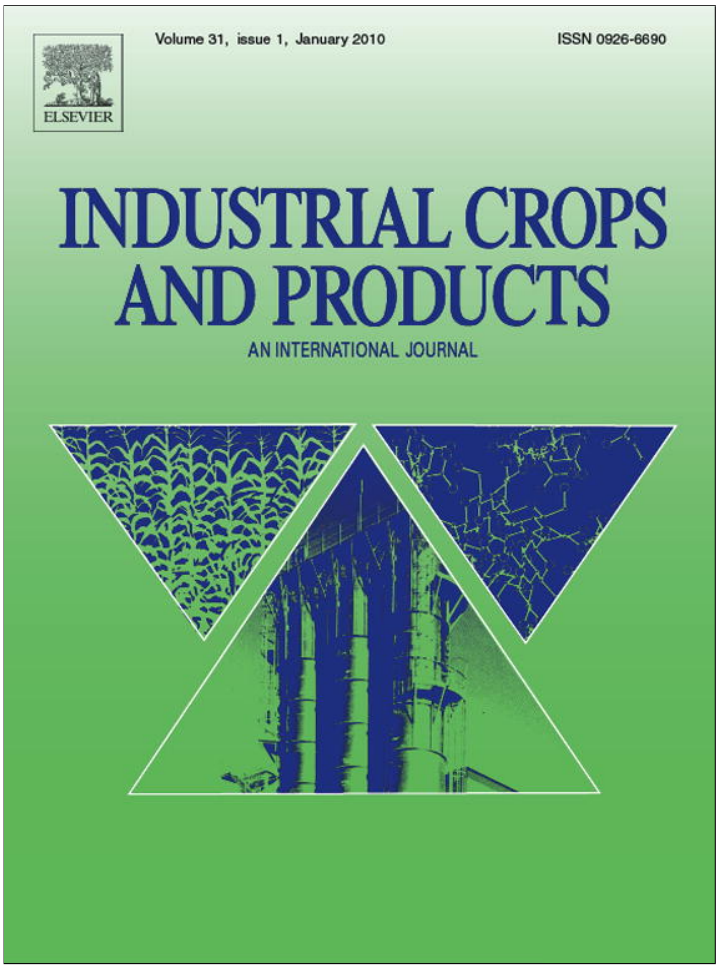

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Differential effects of salt stress on osmotic adjustment and solutes allocation on the basis of root and leaf tissue senescence of two silage maize (*Zea mays* L.) varieties

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ABSTRACT

The comparative effects of salt stress on osmotic adjustment and solutes accumulation in relation to root–leaf tissue senescence of two silage maize varieties were examined. Studies were carried out with seedlings of two forage maize varieties (*Aristo* and *Arper*) subjected to 0, 34, 68 and 102 mM NaCl for 6 weeks under glasshouse conditions. Osmotic potential (OP), osmotic adjustment (OA) and solutes accumulation were quantified in primary roots and in three leaf stages (young, mature and senescent leaves). Moreover, in order to assess the distribution of proline and glycine betaine during root development, the two components were analyzed at different position from the primary root apex of both varieties.

The total dry matter was significantly dropped with increasing salinity and reduction was greater in *Aristo* than in *Arper*. Salt stress impact in terms of ionic status was more pronounced in roots than in leaves and in older leaves than younger ones. In this setting, *Aristo* displayed a more sensitivity than *Arper*. A close relationship between the age of root–leaf tissue and proline and glycine betaine allocation, as salinity response, was shown. During the stress treatment, the accumulation of the two components was higher in growing regions of roots and in young leaves. While total free amino acids (FAA) and sugars were accumulated in roots as well as leaves but preferentially in the mature leaves. The capacity of OA was greater in young than in mature and/or senescent leaves and the contribution efficiency of organic solutes to this occurrence tended to be higher in *Arper* than in *Aristo*. Moreover, glycine betaine and proline appeared to be the main solutes that contributed ably to OA mainly in growing regions followed by sugars and other FAA. Inorganic solutes (K^+ and Ca^{2+}), however, did not seem to play an important role in OA since their amounts were often reduced in response to salt tolerance.

1. Introduction

Soil and water salinity have been considered a limiting factor to crop production in arid and semiarid regions of the world (Denden et al., 2005). Coping with salt stress is a global matter to ensure agricultural survival and sustainable food production. Salinity effects on plants include two distinct types of stress: water stress, caused by the greater difficulty of water absorption, and ionic stress, related to the sodium ion effect on the diverse cellular functions, decreased nutrient absorption, enzyme activities, photosynthesis and metabolism (Zhu, 2001; Hajlaoui et al., 2006). The contribution of each factor to the inhibition of growth depends on species, varieties and plant stage development (Munns and Termaat, 1986).

The adaptation to salinity is accompanied by accumulation of organic osmotically active compounds called osmolytes as a result of alterations in intermediary and secondary metabolism of nitrogen or of carbon (Greenway and Munns, 1980; Hoque et al., 2007). This phenomenon, known as osmotic adjustment (OA), is considered to be an important component of salinity tolerance mechanisms in plants (Neocleous and Vasilakakis, 2007). According to Blum et al. (1996), OA is usually defined as a decrease in cell sap osmotic potential resulting from a net increase in intracellular solutes rather than from a loss of cell water. The former may operate through the concentration accretion of inorganic and/or organic solutes such as proline, glycine betaine, free amino acids, sugars, polyamines and polyphenols (Ben Khaled et al., 2003). It has been suggested that organic solutes play a double role (Greenway and Munns, 1980). Indeed, dealing with their contribution to the

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osmotic balance, they can have a protective effect on enzymes in the presence of high electrolytes in the cytoplasm. However, there remains assumption about the primary roles of these solutes. Whether it is one of the storage of reduced carbon and/or nitrogen, or in the osmotic balance of the cell as a whole (Greenway and Munns, 1980). Glycine betaine and proline are keys of osmolytes contributing toward osmotic adjustment (Soudry et al., 2005; Iqbal et al., 2008). Numerous *in vitro* (Allakhverdiev et al., 1996) and *in vivo* studies (Sakamoto and Murata, 2002) have demonstrated that glycine betaine and its related osmotic effect contribute to the protection of PSII complex from photodamage under low temperature and salt stress. In addition, an *in vivo* study in a transgenic *Synechococcus* has shown that glycine betaine protects Rubisco from inactivation under salt stress condition (Nomura et al., 1998) and it can stabilize membranes under stress conditions (Deshnium et al., 1997). In the same context, it has been reported that proline, in addition to its osmoregulation role, can also serve as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger and acts as storage compound and nitrogen source for rapid growth after stress (Matysik et al., 2002; Sairam et al., 2002).

