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## Photosynthetic Response to the Low Temperature in Elephant Grass (*Pennisetum purpureum*) and *Zea mays*

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**Abstract:** Photosynthetic CO<sub>2</sub> uptake and the quantum efficiency of PSII photochemistry ( $F_v/F_m$ ) of C<sub>4</sub> grasses (*Pennisetum purpureum* and *Zea mays*) were studied at 14 and 25°C. *P. purpureum* showed superior photosynthetic rates at chilling temperature (14°C) than *Z. mays*. Growth at a chilling temperature compared to 25°C had no significant effect on the light saturated assimilation rate ( $A_{sat}$ ), the quantum yield ( $\phi$ ), the CO<sub>2</sub> saturated rate of CO<sub>2</sub> uptake ( $A_{max}$ ) and the carboxylation efficiency of *P. purpureum* but caused more than 78% loss of those occurred in *Z. mays*. The values of  $F_v/F_m$  were significantly reduced only in *Z. mays* at 14°C compared to 25°C and as a result, the photosynthetic apparatus of *P. purpureum* was more resistant to chilling temperature than of *Z. mays*.

**Key words:** *Zea mays*, *Pennisetum purpureum*, chilling stress, gas exchange,  $F_v/F_m$

### INTRODUCTION

Low temperature is one of the most important factors which limit the growth, the distribution and the productivity of plants (Tambussi *et al.*, 2004; Van Heerden *et al.*, 2004; Hu *et al.*, 2006). Most C<sub>4</sub> plants are restricted largely to warmer climates because their physiological processes are adversely affected by low temperatures (Long *et al.*, 1983; Hund *et al.*, 2007). However, few C<sub>4</sub> plants have been found to be adapted and naturally grow in the cool climates (Beale and Long, 1995). The ability of these plants to activate or to increase their physiological and photosynthetic processes at low temperatures enabled them to survive in these cooler environments (Long, 1999; Naidu *et al.*, 2003; Sowinski *et al.*, 2005).

The photosynthetic production of C<sub>4</sub> crops is 40% higher than C<sub>3</sub> plants under the optimum climate (Long *et al.*, 1983). However, this advantage is not realised in C<sub>4</sub> plants under low temperature conditions. Low temperature damages thylakoid membranes, breaks down chlorophyll and induces reduction in the photosynthetic capacity of C<sub>4</sub> plants such as maize (Tambussi *et al.*, 2004; Sowinski *et al.*, 2005). The reduction in the photosynthetic capacity of *Z. mays* leaves at cool temperatures is associated with reductions in both the light-saturated rate of carbon dioxide assimilation ( $A_{sat}$ ) and the maximum quantum yield ( $\phi$ ) (Fryer *et al.*, 1998; Foyer *et al.*, 2002; Sowinski *et al.*, 2005; Hund *et al.*, 2007).

Photoinhibition of photosynthesis may occur when the absorption of light is in excess of that required by the

plant photosynthetic demands (Savitch *et al.*, 2000). Many studies have shown that plants subjected to photoinhibition at low temperatures, both in controlled environments and in the field (Haldimann, 1999; Tambussi *et al.*, 2004; Van Heerden *et al.*, 2004; Sowinski *et al.*, 2005; Hu *et al.*, 2006; Hund *et al.*, 2007). The phenomenon of photoinhibition is characterized by a reduction of the quantum yield of CO<sub>2</sub> uptake ( $\phi$ ) and the ratio of variable to maximum chlorophyll a fluorescence ( $F_v/F_m$ ) (Fryer *et al.*, 1998; Hu *et al.*, 2006). The reduction in  $F_v/F_m$  of dark-adapted leaves indicates photoinhibition of PSII. In addition, the percentage of reduction depends upon the environmental conditions prior to the photoinhibitory treatment and genotypic variability (Lee *et al.*, 2002; Sowinski *et al.*, 2005; Hund *et al.*, 2007).

