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Effects of salt stress on photosynthesis, PSII photochemistry and thermal energy dissipation in leaves of two corn (*Zea mays* L.) varieties

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Abstract

The effect of four different NaCl concentrations (from 0 to 102 mM NaCl) on seedlings leaves of two corn (*Zea mays* L.) varieties (Aristo and Arper) was investigated through chlorophyll (Chl) *a* fluorescence parameters, photosynthesis, stomatal conductance, photosynthetic pigments concentration, tissue hydration and ionic accumulation. Salinity treatments showed a decrease in maximal efficiency of PSII photochemistry (F_v/F_m) in dark-adapted leaves. Moreover, the actual PSII efficiency (Φ_{PSII}), photochemical quenching coefficient (q_p), proportion of PSII centers effectively re-oxidized, and the fraction of light used in PSII photochemistry (%P) were also dropped with increasing salinity in light-adapted leaves. Reductions in these parameters were greater in Aristo than in Arper. The tissue hydration decreased in salt-treated leaves as did the photosynthesis, stomatal conductance (g_s) and photosynthetic pigments concentration essentially at 68 and 102 mM NaCl. In both varieties the reduction of photosynthesis was mainly due to stomatal closure and partially to PSII photoinhibition. The differences between the two varieties indicate that Aristo was more susceptible to salt-stress damage than Arper which revealed a moderate regulation of the leaf ionic accumulation.

Additional key words: chlorophyll fluorescence; photosynthesis; ion accumulation; salinity; *Zea mays* L.

Introduction

High concentrations of salt resulting from natural processes or mismanagement in irrigated agriculture pose major challenges to crop production around the world (Jiang *et al.* 2006). Coping with salt stress is a global matter especially in many arid or semi-arid regions to ensure agricultural survival and sustainable food production (Demiral and Turkan 2006). Eventually the growth of plants is reduced by salinity stress although plant species differ in their tolerance to salinity (Munns and Termaat 1986). The decline in growth observed in many plants subjected to salt stress is often associated with a decrease in their photosynthetic activity (Hajlaoui *et al.* 2006). The decrease in photosynthesis induced by salt stress can be associated with the partial stomatal closure and/or the nonstomatal factors (das Neves *et al.* 2008). Although there are several studies (Lawlor 2002, Long and Bernacchi 2003, Steduto *et al.* 2000) on elucidating the latter causes of the decreased photosynthetic capacity, no clear mechanisms of the inhibited photosynthesis have emerged. Thus photosystem II (PSII) is considered to play a key role in the response of leaf photosynthesis to environmental perturbations (Baker 1991). In this case, the analyses of fluorescence quenching provide information on the fundamental processes of energy absorption, utilization, and dissipation, and electron transport in PSII (Schreiber *et al.* 1986). Previous reports of salinity effects on the photochemical efficiency of PSII of diverse plant organ, tissue, and cell preparation are limited and conflicting. Some studies have demonstrated that salt stress inhibits PSII activity

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Abbreviations: A_N – net assimilation rate; Car – carotenoid; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; DM – dry mass; F_m – maximal fluorescence of dark-adapted state; F_m' – maximal fluorescence of light-adapted state; $(F_m' - F_o)/F_m'$ – proportion of PSII centers effectively re-oxidized; F_o – minimal fluorescence of dark-adapted state; F_o' – minimal fluorescence of light-adapted state; F_s – steady-state fluorescence of light-adapted leaves; F_v – variable fluorescence; F_v/F_m – maximal efficiency of PSII; g_s – stomatal conductance; LWC – leaf water content; NPQ – non-photochemical quenching; $(1 - q_p)/NPQ$ – susceptibility of PSII to high irradiance; PSII – photosystem II; Q_A – primary quinone acceptor of PSII; q_p – photochemical quenching; %D – thermal energy dissipation; %P – fraction of energy allocated to PSII photochemistry; %X – excess of energy excitation; Φ_{PSII} – actual PSII efficiency.

(Santos 2004, Jiang *et al.* 2006); whereas others have indicated that salt stress has no effect on PSII (Lu *et al.* 2003, Demiral and Türkan 2006). The decline in Chl and carotenoids (Car) content is another commonly reported aspect of the salinity effects on plants. Thus, lower contents of photosynthetic pigments due to salinity were reported and therefore they have been proposed as one of the attributes of salt tolerance in crops (Romero *et al.* 1997, Juan *et al.* 2005). While some studies reported changes in pigments composition of leaf tissues, few investigated their relationship with PSII efficiency in salt-treated leaves.

Under field conditions, multiple environmental stresses co-occur frequently. The responses of plants to several simultaneous stresses are complex and the combination of different environmental stress factors can result in intensification, overlapping or antagonistic effects (Netondo *et al.* 2004). Pasternak *et al.* (1995) reported that high salt concentrations in soil solution negatively affect maize growth and, consequently, produce a large drop in yield. Salinity causes both hyperionic and hyperosmotic stress. On one hand, NaCl presence in soil solution affects crop water relations, and causes an osmotic stress in maize plants (Hasegawa *et al.* 2000). On the other hand, shoot Na^+ concentration in maize increases with NaCl increments in soil solution, which involves ionic balance alterations (Shabala *et al.* 1998). However, the underlying mechanism of the photosynthetic response of plants, particularly the C_4 ones, to salt stress is still being studied in both lab and field experiments. Previous studies provided evidence that salt stress decreases the CO_2 assimilation rate and the PSII photochemistry of maize leaves (Yang and Lu 2005, Hasan *et al.* 2006). Moreover Hasan *et al.* (2006) in their recent ultrastructural study on the leaves of maize plants exposed to salinity stress suggested that salinity induced damages in chloroplasts and these are dependent on light and mediated reactive oxygen species. Further understanding of the pattern of maize leaf photosynthesis, particularly the PSII photochemistry, may help to elucidate the mechanisms of tolerance of this plant to salinity.

