldoticum sardoum*.

Materials and methods

Uniform seeds of ×*Haynaldoticum sardoum* Meletti *et* Onnis of the CP and CV lines were surface-sterilized in 1 % NaOCl, thoroughly washed with distilled water, and then germinated in trays at 23 °C in the dark. After 2 d, uniform plants were transferred to hydroponic culture in 4 dm^3 containers. They were then kept in a phytotron with an irradiance of 200 μmol(photon) m^{-2} s^{-1} at shoot level for 12-h photoperiod, a day/night temperature of 25/15 °C and relative humidity 60 - 80 %. Hoagland nutrient solution was continuously aerated and renewed every 3 d to avoid ion depletion. The water loss was compensated daily. NaCl treatments were initiated 6 d after the plants were transferred to nutrient solution; 50, 100, 150 and 200 mM NaCl was added, and growth continued for 3 d. Plants were harvested every day from the start of salinization, and separated into roots and shoots, which were immediately weighed (fresh mass; f.m.) and then dried at 65 °C for 48 h to determine dry mass (d.m.).

Leaf water potential (ψ_w) was measured following Scholander *et al.* (1965). Each leaf was enclosed in a plastic bag and placed in the chamber within 20 s of collection. Osmotic potential (ψ_π) was measured by a cryoscopic method using a digital osmometer (*Osmomat 030*, *Gonotec*, Berlin, Germany). Leaves were cut into small pieces, and put into a plastic microtest tube, without the tip, which was already inserted inside a larger tube. The plant material was frozen, thawed and centrifuged by collecting the cellular sap used for the osmotic potential determination. Leaf pressure potential (ψ_p) was calculated by subtraction. The relative water content was measured on shoot portions of plants harvested at the end of the salinization treatment following Richter (1978).

Leaf sodium content was determined by a flame photometer (*Jenway PFP7*, Dunmow, England) on cellular sap samples obtained with the same procedure described earlier for osmotic potential determination.

Leaf chlorophylls (Chl) were extracted with N,N-dimethylformamide at a ratio 2:100 (m/v) according to Moran and Porath (1980). Chlorophyll *a* and *b* content was determined spectrophotometrically according to Inskeep and Bloom (1985).

The proteolytic activities were determined on frozen and lyophilized leaves (50 mg) homogenized in a cold mortar with 0.05 M sodium phosphate buffer (pH 7) at a ratio 1:100 (m/v) and extracted for 10 min at 4 °C. The homogenate was centrifuged at 40 000 *g* and 4 °C for 10 min, and the supernatant used as an enzymic

source. The proteinase activity was assayed with azocasein: 0.150 cm^3 of enzyme solution was mixed with 0.850 cm^3 of 0.1 M sodium phosphate buffer (pH 7.5). After 10 min of preincubation at 30 °C, the reaction was started by adding 1 cm^3 of 1 % (m/v) azocasein that had been resuspended in the assay buffer. The reaction was stopped by adding 1 cm^3 of 24 % (m/v) trichloroacetic acid and vortexing. After standing at 4 °C for 10 min, the solution was cleared in a bench centrifuge. Soluble peptides in the supernatant were determined by absorbance at 330 nm. Controls for each sample were similarly treated, but without incubation. One unit (U) of proteinase activity was defined as the amount of enzyme required to produce an absorbance change of 1 in a 1 cm cuvette under the conditions of the assay, compared with the blank. Aminopeptidase and carboxypeptidase activities were determined as previously described (Del Zoppo *et al.* 1998).

The protein content was estimated following Lowry *et al.* (1951), using bovine serum albumin as the standard.

The results were analysed by a one-way analysis of variance, using the *CoStat* statistical package (*CoHort 2 Software* 1986, 1990).

Results

Root growth in CP plants was already affected after 24 h in the presence of 50 mM NaCl (Table 1). This effect was maintained during the other two days of salt treatment, and caused a strong reduction in the growth at the highest NaCl concentration, of about 50 %. Shoot length was less negatively affected than root length during the first day of treatment (Table 1). Root and shoot growth was severely inhibited or stopped at the highest NaCl concentration used. The growth in CV plants showed a similar behaviour to that found in CP plants (Table 1).

Table 1. Root and shoot length [cm] of 8-d-old CP and CV plants of *×Haynaldoticum sardoum* treated for 24, 48 and 72 h with NaCl at concentration 0 to 200 mM. Means ± SE of two replicates of 30 seedlings each. Values in the same column followed by different letters are significantly different ($P < 0.01$).

