*Full Length Article*

# Water- and Nitrogen-use Efficiencies of Sweet Sorghum Seedlings are Improved under Water Stress

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## Abstract

The physiological mechanism underlying the high productivity of sweet sorghum (*Sorghum bicolor* (L.) Moench) under drought is not well understood. Water and nitrogen utilization features of leaves can play key roles in plant drought tolerance. The objective of this study was to investigate the water and nitrogen utilization of sweet sorghum seedlings under prolonged water stress within controlled environmental conditions. Under prolonged water stress, leaf area and total water loss per plant decreased significantly, leaf dry mass per unit area increased but the dry mass of the whole plant was unaffected. Total leaf nitrogen, leaf nitrogen per unit area and leaf nitrogen concentration of stressed plants decreased significantly, indicating a reduced accumulation of leaf nitrogen. The photosynthetic rate of stressed plants recovered completely three days after water stress treatment, while transpiration rate was consistently lower than the controls, resulting in improved instantaneous water-use efficiency (WUE). Photosynthetic nitrogen-use efficiency (PNUE) increased significantly, which may contribute to the increase in biomass based nitrogen-use efficiency and WUE correlated positively with PNUE. In conclusion, improved water- and nitrogen-use efficiencies under water stress may both contribute to the high degree of physiological acclimation of sweet sorghum to drought. © 2014 Friends Science Publishers

**Keywords:** Sweet sorghum; Photosynthetic nitrogen-use efficiency; Water-use efficiency; Drought resistance
**Abbreviations:** DM, dry mass per plant; LT, leaf transpiration rate per plant; $g_s$, leaf stomatal conductance; LA, total leaf area per plant; LMA, leaf dry mass per unit area; LN, total leaf nitrogen per plant; LNC, leaf nitrogen concentration; $N_a$, leaf nitrogen per unit area; NUE, nitrogen-use efficiency; $P_n$, leaf photosynthetic rate; PNUE, photosynthetic nitrogen-use efficiency; WUE, instantaneous water-use efficiency.

## Introduction

As alternative sources of energy, biofuels have received strong interest in the current global energy crisis due to the inevitable depletion of fossil fuels. Plants with high capacities for the accumulation of dry mass, such as maize, sugarcane and sugar beets, are potentially good economical and renewable sources of energy and have been widely studied for such purposes (Foteinis *et al.,* 2011; García *et al.,* 2011; Grahovac *et al.,* 2012(Foteinis *et al.,* 2011; García *et al.,* 2011; Bibi *et al.,* 2013). However, the use of corn as an ethanol feedstock is limited by corn's use as food for humans and livestock (Tamang *et al.,* 2011); corn, sugarcane and sugar beets have the disadvantage that they need high requirements for water and nitrogen during growth (Prasad *et al.*, 2007; Tamang *et al.*, 2011).

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a $C_4$ annual crop commonly grown for the production of sugar (Gnansounou *et al.,* 2005), but its superior attributes as a biofuel feedstock have been shown in many studies. Firstly, sweet sorghum has high photosynthetic capacity, growth rate, productivity, and radiation and water use efficiency (Mastrorilli *et al.*, 1995; Steduto *et al.*, 1997; Ali *et al.*, 2009; Bibi *et al.*, 2010). Secondly, low amounts of nitrogen fertilization are required during cultivation for ethanol production (Tamang *et al.,* 2011), which may help to mitigate the crises of pollution from nitrogen fertilization and/or of desiccation in deep soil layers in agro-ecosystems. Thirdly, due to its low input requirements and high stress tolerances, sweet sorghum can be cultivated on marginal or even non-arable land that are unsuitable for major food crops such as rice or wheat (Reddy *et al.,* 2005). Planting sweet sorghum on semi-arid and other marginal land would thus improve the use of agricultural land. Global climate change is also likely altering the patterns of precipitation, which may increase soil and atmospheric water stresses (IPCC, 2007). Studies on sweet sorghum, which predominated in hot and arid regions, where drought frequently occurs may highlight the physiological bases of high productivity under drought conditions.