Maize is an important crop characterised by the multiplicity of its agro-industrial uses. In Tunisia, some varieties have been considered as a good quality forage for livestock. However, in many areas of the country, their productivity is limited because of the water or/and soil salinization. With this background, we considered that the understanding of the physiological responses of crop plants to salt stress will help plant breeders in further genotype improvement and crop physiologists and agronomists in developing best management options to cope with saline conditions.

The objective of this study was to explore if PSII in two maize hybrids (Aristo and Arper) is capable to adapt the high salinity stress. For this reason, we examined (*1*) how salt stress affects PSII photochemistry; (*2*) if salt stress leads to increased susceptibility of PSII to photoinhibition; and (*3*) whether salt stress induces a change in gas exchange parameters, ion accumulation, tissue hydration and photosynthetic pigment composition in corn leaves.

Materials and methods

Plant material and growth conditions: Two hybrids of forage maize were used, Aristo and Arper. The seeds of both varieties were surface sterilized with 5% sodium hypochlorite and then germinated in dark chamber at 28 °C. After germination, when cotyledons fully emerged, seedlings were transferred in plastic pots (45, 66, and 23 cm) filled with peat: perlite mixture (2:1, v/v). Growth took place in a glazed greenhouse which has sides constructed of fiberglass screening and a roof of translucent corrugated fiberglass. The temperature for day/night was 35/23°C, the relative humidity was 60–80% and the average of photosynthetically-active radiation was 500 μmol m^{-2} s^{-1} with a photoperiod of 14 h d^{-1}. 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 after 6 weeks of culture on saline environment, when plants reached their maximal vegetative growth, just before flowering.

Chl fluorescence measurements: For each salinity treatment, the parameters of Chl fluorescence were measured independently on five plants. Measurements were taken on the mature leaves (fifth leaf after the terminal bud) of each of the chosen plants. Before measurements (2 days), pots with plants were brought to laboratory and kept in a small growth chamber equipped with a computerized electronic system controlling the microclimatic conditions. Measurements were made on upper (adaxial) surface of leaves. Chl fluorescence was determined with a portable fluorometer (Fluorescence Induction Monitor *FIM, ADC Bioscientific Ltd.,* Hoddesdon, UK). The experimental protocol of van Kooten and Snel (1990) was basically followed. Leaves had been predarkened for at least 30 min in order to determine the minimal and the maximal fluorescence (F_o and F_m, respectively). The leaves were then continuously illuminated with white actinic light at the intensity of 500 μmol m^{-2} s^{-1}. The steady-state value of fluorescence (F_s) and the maximal fluorescence level in light-adapted leaves (F_m') were recorded after a series of saturating pulse. The minimum fluorescence yield in light-adapted state was calculated assuming that F_o quenching results from increases in energy dissipation in PSII centers as follows:

$F_o' = F_o/[(F_v/F_m) + (F_o/F_m')]$ (Oxborough and Baker 1997, Baker 2008).

By using fluorescence parameters determined in both light- and dark-adapted leaves, the potential maximum efficiency of PSII (F_v/F_m) of dark-adapted leaves was calculated as $F_v/F_m = (F_m - F_o)/F_m$. The actual PSII efficiency (Φ_{PSII}) was calculated as $(F_m' - F_s)/F_m'$ (Genty *et al.* 1989). Calculations of quenching due to a non-photochemical dissipation of absorbed light energy (NPQ), was determined according to the equation $NPQ = (F_m - F_m')/F_m'$ (Bilger and Björkman 1991). The coefficient for photochemical quenching (q_p) which represents the fraction of open PSII reaction centers was calculated as $(F_m' - F_s)/(F_m' - F_o')$ (Schreiber *et al.* 1986). The ratio $(1 - q_p)$/NPQ was used as an estimation of photon excess and, therefore, as the susceptibility of PSII to high irradiance (Park *et al.* 1995). The proportion of PSII centers, which are effectively re-oxidized, was estimated as $(F_m' - F_o)/F_m'$ (Genty *et al.* 1989).

To get more information about the divergent parameters of corn leaves, we calculated the fractions of the excitation energy absorbed in the PSII antennae allocated to PSII photochemistry (P), thermal dissipation (D) and excess excitation (X) according to Demmig-Adams *et al.* (1996). The fractions of the absorbed light dissipated in the PSII antennae (%D) and those utilized in PSII photochemistry (%P) were estimated as $1 - (F_v'/F_m')$ and $(F_v'/F_m') \times q_p$, respectively. The fraction of absorbed light by PSII, which was neither used in photochemistry nor dissipated in the PSII antenna (%X) was estimated as $(F_v'/F_m') \times (1 - q_p)$.

