

*Full Length Research Paper*

# Growth and physiological response of tall oat grass to salinity stress

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**In order to evaluate the responses of tall oat grass plants to increasing salinity, we measured plant growth, ion contents, photosynthetic gas exchange, lipid peroxidation, and proline accumulation in four salt concentrations. Two tall oatgrass genotypes, ZXY03P-367 and ZXY03P-443, were grown for 14 days in greenhouse conditions and after 14 days treated with four NaCl treatments (0, 65, 100, and 135 mM) for 21 days. Most parameters for the two genotypes were significantly different when they were subjected to 100 and 135 mM NaCl. Salt treatment led to decreases in root and shoot biomass, photosynthetic rate ( $A$ ) and stomatal conductance ( $g_s$ ), and  $K^+$  content, and a concurrent increase in  $Na^+$  content. Larger reductions in the parameters occurred in ZXY03P-443. A significant accumulation of lipid peroxidation and proline in leaves was found during the period of intensive leaf growth. These organic compounds likely played a role in leaf osmotic adjustment and in the protection of membrane stability at severe salinity levels. Our results indicated that the two tall oatgrass genotypes differ in their sensitivity to salinity, with ZXY03P-336 classified as relatively salt tolerant and ZXY03P-443 as sensitive.**

**Key words:** Growth, physiological responses, salinity stress, tall oatgrass

## INTRODUCTION

Salinity is the major environmental factor limiting plant growth and productivity. It also severely affects both irrigated and dryland agricultural areas in China. Data suggest that up to  $4.0 \times 10^7$   $hm^2$  of land are currently affected by salinity (Wu et al., 2007). With increasing amounts of arable land undergoing salinization and increasing food demand from the growing human population, the need to develop salt-tolerant crops is increasing. Thus, we need to identify the degree of salinity tolerance within crops and their wild relatives (Rogers, 2007). Using salt-tolerant forage species is one solution to maintaining agricultural production in saline soils. Consequently, there is a need to identify plant species for saline areas in both dryland and irrigated lands that will provide both groundcover and agricultural production (Rogers et al., 2005).

Salinity affects the plant's morphological, physiological and biochemical processes. Salinity stress results in

stunting of plants (Takemura et al., 2000; Alshammary et al., 2004; Yamaguchi and Blumwald, 2005). In such conditions, shoot and roots dry weights are decreased (Heidari-Sharifabada and Mirzaie-Nodoushan, 2006). Zhao et al. (2007) reported that salt stress applied at their lowest treatment level (50 mM) reduced total leaf area of naked oat (*Avena nuda* L.) by 35% and plant dry matter by 52%. With a higher salinity level (250 mM), plant growth was further suppressed, causing decreases of 91 and 86% in total leaf area and plant dry matter, respectively (Zhao et al., 2007).

One mechanism associated with greater tolerance to moderately saline environments is the ability of plants to exclude  $Na^+$  from the shoot, and concurrently maintain high levels of shoot  $K^+$  (Flowers and Hajibagheri, 2001). Salinity was shown to interfere with  $K^+$  and  $Ca^+$  nutrition (Rengel, 1992) and this can cause nutrient deficiencies. Growing plant in increasing NaCl stress induces increases in  $Na^+$  and  $Cl^-$ , and decreases in  $Ca^{2+}$ ,  $K^+$  and  $Mg^{2+}$  levels in many plants (Heidari-Sharifabada and Mirzaie-Nodoushan, 2006).

Biomass production is a direct reflection of net photosynthesis. Decreased photosynthetic rates may

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result from the closure of stoma, induced by osmotic stress, or from salt-induced damage to the photosynthetic apparatus (Parida and Das, 2005). Wilson et al. (2006) found a highly significant reduction in stomatal conductance ( $g_s$ ), and net photosynthetic rate ( $A$ ) in cowpea (*Vigna unguiculata* (L.) Walp.) cultivars suffering from salinity. Moreover, the net photosynthetic rate was found to be a more sensitive physiological indicator of the level of salinity stress than the leaf area. In rice (*Oryza sativa* L.),  $A$  was decreased by about 35% at 12 ds m<sup>-1</sup> with salt stress, but the reductions in  $g_s$  and transpiration rate ( $T_r$ ) were 74 and 63%, respectively. Conversely, the reduction in intercellular CO<sub>2</sub> concentration ( $C_i$ ) was much lower (15%) at the same stress level (Moradi and Ismail, 2007).