	NaCl	CP			CV		
		24 h	48 h	72 h	24 h	48 h	72 h
Root	0	12.1±0.61a	13.1±0.59a	14.4±0.53a	12.2±0.82a	14.1±0.27a	14.9±0.42a
	50	7.4±0.21b	7.4±0.28b	7.8±0.23b	10.7±0.51b	11.2±0.56b	11.8±0.34b
	100	7.2±0.26b	7.2±0.21b	7.5±0.27b	10.5±0.65b	10.2±0.48b	10.8±0.35c
	150	7.1±0.31b	7.2±0.20b	7.3±0.22b	10.6±0.66b	10.9±0.55b	10.8±0.31c
	200	7.1±0.18b	7.2±0.23b	7.2±0.21b	9.6±0.57b	9.6±0.56b	9.6±0.41d
Shoot	0	16.9±0.51a	18.6±0.64a	20.1±0.71a	16.9±0.46a	19.6±0.35a	20.0±0.51a
	50	15.3±0.21b	16.7±0.31b	17.0±0.40b	16.5±0.31a	18.9±0.32a	19.4±0.32a
	100	14.8±0.18c	15.9±0.22c	16.1±0.25c	15.0±0.26b	16.5±0.31b	16.4±0.22b
	150	14.4±0.33c	15.6±0.31c	15.5±0.18d	14.7±0.27bc	15.7±0.29bc	15.5±0.31c
	200	14.2±0.57c	14.2±0.24d	14.5±0.21e	14.5±0.14c	14.8±0.26c	14.8±0.28d

Root and shoot fresh masses of CP and CV plants decreased by increasing the NaCl concentration (Fig. 1*A*). However, roots and shoots of CP plants appeared to be more sensitive to the lowest salt concentrations than those of CV plants. The dry masses of the two types of plants were reduced even at the lowest NaCl concentration (Fig. 1*B*).

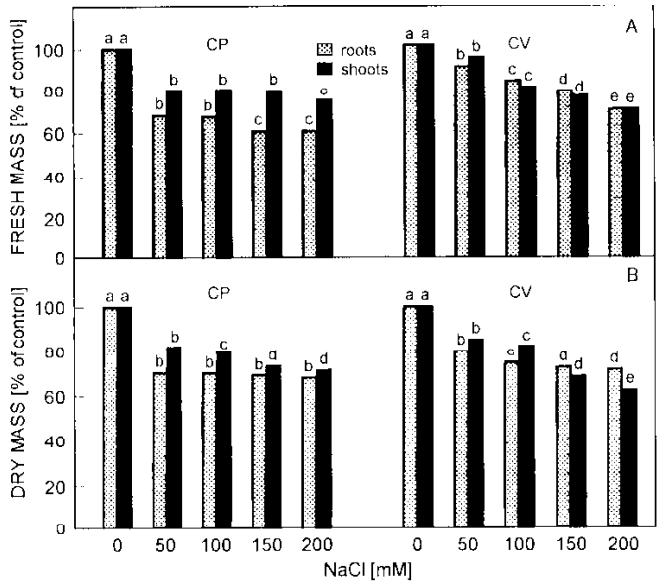

Fig. 1. Effect of 0 to 200 mM NaCl on the fresh mass (*A*) and dry mass (*B*) of roots and shoots in CP and CV plants of ×*Haynaldoticum sardoum* seedlings: after 3 d of salt treatment. Means of 3 replicates of 15 seedlings each.

The amount of Na^+ generally increased with the concentration of NaCl and with the length of treatment, although the behaviour of CP and CV plants was different (Table 2). At the end of the treatment and in the presence of 200 mM NaCl, the CP leaves contained twice as much Na^+ as CV.

NaCl caused a small decrease in chlorophyll *a* and *b* content in CP and CV plants (Table 2). However, the chlorophyll *a*/*b* ratio did not change significantly in either plant.

Leaf water potential progressively decreased due to salinity in both lines (Table 3). The osmotic potential also decreased and the pressure potential increased during salinization both in CP and CV plants (Table 3). The relative water content did not show any significant changes when the NaCl concentration was increased (data not shown).

The aminopeptidase activity increased, particularly at high NaCl concentrations (Fig. 2*A*); a similar pattern was shown by carboxypeptidase activity (Fig. 2*B*), and by proteinase activity (Fig. 2*C*). All three classes of proteolytic enzymes increased at first, and to a higher degree in the CP plants.