Gas exchange measurements: Leaves previously selected for measurement of Chl fluorescence were used for gas exchange measurements. Photosynthetic gas exchange analysis was made using a portable gas exchange system (*LI-6400, LI-COR,* Lincoln, USA), which was equipped with a leaf chamber (*LI-6400-40 LCF*). The net assimilation rate (A_N) [μmol(CO_2) m^{-2} s^{-1}] and the stomatal conductance for water vapour g_s [mmol(H_2O) m^{-2} s^{-1}] were measured at a constant photosynthetic photon flux density (PPFD) (1000 μmol m^{-2} s^{-1}), a fixed CO_2 concentration (C_a of 360 mmol mol^{-1}), air temperature (30 °C), relative humidity (65%) and a flow rate of 500 cm^3 min^{-1}. Stable maximum rates of net photosynthesis were reached between 24 and 30 min after onset of exposition to actinic light.

Leaf hydration, pigments and ionic analyses: The content of pigments as well as ionic accumulation was determined in the same individual leaves used for the Chl fluorescence and gas exchange measurements. Leaf Chl and Cars were determined according to the methods of Akram *et al.* (2003). They were extracted with 85% acetone and the absorbance of extracts was measured at 452.5, 644 and 633 nm with a *Camspec M330 UV/Vis* Spectrophotometer (*Camspec, Ltd*., Cambridge, UK). The equations reported by Akram *et al.* (2003) were used to calculate the Chl *a* and *b* and total Car contents. Cations (Na^+, K^+ and Ca^{2+}) analyses were conducted after digestion by HNO_3 using atomic absorption spectroscopy *Perkin Elmer Analyst 100* (*Perkin Elmer*, Norwalk, CT, USA) and Cl^- was assayed by coulometry (*Buchler Cotlove Chloridometer*, *Buchler Instruments*, *Inc.,* Fort Lee, NJ, USA). The leaf hydration was determined as leaf water content and it was calculated as proposed by Qiujie *et al.* (1997).

Statistical analysis: The experiment was repeated independently and successively at three years (2004, 2005, and 2006). All parameters were measured for at least five plants ($n = 5$). They were taken on the mature leaves (fifth leaf after the terminal bud) of each of the chosen plants. Data were subjected to a two-way analysis of variance (*ANOVA*) in which salinity treatment and varieties of maize represented the main factors: varieties had two levels and salt stress had four ones. *Duncan*'s multiple-range test was used for comparison of means among different levels within a factor and comparisons with p-values < 0.05 were considered significantly different. Statistical analyses of the data were made with *SPSS for Windows 13.0* (*SPSS*, Chicago, IL, USA). In all the figures, the spread of values is shown as error bars representing standard errors of the means.

Results

Salinity effects and differences among corn varieties were obvious for most parameters (Table 1). Significant effects due to salinity treatment were detected for all parameters except for the ratios $(1 - q_p)$/NPQ and the Chl *a*/*b* ratio. Differences among varieties were found for all parameters except those mentioned previously in addition to Chl *b* and Car content. We did not find significance of interaction between varieties and salinity treatment with the exception of A_N, Φ_{PSII}, %P, leaf tissues hydration and Na^+ and K^+ contents.

PSII photochemistry: The changes in PSII photochemistry were first investigated in dark-adapted state in salt-treated leaves. In both varieties the potential efficiency of PSII photochemistry (F_v/F_m) was reduced with increasing salinity. It dropped at 102 mM NaCl, by about 14% for Aristo, and 7% for Arper (Fig. 1). The reduction of F_v/F_m was accompanied by a decline in F_m but no changes were observed in F_o (data not shown). We further investigated PSII photochemistry in the light-adapted leaves, *i.e*. under steady state photosynthesis. The variation of the corresponding parameters, according to

Table 1. Observed significance levels (p-values) from analyses of variance for effects of salinity treatment and corn variety and their interactions on gas exchange and chlorophyll fluorescence parameters, ionic accumulation, tissue hydration and photosynthetic pigments concentration. A_N – net assimilation rate; g_s – stomatal conductance; F_v/F_m – maximal efficiency of PSII; Φ_{PSII} – actual PSII efficiency; q_p – photochemical quenching; NPQ – non-photochemical quenching; $(1 - q_p)/NPQ$ – susceptibility of PSII to high irradiance; $(F_m' - F_o)/F_m'$ – proportion of PSII centers effectively re-oxidized; %P – fraction of energy allocated to PSII photochemistry; %D – thermal energy dissipation; %X – excess of energy excitation; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; Cars – carotenoids.