Tall oatgrass (*Arrhenatherum elatius* L.), native to Europe, is cultivated in the grasslands of Central and Northern USA, Western Eurasia, East Asia, and the Mediterranean Basin (Mitchell et al., 2003). Tall oatgrass is a useful conservation grass for cover and forage on surface mined lands and marginal pastureland, and can also be used for livestock forage beginning in its second growing season. Because of its considerable drought and winter hardiness and high resistance to diseases and insects, tall oatgrass was often used as a tertiary germplasm resource for improving oat which is the most closely related genera to *Arrhenatherum* within the tribe Aveneae (Leggett, 1992). The Poaceae family (grasses) is known to include genera that range from moderately salt sensitive to highly salt tolerant (Maas, 1990). Little information is available on the responses of tall oatgrass to salinity (Yang et al., 2006). Understanding the mechanisms underlying the growth and physiological responses to salinity would be valuable in selecting and improving tall oatgrass strains tolerant or adapted to salt stress. The objective of the present study was to determine the influence of salinity stress on seedling growth, ion content, photosynthetic productivity and metabolism of different tall oatgrass genotypes.

## MATERIALS AND METHODS

A large amount of tall oatgrass genotypes were initially screened for salt tolerance, and two tall oatgrass genotypes, ZXY03P-367 (the relatively salt tolerance) and ZXY03P-443 (relatively salt sensitive), were selected for the study. A 2×4 factorial experiment, arranged in a completely randomized design with three replications, was conducted. 20 seeds were planted in two-liter pots filled with silica sand and thinned to ten plants per pot after emergence. Pots were irrigated with distilled water for 7 days and then fertilized with a salt-free Hoagland's solution. Salinity stress was imposed on seedlings at 14 days of age by adding 65, 100, and 135 mM NaCl in salt-free Hoagland's solution, while taking the salt-free Hoagland's solution itself as the control treatment. For each pot, 200 ml of salt solution was applied daily to ensure that all seedlings received an equal volume of treatment solution and to prevent additional drought stress. The experiment was performed in a greenhouse with a temperature of 25/16°C (day/night) and a 16-h photoperiod with 300 μmol m<sup>-2</sup> s<sup>-1</sup> illumination. Relative humidity was maintained at about

70%. The plants were then subjected to the salt treatments for 21 days before the following measurements were taken.

### Estimation of plant growth

Plant shoots and roots were harvested after 21 days of salinity treatment, and dried at 60°C for 48 h for the determination of dry biomass and further analysis.

### Estimation of ion content

Dried leaf samples were ground to a fine powder and approximately 0.1 g was transferred to a test tube. Ions were extracted by adding 10 ml of 0.1 N acetic acid and heating in a water bath at 80°C for 2 h. The extraction solution was cooled at room temperature and left overnight, and then filtered using Whatman filter paper No. 40. Sodium and potassium concentrations were then determined using an atomic absorption spectrometer (Perkins Elmer, Norwalk, CT, USA).

### Estimation of photosynthetic parameters

Net photosynthetic rate ( $A$ ), and stomatal conductance ( $g_s$ ) were measured on the same leaf samples as the leaf greenness measurements using an infrared, open gas exchange system LI-6400 (LI-COR) following the manufacturer's instructions. The area of each leaf in the photosynthetic meter chamber was determined manually. The measurements were performed with light levels of (1000 μmol m<sup>-2</sup> s<sup>-1</sup>). Data were manually recorded when the gas exchange parameters became stable.

### Estimation of proline and lipid peroxidation

Free proline was quantified spectrophotometrically using the method of Bates et al. (1973). Lipid peroxidation was estimated by measuring the content of 2-thiobarbituric acid-reactive substances in 0.2 g leaf fresh weight according to Madhava Rao and Sresty (2000). Malondialdehyde (MDA) content was determined spectrophotometrically at A<sub>532</sub> and corrected for non-specific turbidity at A<sub>600</sub> and A<sub>450</sub>.

