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Photosynthetic Response of Elephant Grass (*Pennisetum purpureum*) to NaCl Salinity

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Abstract: Photosynthetic responses of the C₄ grass (*Pennisetum purpureum*) to three NaCl salinity concentrations (100, 200 and 400 mM) along with control (0 mM NaCl) were studied. The results showed that all the three salinity treatments did not affect the quantum efficiency of PSII photochemistry (F_v/F_m) compared to control. However, only the highest salinity concentration of 400 mM, significantly reduced ($p < 0.001$) the light saturated assimilation rate (A_{sat}), the quantum yield (ϕ), the CO₂ saturated rate of CO₂ uptake (A_{max}) and the carboxylation efficiency of *P. purpureum*, compared to control. Therefore, it is possible for *P. purpureum* to attain high rates of photosynthesis under low and medium salinity levels.

Key words: Elephant grass, *Pennisetum purpureum*, NaCl salinity, CO₂ uptake, photochemistry

INTRODUCTION

The salinity is considered as one of the major causes that influence the photosynthetic efficiency of plants either in the controlled environments or in the field (Munns *et al.*, 2000; Gibberd *et al.*, 2002; Blindow *et al.*, 2003; Lycoskoufis *et al.*, 2005; Youssef, 2007). Most of the commercially important crops are salt sensitive and as a consequence of the salinity may limit their growth and production (Flowers, 2004). A previous study of photosynthetic gas exchange in the C₄ plant *Atriplex lentiformis* (Torr.) showed high rates of photosynthesis could be attained at low levels of NaCl salinity (Zhu and Meinzer, 1999). In addition, the high levels of NaCl salinity impaired the CO₂ assimilation in this C₄ plant. The inhibition of photosynthesis by high NaCl concentration is usually associated with the reduction of both the light-saturated rate of carbon dioxide assimilation (A_{sat}) and the maximum quantum (ϕ) yield (Lycoskoufis *et al.*, 2005; Lopez-Climent *et al.*, 2008; Youssef, 2007).

The phenomenon of photoinhibition is typically manifested as a light-dependent decrease in the quantum yield of photosystem II (PSII) photochemistry (Hu *et al.*, 2006). The linear relationship between quantum yield and the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) (Adams *et al.*, 1990) indicate that F_v/F_m can monitor the photosynthetic carbon assimilation (Björkman, 1987). The combination of chlorophyll fluorescence measurement together with the net gas exchange parameters provides a good way of the evaluation of the photosynthetic performance in the stressed plants (Jimenez *et al.*, 1997) and to gain an insight into the

behavior of the photosynthetic machinery under such stress (Maxwell and Johnson, 2000). Several plants have been subjected to photoinhibition at salinity treatment, but others showed no change in the F_v/F_m values among salinity treatments (Arfan *et al.*, 2007; Broetto *et al.*, 2007; Lopez-Climent *et al.*, 2008; Neocleous and Vasilakakis, 2007).

Elephant grass (*P. purpureum*) is a fast-growing C₄ grass (Wang *et al.*, 2002) cultivated for cattle feeding in the Kingdom of Saudi Arabia. Salinity is the main concern for the potential commercial production of elephant grass in the arid regions of the world (Wang *et al.*, 2002). The previous results of Al-Shoaibi and Al-Sobhi, (2004) confirmed that the salinity arising from irrigation caused a major problem for *P. purpureum* growth in Al-Madinah Al-Munawwarah in the western part of the Kingdom of Saudi Arabia. This lower growth rate at high salinity levels could be as a result of the reduction in the photosynthetic capacity. Since there is a lack of the knowledge via published reports about the influence of salinity on gas exchange of *P. purpureum*, this study was carried out to demonstrate the response of the photosynthetic CO₂ uptake of *P. purpureum* to NaCl salinity.

MATERIALS AND METHODS

Plant and growth conditions: This research was conducted in Biology Department, Faculty of Science, Taibah University during 2006. Rhizomes of *Pennisetum purpureum* originally derived from Africa were planted in 12 L pots filled with coarse sand and grown in high-light,

in a controlled environment chamber (Fitotron SGC066. CHX, Sanyo Gallenkamp PLC, Leicester, UK.), at day/night temperatures of about 25/20°C. The VPD was kept below 1 kPa. Photon flux density at leaf height was 600 $\mu\text{mol m}^{-2}\text{sec}^{-1}$ and the photo period was 14 h.

