Osmotic adjustment in triticales grown in presence of NaCl

A. MORANT-AVICE*, E. PRADIER* and R. HOUCHI**

Laboratoire de Physiologie Végétale, Université du Maine,
*Avenue Olivier Messiaen, F - 72085 Le Mans Cedex 9, France**
Laboratoire de Physiologie Végétale, Institut d'Agronomie, Université Mouloud Mammeri,
*15000 Tizi Ouzou, Algérie***

Abstract

Growth and Na^+, K^+, Cl^-, proteins, sugars and proline concentrations were measured in three triticale genotypes M2A, DF99 and Asseret grown on nutrient solution with or without 75 mM NaCl. In saline conditions, leaf area of the three triticales was reduced by 50 % and dry to fresh mass ratio increased. Total protein concentration was diminished by 10 %. K^+ concentration decreased whereas Na^+ and Cl^- accumulated in roots and shoots of salt-stressed plants. This ion accumulation was greater in roots of Asseret than in roots of the other triticales. Soluble sugar concentration increased in M2A and Asseret and decreased in DF99. Proline concentration increased in M2A and DF99 and decreased in Asseret. Osmotic adjustment was essentially realized by Na^+ and Cl^- uptake. Non-reducing sugars and proline contributed too, but to a lesser extent.

Additional key words: proline, proteins, sodium, sugars.

Introduction

Triticale is a choice cereal of growth in saline areas because of the combination of high yield quality of wheat and resistant traits of rye. However, experiments in laboratory and in field have shown that only some specific wheat/rye combinations allow the appearance of the desired characters (Lelley 1992).

Physiological criteria suggested for selection of salt tolerant cultivars are osmotic adjustment (Weimberg 1987, Salim 1989), water-use efficiency (Houchi and Coudret 1994 a), Na^+/K^+ selectivity (Bizid *et al.* 1988, Salim 1989, Gorham 1990), cytokinin concentration (Kuiper *et al.* 1990) or proline content (Mumtaz *et al.* 1995, Günes *et al.* 1996, Trotel *et al.* 1996).

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Phone: (+2 43) 833243, fax: (+2 43) 833245, e-mail: avice@aviion.univ-lemans.fr

In order to precise the behaviour of triticales in presence of a moderate salinity, we have measured the contribution of some solutes to osmotic adjustment in three genotypes grown in presence of 75 mM NaCl (osmotic potential was then lowered by -0.403 MPa). The three genotypes were able to continue growth under this salinity during 10 - 12 d. Some cereals can grow in presence of stronger salt stresses, *e.g.*, *Triticum aestivum* and *T. turgidum* at osmotic potential -1.2 MPa (Weimberg 1987), or some triticales at 200 mM NaCl (Bizid *et al.* 1988). This work completed results previously obtained (Houchi and Coudret 1994b) with pre-selected triticales from the cereal experimental station of Constantine (Algeria).

Materials and methods

Plant growth: Seeds of three hexaploid triticales M2A/JAIN, DF99/Yogu"S5" and Asseret were supplied by the station of cereal cultivation of Constantine, Algeria. Seeds were germinated in Petri dishes in presence of distilled water before transfer to hydroponic culture. The plantlets were grown on aerated Coic and Lesaint (1973) nutrient solution (M0) or on nutrient solution complemented with 75 mM NaCl (M75). Root media were replaced every 4 d. The osmotic potential of M0 was -0.077 MPa and that of M75 was -0.48 MPa. The plants were grown at irradiance 150 μmol(photon) m^{-2} s^{-1} (measured by quantum meter (*Li-189, Li-Cor*, Lincoln, USA) provided by 400 W *Phytoclaude* lamps. The photoperiod was 16 h; the air temperature and water vapour pressure deficit were 28 °C and 26.2 hPa during the light period and 23 °C and 18.5 hPa during the dark period.

Plants with five leaves, 18-d-old, were harvested. Leaves and roots were quickly and separately weighed before being dried in an oven at 110 °C for 2 d. Leaf areas were calculated using a scanner and an area programme.

Mineral composition: Na^+ and K^+ were essayed by flame photometry (*Eppendorf*, Raucaire, Cowetaboeuf, France) and Cl^- by potentiometric titration with $AgNO_3$ (*Metrohm,* Herisau, Switzerland) in aqueous extracts of aerial parts and roots according to Férard and Coudret (1982).

Organic solutes: Proline was detected according to Bates *et al.* (1973), reducing sugars by cuprimetric method of Somogyi as well as soluble non-reducing sugars hydrolysed with H_2SO_4. Total proteins were determined according to Hartree (1972).