The previous results (AL-Shoaibi, 2007) showed that *P. purpureum* had significantly higher growth rate at 14°C compared to *Z. mays*. This higher growth rate of *P. purpureum* could be, in part, is due to the consequence of differences in photosynthetic capacity between the two C<sub>4</sub> grasses (AL-Shoaibi, 2007). Therefore, this study was carried out to determine the effect of low temperature on photosynthetic CO<sub>2</sub> uptake of both *P. purpureum* and *Z. mays* and to compare the responses of photosynthesis of the two grasses to low temperature.

### MATERIALS AND METHODS

**Plants 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 and *Zea mays* cv. LG 80 were planted in pots containing a peat-based compost (F2, Levington Horticultural Ltd., Ipswich, UK.) and grown in high-light controlled environment chambers (Fitotron SGC066. CHX, Sanyo Gallenkamp PLC, Leicester, UK.), at day/night temperatures of 25/20°C and 14/12°C. Fertilisation was provided once a week by irrigating with Hoagland's nutrient solution (Arnon and Hoagland, 1940). The Vapour Pressure Deficit (VPD) was kept below 1 kPa. Photosynthetic Photon Flux Density (PPFD) at leaf height was 600  $\mu\text{mol m}^{-2}\text{sec}^{-1}$  and the photo period was 14 h.

#### Gas exchange and chlorophyll fluorescence measurements:

The  $\text{CO}_2$  uptake was measured with an open gas-exchange system incorporating open path infrared,  $\text{CO}_2$  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  $\text{CO}_2$  uptake at PPFD of 0-2000  $\mu\text{mol m}^{-2}\text{sec}^{-1}$ . Net photosynthesis per unit leaf area and intercellular  $\text{CO}_2$  concentration ( $c_i$ ) were determined using the equations of Von Caemmerer and Farquhar (1981). The light saturated photosynthesis ( $A_{\text{sat}}$ ) was determined at saturating PPFD (1500  $\mu\text{mol m}^{-2}\text{sec}^{-1}$ ) and at the ambient  $\text{CO}_2$  concentration of 360  $\mu\text{mol mol}^{-1}$ . 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  $(F_m - F_0)/F_m$  as described by Öquist and Wass (1988). The  $F_v/F_m$  ratio was measured weekly for four replicates of each plant using the youngest fully expanded leaves.

**Statistical analyses:** The data obtained from various analyses and measurements were statistically analysed using analysis of variance (Systat, Inc., Evanston, Illinois, USA).

## RESULTS

Leaves of *Z. mays* growing at 14°C showed a significant reduction in  $F_v/F_m$  ( $p < 0.001$ ) compared to those of *Z. mays* and *P. purpureum* grown at 25°C (Fig. 1).

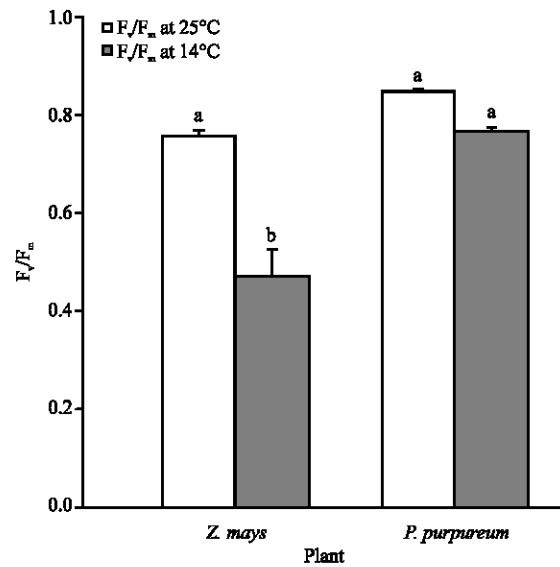


Fig. 1: The dark adapted  $F_v/F_m$  of *P. purpureum* and *Z. mays*. The data represent the mean of  $n = 3-6$  replicate shoots ( $\pm$ SE). Different letter(s) shows the significant different between low temperature treatments ( $p < 0.05$ )

Furthermore, the  $F_v/F_m$  of *P. purpureum* leaves grown at 14°C was 64% greater than that of *Z. mays* leaves ( $p < 0.001$ ) growing at the same temperature (Fig. 1).