These advantages have led to studies of the productivity of sweet sorghum under field conditions. Steduto *et al.* (1997) reported that sweet sorghum had higher leaf and canopy water-use efficiencies than maize and grain sorghum during water stress, which may account for the high water-use efficiency based on biomass ($WUE_b$) found by Mastrorilli *et al.* (1995). Different regimes of irrigation did not significantly affect $WUE_b$ (Curt *et al.,* 1995) and temporary soil-water stress at the stem stage improved $WUE_b$ (Mastrorilli *et al.,* 1999), showing its high drought tolerance and productivity. $C_4$ plants can have higher nitrogen-use efficiency (NUE) than $C_3$ plants (Gardner *et al.,* 1994), and sweet sorghum belongs to the NADP-malic enzyme (NADP-ME) subtype of $C_4$ plants that often have a lower leaf nitrogen content and a higher NUE and instantaneous photosynthetic nitrogen-use efficiency (PNUE) than do plants of the NAD-malic enzyme (NAD-ME) subtype (Ghannoum *et al.,* 2005). Cosentino *et al.* (2012) reported that different levels of nitrogen supply did not affect the final yield or $WUE_b$ of sweet sorghum, which also confirms the high nitrogen-use efficiency of sweet sorghum. The high productivity, drought tolerance and nitrogen-use efficiency of sweet sorghum in the field is widely known, but the physiological relationships underlying these features are not well understood.

Photosynthesis in $C_3$ plants tends to be negatively affected by water stress in the stomatal and non-stomatal phases (Lawlor and Cornic, 2002; Flexas *et al.*, 2004). $C_4$ crops, such as maize and sorghum, are very important for the security of the global food supply and for the production of biofuel, but photosynthesis features under water stress has been less well studied in $C_4$ than in $C_3$ plants (Ghannoum, 2009). Drought can reduce the leaf photosynthetic rate ($P_n$) in many plants, and leaf photosynthesis is linearly dependent on leaf nitrogen per unit area ($N_a$) in both $C_3$ and $C_4$ plants (Sage and Pearcy, 1987). Leaf area is reduced under water stress to reduce water loss, so an increased $N_a$ is logically required to optimize the use of nitrogen and water (Farquhar *et al.,* 2002). Water stress, however, can significantly reduce the activity of nitrate reductase, transport-associated processes and consequently the $N_a$ in wheat and maize (Larsson *et al.,* 1989; Foyer *et al.,* 1998; Shangguan *et al.,* 2000), which can subsequently affect plant growth and function (McDonald and Davies, 1996). Fundamentally high or artificially improved nitrogen utilization ability is thus very important for maintaining productivity under water or nitrogen deficits conditions. Studies show that different conditions of soil water or nitrogen fertilizer could not affect biomass yield of sweet sorghum (Cosentino *et al.*, 2012; Erickson *et al.*, 2012; Zegada-Lizarazu and Monti, 2012), but the physiological mechanisms underlying the use efficiencies of both water and nitrogen under water stress are not well understood.

So the objective of this study is to identify the physiological relationships among biomass accumulation,

leaf nitrogen utilization and water use efficiency of sweet sorghum during prolonged water stress under controlled environmental conditions. Our results suggest that improved instantaneous water-and nitrogen-use efficiencies may contribute to the maintenance of biomass accumulation in sweet sorghum under drought conditions.

## Materials and Methods

### Plant Material and Treatments

The experiment was conduct in a phytotron of the Institute of Soil and Water Conservation located in Yangling, Shaanxi, China. Seeds of a commercial hybrid variety of sweet sorghum (*Sorghum bicolor* L. Moench, cv. Yajin19) were disinfected with 2% sodium hypochlorite for 30 min to prevent fungal infection, rinsed with distilled water and then germinated on moist filter paper in a dark chamber at 30°C for about two days. After emergence, the seedlings were grown hydroponically in an artificial climate chamber (AGC-D001P, Qiushi Corp., Hangzhou, China). The environmental conditions were set as: lights for 13 h (25°C, RH 50%, 600 μmol photons $m^{-2}$ $s^{-1}$) and dark for 11 h (18°C, RH 60%). The length, width and height of pots used in this study is 40 cm, 15 cm and 20 cm. Each pot can contain 10 L nutrient solution and three plants were grown. Sixty plants within twenty pots were used in the whole study. Half-strength modified Hoagland nutrient solution with enhanced $Fe^{2+}$ (pH 5.5) was used and aerated by aquarium diffusers. Nutrient solutions were renewed every two days in the evening to maintain a consistent supplementation of nutrients. When the seedlings had six fully expanded leaves (six leaf stage), half of the plants were transplanted to the 1/2 Hoagland solution containing 10% PEG 6000 (−0.3 M Pa) to simulate water stress at 12:00 and were sustained for 15 days until ten leaf stage. The remaining plants, as controls, continued to grow in the 1/2 Hoagland solution without PEG. So this study had two treatments: controls and water stressed plants. The following parameters were measured both on the controls and water stressed plants after the 1st, 3rd, 6th, 9th and 15th d of water stress treatment.