Parameter	Treatment	Variety	Interaction
A_N	<0.0001	<0.0001	0.005
g_s	<0.0001	0.001	0.944
F_v/F_m	<0.0001	0.010	0.456
Φ_{PSII}	<0.0001	<0.0001	0.036
q_p	<0.0001	<0.0001	0.096
NPQ	0.001	0.002	0.190
$(1 - q_p)/NPQ$	0.640	0.672	0.998
$(F_m' - F_o)/F_m'$	<0.0001	0.001	0.130
%P	<0.0001	<0.0001	0.036
%D	<0.0001	0.001	0.263
%X	0.006	0.012	0.785
Na^+	<0.0001	<0.0001	<0.0001
K^+	<0.0001	<0.0001	<0.0001
Ca^{2+}	<0.0001	<0.0001	0.382
Cl^-	<0.0001	<0.0001	0.744
Water content	<0.0001	<0.0001	<0.0001
Chl *a*	<0.0001	0.017	0.942
Chl *b*	<0.0001	0.580	0.972
Chl (*a* + *b*)	<0.0001	0.023	0.977
Chl *a*/*b* ratio	0.544	0.389	0.980
Cars	<0.0001	0.204	0.982

salt stress, is not of the same extent in both varieties. For Aristo, the fraction of open PSII reaction centers (q_p) began to decrease significantly when plants were exposed to 68 mM NaCl or higher. Arper was remarkably more tolerant to salt stress and this parameter showed a reduction solely at 102 mM NaCl. Salt stress induced also an increase in nonphotochemical quenching coefficient (NPQ). The increasing trend of NPQ in Arper was gentler than in Aristo. When the level of salinity reached 102 mM, values of NPQ were about 0.57 and 0.41 for Aristo and Arper, respectively (Fig. 1). Concomitantly, the Φ_{PSII} declined differently between the two varieties. For example, at 102 mM NaCl, the Φ_{PSII} was reduced by 47% for Aristo and 25% for Arper compared with the controls (Fig. 2).

Since plants are exposed to photon flux densities which can lead, with salt stress conditions, to the saturation of electron transport chain in thylakoid membranes and, in a successive stage, the degradation of reaction centres. In order to verify this hypothesis we have proposed to determine the ratio $(1 - q_p)/NPQ$ which estimates the susceptibility of PSII to high irradiance and

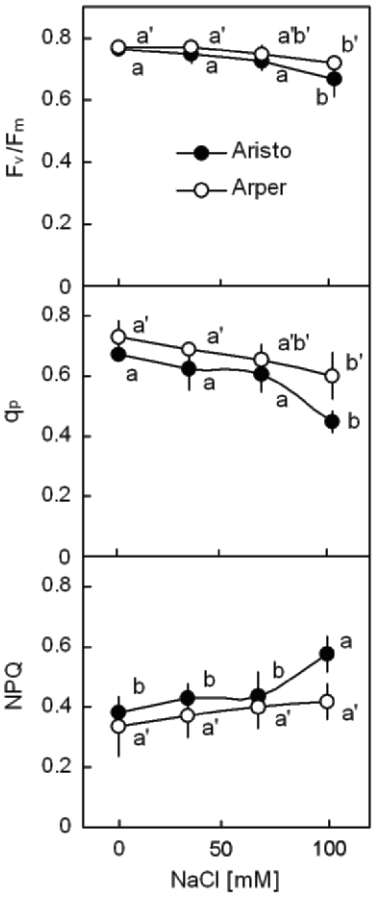

Fig. 1. Changes in maximal efficiency of PSII (F_v/F_m), photochemical quenching (q_p) and nonphotochemical quenching (NPQ) of chlorophyll fluorescence in salt-treated leaves of two corn varieties (Aristo and Arper). Means ± SD of five independent measurements. Values of each variety followed by the same letter indicate no significant differences (p<0.05) according to *Duncan* test.

the proportion of PSII centers effectively re-oxidized $[(F_m' - F_o)/F_m']$. Fig. 2 shows the response of these parameters to salinity treatment. Analysis showed that, for Aristo, a NaCl concentration range of 0–68 mM had no significant effects on the proportion of PSII centers effectively re-oxidized. For Arper, the ratio $(F_m' - F_o)/F_m'$ decreases when the concentration of NaCl exceeds 34 mM, but the recorded values for all salinity treatments are notably higher than those of Aristo. The $(1 - q_p)/NPQ$ ratio which estimates the excess of light during photosynthesis induction showed, in both varieties, no significant differences between control plants and NaCl-treated plants.

Distribution of absorbed energy: Our data (Table 2) indicate that P was remarkably reduced due to salinity. However, energy dissipated as heat or used in other pathways increased linearly with the increase of salt stress. Considerable differences were observed between the average values of varieties in control and salt-treated

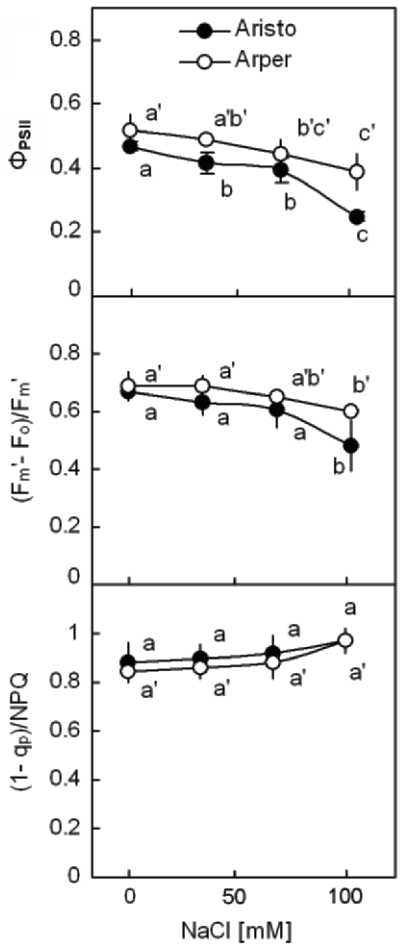

Fig. 2. Changes in actual quantum yield of PSII (Φ_{PSII}), proportion of PSII centers effectively re-oxidized [(F_m' – F_o)/F_m'] and susceptibility of PSII to high irradiance [(1 – q_p)/NPQ] in salt-treated leaves of two corn varieties (Aristo and Arper). Means ± SD of five independent measurements. Values of each variety followed by the same letter indicate no significant differences (p<0.05) according to *Duncan* test.