### Statistical analysis

All data were subjected to analysis of variance using the general linear model procedure of SAS (SAS, 1996). Treatment mean differences were separated by the least significant difference (LSD<sub>0.05</sub>) test if F tests were significant (P ≤ 0.05).

## RESULTS

### Plant growth response to salinity

The shoot biomass of the two tall oatgrass genotypes was significantly decreased with increased NaCl concentration (Table 1). Average of 29% reduction in shoot biomass was found at 65 mM salinity level compared with the control, and further decreased with higher NaCl concentration. In 65 to 135 mM NaCl stress, the decrease in shoot biomass ranged from 28 to 71% and

**Table 1.** Shoot and root dry weight of two tall oatgrass genotypes under four salt treatments measured during seedling stages.

Parameter	Shoot biomass (mM)				Root biomass (mM)			
	0	65	100	135	0	65	100	135
ZXY03P-367	1.96±0.03	1.42±0.03	1.02±0.06	0.56±0.05	1.25±0.02	0.87±0.05	0.43±0.04	0.20±0.04
ZXY03P-443	1.52±0.04	1.07±0.09	0.73±0.07	0.38±0.08	1.00±0.02	0.70±0.04	0.34±0.05	0.12±0.02
Genotype (G)		*				*		
Salinity (S)		***				***		
G×S		ns				ns		

\*, \*\*\* Effects that are significant at  $p < 0.05$ , and  $0.001$  respectively; ns, effects that are not significant at  $p < 0.05$ .

30 to 80% for ZXY03P-367 and ZXY03P-443, respectively. Moreover, significant differences in aboveground biomass production were observed, both between genotypes ( $P < 0.05$ ) and salinity stress ( $P < 0.001$ ). No obvious genotype  $\times$  salinity interaction measurements were found.

Root biomass of the two tall oatgrass genotype exhibited a similar trend to shoot biomass with salt stress (Table 1). On average, 30, 65, and 86% reduction in root biomass occurred in 65, 100, and 135 mM salinity levels, respectively, compared with the control.

### Sodium and potassium content

With salinity stress, leaf  $\text{Na}^+$  contents increased significantly ( $P < 0.001$ ) with increasing NaCl concentrations (Figure 1a). High  $\text{Na}^+$  accumulation was observed in the leaves of ZXY03P-367 and ZXY03P-443. Compared with the control,  $\text{Na}^+$  accumulation in ZXY03P-443 increased by about 3.2, 15, and 24.7 fold in 65, 100, and 135 mM NaCl stress, respectively, while these values were 2.7, 13, and 23 fold for ZXY03P-367. Moreover, in ZXY03P-443, the increase in  $\text{Na}^+$  content was comparatively higher than ZXY03P-367 in all the salt treatments (Figure 1a).

Increasing salt levels led to a significant reduction in leaf  $\text{K}^+$  concentration (Figure 1b). The reduction of  $\text{K}^+$  in ZXY03P-443 was greater than ZXY03P-367 at all the salt levels (Figure 1b). As a result, leaf K contents were slightly higher in ZXY03P-367 than in ZXY03P-443. However, no difference between the two species was found in any salt treatments.

### Photosynthetic gas exchange

Leaf photosynthetic rate ( $A$ ) and stomatal conductance ( $g_s$ ) were significantly higher in ZXY03P-367 than in ZXY03P-443 (Figures 2a and b). With salinity stress, leaf photosynthetic rate was reduced significantly ( $P < 0.01$ ) following the increased NaCl concentration (Figure 2a). Significant reduction in  $A$  was observed even at the low

salinity levels (Figure 2a). The  $A$  decreased by about 21, 57 and 76% when seedlings were subjected to salt stress of 65, 100, and 135 mM, respectively; however, reductions in  $g_s$  were slightly lower than in  $A$ , amounting to 17, 31 and 61% with the same salt levels, respectively (Figure 2b).