### Measurements of Leaf Gas Exchange

Leaf photosynthetic rate ($P_n$), stomatal conductance ($g_s$) and transpiration rate ($E$) were measured between 11:30 and 12:30 at the center of the newest fully expanded leaf under ambient conditions with a portable gas-exchange system (Li-6400, LI-COR Inc., Lincoln, USA). Five randomly plants were sampled for each gas exchange measurement. Then the instantaneous water-use efficiency (WUE) was calculated as the ratio of $P_n$ to $E$.

### Measurements of Leaf Area and Biomass

The leaf laminar area per plant (LA) was subsequently

obtained with a scanner (Epson Perfection V700, Seiko Epson Crop., Suwa, Japan) and analyzed with Win RHIZO (Regent Instrument Inc., Quebec, Canada). The leaf transpiration rate per plant (LT) was calculated as the product of $E$ and LA. Fresh leaves, sheaths and roots were then separated, dried at 70°C in an air oven for at least 72 h and weighed with an electronic balance (model AUX220, ±0.1 mg sensitivity, Shimadzu Crop., Kyoto, Japan). The dry mass per plant (DM) was calculated as the sum of the weights of the leaves, sheaths and roots. The root-shoot ratio was calculated as the ratio of the weights of leaves and sheaths to roots. Five randomly plants were used in each measurement.

## Measurements of Leaf Nitrogen Content

Leaf samples were pulverized to fine powders, and leaf nitrogen concentrations (LNC) were analyzed by the Kjeldahl method (Kjeltec 2300 Auto analyzer, Foss Tecator AB, Hoganas, Sweden). The total leaf nitrogen per plant (LN) was calculated as the product of the dry mass of leaves and nitrogen content. $N_a$ was calculated as the ratio of LN to LA. NUE was calculated as the ratio of DM to LN. PNUE was calculated as the ratio of $P_n$ to $N_a$.

## Statistical Analysis

Means ± standard deviation (SD) of the measurements are presented in the figures. Differences between the controls and the water stressed plants were assessed using independent $t$-tests. Analyses were performed with SPSS version 13.0 (SPSS Inc., Chicago, USA). Figures were drawn and correlation analysis was performed by Sigma Plot version 12.0 (Systat Inc., San Jose, USA).

## Results

### Dry-mass Accumulation and Leaf Area

The DM of water stressed seedlings was significantly different from that of the controls on the 9th d but not on the 3rd, 6th or 15th d of PEG treatment (Fig. 1a). The root-shoot ratios were not significantly different between water stressed plants and the controls throughout the treatment (Fig. 1b). After six days of PEG treatment, the LA of water stressed plants was significantly lower than that of the controls (Fig. 2a). On the 15th d of PEG treatment, the leaf dry mass per unit area (LMA) of water stressed plants was significantly higher than that of the controls (Fig. 2b).

### Leaf Gas Exchange and Water Use

The $P_n$ of water stressed plants decreased significantly on the first day of treatment but completely recovered after three days (Fig. 3a). The $g_s$ of water stressed plants, however was consistently and significantly lower than that of the controls within the whole study (Fig. 3b).
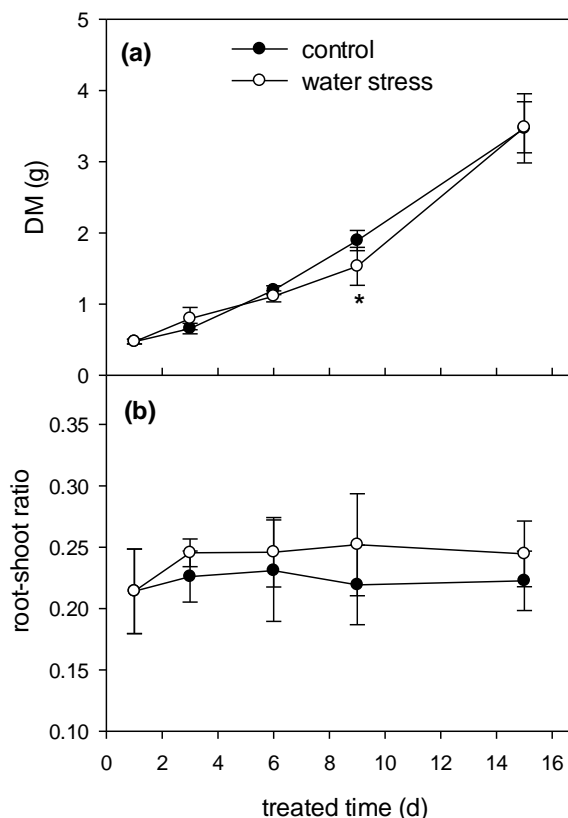


**Fig. 1a:** Dry mass per plant (DM) and (**b**) root-shoot ratio of sweet sorghum seedlings under water stress. Filled circles represent the controls and open circles represent water stressed plants. Error bars indicate means ± SD of five replicates and ∗ indicates a significant difference at $P < 0.05$

The WUE of the controls decreased during growth, but that of water stressed plants did not decrease and was significantly higher than the controls after the 3rd d of PEG treatment (Fig. 4a). Water stressed plants had significantly lower LT than the controls, suggesting decreased water loss in stressed plants (Fig. 4b).