Three NaCl concentrations, 100, 200 and 400 mM, in full strength Hoagland nutrient solution were used (Arnon and Hoagland, 1940). The control was Hoagland nutrient solution without addition of NaCl. All pots were irrigated every other day for 7 days with full strength Hoagland nutrient solution. NaCl treatments begun 9 days after the start of the experiment. The NaCl concentration was increased in steps of 100 mM on alternate days until the appropriate salt treatments were reached.

Gas exchange and chlorophyll fluorescence measurements:

The CO_2 uptake was measured using an open gas-exchange system incorporating open path infrared, CO_2 and water vapour analysers (LI-6400, LI-COR Inc., Lincoln, USA). A Peltier cooling system maintained the leaf temperature at 20°C as described by Long *et al.* (1996) and VPD was controlled between 1 and 1.5 kPa. The most recent fully expanded leaf was used to measure photosynthetic CO_2 uptake at PPFD of 0-2000 $\mu\text{mol m}^{-2} \text{sec}^{-1}$. Net photosynthesis per unit leaf area and intercellular CO_2 concentration (c_i) were determined using the equations of Von Caemmerer and Farquhar (1981). The light saturated photosynthesis (A_{sat}) was determined at saturated PPFD (1500 $\mu\text{mol m}^{-2} \text{sec}^{-1}$) and at the ambient CO_2 concentration of 360 $\mu\text{mol mol}^{-1}$. As well, The light-limited photosynthetic capacity was measured at the ambient CO_2 concentration by increasing PPFD in three steps to 150 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ (0-50, 50-100 and 100-150 $\mu\text{mol m}^{-2} \text{sec}^{-1}$). Carbon dioxide response curves were made over the range of 50-550 $\mu\text{mol mol}^{-1}$ using PPFD of 1500 $\mu\text{mol m}^{-2} \text{sec}^{-1}$, at a leaf temperature of 20°C. These curves were analysed according to the model of Collatz *et al.* (1992).

Chlorophyll fluorescence was measured using a portable fluorimeter (PEA, Hansatech, Kings Lynn, Norfolk). The initial (F_0) and maximum (F_m) fluorescence emissions were measured after 20 min of dark adaptation and the ratio of variable to maximum fluorescence (F_v/F_m) was calculated as described by Öquist and Wass (1988) from the following equation: $F_m - F_0 / F_m$. The F_v/F_m ratio was measured weekly for 4 replicates of each treatment, using the youngest fully expanded leaves.

Statistical analyses: One-way analysis of variance was used to study the effect of salinity on F_v/F_m , A_{sat} , ϕ , A_{max} and the carboxylation efficiency of *P. purpureum*. Post hoc pairwise comparisons by Scheffe's test were

performed to detect differences between individual means (Systat, Inc., Evanston, Illinois, USA). The effect was described as significant where $p < 0.05$.

RESULTS

The values of F_v/F_m were not significantly affected by the three salinity treatments compared to control. They always remained above 0.8 (Fig. 1).

The results shown in Fig. 2 revealed the response of photosynthetic CO_2 uptake (A) to photon flux (Q) for *P. purpureum* at different salinity levels. The light-saturated (A_{sat}) of *P. purpureum* leaves grown at 400 mM NaCl concentration was significantly decreased ($p < 0.001$), compared to leaves grown control (Fig. 2). The reduction percentage was 36%, compared to the control reading. Also, the highest salinity concentration significantly reduced ($p < 0.001$) the light-limited photosynthetic capacity (ϕ) of *P. purpureum* leaves, compared to leaves grown at control (Fig. 3). The reduction percentage was 21%, compared to control record. On the other hand, the A_{sat} and ϕ of *P. purpureum* leaves grown at 100 and 200 mM NaCl were similar to those grown at control (Fig. 2-3).