plants. It appeared that Arper used the absorbed light energy for photosynthesis better than Aristo. Indeed, control leaves used approximately 46% and 51% of the light absorbed by PSII respectively for Aristo and Arper. Leaves exposed to high salinity (102 mM NaCl) used about 38% of the absorbed light in primary photochemistry in Arper and only 25% in Aristo. Thermal energy dissipation (D) was at the range of 28% (Arper) and 30% (Aristo) in control leaves and increased significantly in leaves exposed to the different stresses. At 102 mM NaCl, it closed to 35% and 43% respectively for Arper and Aristo. A similar pattern was observed in the third fraction (X). At the sternest salinity treatment (102 mM) values were around 25% in Arper and 31% in Aristo.

Gas exchange parameters: Increased salt stress significantly reduced A_N of both maize varieties. This effect was accompanied by consistent decreases in g_s. For all treatments, averages of A_N and g_s in Arper were significantly higher than those observed in Aristo (Table 3). The reduction of net photosynthesis can be caused by the decline of g_s and/or to the PSII photoinhibition. In order to determine the contribution of each of these factors, a multivariate regression analysis was realised between A_N as dependent variable and g_s and Φ_{PSII} as independent variables. Multivariance regression analysis was performed for the two varieties using the formula $A_N = a\ g_s + b\ \Phi_{PSII} + c$, where a, b, and c are the unstandardized regression coefficients of the model (*see* values in Table 4).

The results of regression are shown in Table 4. It showed that the square of total correlation coefficient (R^2) values of the model, in both varieties, were close to 0.9, indicating a high linear correlativity between each one of the two independent variables (g_s, Φ_{PSII}) and A_N.

The importance of each one of the two independent factors was compared according to their standardized

Table 2. Changes in the fractions of light absorbed by the PSII antennae used in photochemistry (P), thermally-dissipated (D) and not used in photochemistry nor dissipated in the antenna (X) in both corn varieties (Aristo and Arper) grown at different NaCl concentrations. Means ± SD of five independent measurements. Values in each row followed by the same letters indicate no significant differences (p<0.05) according to *Duncan* test.

	NaCl [mM]			
	0	34	68	102
Aristo				
P [%]	46.88 ± 1.48^{a}	41.82 ± 3.45^{b}	39.30 ± 3.79^{b}	25.02 ± 1.47^{c}
D [%]	30.18 ± 1.06^{b}	32.91 ± 3.76^{b}	35.15 ± 4.49^{b}	43.62 ± 6.33^{a}
X [%]	22.94 ± 1.19^{b}	25.27 ± 5.83^{ab}	25.55 ± 5.11^{ab}	31.36 ± 5.65^{a}
Arper				
P [%]	$51.79 \pm 4.99^{a'}$	$48.96 \pm 1.01^{a'b}$	$44.55 \pm 4.12^{b'c}$	$38.81 \pm 5.76^{c'}$
D [%]	$28.81 \pm 4.02^{b'}$	$28.91 \pm 3.29^{b'}$	$31.88 \pm 2.03^{a'b'}$	$35.66 \pm 1.94^{a'}$
X [%]	19.39 ± 3.73^{b}	$22.13 \pm 2.44^{a'b}$	$23.56 \pm 3.73^{a'b'}$	$25.52 \pm 4.38^{a'}$

Table 3. Changes of net CO_2 assimilation rate (A_N [μmol(CO_2) m^{-2} s^{-1}]) and stomatal conductance (g_s [mmol(H_2O) m^{-2} s^{-1}]) in leaves of two corn varieties (Aristo and Arper) treated with different salt concentrations. Means ± SD of five independent measurements. Values in each line with different superscripted letters are significantly different according to *Duncan* test ($p < 0.05$).

	NaCl [mM] 0	34	68	102
A_N				
Aristo	5.33 ± 0.16^{a}	4.68 ± 0.22^{b}	2.58 ± 0.52^{c}	1.63 ± 0.20^{d}
Arper	5.68 ± 0.17^{a}	5.58 ± 0.32^{a}	$2.80 \pm 0.12^{b'}$	1.63 ± 0.20^{c}
g_s				
Aristo	64.78 ± 5.58^{a}	59.9 ± 7.58^{a}	33.54 ± 3.74^{b}	28.08 ± 1.62^{b}
Arper	$72.78 \pm 5.66^{a'}$	$67.9 \pm 6.88^{a'}$	$40.54 \pm 5.96^{b'}$	$33.16 \pm 3.29^{b'}$

Table 4. Results of multiple regression between net photosynthetic assimilation (A_N), stomatal conductance (g_s) and actual PSII efficiency (Φ_{PSII}) of two corn varieties (Aristo and Arper) seedlings growing in different NaCl concentrations. Dependent variable: A_N. Predictors: Φ_{PSII} and g_s. A_N = a g_s + b Φ_{PSII} + c. a, b, and c – unstandardized regression coefficients; a′ and b′ – standardized regression coefficients corresponding respectively to g_s and Φ_{PSII}; R^2 – the square of total correlation coefficient, and p – statistical significance of the model.