### Leaf Nitrogen Accumulation and Use Efficiency

The LN of water stressed plants increased more slowly and was significantly lower than the controls after six days of treatment, 42.6% reduced on the 15th d (Fig. 5a). The LNC of the controls remained at about 3.5% during growth, but that of water stressed plants decreased significantly, 40% reduced on the 15th d (Fig. 5b). The $N_a$ of the controls increased during growth, while that of stressed plants was more consistent. So the $N_a$ of stressed plants was significantly lower than the controls after six days of treatment (Fig. 5c). $N_a$ had a significantly linear relationship with LMA in well-watered plants but did not change significantly with LMA in water stressed plants (Fig. 6).
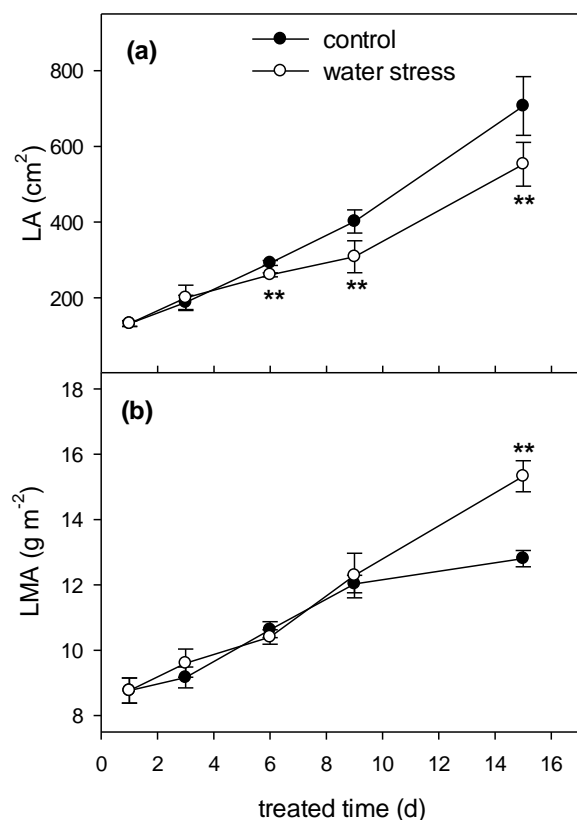
**Fig. 2a:** Total leaf area per plant (LA) and (**b**) leaf dry mass per unit area (LMA) of sweet sorghum seedlings under water stress. Filled circles represent the controls and open circles represent water stressed plants. Error bars indicate means ± SD of five replicates, and ∗∗ indicates a significant difference at $P < 0.01$



**Fig. 3:** Leaf gas exchange of sweet sorghum seedlings under water stress. (**a**), photosynthetic rate ($P_n$). (**b**), stomatal conductance ($g_s$). Filled circles represent the controls and open circles represent water stressed plants. Error bars indicate means ± SD of five replicates, and ∗∗ indicates a significant difference at $P < 0.01$

The biomass based NUE of water stressed plants increased during growth, but that of the controls did not, so the NUE of water stressed plants was significantly higher than the controls after three days of treatment, 73.9% increased on the 15th d (Fig. 7a). As with the changes in WUE, the PNUE of the controls decreased during growth. On the first day of PEG treatment the PNUE of water stressed plants decreased significantly but was significantly higher than the controls after six days of treatment (Fig. 7b).

For both the controls and water stressed plants, NUE had a very significantly negative correlation with LNC (Fig. 8). PNUE significantly decreased as $N_a$ increased, showing a nonlinear relationship (Fig. 9). And the PNUE of sweet sorghum seedlings had a significantly positive relationship with WUE (Fig. 10).