Growth at chilling temperature (14°C), compared to 25°C, had no significant effect on the light-saturated ( $A_{\text{sat}}$ ) or light-limited photosynthetic capacity ( $\phi$ ) of *P. purpureum*, but caused 78% loss ( $p < 0.001$ ) of both in *Z. mays* (Fig. 2-4). For leaves of *P. purpureum* grown at 14°C, the  $A_{\text{sat}}$  and  $\phi$  were significantly greater than those of *Z. mays* grown at the same temperature (Fig. 3-4).

The plateau of the  $A/c_i$  curve ( $A_{\text{max}}$ ) is co-limited by the amount of *in vivo* Rubisco or/and PPDK activity (Collatz *et al.*, 1992). Decreasing temperature from 25°C to 14°C did not significantly affect the  $A_{\text{max}}$  of *P. purpureum*, but caused 78% loss ( $p < 0.001$ ) of *Z. mays* leaves compared to those grown at 25°C (Fig. 5-6). In addition, the  $A_{\text{max}}$  of *P. purpureum* leaves grown 14°C was more than 7 times greater than that of *Z. mays* leaves growing at the same temperature (Fig. 5-6).

The carboxylation efficiency was calculated from the initial slope of  $A/c_i$  curve (Fig. 5). Growth at 14°C, relative to 25°C, had no significant effect on the carboxylation efficiency of *P. purpureum*, but caused 83% loss ( $p < 0.001$ ) of *Z. mays* (Fig. 7). For leaves of *P. purpureum* grown at 14°C, the carboxylation efficiency was significantly greater ( $p < 0.001$ ) than those of *Z. mays* grown at the same temperature (Fig. 7).

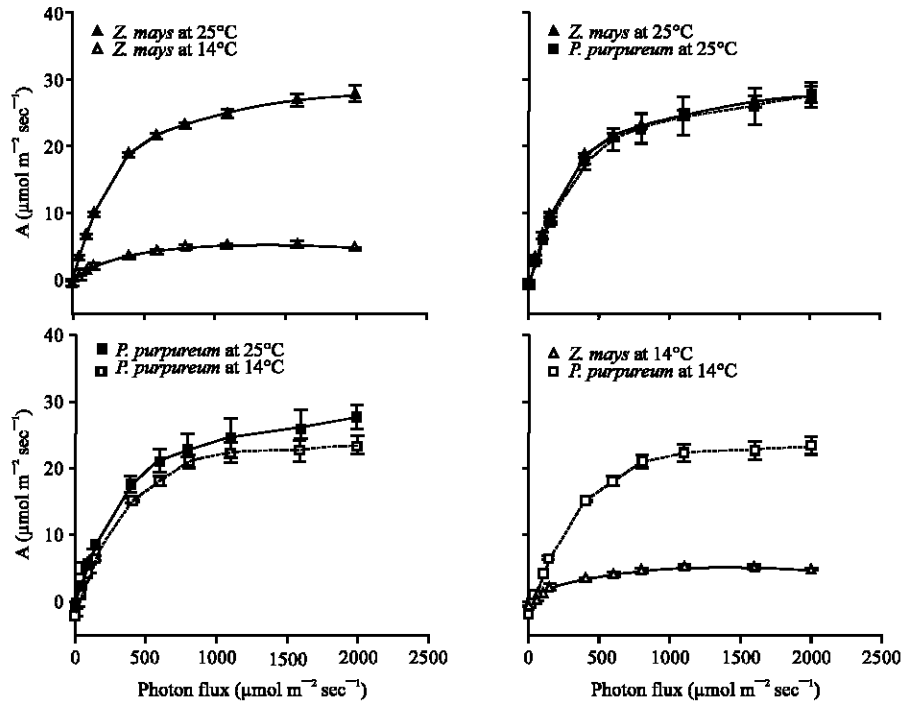


Fig. 2: The response of photosynthetic CO<sub>2</sub> uptake (A), per unit leaf area, to photon flux (Q) for *P. purpureum* and *Z. mays* leaves. Measurements of CO<sub>2</sub> uptake were all made at 20°C and a c<sub>2</sub> of 360 μmol mol<sup>-1</sup>. The data represent the mean of n = 3-6 leaves (±SE)