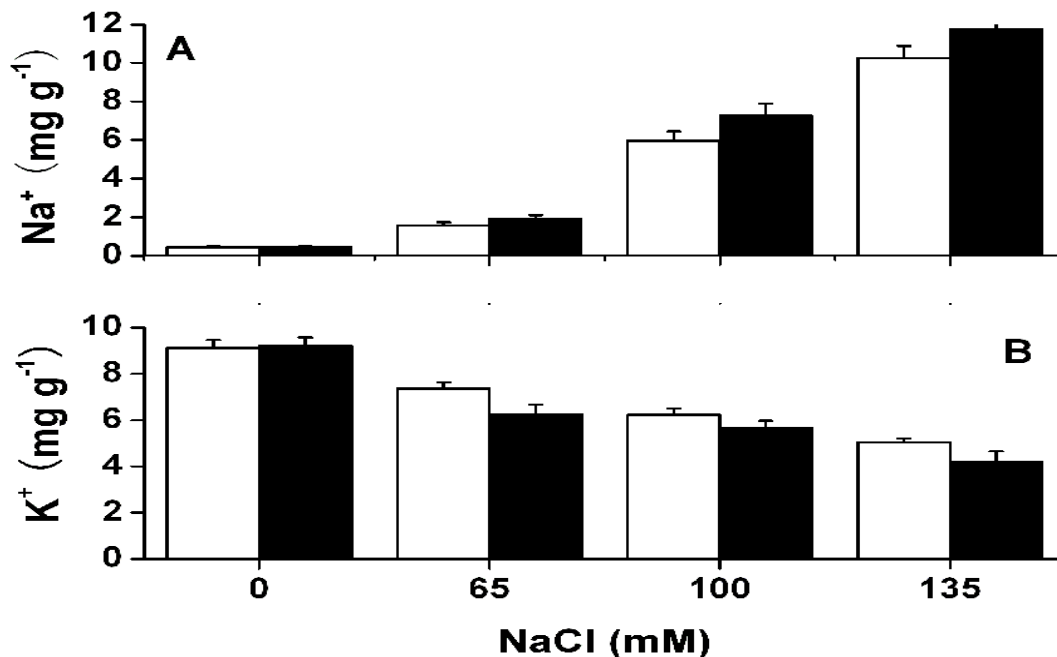
### Accumulation of proline and MDA

Proline concentration was measured at the seedling stage to determine its association with salinity tolerance. With salt stress, proline concentration increased significantly in the two genotypes (Figure 3a). On average, 83, 244, and 262% increase in proline content was found at 65, 100, and 135 mM salinity levels, respectively, compared with the control.

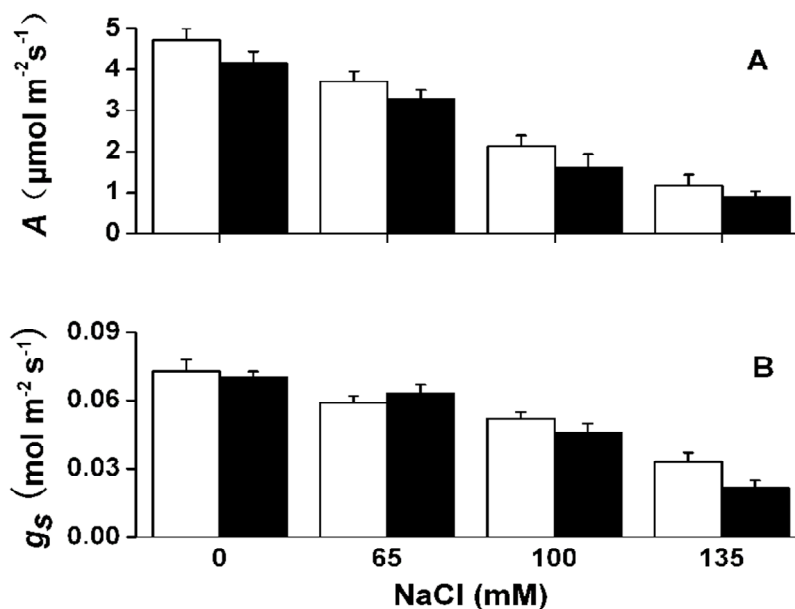
NaCl treatments significantly increased MDA content in both tall oatgrass genotypes, with the higher relative increase observed in ZXY03P-443, in which it increased by 18.1 to 97.2%, whereas a much lower increase was observed in ZXY03P-336 (6.3 to 48.4%) (Figure 3b). This indicated a higher level of lipid peroxidation in ZXY03P-443 due to salt stress.