Regression parameters	Aristo	Arper
a	0.073	0.078
b	5.33	3.21
c	–2	–1.56
a′	0.73	0.85
b′	0.25	0.13
R^2	0.91	0.89
p	$p<0.001$	$p<0.001$

regression coefficients (a′ and b′ for g_s and Φ_{PSII}, respectively) which determine the relative contribution of g_s and Φ_{PSII} to the photosynthetic limitation. In both varieties, the absolute standardized regression coefficients of g_s were greater than those of actual PSII efficiency, seemingly, indicating that A_N at both varieties was most closely correlated with g_s than actual PSII efficiency.

Ion accumulation and tissue hydration: Maize seedlings grown in the presence of NaCl accumulated large amounts of both Na^+ and Cl^- ions. The observed accumulation was greater in Aristo than in Arper. In fact at 102 mM NaCl the Na^+ concentration in leaf tissues was close to 5 and 7 mmol g^{-1}(DM), while Cl^- concentration was close to 3.8 and 4.2 mmol g^{-1}(DM) for Arper and Aristo, respectively (Table 5). Salt treatments also resulted in a decrease of K^+ and Ca^{2+} concentration in leaves of both varieties. However NaCl treatments of the variety Arper reserved the higher levels of K^+ as well as Ca^{2+}. For example at 102 mM NaCl, the content of K^+ and Ca^{2+} in the leaves of Arper was almost 2 times more elevated than those of Aristo. The elevated NaCl in the growth medium induced tissues dehydration in leaves of

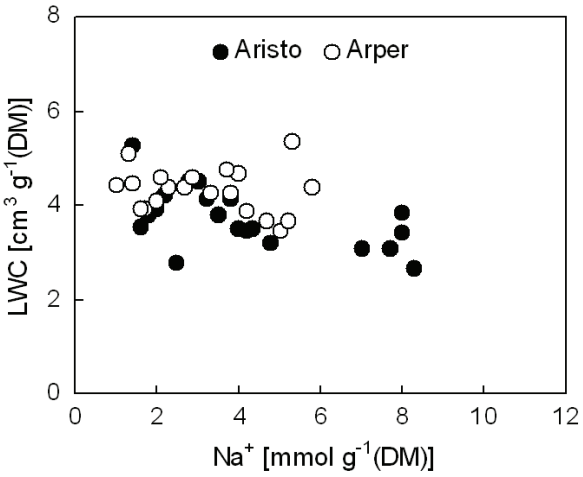

Fig. 3. Leaf water content (LWC) as a function of Na^+ accumulation in leaves of two corn varieties (Aristo and Arper) treated with different NaCl concentrations. The difference between varieties was significant at $p<0.05$.

Aristo but not in those of Arper (Table 5). The leaf dehydration was correlated with the accumulation of the major ion Na^+. Indeed, Fig. 3 revealed that increase in Na^+ content was associated with large decrease in water content especially in leaves of Aristo. For this variety, because the accumulation of Na^+ ion resulted in tissue dehydration, it is likely that this ion was deposited in the leaf apoplast rather than being compartmentalized inside the cells (Flowers *et al.* 1991).

Photosynthetic pigments concentration: The two varieties displayed maximum and minimum contents of Chl *a* and Chl *b* at control plants and those treated with 102 mM NaCl, respectively. Nevertheless, the trend of reduction was more pronounced in Chl *a* than Chl *b* (Table 6). Moreover, the first significant detectable decrease of total Chl was observed at concentrations of 34 and 68 mM NaCl for Arper and Aristo, respectively. However, the difference between means of Chl *a*/*b* ratios were not significant neither between salinity levels nor between varieties (Tables 1 and 6). Cars are responsible

Table 5. Effects of salt stress on ionic contents [mmol g^{-1}(DM)] and water content [mmol(H_2O) g^{-1}(DM)] of leaves of both corn varieties: Aristo and Arper. Means ± SD of five independent measurements. Values in each column followed by the same letters indicate no significant differences (p<0.05) according to *Duncan* test.