Considerable differences have been reported between species and cultivars in terms of OA capacity and with respect to the nature of the major solutes contributing to osmotic potential (Rhodes and Samaras, 1994). In fact, the degree of osmotic adjustment could be affected by the rate of stress development (Shangguan et al., 1999) and most particularly by organ type and age (Kameli and Lösel, 1995). For example, it has been reported in salt treated *Anacardium occidentale* that proline accumulation was higher in shoots than roots (de Lacerda et al., 2003). In other studies, Silveira et al. (2003) showed in cashew leaves that organic solutes accumulation, mainly proline and glycine betaine, was higher in the apical zone of the leaf and occurred when the leaves practically had reached their final size. In *Zea mays*, proline concentration increases greatly in the growing region of maize primary roots at low water potentials, as a result of preferentially increased net rate of proline deposition (Voetberg and Sharp, 1991; Verslues and Sharp, 1999). In contrast, increased deposition for hexoses occurred primarily in the basal regions of the elongating root zone (Sharp et al., 1990).

The stress dynamics interact with the ontogeny dynamics of structure and function in growing tissues, resulting in very different responses to the stress in leaves and roots at particular development stages (Delpérée et al., 2003). Salinity applied at the seedling often induces premature senescence of leaves (Lutts et al., 1996) and the most prominent features of salt stress-induced senescence seem to have decreased protein, nitrogen and chlorophyll concentrations (Chen et al., 1991). Indeed, salinity decreases protein synthesis and increases its hydrolysis in some plants, resulting in the production of free amino acids (Kozlowski, 1997).

The association between salt stress damage and senescence, on one hand, and osmotic adjustment mechanisms on the other, are little considered. Several recent studies focus on saltiness effects and plant adaptation of fully expanded, just matured leaves in the upper canopy. However, we hypothesized that solute accumulation and allocation under salinity conditions depend on aging process of plant tissue. The present work was conducted in order to assess two maize varieties for their salt tolerance and to give more information on the significance of osmotic adjustment and osmolytes accumulation under salt stress. In this case, the following questions were therefore addressed: (i) how these processes are influenced by root–leaf tissue senescence; and (ii) what is the degree of tolerance of the two tested varieties? For this purpose we investigated the changes in ionic status and solutes accumulation especially proline, glycine betaine and sugars in maize roots and shoots of different tissue maturity, and to relate them to defence strategies in terms of osmotic adjustment of each variety against salt stress.

2. Material and methods

2.1. Plant material and growth conditions

Two hybrid corn (*Zea mays* L.) varieties destined for forage ensilage are used: *Aristo* and *Arper*. The seeds of both varieties were surface sterilized with 5% sodium hypochlorite for 5 min and then thoroughly rinsed with distilled water before further experimentation. Seeds were germinated in Petri dishes containing two sheets of Watman No. 1 filter moistened with half strength Hoagland's nutrient solution. Germination chamber was set at 28 °C and with dark conditions. After germination, when cotyledons fully emerged, seedlings were transferred in plastic pots (45, 66, 23 cm) filled with peat/perlite mixture (2:1, v/v). Growth took place in a glazed greenhouse where the temperature for day/night was 35/24 °C, the relative humidity was 60–80% and the average of photosynthetically active radiation was 500 μmol/m^2/s with a photoperiod of 14 h/day. Salt treatment was started 20 days after planting. Sodium chloride was added to Hoagland nutrient solution to provide final concentrations of 0 (control), 34, 68 and 102 mM and plants were watered three times per week with approximate 0.5 l of salt solution. All measurements were made 6 weeks after final treatment concentrations were reached, when plants had achieved a steady state.

2.2. Calculation of osmotic potential and osmotic adjustment

The leaf and root samples collected from both varieties were frozen in liquid nitrogen and stored at −20 °C in order to disrupt cell membranes. Leaf tissues were thawed and centrifuged at 1200 × g for 25 min at 4 °C to extract the cell sap. The osmotic potential (OP) of the sap was measured using a vapor pressure osmometer (model 5500, Wescor, Logan, UT, USA). Osmotic adjustment (OA) was calculated as the difference in OP between salinized and control plants.

2.3. Biochemical analysis

The analysis of organic and inorganic solutes was performed in roots and at three leaf stages during the progression of senescence: young leaves (3rd leaf after the terminal bud), mature leaves (5th leaf after the terminal bud) and senescent leaves (7th leaf after the terminal bud). Moreover, in roots, the proline and glycine betaine concentrations were determined at 2, 4, 6, 8 and 10 cm from the primary root apex.

Glycine betaine was estimated by the periodide colorimetric method according to Grieve and Grattan (1983) using standard curve plotted with known concentration of glycine betaine at 365 nm with a Camspec M330 UV/Vis Spectrophotometer.

The proline concentration was estimated according to the method of Monneveux and Nemmar (1986). L-Proline (Sigma) was used for the preparation of standard curve at the absorbance of 528 nm.

Total free amino acids (FAA) content was estimated as described by Mukherjee and Choudhuri (1983) with little modification. Fresh leaf and root samples conserved in liquid nitrogen were extracted with 10 ml of 6% trichloroacetic acid. Four milliliter of each extract sample were mixed with 2 ml of 2% dinitrophenylhydrazine (in acidic medium) followed by the addition of one drop of 10% thiourea prepared in 70% aqueous ethanol. The resulting reaction mixture was refluxed during 15 min using a water bath and then immersed into an ice bath to cool down to around 0 °C. Further, it was mixed with 5 ml of 80% (v/v) H_2SO_4. The absorbance was recorded at 530 nm and the concentration of FAA was calculated from a standard curve plotted with known concentrations of leucine.

Soluble sugars were extracted from 50 mg of lyophilized powder using the alcohol extraction method described by Bartolozzi et al. (1997). They were analyzed using a Hewlett-Packard 5890 series II gas chromatograph equipped with flame ionisation detection (FID) system and a HP-5MS capillary column (30 m × 0.25 mm). Injector temperature was fixed to 250 °C. The temperature of capillary column was programmed to increase from 180 to 300 °C at a rate of 5 °C per min. Identification of individual carbohydrates was achieved by use of the relative retention times, i.e., in comparison to that of the standard. These were compared to those identified earlier by GC–MS. Only the total soluble carbohydrates are presented in this study.