Osmotic potential: Leaves and roots were immediately frozen in liquid nitrogen and put in plastic syringes. Leaves and roots were thawed and the cell sap was obtained by extrusion. Osmotic potentials of crude extracts were determined using a microosmometer (*Roebling*, Berlin, Germany). The estimated osmotic potential for each solute species was obtained using the total tissue water and solute content with the van't Hoff equation.

Results

Plant growth: In salinized medium, leaf area decreased (Fig. 1) in relation to control plants. M2A was more affected by NaCl than the other triticales. Dry mass/fresh mass ratios (DM/FM) of plants grown in presence of NaCl increased (Table 1). The reduction of plant dry biomass was essentially due to reduction of leaf dry mass because root dry mass production was little affected by NaCl.

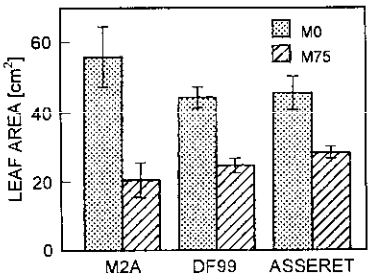

Fig. 1. Leaf area of three triticales grown on nutrient medium (M0) or on nutrient medium complemented with 75 mM NaCl (M75). Means of 6 plants ± SE (*vertical bars*).

Table 1. Root and leaf dry mass [g plant^{-1}] and dry mass/fresh mass ratio of triticales M2A, DF99 and Asseret grown on nutrient medium (M0) or on nutrient medium complemented with 75 mM NaCl (M75).

Parameter	Genotype	Leaves M0	M75	Roots M0	M75
Dry mass	M2A	0.202 ± 0.030	0.100 ± 0.036	0.750 ± 0.010	0.065 ± 0.020
	DF99	0.140 ± 0.012	0.088 ± 0.010	0.041 ± 0.008	0.051 ± 0.003
	Asseret	0.108 ± 0.017	0.075 ± 0.008	0.029 ± 0.005	0.040 ± 0.005
DM/FM	M2A	12.97 ± 0.50	14.51 ± 0.61	7.90 ± 0.30	8.81 ± 0.89
	DF99	10.36 ± 0.40	13.55 ± 0.20	4.18 ± 0.40	5.17 ± 0.26
	Asseret	11.52 ± 0.31	14.28 ± 0.26	3.30 ± 0.16	4.61 ± 0.40

Ion concentration: Leaf K^+ concentration of NaCl treated plants were lower than in control plants (Fig. 2). Decrease of shoot K^+ concentration was 36 % in DF99; 34.7 % in Asseret and 26.6 % in M2A. Leaf K^+ concentration was equal in the three salt-stressed triticales. Under control conditions (M0), root K^+ concentration was very different according to genotypes. Root K^+ concentration of DF99 was twice than M2A and that of Asseret was three times greater than M2A. In presence of NaCl, root K^+ concentration was reduced by 58.1 % in M2A, 64.5 % in DF99 and 68.7 % in Asseret. Presence of NaCl in root medium more affected K^+ concentration in roots than in shoots in the three triticales.

In M2A and DF99 control plants, Na^+ and Cl^- concentrations in roots and in shoots were lower (Fig. 2) than that in Asseret. The salinization of root medium considerably increased Na^+ concentration in roots and in shoots in the three triticales. Leaf Na^+ concentration was 11 to 24 times greater than in control plants; root Na^+ concentration was 6 to 17 times greater than in control plants. Na^+ and Cl^- accumulation was higher in roots of Asseret than in DF99 and M2A.