## Discussion

From the six leaf stage to the ten leaf stage of sweet sorghum, $P_n$ did not change but $g_s$ increased significantly in the newly unfolded le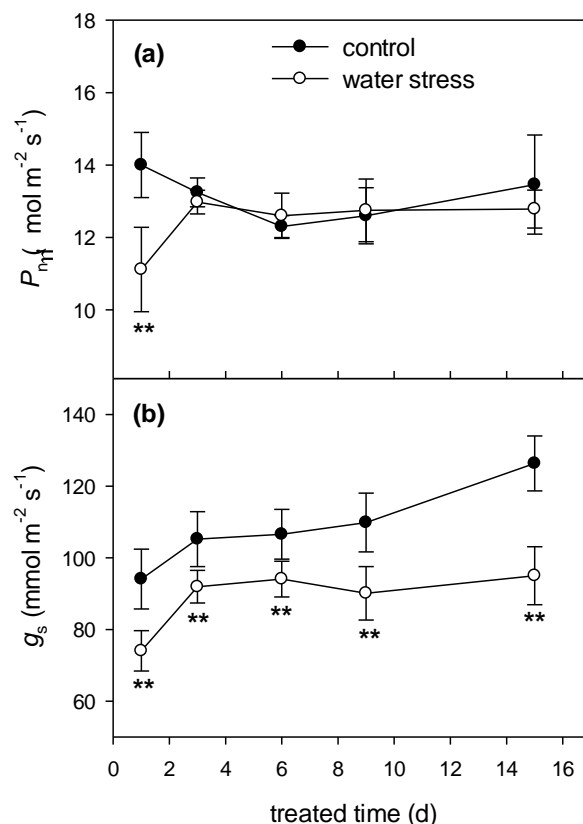aves of well-watered plants, leading to a decreased WUE (Fig. 4a). Prolonged water stress significantly reduced both $g_s$ and LA (Fig. 2a; Fig. 3b), so the LT of stressed plants decreased very significantly and was 38% lower than the controls on the 15th d after treatment (Fig. 4b). Due to the unchanged $P_n$ and reduced leaf transpiration, the WUE of stressed plants was significantly higher than the controls (Fig. 4a). This result is consist with the observations of previous studies concerned on other crops (Zhao *et al.*, 2004; Blum, 2005). Nitrogen concentrations of plant tissues often decrease during growth (Coleman *et al.,* 1993), but our results show that the LNC of well-watered sweet sorghum did not change significant during growth, and $N_a$ progressively increased (Fig. 5bc). These conflicting observations may be due to the sufficient nitrogen resources in Hoagland nutrient solution used in this study. As with LNC, the biomass based NUE of the controls did not change significantly during growth, while PNUE decreased progressively (Fig. 7). Gardner *et al*. (1994) reported that sweet sorghum cultivars with low LNC had high NUE. Our results more clearly show the significantly negative correlations between LNC and NUE and between
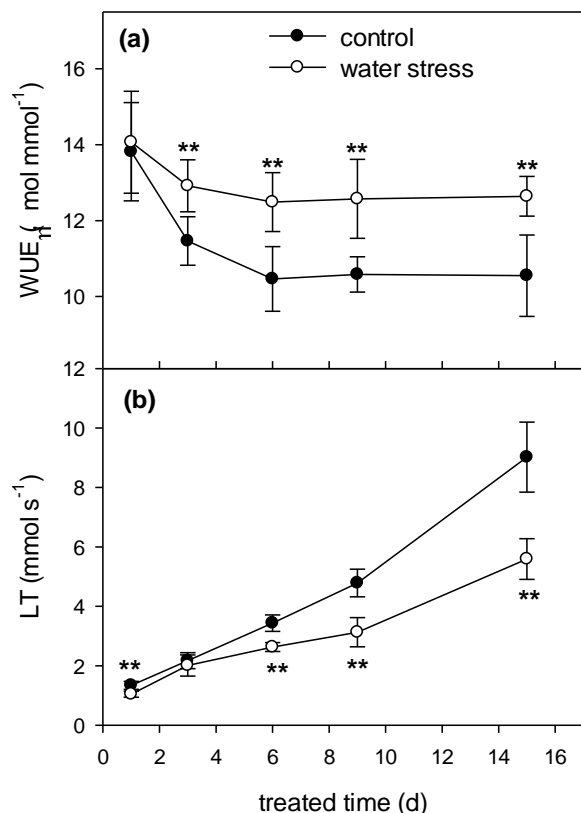
**Fig. 4a:** Leaf instantaneous water-use efficiency (WUE) and (**b**) leaf transpiration rate per plant (LT) of sweet sorghum seedlings under water stress. Filled circles represent controls and open circles represent water stressed plants. Error bars indicate means ± SD of five replicates, and ∗∗ indicates a significant difference at *P* < 0.01

$N_a$ and PNUE in sweet sorghum (Fig. 8; Fig. 9).