### DISCUSSION

Shoot and root growth inhibition is a common response to salinity, and plant growth is one of the most important agricultural indices of salt stress tolerance as indicated by different studies (Kao et al., 2006; Hulusi et al., 2007). Salt stress significantly reduced the growth of the two tall oatgrass genotypes during the seedling stage. The observed reduction in shoot and root biomass is likely to be due to a combination of slower growth and development as a result of osmotic stress (Shani and Ben-Gal, 2005) and an inhibition of photosynthesis either as a result of the direct effects of salinity on the photosynthetic apparatus or the indirect effects of a reduction in sink capacity (Kato and Takeda, 1996). ZXY03P-443 was found to be more sensitive than ZXY03P-336 to salinity treatments. However, the differential response of growth



**Figure 1.** Effects of increasing NaCl concentration on shoot Na<sup>+</sup> (A), and K<sup>+</sup> (B) content of the two tall oatgrass genotypes; ZXY03P-367 (open columns) and ZXY03P-443 (closed columns), exposed to four NaCl treatments over 21 days. Vertical bars represent ± S.E. of treatment (n = 3).

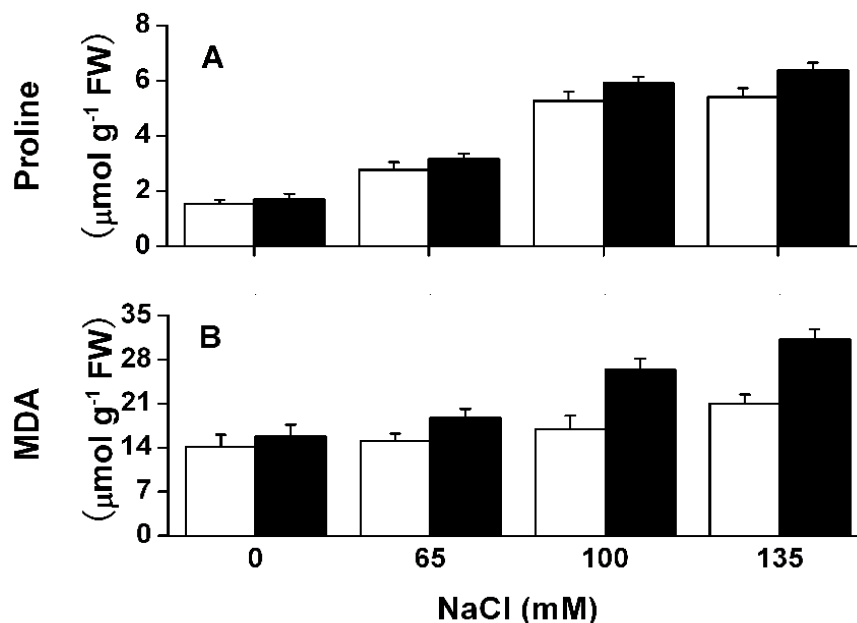


**Figure 2.** Effects of increasing NaCl concentration on photosynthetic rate (A), and stomatal conductance  $g_s$  (B) of the two tall oatgrass genotypes; ZXY03P-367 (open columns) and ZXY03P-443 (closed columns), exposed to four NaCl treatments over 21 days. Vertical bars represent ± S.E. of treatment (n = 3).

to salinity could be due to genotypic differences (Gunasekera et al., 2006). Genotypic differences to salinity have been reported in green gram (Misra and

Dwivedi, 2004), barley (Katerji et al., 2006), and rice (Moradi and Ismail, 2007).