For Na^+, K^+ and Ca^{2+} analyses were conducted after digestion by HNO_3 using a flame photometer (DNMOW ESSEX CM6 3LB., Jenway, UK).

2.4. Statistical analysis

In all experiments, each analysis is the mean of minimum five independent measurements ($n = 5$), expected for soluble sugars analysis where measurements are conducted only at three times. The results were analyzed by one-way ANOVA with a significance level of $P \leq 0.05$. Salinity treatment and varieties of *Zea mays* represented the main factors: varieties had two levels and salt stress had four levels. Duncan's multiple-range test was used for comparison of means among different levels within a factor. Statistical analyses of the data were made with SPSS for Windows: Version 13.0 (standard version). In figures, the spread of values is shown as error bars representing standard errors of the means.

3. Results

3.1. Growth and ionic characteristics

Effect of NaCl on the growth assessed by the total dry matter of plants was studied after 6 weeks of exposure to varying concentrations of NaCl. Our data (Fig. 1) showed that salt stress bring considerable changes in this parameter ($P < 0.001$). The tow varieties displayed maximum and minimum rates of growth, respectively, for plants control and plants treated with the highest

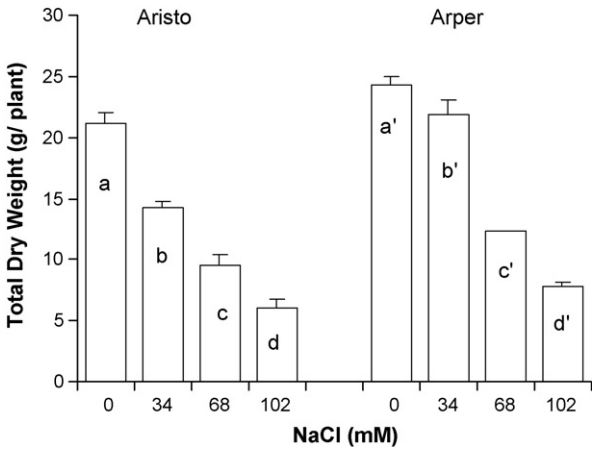

Fig. 1. Effect of different levels of salt stress (0, 34, 68 and 102 mM) on total dry matter of two maize variety (*Aristo* and *Arper*) seedlings. Histograms of each variety followed by the same letter indicate no significant differences ($P \leq 0.05$) according to Duncan test.

NaCl concentration (102 mM). The first detectable changes were occurred at concentration of 34 mM NaCl. They were marked by consistent and significant decreases in the total dry biomass of seedlings. However, it was noted that the trend of rate reduction in *Arper* was gentler than in *Aristo*. Indeed, in *Arper* seedlings, the rates of reduction compared to the control were about 9.46%, 48.75% and 67.5% respectively for 34, 68 and 102 mM NaCl. While in *Aristo*, rates of decrease were higher, they were about 33%, 56% 72.6% respectively for 34, 68 and 102 mM NaCl.

Both varieties indicated a significant ($P < 0.001$) difference for the accumulation of root-Na^+ with increased salinity but K^+ content indicated a concomitant decrease showing a significant interaction of the varieties with salinity (Table 1). The *Arper* variety accumulated markedly less of Na^+ and more of K^+ than *Aristo*. Moreover, the applied salinity reduced the Ca^{2+} content in roots of *Aristo* but no significant changes were observed in those of *Arper*.

Leaf Na^+ content in control and salt stressed seedlings of both varieties increased from the younger leaves to the older leaves, and

Table 1
Inorganic solute contents (mmol/g DW) of roots and leaves of two forage maize varieties (*Aristo* and *Arper*) grown at different NaCl concentrations.

Variety	Parameter	Treatment	Roots	Young leaves	Mature leaves	Senescent leaves
Aristo	Na	0	2.81 ± 0.44 d	0.97 ± 0.23 a	1.8 ± 0.31d	1.81 ± 0.27 d
		34	7.42 ± 0.54 c	1.04 ± 0.38 a	3 ± 0.18 c	5.32 ± 0.39 c
		68	10.40 ± 0.54 b	1.04 ± 0.35 a	4.22 ± 0.16 b	7.63 ± 0.53 b
		102	14 ± 0.35 a	1.12 ± 0.26 a	7.80 ± 0.41a	9.20 ± 0.44 a
	K	0	5.62 ± 0.36 a	8.79 ± 0.44 a	7.81 ± 0.81 a	6.41 ± 0.52 a
		34	3.40 ± 0.52 b	7.61 ± 0.89 b	6.60 ± 0.56 b	3.80 ± 0.43 b
		68	2.86 ± 0.22 b	5.34 ± 0.32 c	4.22 ± 0.43 c	2.43 ± 0.66 c
		102	1.88 ± 0.18 c	4.21 ± 0.45 d	2.41 ± 1.12 d	1.60 ± 0.22 d
	Ca	0	2.62 ± 0.54 a	3.41 ± 0.32 a	3.20 ± 0.62 a	2.90 ± 0.38 a
		34	2.20 ± 0.14 ab	3.29 ± 0.12 a	2.11 ± 0.21 b	2 ± 0.24 b
		68	2 ± 0.16 ab	2.76 ± 0.19 b	1.87 ± 0.14 b	1.83 ± 0.22 b
		102	1.84 ± 0.25 b	2.74 ± 0.33 b	1.22 ± 0.19 c	1.20 ± 0.22 c
Arper	Na	0	2.29 ± 0.44 c′	0.76 ± 0.27 a′	1.40 ± 0.27 d′	1.60 ± 0.27 d′
		34	4.20 ± 0.44 c′	0.92 ± 0.12 a′	2.40 ± 0.39 c′	4.21 ± 0.28 c′
		68	8.60 ± 0.54 b′	0.92 ± 0.22 a′	3.80 ± 0.33 b′	5.42 ± 0.43 b′
		102	9.62 ± 0.54 a′	0.99 ± 0.16 a′	5.21 ± 0.40 a′	7.80 ± 0.32 a′
	K	0	6.21 ± 0.54 a′	9.23 ± 0.27 a′	8.22 ± 0.57 a′	7.59 ± 0.41 a′
		34	3.63 ± 0.63 b′	8.20 ± 0.36 b′	6.62 ± 0.34 b′	4.42 ± 0.24 b′
		68	3.06 ± 0.38 b′	7.41 ± 0.24 c′	6.48 ± 0.29 b′	3.21 ± 0.26 c′
		102	2.41 ± 0.26 c′	7.28 ± 0.43 c′	5.61 ± 0.36 c′	2.50 ± 0.19 d′
	Ca	0	3.62 ± 0.22 a′	3.61 ± 0.24 a′	3.32 ± 0.16 a′	3.22 ± 0.34 a′
		34	3.60 ± 0.17 a′	3.46 ± 0.51 a′	2.60 ± 0.18 b′	2.42 ± 0.27 b′
		68	3.41 ± 0.31 a′	3.43 ± 0.46 a′	2.44 ± 0.31 b′	2.38 ± 0.13 b′
		102	3.19 ± 0.21 a′	3.41 ± 0.26 a′	1.87 ± 0.24 c′	1.85 ± 0.28 b′