	NaCl [mM]	Na^+	K^+	Ca^{2+}	Cl^-	H_2O
Aristo						
	0	1.80 ± 0.31^{d}	7.81 ± 0.81^{a}	3.20 ± 0.62^{a}	1.10 ± 0.22^{d}	4.14 ± 0.66^{a}
	34	3 ± 0.18^{c}	6.60 ± 0.56^{b}	2.11 ± 0.21^{b}	1.74 ± 0.18^{c}	3.94 ± 0.70^{ab}
	68	4.22 ± 0.16^{b}	4.22 ± 0.43^{c}	1.87 ± 0.14^{b}	2.41 ± 0.54^{b}	3.56 ± 0.34^{ab}
	102	7.80 ± 0.41^{a}	2.41 ± 1.12^{d}	1.22 ± 0.19^{c}	4.21 ± 0.43^{a}	3.20 ± 0.42^{b}
Arper						
	0	$1.40 \pm 0.27^{d'}$	$8.22 \pm 0.57^{a'}$	$3.32 \pm 0.16^{a'}$	$0.81 \pm 0.27^{c'}$	$4.35 \pm 0.49^{a'}$
	34	$2.41 \pm 0.39^{c'}$	$6.62 \pm 0.34^{b'}$	$2.60 \pm 0.18^{b'}$	$1.12 \pm 0.22^{c'}$	$4.41 \pm 0.20^{a'}$
	68	$3.80 \pm 0.33^{b'}$	$6.48 \pm 0.29^{b'}$	$2.44 \pm 0.31^{b'}$	$1.82 \pm 0.36^{b'}$	$4.36 \pm 0.35^{a'}$
	102	$5.21 \pm 0.40^{a'}$	$5.61 \pm 0.36^{c'}$	$1.87 \pm 0.24^{c'}$	$3.80 \pm 0.41^{a'}$	$4.10 \pm 0.78^{a'}$

Table 6. Chlorophyll (Chl) content and carotenoids (Car) [mg g^{-1}(fresh mass of leaf tissue)] in two corn varieties (Aristo and Arper) grown at different NaCl concentrations. Means ± SD of five independent measurements. Values in each row with different superscripted letters are significantly different according to *Duncan* test ($p < 0.05$).

	NaCl [mM] 0	34	68	102
Aristo				
Chl *a*	1.42 ± 0.15^{a}	1.39 ± 0.07^{ab}	1.26 ± 0.08^{b}	0.92 ± 0.10^{c}
Chl *b*	0.63 ± 0.10^{a}	0.61 ± 0.09^{a}	0.55 ± 0.05^{b}	0.42 ± 0.04^{b}
Chl (*a* + *b*)	2.05 ± 0.12^{a}	1.99 ± 0.06^{a}	1.81 ± 0.11^{b}	1.34 ± 0.14^{c}
Chl *a*/*b* ratio	2.33 ± 0.56^{a}	2.32 ± 0.40^{a}	2.31 ± 0.20^{a}	2.21 ± 0.09^{b}
Cars	0.63 ± 0.10^{a}	0.60 ± 0.04^{a}	0.54 ± 0.01^{b}	0.43 ± 0.04^{b}
Arper				
Chl *a*	$1.52 \pm 0.15^{a'}$	$1.49 \pm 0.12^{a'b'}$	$1.36 \pm 0.08^{b'}$	$0.97 \pm 0.10^{c'}$
Chl *b*	$0.63 \pm 0.05^{a'}$	$0.62 \pm 0.06^{a'}$	$0.57 \pm 0.05^{a'}$	$0.44 \pm 0.04^{b'}$
Chl (*a* + *b*)	$2.15 \pm 0.17^{a'}$	$2.10 \pm 0.16^{a'b'}$	$1.93 \pm 0.11^{b'}$	$1.41 \pm 0.14^{c'}$
Chl *a*/*b* ratio	$2.44 \pm 0.25^{a'}$	$2.42 \pm 0.21^{a'}$	$2.40 \pm 0.20^{a'}$	$2.22 \pm 0.08^{a'}$
Cars	$0.64 \pm 0.05^{a'}$	$0.63 \pm 0.05^{a'}$	$0.58 \pm 0.01^{a'}$	$0.45 \pm 0.04^{b'}$

for quenching of singlet oxygen, and hence their comparative levels in two varieties determine relative stress tolerance. In Aristo, the onset of decline of Cars quantity was at 68 mM NaCl and this was continued through to 102 mM NaCl. While, in Arper, only the highest level of salt treatment (102 mM NaCl) decreased the Cars content in leaves.

Discussion

In the present study, we have investigated the effects of different NaCl concentrations on two corn varieties by analyzing fluorescence quenching, photosynthetic gas exchange, leaf ionic accumulation, and photosynthetic pigment contents. We intended to use this comparative response study to elucidate the salt tolerance mechanism in maize plants.

The maximum quantum yield of PSII photochemistry (F_v/F_m) is frequently used as an indicator of photoinhibition or of other types of stresses caused to PSII (Calatayud and Barreno 2004, Baker 2008). At 102 mM NaCl, the reduction of this ratio, in both varieties, was accompanied with insignificant changes of F_o and significant decrease of F_m (data not shown). This suggests that the observed photoinhibition was due to photoprotective processes but not to photoinhibitory damage (Krause 1988). The maximal fluorescence decrease may be related to the reduction in the activity of the water-splitting enzyme complex and perhaps also to the concomitant cyclic electron transport within or around PSII (Aro *et al.* 2005). During the light-adapted state, our results disclose a decrease in the activity of PSII photochemistry which was reflected by the reduction of the Φ_{PSII} and q_p in salt-treated leaves. The rate of the

decline of PSII function depends on both salinity level and varieties (Table 1). Salt stress caused more photosynthetic inhibition to Aristo than to Arper (Fig. 1). Some studies demonstrated that salinity in the presence of high light induced significant changes in PSII photochemistry and increased susceptibility of PSII to photoinhibition (Mishra *et al.* 1991, Santos 2004, Jiang *et al.* 2006).