The reduction in photosynthesis was small when plants



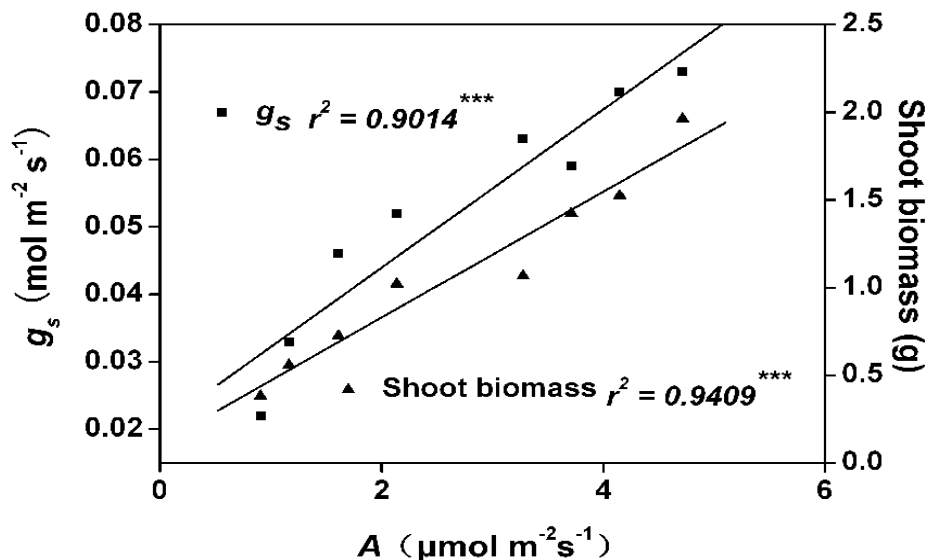
**Figure 3.** Effects of increasing NaCl concentration on proline (A), and MDA (B) content of the two tall oatgrass genotypes; ZXY03P-367 (open columns) and ZXY03P-443 (closed columns), exposed to four NaCl treatments over 21 days. Vertical bars represent  $\pm$  S.E. of treatment (n = 3).

were subjected to salt levels lower than 100 mM (Figure 3), but significant effects occurred at higher salt concentrations. This is in agreement with Zhao et al. (2007) findings for rice. The change in  $g_s$  was similar to that of  $A$  in tall oatgrass. A close relationship was found between  $A$  and shoot biomass and  $g_s$  (Figure 4), suggesting that the severe reduction in growth with salt stress was strongly related to the reduction in leaf gas exchange properties. Analysis of gas exchange measurements revealed that the greater relative reductions in  $A$  than in  $g_s$  (Figures 2a and b) measured in tall oatgrass suggests that non-stomatal inhibition of photosynthesis, caused by direct effects of NaCl on the photosynthetic apparatus independent of stomatal closure, might be responsible for the reduction in photosynthetic rate. Non-stomatal inhibition of photosynthesis by salinity was also reported for several other plant species (Kao et al., 2006). However, some reports that photosynthesis is not slowed down by salinity and is even stimulated by low salt concentrations (Kurban et al., 1999). In *A. pseudoalhagi*, the leaf  $\text{CO}_2$  assimilation rate increases in conditions of low salinity (50 mM NaCl) and is not significantly affected by 100 mM NaCl. It is, however, reduced to about 60% of the control by 200 mM NaCl. The observed decrease in both  $g_s$  and transpiration rate might be among the important adaptive mechanisms conferring tolerance to salinity in rice (Robinson, 1988; Moradi and Ismail, 2007). Decreases in photosynthetic rates are due to several factors including the dehydration of cell membranes which reduce their permeability to  $\text{CO}_2$ , salt toxicity and the

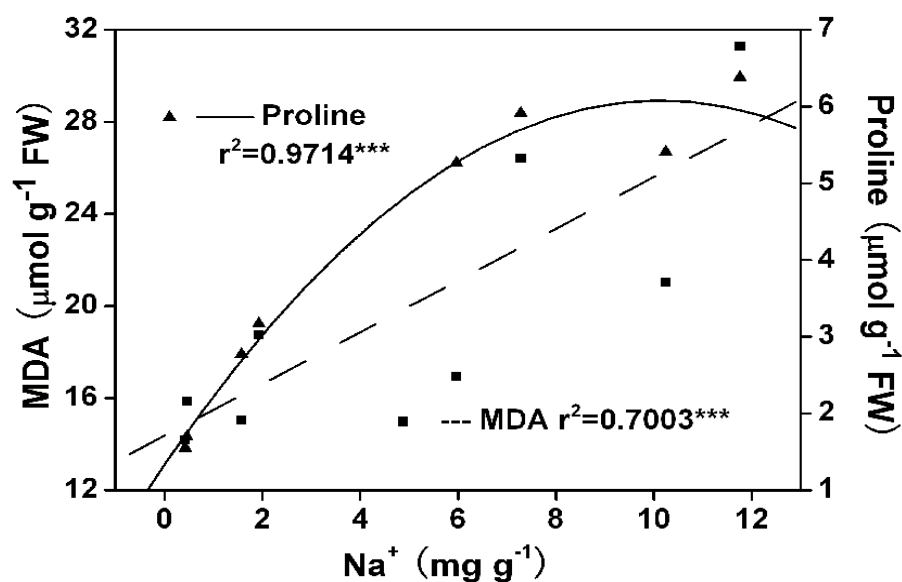
reduction of  $\text{CO}_2$  supply because of the hydroactive closure of the stomata, enhanced senescence induced by salinity, changes in enzyme activity induced by alterations in cytoplasmic structure and negative feedback by reduced sink activity (Iyengar and Reddy, 1996).