The saturated rate of CO_2 uptake (A_{max}) is co-limited by the amount of *in vivo* Rubisco or/and PPDK activities (Collatz *et al.*, 1992). The results (Fig. 5) show that A_{max} was significantly reduced ($p < 0.001$) at the highest level of

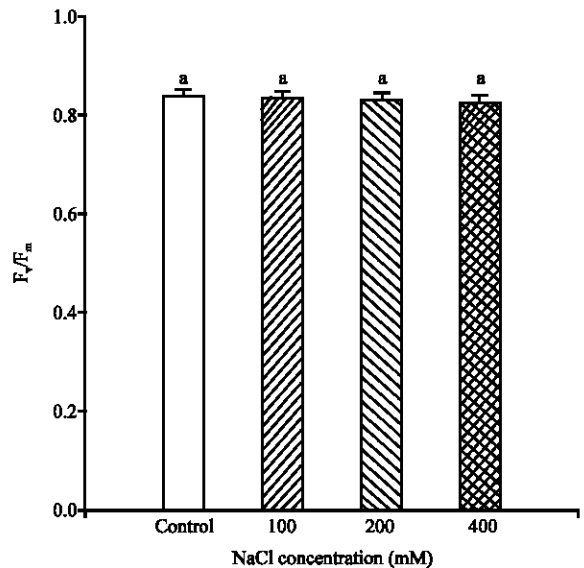


Fig. 1: The dark adapted F_v/F_m of *Pennisetum purpureum*. The data represent the mean of $n = 3-6$ replicate shoots (\pm SE). Different letters show the significant differences between NaCl treatments at $p < 0.05$

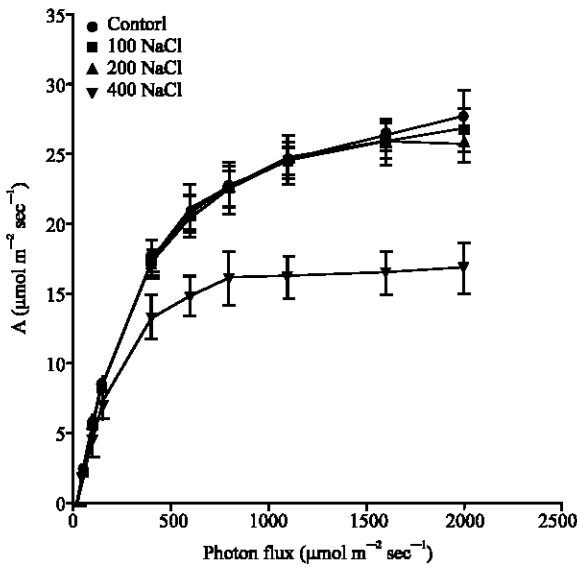


Fig. 2: The response of photosynthetic CO₂ uptake (A), per unit leaf area, to photon flux (Q) for *Pennisetum purpureum*. Measurements of CO₂ uptake were all made at 20°C and C_a of 360 $\mu\text{mol mol}^{-1}$. The data represent the mean of n = 3-6 leaves (\pm SE)

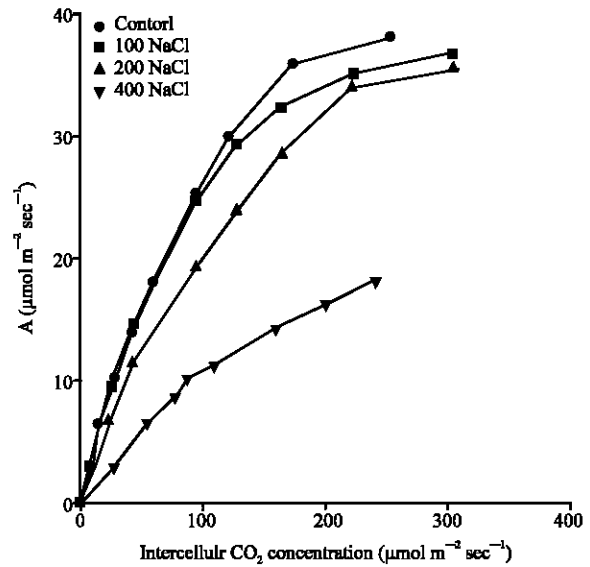


Fig. 4: The response of photosynthetic CO₂ uptake (A), per unit leaf area, to the changes in intercellular CO₂ concentration (c_i) for *Pennisetum purpureum* leaves. Measurements of CO₂ uptake were all made at 20°C and photon flux of 1500 $\mu\text{mol mol}^{-1}$. Data illustrated are for one selected leaf of each plant