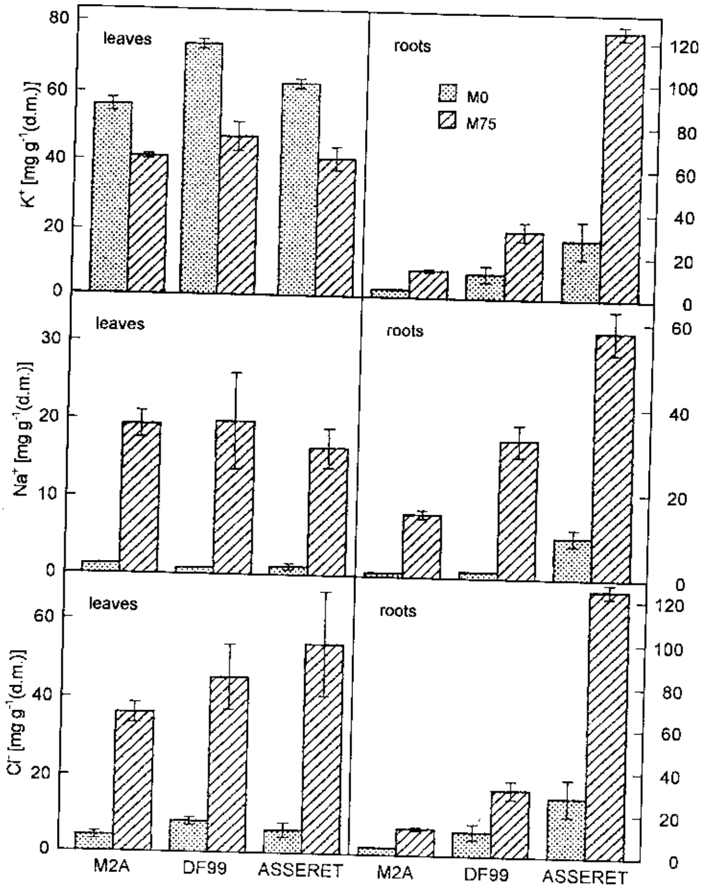

Fig 2. K^+, Na^+ and Cl^- concentrations in leaves and in roots of three triticales grown on nutrient medium (M0) or on nutrient medium complemented with 75 mM NaCl (M75). (d.m. - dry mass). Means of 6 plants ± SE (*vertical bars*).

Organic compounds: Total protein concentration of both shoots and roots (Fig. 3) was little decreased (about 10 %) in the salt-stressed genotypes in relation to control plants. Regardless the growth medium or the genotype, soluble sugar concentration was higher in roots than in shoots (Fig. 4). Total soluble sugar concentration increased in roots and shoots of M2A and Asseret grown on M75. Soluble sugar concentration decreased in DF99. These variations were mainly due to soluble non-reducing sugars.

In control plants, proline concentration (Fig. 4) was 3 to 4 times higher in roots than in shoots. The salinization of root medium induced an increase of proline concentration in roots and shoots of M2A and DF99, while proline concentration decreased in leaves and roots of Asseret.

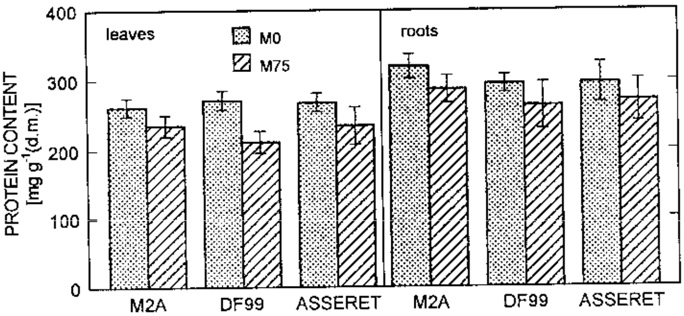

Fig. 3. Total protein concentration in leaves and in roots of three triticales grown on nutrient medium (M0) or on nutrient medium complemented with 75 mM NaCl (M75). Means of 6 plants ± SE (*vertical bars*).

Osmotic adjustment: Under control conditions, contribution of Na^+, Cl^- and K^+ ions to leaf osmotic potential was greater than that of soluble sugars (Table 2). Under salt stress, the contribution of ions to leaf osmotic potential greatly increased whereas that of soluble sugars increased only in Asseret. Measured ionic and organic solutes accounted for about 75 - 80 % of the osmotic potential in leaves of the three salt-stressed triticales.

Discussion

Presence of 75 mM NaCl in the nutrient medium induced a growth decrease of M2A, DF99 and Asseret. NaCl particularly affected shoot growth. Similar results were obtained by Bizid *et al.* (1988) with other triticales.

Decrease of total protein concentration was about 10 % in roots and shoots of salt-stressed plants in comparison with control plants (Fig. 3). According to Serrano (1996), some enzyme systems may be especially sensitive to inhibition by either Na^+ or Cl^- at low concentration. Protein synthesis and important metabolic reactions could then be reduced. Protein synthesis has been considered as a possible primary target of salt toxicity because *in vitro* protein synthesis systems are dependent on physiological potassium and are inhibited by sodium and chloride.

Under salt-stress conditions, plants took up mineral ions, especially Na^+ and Cl^- from nutrient medium for osmotic adjustment. Under saline treatment, Na^+ and Cl^- concentrations extensively increased in roots and shoots of the three triticales. Cl^- was translocated up to the leaves of M2A and DF99 (Table 2).