Values in each column followed by the same letter indicate no significant differences ($P \leq 0.05$) according to Duncan test.

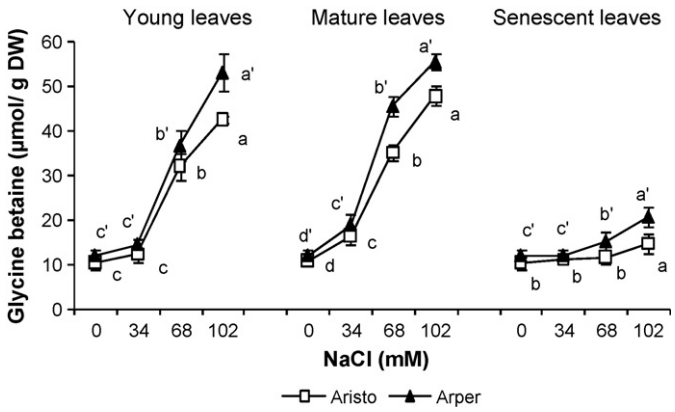

Fig. 2. Glycine betaine accumulation in leaves of two maize variety (*Aristo* and *Arper*) plants treated with different concentrations of NaCl (0, 34, 68 and 102 mM). Duncan test letters indicate significant differences between the means ($P \leq 0.05$).

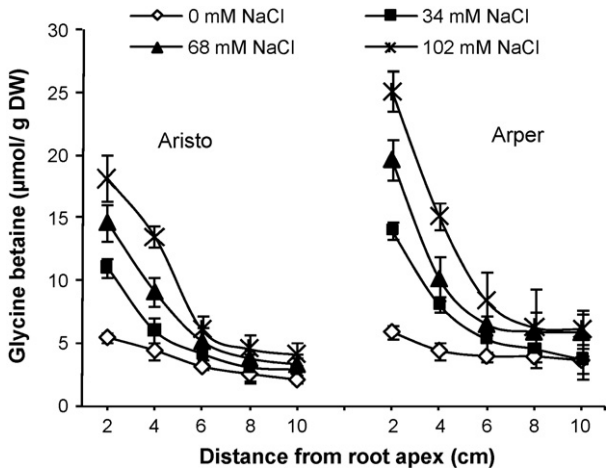

Fig. 3. Spatial distribution of glycine betaine concentration in the apical 10 cm of primary roots of two maize variety (*Aristo* and *Arper*) seedlings growing under different concentrations of NaCl (0, 34, 68 and 102 mM).

this increase was much more apparent in the leaves of salt stressed plants of the *Aristo*. On the other hand, younger leaves of control seedlings of both varieties had a higher K^+ and Ca^{2+} content than the older ones (Table 1). When the seedlings were stressed the mature and senescent leaves from both varieties suffered a reduction in K^+ and ca^{2+} content. This reduction was greater in *Aristo* than in *Arper*, and was more conspicuous in senescent leaves. The salt treated young leaves of both varieties, showed no significant changes in Na^+ content but a decrease in K^+ and ca^{2+} content, exception of those of *Arper* where Ca^{2+} content was steady among all NaCl treatments.

3.2. Glycine betaine

Distribution of the glycine betaine in leaves changed significantly with their age and NaCl concentration ($P < 0.001$). Dealing with all treatments of salinity, the greater accumulation was showed, principally, in young and mature leaves. A more pronounced increase of glycine betaine concentration, especially at high salinity, was observed in the variety of *Arper* compared to *Aristo* (Fig. 2). For example, for the treatment of 102 mM, the glycine betaine concentration of *Aristo* leaves was around 42, 47 and 14 µmol/g DW, respectively, in young, mature and senescent leaves. For this treatment and in the same leaf stages, values of *Arper* were respectively in the range of 52, 55 and 20 µmol/g DW.

The behavior of glycine betaine in roots of two maize varieties showed that his accumulation was raised significantly with the increase of saltiness ($P < 0.001$). This accumulation becomes important especially at the growing region of primary roots (Fig. 3). The raise of glycine betaine concentration, according to salt stress and distance from root apex, is not of the same extent in both varieties. Indeed, at all levels of salt stress and all positions of primary roots, the increasing trend of glycine betaine concentration in *Arper* was greater than in *Aristo*. This difference between varieties was remarkable, at higher salinity, in the first 4 cm of the apical primary roots. For example, in the position of 2 cm from root apex, the glycine betaine content of seedlings treated with 102 mM NaCl was 18.1 and 25.1 µmol/g DW for *Aristo* and *Arper*, respectively.

3.3. Total free amino acids

Results in Fig. 4 showed that total free amino acids generally increased in all leaf stages in both maize varieties under saline conditions. These organic solutes accumulated preferentially in the mature and more injured leaves of both varieties and it was much more apparent with the increasing level of salinity. At all leaf stages, the tendency of FAA rate increase, during stress application, was gentler in *Aristo* than in *Arper*. For example, in middle stage where the accumulation of FAA was more pronounced in both varieties, FAA content of leaves treated with 102 mM NaCl was around 93.6 µmol/g FW for *Arper* and only 91 µmol/g FW for *Aristo*.

Changes of FAA content in roots during salt stress treatment are shown in Fig. 5. The rate of FAA accumulation rose significantly when salt stress increases. The two maize varieties displayed similar behavior to NaCl (P of variety × treatment = 0.094) with a lesser extent of *Aristo* compared to *Arper*. For instance, at the concentration of 102 mM NaCl, FAA content of roots was around 85 and 87 µmol/g FW for *Aristo* and *Arper*, respectively.