Water stress can significantly reduce $N_a$, possibly due to reduced nitrate reductase activities and transport-associated processes (Foyer *et al.*, 1998; Lawlor and Cornic, 2002; Reddy *et al.*, 2004). Our results also show reduced LN, LNC and $N_a$ in osmotically stressed plants (Fig. 5), which confirm these previous observations. The different correlations between $N_a$ and LMA in the controls and water stressed plants in Fig. 6 may also more clearly indicate the reduced accumulation of nitrogen in the leaves of sweet sorghum during prolonged water stress. Different with the decrease in leaf photosynthesis during drought (Turner *et al.*, 1985; Tezara *et al.*, 1999; Flexas and Medrano, 2002), the $P_n$ of sweet sorghum seedlings in this study recovered quickly and had no significant difference with the controls (Fig. 2a), which may be one reason for the unchanged dry mass accumulation. Reich *et al.* (1989) reported that *Ulmus americana* plants with lower levels of leaf nitrogen had a higher PNUE. Findings of this study suggest that PNUE negatively correlates with $N_a$ which is consistent with this report (Fig. 9). Prolonged water stress could decrease $N_a$
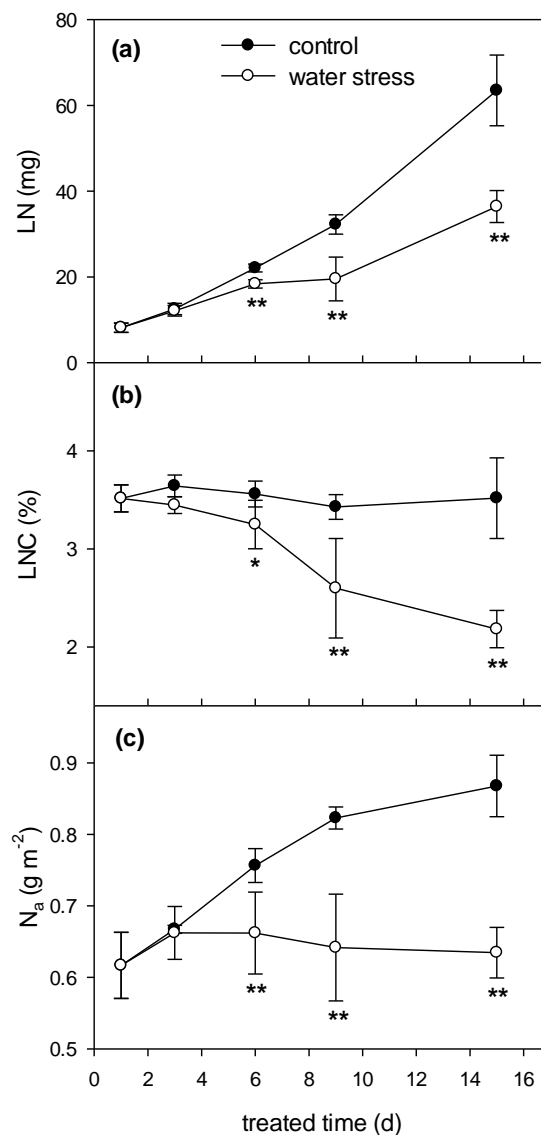


**Fig. 5a:** Total leaf nitrogen per plant (LN), (**b**) leaf nitrogen concentration (LNC) and (**c**) leaf nitrogen per area ($N_a$) of sweet sorghum seedlings under water stress. Filled circles represent the controls and open circles represent water stressed plants. Error bars indicate means ± SD of five replicates. ∗ and ∗∗ indicate significant differences at *P* < 0.05 and *P* < 0.01, respectively