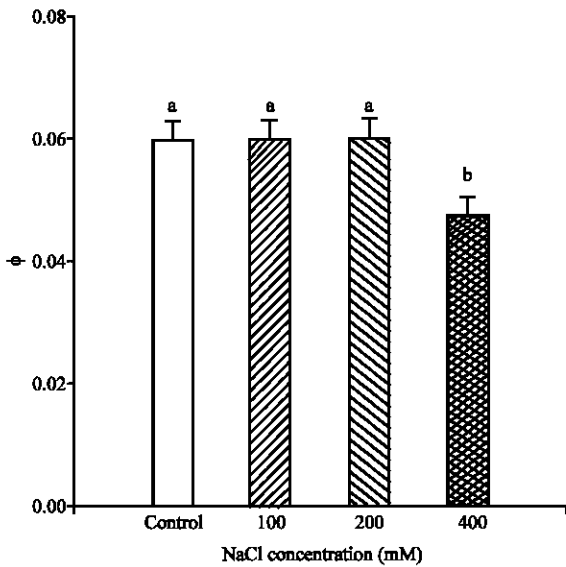


Fig. 3: The mean quantum yield (ϕ), measured at 20°C, for *Pennisetum purpureum*. The data represent the mean of n = 3-6 leaves (\pm SE). Different letters show the significant differences between NaCl treatment at p<0.05

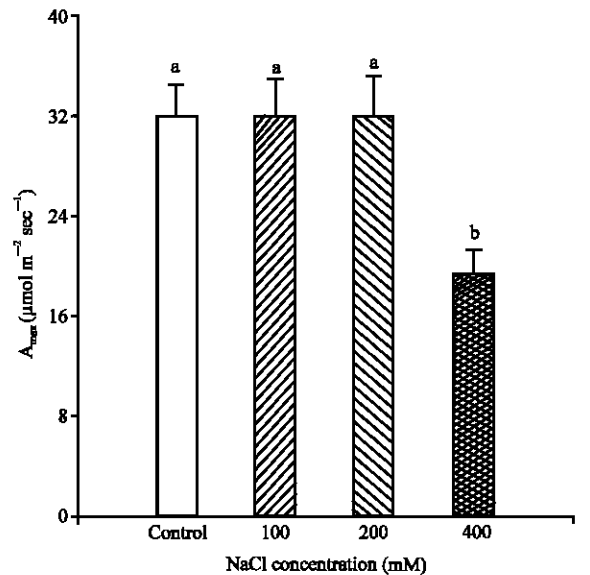


Fig. 5: The mean plateau (A_{max}) per unit leaf area, measured at 20°C and photon flux of 1500 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ for *Pennisetum purpureum*. The data represent the mean of n = 3-6 leaves (\pm SE). Different letter(s) show the significant differences between NaCl treatments at p<0.05

salinity compared to control. The percent of reduction was 40% compared to control (Fig. 4-5). The A_{max} was not significantly decreased by 100 and 200 mM NaCl salinity

compared to control (Fig. 4-5). In addition, growth at the highest salinity treatment, in comparison to control, had

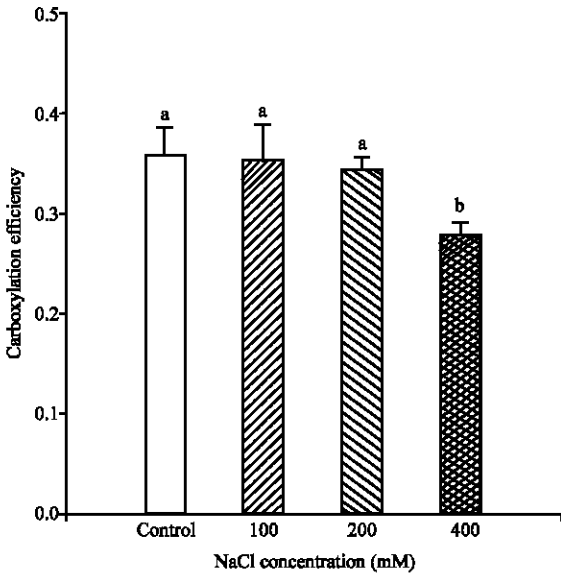


Fig. 6: The mean carboxylation efficiency, measured at 20°C and photon flux of 1500 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ for *Pennisetum purpureum*. The data represent the mean of n = 3-6 leaves (\pm SE). Different letter(s) show the significant differences between NaCl treatments at $p < 0.05$

a significant affect ($p < 0.001$) on the carboxylation efficiency of *P. purpureum* leaves (Fig. 6). The percentage of reduction was about 22%, compared to control. On the contrary, the carboxylation efficiency of *P. purpureum* leaves grown at 100 and 200 mM NaCl salinity were quite similar to those grown at the control (Fig. 6).