3.4. Proline

The free proline content was significantly increased in the stressed plants over control plants of both corn varieties at all stress regimes. Free proline concentration showed obvious differences between both varieties in the respective leaf classes under

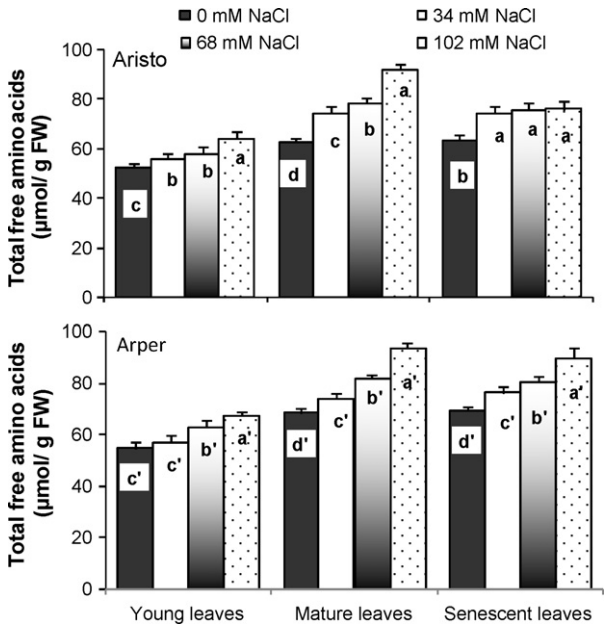

Fig. 4. Total free amino acids content in leaves of two corn varieties (*Aristo* and *Arper*) grown at different NaCl treatments (0, 34, 68 and 102 mM NaCl). Histograms of each variety followed by the same letter indicate no significant differences ($P \leq 0.05$) according to Duncan test.

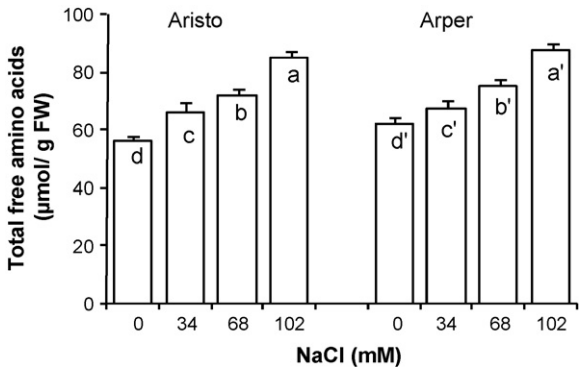

Fig. 5. Effects of salt stress treatments (0, 34, 68 and 102 mM NaCl) on the level of total free amino acids in primary roots of two maize varieties (*Aristo* and *Arper*). Histograms of each variety followed by the same letter indicate no significant differences ($P \leq 0.05$) according to Duncan test.

salt stress treatments (Fig. 6). Indeed, at three leaf classes and at any NaCl treatment, the proline accumulation was greater in *Arper* than *Aristo*. Moreover, proline tended to be higher in young leaves than in mature and senescent leaves under elevated NaCl concentration. For example, at 102 mM NaCl, the average of leaf proline content in *Arper* seedlings was about 38, 25 and 14 µmol/g FW, respectively, for young, mature and senescent leaves. In *Aristo* plants, values were slightly reduced (i.e., 32, 22 and 13 µmol/g FW).

Simultaneously, salt treatment induced significant changes of spatial proline deposition in roots tissue ($P < 0.05$). In both varieties, roots growing at high salinity showed that the proline accumulation in the apical 10 cm was greatly increased, particularly toward the apex (Fig. 7). The maximum accumulation rate of proline was showed at the sternest salinity treatment (102 mM NaCl) and at the distance of 2 cm from root apex, but the averages of proline concentration in *Arper* roots were faintly higher than observed in those of *Aristo*.

In order to evaluate the proportion of proline content among the totality of free amino acids, the ratio proline/FAA (%) was examined in leaves and roots. Data in Fig. 8 present the evolution of this parameter as function of saltiness and leaf tissue senescence. In both varieties, results indicated that the fraction of proline, compared to the rest of amino acids, increased significantly ($P < 0.001$) with salt stress particularly in young leaves. At these last leaves treated with 102 mM NaCl, the fraction of proline in relation to the rest of free amino acids was approximately 50% and 56% correspondingly for *Aristo* and *Arper*. The same evolution of this ratio, according to salt stress, was observed also in roots (Fig. 9). In *Aristo*, the proline percentage among the totality of FAA was increased from 4%, at plants controls, to 22% at 102 mM NaCl. Those of *Arper*, were, respectively 3.3% and 23.4%.

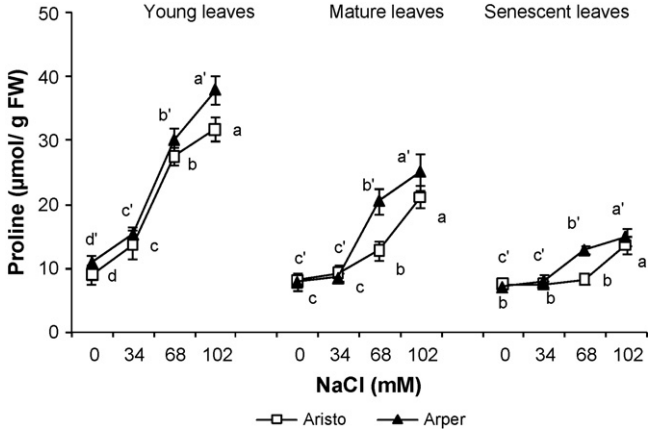

Fig. 6. Effects of salt treatments (0, 34, 68 and 102 mM NaCl) on the proline content of leaves of two corn varieties (*Aristo* and *Arper*). Values of each variety followed by the same letter indicate no significant differences ($P \leq 0.05$) according to Duncan test.