but increase PNUE in this study. But this result differs from observations suggesting that drought can significantly decrease PNUE in some $C_3$ plants (Duan *et al.,* 2009; Zhang *et al.,* 2011). As a member of the NADP-ME subtype of $C_4$ plants, sweet sorghum has lower levels of leaf nitrogen but a higher rate of Rubisco turnover than do plants of the NAD-ME subtype (Ghannoum *et al.,* 2005). $C_4$ plants also often have higher NUEs and lower investments in leaf nitrogen than do $C_3$ plants (Sage and Pearcy, 1987). These traits of sweet sorghum may contribute to the maintenance of $P_n$ and the increase in PNUE during the nitrogen deficit induced by
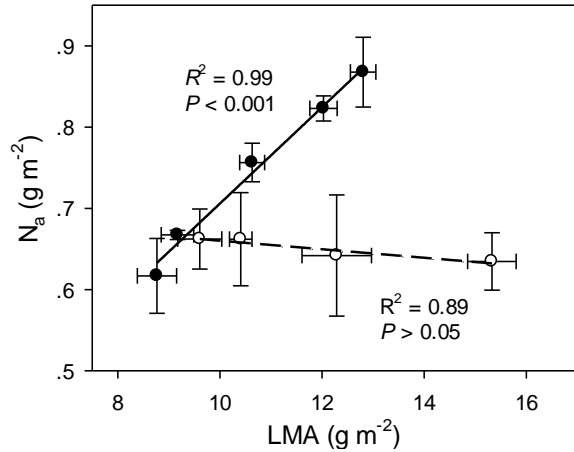
**Fig. 6:** Correlations between leaf nitrogen per unit area ($N_a$) and leaf mass per unit area (LMA) of sweet sorghum seedlings. Filled circles represent the controls and open circles represent water stressed plants. Error bars indicate means ± SD of five replicates
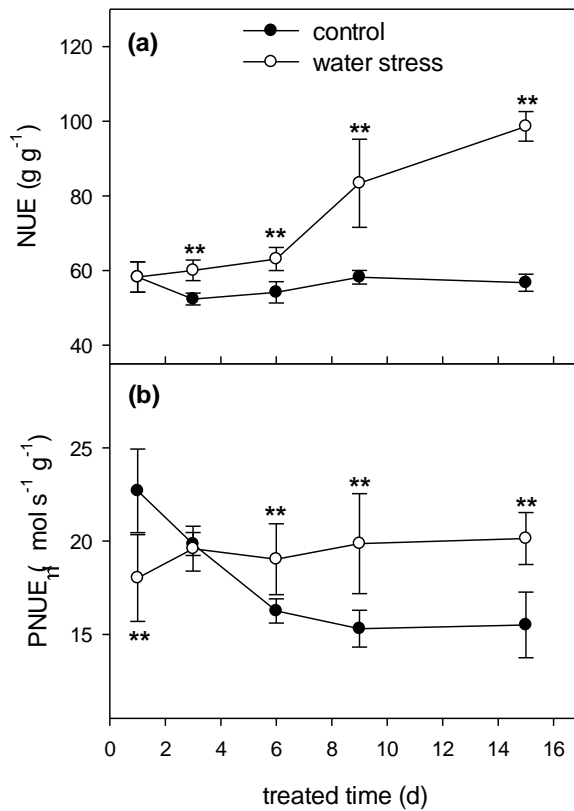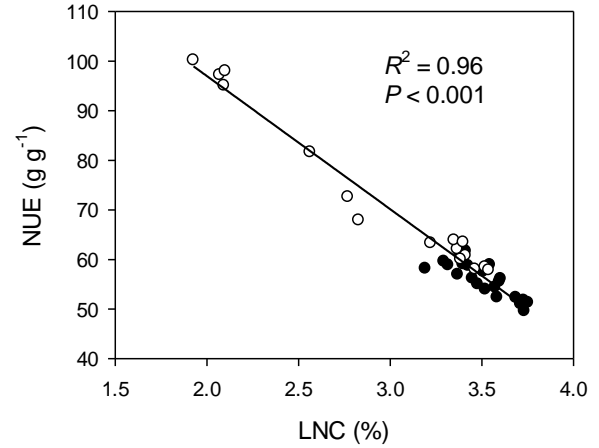


**Fig. 8:** Correlation between nitrogen-use efficiency (NUE) and leaf nitrogen concentration (LNC) of sweet sorghum seedlings. Filled circles represent the controls ($n = 22$) and open circles represent water stressed plants ($n = 16$)
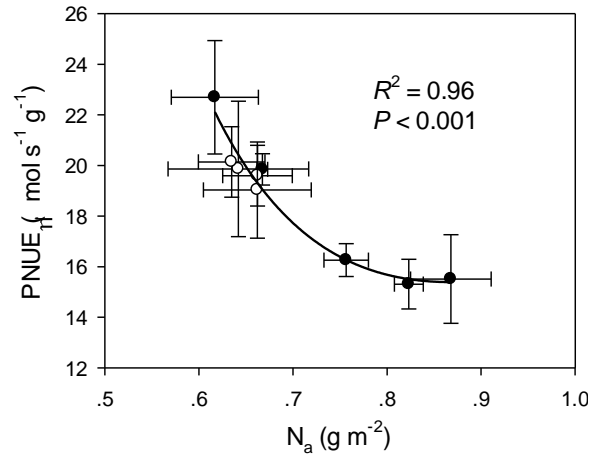


**Fig. 7a:** Whole-plant nitrogen-use efficiency (NUE) and (**b**) photosynthetic nitrogen-use efficiency (PNUE) of sweet sorghum seedlings under water stress. Filled circles represent the controls and open circles represent water stressed plants. Error bars indicate means ± SD of five replicates, and ∗∗ indicates a significant difference at $P < 0.01$



**Fig. 9:** Correlation between leaf nitrogen per unit area ($N_a$) and photosynthetic nitrogen-use efficiency (PNUE) of sweet sorghum seedlings. Filled circles represent the controls and open circles represent water stressed plants. Error bars indicate means ± SD of five replicates

water stress. The higher instantaneous PNUE may also have contributed to the higher NUE and the unchanged accumulation of DM.