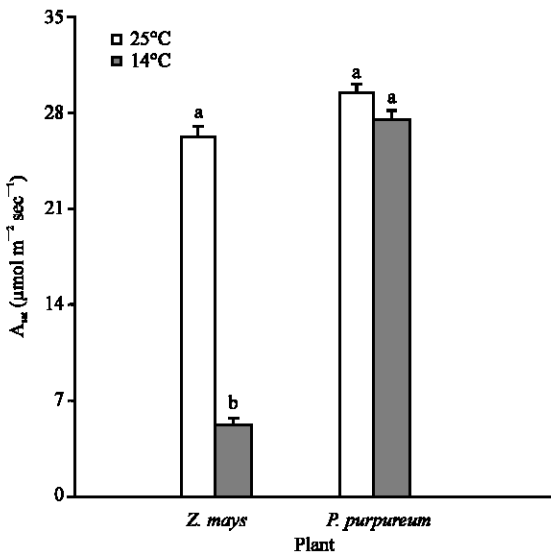


Fig. 3: The light-saturated photosynthetic rate (A<sub>sat</sub>), per unit leaf area, measured at 20°C and a photon flux of 1500 μmol mol<sup>-2</sup> sec<sup>-1</sup>, for *P. purpureum* and *Z. mays*. The data represent the mean of n = 3-6 leaves (±SE). Different letters shows significant difference between temperature treatments at p<0.05

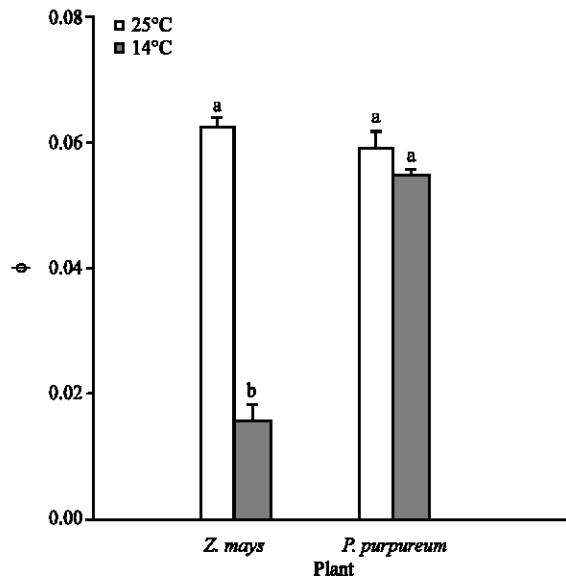


Fig. 4: The mean quantum yield (φ), measured at 20°C for *P. purpureum* and *Z. mays*. The data represent the mean of n = 3-6 leaves (±SE). Different letters shows the significant difference between temperature treatments at p<0.05