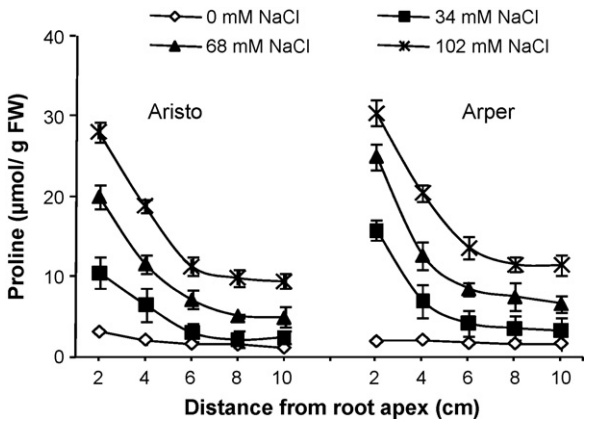

Fig. 7. Spatial distribution of proline concentration in the apical 10 cm of primary roots of two maize varieties (*Aristo* and *Arper*) treated with different NaCl concentrations (0, 34, 68 and 102 mM).

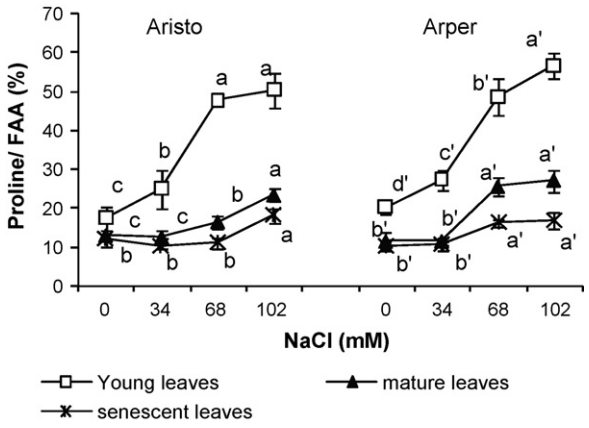

Fig. 8. Changes of the ratio proline/FAA as a function of leaf senescence of two maize varieties (*Aristo* and *Arper*) growing under different concentrations of NaCl (0, 34, 68 and 102 mM). Duncan test letters indicate significant differences between the means ($P \leq 0.05$).

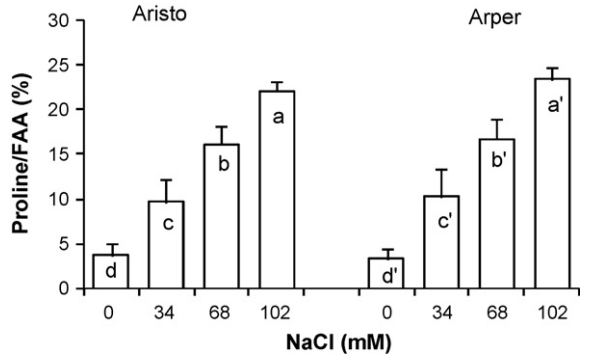

Fig. 9. Variation of the ratio proline/free AA in primary roots of two maize varieties (*Aristo* and *Arper*) treated with different NaCl concentrations (0, 34, 68 and 102 mM). For each variety, histograms followed by the same letter indicate no significant differences ($P \leq 0.05$) according to Duncan test.

Table 2
Changes of soluble sugars concentration (mg/g FW) in roots and leaves of two maize variety (*Aristo* and *Arper*) seedlings treated with different concentrations of NaCl (0, 34, 68 and 102 mM).

NaCl (mM)	0	34	68	102	0	34	68	102
	Aristo				*Arper*			
Roots	13.74 ± 0.21 a	13.26 ± 0.63 a	10.57 ± 0.36 b	7.63 ± 0.31 c	18.46 ± 0.46 a′	18.60 ± 0.34 a′	18.72 ± 0.23 a′	18.52 ± 0.50 a′
Young leaves	18.01 ± 0.97 d	24.80 ± 1.31 c	40.87 ± 1.02 b	47.13 ± 0.80 a	22.50 ± 0.86 d′	25.13 ± 1.50 c′	51.20 ± 1.05 b′	63.50 ± 0.86 a′
Mature leaves	17.36 ± 0.54 d	27.20 ± 0.69 c	49.54 ± 0.50 b	58.75 ± 0.21 a	21.66 ± 0.28 d′	26.34 ± 0.57 c′	65.38 ± 0.53 b′	75.46 ± 0.46 a′
Senescent leaves	16.66 ± 0.57 a	14.57 ± 0.51 b	11.24 ± 1.52 c	10.26 ± 1.27 c	21.23 ± 0.40 a′	21.13 ± 0.23 a′	19.46 ± 0.92 b′	19.06 ± 1.10 b′

Values in each line followed by the same letter indicate no significant differences ($P \leq 0.05$) according to Duncan test.

Table 3
Mean values of osmotic potential (MPa) and osmotic adjustment in roots and leaves of two forage maize varieties (*Aristo* and *Arper*) grown in nutrient solution containing 0, 34, 68 and 102 mM NaCl.

Variety	Parameter	Treatment	Roots	Young leaves	Mature leaves	Senescent leaves
Aristo	OP	0	−0.43 ± 0.06 b	−1.08 ± 0.05 d	−1.04 ± 0.04 d	−1.04 ± 0.03 c
		34	−0.49 ± 0.04 ab	−1.30 ± 0.05 c	−1.28 ± 0.05 c	−1.14 ± 0.09 b
		68	−0.57 ± 0.18 ab	−1.86 ± 0.13 b	−1.73 ± 0.11 b	−1.22 ± 0.07 a
		102	−0.61 ± 0.08 a	−2.02 ± 0.16 a	−1.94 ± 0.07 a	−1.25 ± 0.14 a
	OA	0	–	–	–	–
		34	0.06 ± 0.03 b	0.23 ± 0.03 b	0.25 ± 0.08 c	0.11 ± 0.04 a
		68	0.14 ± 0.12 a	0.79 ± 0.14 a	0.69 ± 0.13 b	0.19 ± 0.11 a
		102	0.18 ± 0.09 a	0.94 ± 0.06 a	0.91 ± 0.10 a	0.21 ± 0.06 a
Arper	OP	0	−0.45 ± 0.07 c′	−1.09 ± 0.11d′	−1.13 ± 0.16 c′	−1.07 ± 0.04 c′
		34	−0.52 ± 0.02 b′c′	−1.76 ± 0.15 c′	−1.76 ± 0.12 b′	−1.16 ± 0.08 b′
		68	−0.62 ± 0.14 a′b′	−1.96 ± 0.04 b′	−1.93 ± 0.07 b′	−1.26 ± 0.05 a′
		102	−0.67 ± 0.06 a′	−2.92 ± 0.09 a′	−2.84 ± 0.08 a′	−1.30 ± 0.10 a′
	OA	0	–	–	–	–
		34	0.06 ± 0.06 b′	0.67 ± 0.06 c′	0.63 ± 0.14 b′	0.10 ± 0.03 b′
		68	0.17 ± 0.12 a′	0.87 ± 0.08 b′	0.79 ± 0.08 b′	0.20 ± 0.04 a′
		102	0.22 ± 0.08 a′	1.83 ± 0.04 a′	1.71 ± 0.11 a′	0.24 ± 0.09 a′

Values in each column followed by the same letter indicate no significant differences ($P \leq 0.05$) according to Duncan test.