WUE had a significant inverse relationship with PNUE in different species of trees and shrubs (Field *et al.,* 1983). This trade-off between WUE and PNUE may be due to the dependence of both processes on stomatal conductance (Warren and Adams, 2006). However, the interrelation of WUE and PNUE is complex. Our results show that PNUE had a significantly positive relationship with WUE in sweet sorghum seedlings (Fig. 10). So the instantaneous use efficiencies of water and nitrogen can both increase during prolonged water stress. This relationship differs from observations in $C_3$ trees or grasses that drought often decreases both $g_s$ and $CO_2$ conductance
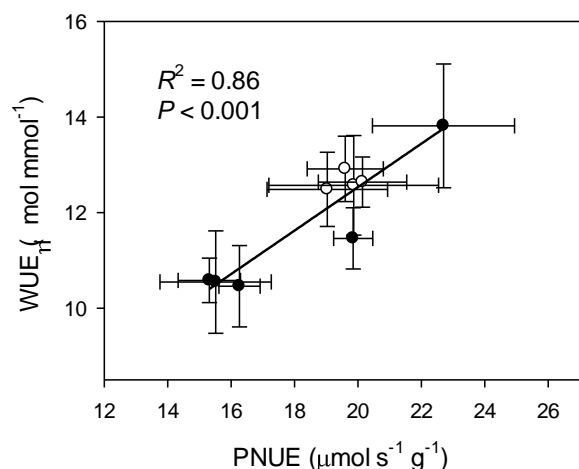
**Fig. 10:** Correlation between photosynthetic nitrogen-use efficiency (PNUE) and water-use efficiency (WUE) of sweet sorghum seedlings. Filled circles represent the controls and open circles represent water stressed plants. Error bars indicate means ± SD of five replicates

($g_m$), and consequently the PNUE (Flexas *et al.*, 2002; Cabrera-Bosquet *et al.*, 2007; Soolanayakanahally *et al.*, 2009; Zhang *et al.*, 2011). And study showed that a finite $g_m$ can decrease both WUE and PNUE (Warren and Adams, 2006), so the reason for both increased WUE and PNUE in sweet sorghum under prolonged water stress may be an increased $g_m$ in the leaves.

Even though the $CO_2$-concentrating mechanism allowing $C_4$ plants to have a higher photosynthetic capacity and WUE than $C_3$ plants (Taiz and Zeiger, 2006), the limited capacity for photorespiration may lead to a sensitivity of $C_4$ photosynthesis to water stress (Ghannoum, 2009). In this study, the $P_n$ and $g_s$ of sweet sorghum seedlings decreased significantly on the first day of water stress (Fig. 3), which confirms the previous knowledge. But different to the reports that $C_3$ plants often have non-stomatal limitations and metabolic inhibitions during prolonged water stress (Lawlor and Cornic, 2002; Flexas *et al.*, 2004), the $P_n$ of sweet sorghum recovered completely three days after water stress in this study. Base the results of this study, two integrative traits that allow sweet sorghum to acclimate to prolonged water stress are suggested. Firstly, the leaf area per plant and $g_s$ decreases significantly, which drastically reduces the water loss of the whole plant. Secondly, a significantly improved PNUE offset the lower accumulation of leaf nitrogen, which may have contributed to the improved NUE and the unchanged accumulation of carbon. The sweet sorghum seedlings could thus increase the utilization efficiency of both water and nitrogen to obtain dry mass during the acclimation to water stress. However, details of leaf nitrogen partitioning and mesophyll conductance during water and/or nitrogen deficit are still needed to understand the exact mechanisms of drought resistance in sweet sorghum.

In crux, under controlled environmental conditions, water stress reduced leaf nitrogen accumulation but the possible negative effects were counteracted by the improved PNUE. For both the controls and water stressed plants, PNUE positively correlated with WUE. Sweet sorghum could improve leaf nitrogen and water utilization efficiency to reduce water loss and maintain carbon fixation, and then dry mass accumulation during prolonged water stress. These results may help us to understand the mechanisms underlying the drought resistance of sweet sorghum.

## Acknowledgments

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