Table 2. Leaf sodium [μmol g^{-1}(d.m.)] and chlorophyll [mg g^{-1}(d.m.)] contents in 8-d-old plants of ×*Haynaldoticum sardoum* treated for 24, 48 and 72 h with NaCl at concentration 0 to 200 mM. Means ± SE of two replicates of 5 seedlings each. Values in the same column followed by different letters are significantly different ($P < 0.01$).

	NaCl	Na^+ 24 h	48 h	72 h	Chl *a* 72 h	Chl *b* 72 h
CP	0	0±0.0a	0± 0.0a	0± 0.0a	1.71±0.02a	0.46±0.02a
	50	4±1.1b	17± 1.3b	65± 6.4b	1.62±0.03ab	0.42±0.01ab
	100	13±1.5c	77± 2.9c	256±12.3c	1.63±0.03ab	0.43±0.01ab
	150	83±8.7d	216±12.3d	479±11.6d	1.65±0.02ab	0.43±0.01ab
	200	94±8.3d	359±11.4e	485±18.8d	1.52±0.02b	0.40±0.01b
CV	0	0±0.0a	0± 0.0a	0± 0.0a	1.63±0.04a	0.44±0.01a
	50	0±0.0a	9± 1.8a	59± 7.4b	1.44±0.04b	0.38±0.01b
	100	11±1.3b	43± 2.7b	109±17.1c	1.45±0.04b	0.39±0.02b
	150	67±3.5c	105±15.8c	151±10.9d	1.49±0.03b	0.40±0.01b
	200	99±6.9d	179±15.8d	220±12.3e	1.42±0.05b	0.38±0.02b

Table 3. Leaf water (Ψ_w), osmotic (Ψ_π), and pressure (Ψ_p) potentials [MPa] in 8-d-old plants of ×*Haynaldoticum sardoum* treated for 24, 48 and 72 h with NaCl at concentration 0 to 200 mM. Means ± SE of two replicates of 5 seedlings each. Values in the same column followed by different letters are significantly different ($P < 0.01$).

	NaCl	CP			CV		
		24 h	48 h	72 h	24 h	48 h	72 h
Ψ_w	0	-0.62±0.008a	-0.66±0.023a	-0.63±0.012a	-0.45±0.010a	-0.51±0.009a	-0.55±0.009a
	50	-0.68±0.008b	-0.69±0.008a	-0.72±0.033b	-0.45±0.012a	-0.53±0.018a	-0.62±0.018b
	100	-0.69±0.009b	-0.76±0.014b	-0.79±0.011c	-0.57±0.024b	-0.69±0.024b	-0.78±0.027c
	150	-0.72±0.020b	-0.76±0.016b	-0.80±0.027c	-0.58±0.026b	-0.69±0.014b	-0.78±0.027c
	200	-0.78±0.012c	-0.78±0.014b	-0.80±0.026c	-0.59±0.014b	-0.70±0.005b	-0.79±0.010c
Ψ_π	0	-0.66±0.042a	-0.70±0.042a	-0.67±0.051a	-0.64±0.082a	-0.67±0.055a	-0.67±0.053a
	50	-0.73±0.063b	-0.82± 0.059b	-0.95±0.089b	-0.69±0.073a	-0.86±0.072b	-0.86±0.092b
	100	-0.74±0.045b	-0.92±0.062c	-1.29±0.093c	-0.84±0.118b	-0.96±0.049c	-1.11±0.088c
	150	-0.94±0.056c	-1.30±0.075e	-1.73±0.104d	-1.02±0.121c	-1.13±0.077d	-1.37±0.085d
	200	-0.99±0.067c	-1.52±0.081e	-2.00±0.124e	-1.13±0.133c	-1.36±0.084e	-1.61±0.079e
Ψ_p	0	0.04±0.007a	0.04±0.007a	0.04±0.008a	0.19±0.007a	0.16±0.007a	0.12±0.006a
	50	0.05±0.007a	0.13±0.025b	0.23±0.021b	0.24±0.015b	0.33±0.031b	0.24±0.012b
	100	0.05±0.008a	0.16±0.017b	0.50±0.009c	0.27±0.017c	0.28±0.020b	0.33±0.010c
	150	0.22±0.007b	0.52±0.015c	0.93±0.010d	0.44±0.025d	0.44±0.024c	0.59±0.009d
	200	0.21±0.019b	0.74±0.018d	1.19±0.015e	0.54±0.036e	0.66±0.021d	0.82±0.017e