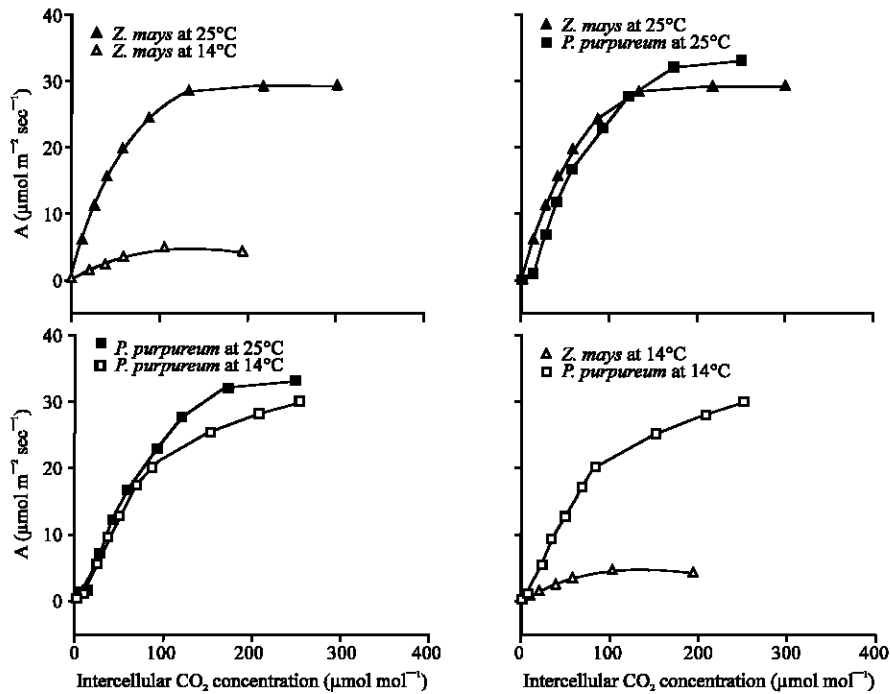


Fig. 5: The response of photosynthetic CO<sub>2</sub> uptake (A), per unit leaf area, to changes in intercellular CO<sub>2</sub> concentration (c<sub>i</sub>) for *P. purpureum* and *Z. mays* leaves. Measurements of CO<sub>2</sub> uptake were made at 20°C and a photon flux of 1500 μmol mol<sup>-1</sup>. Data illustrated are for one selected leaf of each plant

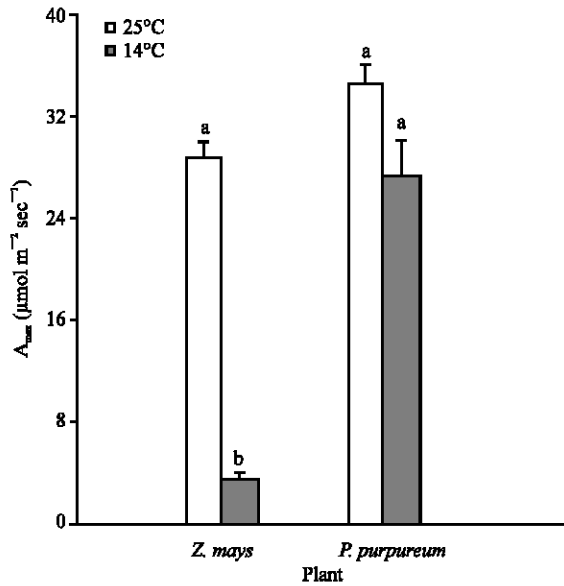


Fig. 6: The mean plateau (A<sub>max</sub>), per unit leaf area, measured at 20°C and a photon flux of 1500 μmol mol<sup>-2</sup> sec<sup>-1</sup>, for *P. purpureum* and *Z. mays*. The data represent the mean of n = 3-6 leaves (±SE). Different letters shows significant differences between temperature treatments at p<0.05

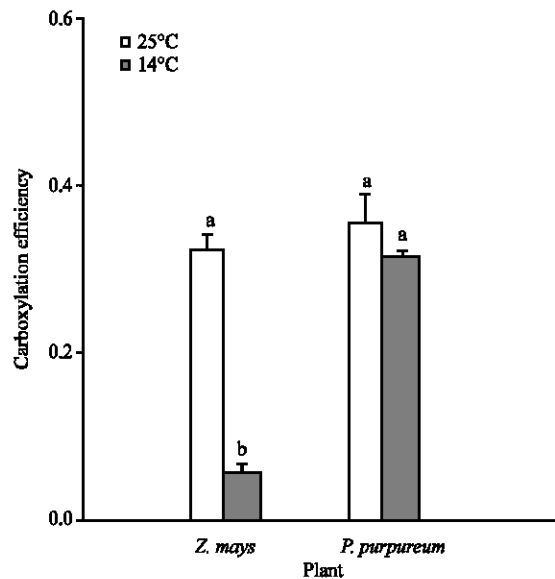


Fig. 7: The mean carboxylation efficiency, measured at 20°C and photon flux of 1500 μmol mol<sup>-2</sup> sec<sup>-1</sup>, for *P. purpureum* and *Z. mays*. The data represent the mean of n = 3-6 leaves (±SE). Different letters shows significant differences between temperature treatments at p<0.05