Photochemical quenching indicates the oxidation-reduction state of the primary acceptor (Q_A) for PSII (Baker 2008). A decrease in q_p values indicates an increase in the fraction of reduced Q_A (Q_A^-) of PSII, which means there is an increase in susceptibility to photoinhibition (Lu and Lu 2004). On the other hand, the increased level of NPQ suggests that it may become established in the salt-treated leaves of maize varieties as shown in Fig. 1. Compared to Aristo, the variety Arper allocated a reduced amount of light energy to NPQ pathways, as suggested by the lower values of NPQ. In fact, in this variety NPQ has no significant changes even at the highest NaCl concentration. It was as low as in controls (Fig. 1). Thus, Arper appears to be equipped with an efficient defence system, independently of NPQ, which prevents damages to the photosynthetic mechanism under stress conditions (Hajlaoui *et al.* 2009). Cushman and Bohnert (1999) report that some plants show several strategies to protect their photosynthetic capacity during stress situations. In reality once plants are exposed to high salt stress, Na^+ and Cl^- concentrations increase within chloroplasts to a smaller extent than in the total cell volume (Demmig and Winter 1988). Furthermore, in the absence of efficient internalization of the ions by leaf cells as in the case of Aristo variety, their concentration in the leaf apoplast may reach excessive values, leading to leaf cell dehydration (Flowers *et al.* 1991), stomata closure and photosynthesis inhibition (Meloni *et al.* 2003). In fact, Na^+ and Cl^- were accumulated at high concentrations in leaves of both varieties (Table 5), but this accumulation appears to be more regulated in leaves of Arper compared to those of Aristo. The increased levels of Na^+ and Cl^- were accompanied by a reduction in K^+ and Ca^{2+} accumulation, a response characteristic to many glycophytes. However, at all NaCl treatments, the Arper variety preserved the highest amounts of K^+ as well as Ca^{2+}.

The contribution of photochemical and non-photochemical quenching to the photoinactivation of PSII can be conveniently assessed by the ratio $(1 - q_p)/NPQ$. In both varieties, the constancy of this ratio between NaCl treatments indicate that $(1 - q_p)$ increased with respect to the control, and also that the values of NPQ were high enough to maintain this ratio and prevent the damage caused to PSII (Calatayud and Barreno 2004).

Both D and X increased with the decrease of P when salt stress was increased. In both varieties, the elevated fraction of thermal dissipation showed that this pathway generally played an important role in preventing photodamage in corn. The salt stress limited the fraction of light absorbed in the PSII antennae and used in PSII photochemistry more in Aristo than in Arper (Table 2). Such phenomenon was also found in other species (Ort 2001). So energy, which is not used in photochemical pathways or dissipated thermally, may cause photoinhibition (Lichtenthaler and Burkart 1999). In our study, when photochemical energy use was significantly reduced by rising salinity, photoinhibition increased, which in turn resulted partially in a photosynthetic activity decline. The data on A_N and g_s for the two corn varieties showed that both variables declined considerably under saline conditions (Table 3). However, photosynthesis in Aristo was limited more than in Arper. Similar results were reported in tomato (Sonneveld and Voogt 1990) and also in cucumber and pepper (Kaya *et al.* 2003). The reduction of A_N in salt-treated leaves of both maize varieties seems to be more closely related to stomatal limitations than to photoinhibition (Table 4). In the mean time, changes in Chl fluorescence confirm that nonstomatal limitations may also have been responsible for the reduction in photosynthesis. The decline of photosynthetic capacity under salt stress was associated also with the reduction of photosynthetic pigments content. In both varieties, the content of total Chl and Cars was significantly reduced with increasing salinity. Separation of total Chl into Chl *a* and *b* revealed a constant ratio of both pigments and thus an almost parallel decreasing in both pigments in salt-treated leaves. The less reduction of Chl *a*, Chl *b* and Cars with increasing salinity was shown in Arper seedlings leaves. Such reduction has been described for maize plants exposed to NaCl (Yang and Lu 2005) or for other species under severe stress (Genty *et al.* 1989, Yang *et al*. 2008). The decrease of Chl content may be due to the increase of Chl degradation and/or to the decrease of Chl synthesis. During the process of Chl degradation, Chl *b* is converted to Chl *a* and this may explain the constancy of the ratio Chl *a/b* in stressed leaves together with the depression of Chl content (Santos 2004).

Our study represents one of the few attempts to coordinate the effects of salinity on light-harvesting characteristics and carbon fixation in C_4 plant. Our results indicate how photosynthesis in C_4 plants may be adapted to soil salinity. High salinity causes some decrease in carbon assimilation. The salt stress predisposes photoinhibition in the two corn varieties when exposed to high salinity. Notable distinction between the two tested varieties was observed in the present study. The Arper variety showed more tolerance to salt stress than Aristo as indicated by the lower reduction of photosynthesis and PSII photochemistry, more regulated leaf ionic status and lower leaf tissues dehydration. The various parameters explored in both varieties, with their marked effects in the photosynthetic apparatus, vary in their relative contributions. It can, therefore, be concluded that g_s was the main factor responsible for reduction in A_N coupled with photoinhibition.

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