Discussion

In the presence of NaCl, the growth of roots and shoots decreases in both lines of *×Haynaldoticum sardoum* plants, although the shoots of the CV plants show a minor sensitivity to the lowest salt concentration. Moreover, the fresh mass and the dry mass of shoots and roots decrease, even at low NaCl concentrations. Although CV plants appear to be less sensitive to low NaCl concentrations, this difference disappears when the NaCl concentration is increased. Under salt stress the leaf water potential and the osmotic potential decrease in both lines. As pressure potential increases by salinity osmotic adjustment may be involved. CP plants show high

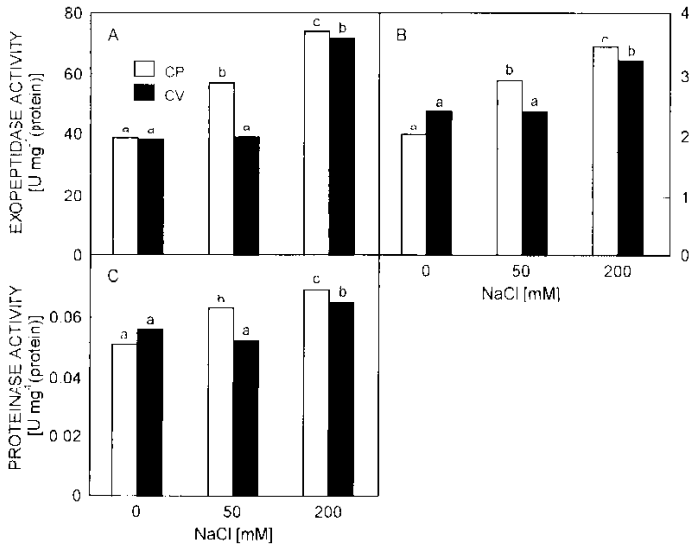

Fig. 2. Exopeptidase activities hydrolysing Leu-pNA (*A*) and CBZ-Phe-Ala (*B*) and proteinase activity hydrolysing azocasein (*C*) in CP and CV leaves of *×Haynaldoticum sardoum* grown for 3 d at different NaCl concentrations. Means of 8 replicates.

content of Na^+ in leaf tissues, both as a result of increasing the salt concentration of the external medium or in relation to the duration of the salt treatment. Thus, osmotic adjustment would seem to be achieved above all by an increase in sodium. This strategy appears to be common (Greenway and Munns 1980, Cachorro *et al.* 1993, Abd El-Samad and Shaddad 1997). However, high concentrations of sodium might negatively influence many processes in the leaf, *e.g.*, the synthesis of lipids, nucleic acids, proteins (Mansour 1995). Although excessive Cl^- uptake can be also toxic, Na^+ is mainly responsible for the toxicity caused by NaCl in wheat, rice, barley, and sorghum (Flowers *et al.* 1991, Yeo 1993). The chlorophyll *a*/*b* ratio remains constant in CP and CV plants under increasing NaCl concentration, as found in *Spinacia oleracea* (Robinson *et al.* 1983), in *Brassica juncea* (Alia *et al.* 1992) and in *Panicum miliaceum* (Li and Nothnagel 1989).

The endopeptidase and exopeptidase activities increase with increasing NaCl concentration. This is the opposite of what has been found in the endosperms from germinating seeds of ×*Haynaldoticum sardoum*, where the decrease of the proteolytic activity leads to the impaired capacity of the seed to mobilize storage proteins, and hence deprive young seedlings of nutrients prior to the development of the autotrophic capacity (Del Zoppo *et al.* 1998). However, an increase in proteolytic activities was also found in rice leaves (Dubey and Rany 1990) and mung bean seedlings (Zayed and Zeid 1997/98). The increase in proteolytic activities, found in ×*Haynaldoticum sardoum* leaves under salt stress, might represent a strategy to produce amino acids for osmotic adjustment (Flowers *et al.* 1991). Dubey and Rany (1990) suggest that the salt tolerance of certain rice cultivars might be due to higher levels in proteinase and aminopeptidase activities. Our study gives a different interpretation: since the proteolytic activities increase at the maximal salt concentration in a similar manner in both lines, the osmotic adjustment do not seem to be due to proteolysis. Since the osmotic adjustment appears to depend from an increased uptake and translocation of NaCl to the leaves, the CP plants might be less sensitive to the toxic effects caused by the highest sodium concentration. However, this capacity do not benefit them because they grow similarly to the CV plants.

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