Presence of salt in root medium induces a decrease of potassium absorption in numerous plants. Then, K^+ ions were preferentially transported to aerial parts of the triticales. Potassium is beneficial for salt tolerance because K^+ counteracts the inhibitory effects of Na^+ on enzymatic systems (Serrano 1996). The three triticales have the same discrimination between Na and K in transport to the shoots (K^+/Na^+ ratio) and could not be used here as a criterion for triticale discrimination facing NaCl.

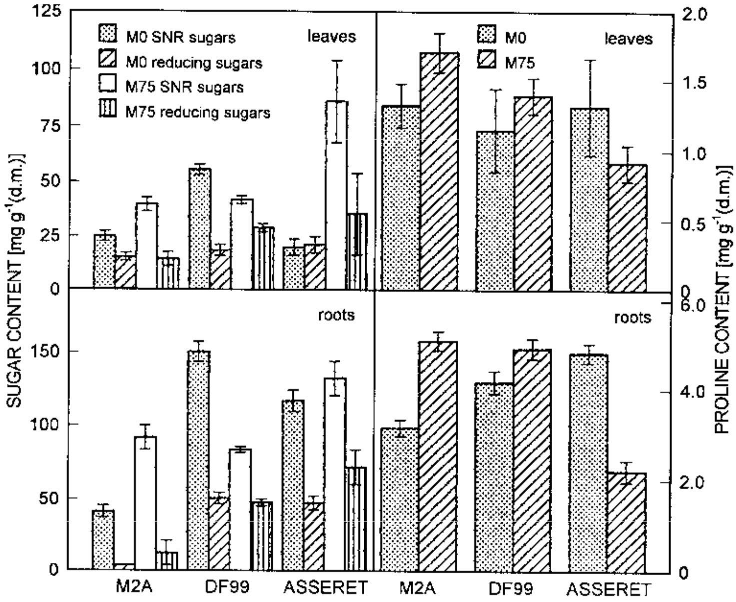

Fig. 4. Soluble sugar and proline concentrations in leaves and in roots of three triticales grown on nutrient medium (M0) or on nutrient medium complemented with 75 mM NaCl (M75). (SNR sugars - soluble non-reducing sugars). Means of 6 plants ± SE (*vertical bars*).

Table 2. Osmotic potential [-MPa], Na^+, Cl^-, K^+ and soluble sugars concentrations [mol dm^{-3}] and their contribution [%] to osmotic potential in leaves of triticales M2A, DF99 and Asseret grown on nutrient medium (M0) or on nutrient medium complemented with 75 mM NaCl (M75).

	M2A		DF99		Asseret	
	M0	M75	M0	M75	M0	M75
osmotic potential	1.30 ± 0.09	1.70 ± 0.21	0.91 ± 0.28	1.70 ± 0.14	1.12 ± 0.15	1.86 ± 0.48
K^+, Na^+, Cl^- conc.	0.244	0.497	0.250	0.521	0.239	0.543
contribution	45.38	70.88	66.59	74.35	51.78	70.80
soluble sugars conc.	0.022	0.33	0.030	0.043	0.022	0.074
contribution	4.15	4.70	8.06	6.17	4.82	9.62

During salt stress, the concentration of proline and sugars could also increase in plants (Weimberg 1987). On M75, soluble sugars concentration increased in roots and shoots of M2A and Asseret. On the contrary, it decreased in DF99 (Fig. 4). When reducing sugars were distinguished from soluble non-reducing sugars, it appeared that the latter were essentially responsible for concentration variations (Fig. 4). Weimberg (1987) has also observed an increase of sucrose content in *Triticum turgidum* and *T. aestivum* submitted to different levels of salinity. On the other hand, Kameli and Lösel (1995) have shown that sugars, particularly glucose made the largest contribution to osmotic adjustment of *Triticum durum* submitted to water stress. In *Agropyron elongatum*, reducing sugars content does not vary when grown on saline media (Weimberg 1986).

In control and in salt-treated triticales, proline concentration (Fig. 4) was greater in roots than in shoots. Salinization of root medium induced an increase of proline concentration in M2A and DF99 and a decrease in Asseret. Proline accumulated in numerous plants undergoing saline and other types of stress. Proline is a compatible osmolyte and can protect proteins from inhibitory effects of ions (*e.g.*, Olmos and Hellin 1996).

Considering our results, it is clear that osmotic adjustment was widely realized by absorption of Na^+ and Cl^- in the three triticales grown on M75. In Asseret ion accumulation in its roots was greater. Non-reducing sugars also took part in osmotic adjustment in M2A and Asseret, but in a lesser extent. At last, proline was a stress indicator only in M2A and DF99; its concentration was too low in relation to other solutes to be counted in osmotic adjustment. This study showed that, in controlled environment, the three triticales were salt-tolerant with some different physiological responses.

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