An interesting characteristic of most plants growing in saline environments is the accumulation of proline (Hasegawa et al., 2000). Our results showed that a strong increase in proline concentration with less than 100 mM NaCl treatment. A significantly high accumulation of proline was probably associated with osmotic adjustment and protection of membrane stability (Maggio et al., 2000). However, Lutts et al. (1996) observed that proline did not appear to be involved in osmotic adjustment in salt stressed rice plants. In some studies, the accumulation of proline has been related to the ionic status of the plants (Chaudhary et al., 1997; Rout and Shaw, 1998). Colmer et al. (1996) showed that high proline content was associated with the maintenance of a more favourable  $\text{K}^+$  to  $\text{Na}^+$  ratio in the NaCl-treated root tips supplied with  $\text{Ca}^{2+}$ . In this study, a high positive correlation was found between proline and  $\text{Na}^+$  concentration in leaves (Figure 5), suggesting the positive role of  $\text{Na}^+$  in proline accumulation. The same result was also observed in *Sesuvium portulacastrum* (Slama et al., 2007). The contribution of proline to osmotic adjustment becomes quite significant by the fact that this compatible osmolyte is concentrated mostly in the cytosol and the chloroplasts (Aubert et al., 1999).

Peroxidation of membrane lipids is an indication of



**Figure 4.** Relationship between photosynthetic rate ( $A$ ), and stomatal conductance ( $g_s$ ) (■) and photosynthetic rate ( $A$ ) and shoot biomass (▲) in the tall oatgrass. \*\*\* Significant at the 0.001 probability level, respectively.



**Figure 5.** Relationship between  $\text{Na}^+$  content and proline (▲), and  $\text{Na}^+$  content and MDA (■) content in the tall oatgrass. \*\*\* Significant at the 0.001 probability level, respectively.

membrane damage and leakage with salt stress conditions (Katsuhara et al., 2005). Salt stress affected both genotypes by means of lipid peroxidation but, ZXY03P-443 had higher incremental rates with all the NaCl treatments. Growth inhibition with salinity in ZXY03P-443 was in good correlation with increased lipid peroxidation levels. Lower MDA level was remarkable in ZXY03P-336 even at the highest NaCl concentrations (Figure 2b). It is

presumed that the extent of membrane damage was not so severe in ZXY03P-336 due to salinity. This is consistent with the results obtained for  $\text{Na}^+$  and  $\text{K}^+$  content. Similar results occurred in salt tolerant barley cultivars (Liang et al., 2003), and salt resistant tobacco plants (Ruiz et al., 2005) also had lower levels of lipid peroxidation which is an important sign of higher oxidative damage limiting capacity with salinity.

However, salt sensitive rice varieties had higher MDA content and electrolyte leakage in response to salt stress (Moradi and Ismail, 2007). In addition, a high positive correlation was established between MDA and Na<sup>+</sup> concentration in leaves (Figure 5), suggesting the positive role of Na<sup>+</sup> in MDA accumulation.

In conclusion, salinity stress significantly inhibited the growth of tall oatgrass. Between the two genotypes in their responses to salinity, there are significant differences which were consistent with their physiological responses measured in different salinity levels. The general growth, gas exchange, lipid peroxidation and proline accumulation of two tall oatgrass genotypes are also in good correlation with each other, indicating that ZXY03P-443 is more sensitive to salinity than ZXY03P-336.

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