## DISCUSSION

The results of this research provide a clear evidence that *P. purpureum* showed significantly greater resistance to photoinhibition at low temperatures than *Z. mays*. The low  $F_v/F_m$  in *Z. mays* grown at low temperature may not be related to the photoinhibition, but to the impaired development of the photosynthetic apparatus. Nie and Baker (1991) and Nie *et al.* (1995) showed that several chloroplast polypeptides were poorly expressed in *Z. mays* leaves developed at 14°C. These lesions, which included the D1 protein, could account for the low  $F_v/F_m$  even in the absence of direct photoinhibition.

The photosynthetic rates for all leaves grown at 25°C of both *P. purpureum* and *Z. mays* were similar and close to the rates recorded previously for healthy leaves of a range of NADP-malic enzyme type  $C_4$  grasses (Ehleringer and Pearcy, 1983). This indicates that these plants were unstressed and not suffering any photoinhibition at the optimal growth temperatures and as indicated by high  $F_v/F_m$ . When grown at 25°C, *P. purpureum* had similar  $A_{sat}$ ,  $\phi$ ,  $A_{max}$  and carboxylation efficiencies to *P. purpureum* grown at 14°C, but *Z. mays* showed a very substantial decrease in all these measures. Moreover, rates of  $CO_2$  uptake of *P. purpureum* grown at 14°C were in excess of *Z. mays* grown at 14°C at all light levels. The decrease in the photosynthetic performance in *Z. mays* grown at chilling temperatures was observed previously in controlled environments and in the field (Nie and Baker, 1991; Nie *et al.*, 1992; Haldimann *et al.*, 1996; Prioul, 1996; Fryer *et al.*, 1998).

The sensitivity of  $C_4$  photosynthesis to low temperature is frequently associated with the reduction in the rate of enzymatically controlled reactions (Long *et al.*, 1983). This was investigated *in vivo* by A/c<sub>i</sub> analysis. 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). Also, the regulatory enzyme Pyruvate, Pi dikinase (PPDK) is another limitation for  $A_{max}$  decreased (Furbank *et al.*, 1997). PPDK is known as a cold sensitive enzyme (Usami *et al.*, 1995; Du *et al.*, 1999). Research work with *Flaveria bidentis* and *Amaranthus edulis* indicates that, at 25°C, Rubisco accounts for 50-70% of the metabolic control over A at 360  $\mu$ bar  $CO_2$ , while PPDK and PEPC account for only 20-30% (Dever *et al.*, 1997; Furbank *et al.*, 1997; Von Caemmerer *et al.*, 1997). Results of the  $A_{max}$  and carboxylation efficiency of the 14°C grown *P. purpureum* leaves were similar to leaves grown at 25°C (Fig. 5-7). In contrast, the  $A_{max}$  and carboxylation efficiency of the 14°C grown *Z. mays* leaves suggested a reduction of 84% in the

*in vivo* activity of these enzymes compared to leaves grown at 25°C. This suggests that in contrast to *Z. mays*, *P. purpureum* has similar activity of PEPC, PPDK and Rubisco regardless of whether it is grown at 25 or 14°C.

In conclusion, *P. purpureum* has superior photosynthetic rates at chilling temperatures than *Z. mays*. The values of  $F_v/F_m$  were significantly reduced only in *Z. mays* at 14°C compared to 25°C and as a result, the photosynthetic apparatus of *P. purpureum* was more resistant to chilling temperature than that of *Z. mays*. This high capacity of photosynthetic rates of *P. purpureum* at chilling temperatures may be responsible for the high growth rates of this grass at chilling temperatures.

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