DISCUSSION

The photosynthetic response of *P. purpureum* to the salinity was investigated under salinity stress conditions. The results obtained in the present study showed that the quantum yield of PSII (F_v/F_m) was not significantly changed due to salt stress applications. The values of F_v/F_m always remained above 0.8 and there was no acute photoinhibition (Broetto *et al.*, 2007). These high values of F_v/F_m provided a clear evidence of *P. purpureum* resistance to the photoinhibition under different salinity treatments (Fig. 1). Photoinhibition occurs when the leaves are exposed to irradiances in excess of what can be utilized in photosynthesis (Powles, 1984). Similar results were previously reported for two wheat cultivars differing in their salinity tolerance (Arfan *et al.*, 2007). Thus, this result suggests that the quantum yield of PSII (F_v/F_m) cannot be considered as one of the factors to regulate photosynthesis in *P. purpureum* under salinity treatments.

When *P. purpureum* grown under 100, 200 mM NaCl, all leaves showed similar A_{sat} and ϕ to control (Fig. 2-3). The rates of A_{sat} and ϕ were close to that recorded previously for healthy leaves of a range of NADP-malic enzymes type C_4 grasses (Ehleringer and Pearcy, 1983). On the other hand, the 400 mM NaCl significantly reduce the A_{sat} and ϕ of *P. purpureum*. However, rates of A_{sat} and ϕ of *P. purpureum* grown at 400 mM NaCl were in excess of *Zea mays* grown at 200 mM of NaCl (Data not shown). This depression in the photosynthetic performance of *P. purpureum* could be due to the stomatal conductance or ion toxicity or both (Ashraf, 2004; Sudhir and Murthy, 2004). Another possibility is that the significant reduction in the photosynthetic capacity of *P. purpureum* at the highest concentration of salinity could be a result of reduction in the leaf area (Munns *et al.*, 2000).

Analysis of A/c_i curves indicated that the 400 mM NaCl treatment caused a significant reduction in A_{max} and carboxylation efficiency of *P. purpureum* (Fig. 4-6). Similar results were recorded for three wild soybean species regarding the difference in their response to NaCl treatments (Crosbie and Pearce, 1982; Kao *et al.*, 2006) in response to salinity. The A_{max} and carboxylation efficiency are controlled by activity of Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and activity of Phosphoenol pyruvate carboxylase enzymes (PEPC), respectively (Collatz *et al.*, 1992). Therefore, the inhibition of A_{max} by high NaCl salinity may cause a reduced efficiency of ribulose-1,5-bisphosphate (RuBP) carboxylase, or a reduction of RuBP regeneration capacity, or the sensitivity of PSII to NaCl concentration (Ball and Anderson, 1986). On the other hand, the reduced efficiency of PEPC enzyme may cause the inhibition of carboxylation efficiency by high NaCl salinity. In contrast, the rates of A_{max} and carboxylation efficiency of *P. purpureum* grown at 100 and 200 mM NaCl were similar to control. This may suggest that *P. purpureum* contains similar levels of Rubisco and PEPC regardless whether the growth at low or medium salinity.

In conclusion, the photosynthetic response of *P. purpureum* to the salinity was investigated under salinity stress conditions. The values of F_v/F_m for *P. purpureum* leaves grown at 100, 200 and 400 mM NaCl were similar to control and always remained above 0.8. Only the highest salinity concentration of 400 mM, significantly reduced the light saturated assimilation rate (A_{sat}), the quantum yield (ϕ), the CO_2 saturated rate of CO_2 uptake (A_{max}) and the carboxylation efficiency of *P. purpureum*, compared to control. Therefore, *P. purpureum* can attain high photosynthetic rates under low and medium salinity levels.

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