3.5. Soluble sugars

The salt stress led to reductions of the total soluble sugars content in roots of *Aristo* while no significant changes are observed in roots of *Arper* (Table 2). In leaves of both varieties, the accumulation of total soluble sugars raised with increasing salinity with an advantage for the *Arper* variety. Moreover, these organic solutes accumulated preferentially in mature leaves of both varieties and it was much more apparent in salt stressed seedlings. For example, at 102 mM NaCl, the concentration of total soluble sugars in the young, mature and senescent leaves, was, respectively 63.5, 75.46 and 21.23 mg/g DW for *Arper* against 47.13, 58.75 and 16.66 mg/g DW for *Aristo*. In senescent leaves, the two varieties exhibited a reduction in their content of total soluble sugars with increasing salinity. The trend of reduction was more pronounced in senescent leaves of *Aristo* than those of *Arper*.

3.6. Osmotic potential and osmotic adjustment

On the one hand, the salinity reduced the leaf and root OP of the two corn varieties and on the other hand it increased their OA (Table 3). Regardless of culture medium and varieties, the leaves showed lower OP and higher OA than roots. Moreover, in control plants of both varieties, values of OP as well as OA were similar between the three leaf stages. However, in salt stressed seedlings, the OP decreased from older to the younger leaves. As a result of the OP variation with salt stress, the young leaves showed to have the maximum OA comparably to mature and senescent leaves. The difference, in terms of OP and OA, between varieties was more conspicuous especially at higher NaCl treatments (68 and 102 mM). Generally, the *Arper* variety exhibited the more negative osmotic potential and the maximum osmotic adjustment.

4. Discussion

Plants have developed various combating mechanisms to survive with the deleterious effects of salt stress. Among these, osmotic adjustment is one of the strategies that have been a potential defense toward NaCl. However, we supposed that this phenomenon depends on cultivars, organ type and on aging process of plant tissue. In the current study, two varieties of maize were used to test their differential response to salinity on the basis of osmotic adjustment at whole plant level. Comparative response studies can illuminate the salt tolerance mechanism in the maize varieties.

The results obtained in these experiments could indicate that the *Arper* variety was more tolerant to salt stress than *Aristo*. The decrease in total dry matter of seedlings under salinity stress in both varieties is in confirmation of already reported results (Cramer et al., 1994; Mansour et al., 2005). The variety of *Arper* reserved higher total dry mass at all NaCl concentrations. Changes in ionic status of green leaves are crucial to adjudge the sustenance of the active photosynthetic canopy in giving higher dry matter yield (Francois and Maas, 1999). In this study, the accumulation of Na^+ and reduction in K^+ and Ca^{2+} took place, but with a substantial difference ($P < 0.05$) between the two varieties (Table 1). This result is in accordance with Cramer et al. (1994). However, for these authors, the lack of correlation between accumulation of Na^+, decreasing of K^+, Ca^{2+} and salt sensitivity in the maize cultivars led them to the conclusion that mineral nutrition of maize is not correlated with salt tolerance and that the growth response of maize to salinity may be primarily affected by osmotic factors. In our study we think that lower content of Na^+ and higher content of K^+ and Ca^{2+} in the roots and leaves of *Arper* is crucial for better growth and tillering, which was hardly displayed by *Aristo*. Furthermore, the improved maintenance of Ca^{2+}contents in roots and young leaves of *Arper* contrarily to those of *Aristo* (Table 1) revealed reduced perturbation of their

ionic contents and it may be related to salt tolerance of this variety and this points to its possible regulation of sodium uptake at the root or phloem level.

The pattern of glycine betaine distribution in plants of two maize varieties depended on the NaCl concentration in the growth medium and the root–leaf tissue age (Figs. 2 and 3). Although there was a substantial accumulation of glycine betaine in both types of organs of maize plants, the tissue responses were much more pronounced on the leaf than in the primary root. Furthermore, a negative correlation between magnitude of glycine betaine accumulation and tissue senescence was observed. Indeed, in primary root of either variety, glycine betaine deposition was greater in the growing region, principally at the distance of 2 cm from the apex. In the same meaning severe reduction in leaf glycine betaine content of both varieties occurred at senescent leaves as compared with other leaf stages. This translocation behavior suggests that glycine betaine is one of the more mobile osmolyte. Under salt stress it accumulates preferentially and swiftly in young tissue of roots and leaves. Increase of glycine betaine under salt stress has previously been reported for *Zea mays* (Yang and Lu, 2005) and other crop species (Sairam et al., 2002; Moghaieb et al., 2004) but as far as we know, few studies were focussed on differences between leaves of the same plant. Bajji et al. (2001) reported that glycine betaine levels increased under water deficit conditions in young mature and in growing leaves of durum wheat cultivars, but it remained unchanged in aged mature leaves. It had been reported also that the accumulation of glycine betaine was found to be high in salt tolerant cultivars of maize while salt sensitive cultivars exhibited a low magnitude of glycine betaine accumulation (Mansour et al., 2005). In the present study, the higher accumulation of glycine betaine was found in *Arper* than in *Aristo* suggesting the salt tolerant nature of the first cited variety.

Another contributor to OA in salt stressed maize was proline. The present findings showed that in both varieties, the greatest increase in proline concentration at high level of salt stress occurred in the growing tissue of primary roots (Fig. 7). Similar results have been reported in plants of *Zea mays* subjected to water stress (Voetberg and Sharp, 1991; Ober and Sharp, 1994). Studies of these researchers showed that the highest proline concentration occurs within 4 mM of the apex, where the meristem and newly elongating cells are located. Proline makes a significant contribution to the osmotic potential in this region (Voetberg and Sharp, 1991) reaching 0.1 mol. Some authors suggest that increases in solute concentration in growing regions may be simply a consequence of sustained solute deposition when growth is inhibited (Steponkus et al., 1982). Whereas, others contribute the very high proline concentrations in the root tip to the increased levels of abscissic acid (Ober and Sharp, 1994). They supposed that increased abscissic acid is required for high rates of proline deposition and, thereby, elevated proline concentrations in root tip which encompassed the elongation zone. The level of proline accumulation in leaves of both varieties has the same trend of remobilization observed in roots. In fact, all leaf stages increased their proline content with salt stress but accumulation was higher in the youngest leaves (Fig. 6). A similar behavior was observed in salt treated tomato leaves (Cuartero and Fernandez-Munoz, 1999; Hernandez et al., 2000). Proline accumulation resulting from high salt treatment was one of the earliest observations of the biochemical process in higher plants and has now been documented for a large number of species but its role as an adaptive process is still a matter of debate. On one hand, a positive correlation between magnitude of free proline accumulation and salt tolerance has been suggested as an index for determining salt tolerance potentials between cultivars (Kumar et al., 2003). On the other hand, it has been also reported that the salt sensitive cultivars accumulated significantly higher levels of proline compared to the tolerant ones (Lutts et al., 1999; de Lacerda et al., 2003). In this article, we have reported a positive correlation between proline accumulation and salt tolerance. The variety that appears more tolerant (*Arper*) always maintained higher level of free proline contents than the sensible one (*Aristo*). Proline accumulation in plant tissue under stressful conditions has been suggested to be result of a decrease in proline degradation (Hare et al., 1999), increase in proline biosynthesis (Lutts et al., 1999), a decrease in protein synthesis or proline utilization and increased hydrolysis of proteins (Viégas and Silveira, 1999).

The content of the total free amino acids increased in salt stressed plants whatever in roots or in leaves (Figs. 4 and 5). In previous study, Abd El Baki et al. (2000) showed that with increasing external salt concentration, amino acids accumulate in leaves and roots of *Zea mays*, with concentrations in leaves exceeding those in roots. However in other study with mulberry, Agastian et al. (2000) demonstrate that free amino acids increase at low salinity but decrease at high salinity. Among leaves of both varieties, the most pronounced accumulation was observed, unlike proline, particularly in mature leaves and after with less degree in old leaves (Fig. 4). In both varieties, the proportion of proline among the totality of FAA increased with saltiness and decreased with leaf tissue senescence. Generally the steady-state levels of free amino acids, among them the proline, during leaf ontogeny, are dependent on the rate of their release during protein degradation and on the rate of efflux into growing structures (Soudry et al., 2005).

A strong correlation between sugar accumulation and osmotic stress tolerance has been widely reported, including transgenic experiments (Abd El Baki et al., 2000; Taji et al., 2002). In our study, the *Arper* variety when compared with *Aristo* not only accumulated more soluble carbohydrates in leaves, but also partitioned more sugars to roots. Nevertheless, the reduced levels of sugars in *Aristo* roots (Table 2) indicate that photosynthate translocation from leaves (the source) to roots (the sink) was repressed under salt stress. As explanation, we thought that the reduction in root growth and shoot elongation of this variety as a salinity response (Fig. 1) may lead to a decrease in roots carbohydrates demand, which may be one cause of inhibition of photosynthates transport to salt stressed roots. In leaves, the two varieties showed that the levels of soluble sugars were higher in mature and young leaves than senescent leaves. Various hypotheses were proposed to account for the varying degree of sugars accumulation in stressed leaves. At the beginning of its development, the young leaves function as a sink and an accumulation of sugars at this level may result from a reduction in the utilization of assimilates induced by salt stress in relation to an inhibition of sucrose synthase or invertase activities (Sturm and Tang, 1999). In mature source leaves, accumulation may be due to a stress-induced inhibition of phloem loading (Daie, 1989) and/or to a stimulation of sucrose–phosphate–synthase activities (Huber and Huber, 1996). In fact the lower levels of sugars in senescent leaves than young and mature ones may be a result of a decrease in their net photosynthesis (Veneklaas and van den Boogaard, 1994).

Osmotic adjustment is a mechanism used for maintaining turgor and reducing the deleterious effects of salt stress on vegetative and reproductive tissue. In the present study, the reduction in cell OP of both varieties in response to NaCl stress is in agreement with previous reports with other maize cultivars (Mansour et al., 2005). Generally, *Arper* exhibited more negative osmotic potentials than *Aristo*. In addition, the observed difference in terms of OP between leaves at three different physiological stages was associated with the capacity of the leaves for OA maintenance, which was higher in young leaves (Table 3) than in mature and senescent leaves. The relative magnitude of OA reported here corroborate the finding of Kameli and Lösel (1995) who also reported a greater OA, in response to water stress, in growing than in expanded durum wheat leaves. In both varieties, the progressive loss in the capacity of OA as the leaf ages seemed to be related to the accumulation and allocation of

proline, glycine betaine and with less degree sugars. In both control and stressed plants their concentrations were indeed lower in older leaves. Unlike organic solutes, inorganic solutes (K^+ and Ca^{2+}) did not seem to play an important role in OA given that their amounts were either unaffected or reduced in salt stressed leaves (Table 1). It has been hypothesized that K^+ contributes to OA but experimental results are inconsistent (Bajji et al., 2001; de Lacerda et al., 2003). Comparing the two varieties revealed that the changes in OA were different depending on the variety. The accretion of osmotic adjustment in roots as well as in leaves, induced by salinity, was greater in *Arper* and lowest in *Aristo*. These findings suggest that the microenvironment existing within the cells of the *Arper* variety seems to favor a more efficient metabolism with a consequent better growth under salt stress conditions than in *Aristo*.

5. Conclusion

As conclusion, in many studies, the different organs of plant (roots or leaves) are considered as if they all had the same properties, despite the fact that they form an age-structured population and thus are likely to present differences in their functioning and in their reaction to alterations of the environment. In salt treated maize, the accumulation and remobilization of the major solutes contributing to the osmotic adjustment were shown to be affected by leaf and root tissue senescence. The later growth stages, of both organs seem to be more protected but sometimes more reactive toward the application of salt stress. Results of this research suggest also that OA could be a part of the salt resistance mechanisms developed by maize and could be exploited in breeding programs for improved salt stress tolerance.

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