Photosynthesis and chlorophyll fluorescence in two hybrids of sorghum under different nitrogen and water regimes

I. Cechin*

Universidade Federal de São Carlos. Departamento de Botânica.
Via Washington Luis, km 235 CP: 676, São Carlos, SP 13565-905, Brasil

Abstract

In two hybrids of sorghum (Sorghum bicolor Moench.), C51 and C42, high nitrogen concentration (HN) increased net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), and transpiration rate ($E$) of well watered (HW) plants. Water stressing (LW plants) resulted in low $P_N$, $g_s$, and $E$ in both hybrids, but the values were still higher in HN plants as compared to low nitrogen-grown (LN) plants. Intercellular CO$_2$ concentration ($C_i$) increased in droughted plants. This increase was much higher in LN plants as compared to HN plants. Instantaneous water use efficiency was lower in LN plants as a consequence of a greater effect of water stress on photosynthesis. Leaf water potential was reduced by water stress in all treatments. Analysis of chlorophyll $a$ fluorescence at room temperature showed that photosystem 2 (PS2) was rather tolerant to the water stress imposed. Water stress caused a slight decrease in the efficiency of excitation capture by open PS2 reaction centres ($F_v/F_m$). The in vivo quantum yield of PS2 photochemistry ($\Phi_{PS2}$) and the photochemical quenching coefficient ($q_P$) were slightly reduced, while the nonphotochemical quenching coefficient ($q_N$) was increased under the water stress. However, in hybrid C42 these characters were little or not affected by the water stress.

Additional key words: drought; intercellular CO$_2$ concentration; leaf water potential; photosystem 2; Sorghum bicolor; stomatal conductance; transpiration rate; water use efficiency.

Introduction

High nitrogen fertility results in an increased rate of carbon assimilation also of C$_4$ plants, which can be partially attributed to high investment of nitrogen into the photosynthetic machinery (Sugiyama et al. 1984, Sage et al. 1981, Huber et al. 1989,

Received 8 July 1997, accepted 20 November 1997.

*Fax: (016) 260 8308, e-mail: p-cechin@power.ufscar.br

Acknowledgments: The author thanks CNPq-Centro Nacional de Desenvolvimento Científico e Tecnológico-Brazil for financial support. The author also is grateful to Sementes Cargil S.A. which supplied the seeds.
Sugiharto et al. 1990, Ranjith et al. 1995). Water availability is a common environmental limitation to photosynthesis in most mesophytic plants. The negative effect of water stress on photosynthesis is attributed to stomatal limitations and to limitations independent of stomatal functioning laying at the level of chloroplasts. Biochemical dysfunctions of the chloroplasts under water stress include reduction in the Calvin cycle activity (Salindra et al. 1996) and impairment of the primary photochemistry associated with PS2 (Loreto et al. 1995).

Under field conditions, plants have frequently to cope with a combination of several stresses. Studies on the influence of nitrogen on the stomatal response to water stress have shown conflicting results. Radin and Ackerson (1981) showed that cotton plants grown under low N concentration had the greatest stomatal sensitivity to water stress. This is opposite to the effects found for wheat (Morgan 1986). Conversely, Green and Mitchell (1992) found that nitrogen fertilization did not cause any change in stomatal sensitivity to water stress in conifers in the field.

The aim of the present study was to investigate how nitrogen supply and water stress affect gas exchange characteristics of two hybrids of sorghum growing in a greenhouse. Particular attention was placed on any effect N supply and water stress might have on chlorophyll (Chl) fluorescence emission as this process is closely related to CO2 assimilation.

Materials and methods

Plants: Seeds of sorghum (Sorghum bicolor Moench.) hybrids C51 and C41 were sown in 4 000 cm3 pots containing sand. Seedlings were thinned to one per pot after emergency and were grown in a greenhouse from September to November. Day and night temperatures were maintained close to 34 and 16 °C, respectively. Plants were watered with 40% of full strength nitrogen-free Long Ashton solution (Hewitt 1966), containing either 1 (LN) or 3 (HN) mol m−3 ammonium nitrate. 200 cm3 of the solution per pot were supplied three times a week, and the plants were watered with tap water on other days.

Forty nine days after sowing, two levels of irrigation were established. These consisted of (1) irrigation with tap water every day (HW), and (2) no water during a 7 d period (LW). For HN plants, a small amount of tap water was added to the pots during the drought period in order to obtain a dehydration rate comparable to that of the LN plants.

Photosynthetic measurements: PN was measured on the youngest fully expanded leaf at the end of water stress treatment (55 d after sowing). A portable infra-red gas analyser (LCA-2, Analytical Development Co., Hoddesdon, UK) was used. Measurements were made in the laboratory. Photosynthetically active radiation (PAR) of 1700 μmol m−2 s−1 was supplied by a halogen lamp with an infra-red absorbing filter (portable light unit type PLU-002, ADC). The partial pressure of CO2 and relative humidity of air entering (from outside of the laboratory) and leaving the leaf chamber (ADC, PLC-B) were recorded using a data logger, together with the
flow rate (66.6 cm$^3$ s$^{-1}$), air temperature, and PAR. $P_N$, $g_s$, $E$, and $C_i$ were calculated using the equations described by Caemmerer and Farquhar (1981). Instantaneous water use efficiency (WUE) was calculated by dividing $P_N$ by $E$.

**Chl a fluorescence** was measured with a portable fluorometer (PAM-2000, Walz, Effeltrich, Germany) after a 15-min dark period at ambient conditions in the laboratory. Measurements of the minimal ($F_0$) and maximal ($F_{m}$) fluorescence yields were made after 15 min of dark adaptation, thus enabling determination of the ratio of variable to maximal fluorescence ($F_v/F_{m}$). $F_v/F_{m}$ is a measure of potential efficiency of PS2 in darkness. For tissues exposed to actinic radiation, $\Phi_{PS2}$ was calculated as $(F_{m} - F_0)/F_{m}$, in accordance with the method of Genty et al. (1989). Photochemical ($q_P$) and total non-photochemical ($q_N$) quenching coefficients were estimated after approximately 5 min under actinic irradiation when the leaf had reached steady-state of fluorescence. The $q_P$ is defined as $(F_{m} - F_0)/(F_{m} - F'_0)$, whereas $q_N$ is defined as $(F_{m} - F'_0)/(F_{m} - F'_0)$, where $F_{m}$, $F_0$, and $F_0'$ are the maximal fluorescence, the steady-state fluorescence, and the minimal fluorescence of the leaf adapted to irradiation, respectively. $F'_0$ was determined by exposing the leaf to far-red radiation on removal of the actinic radiation (Genty et al. 1989).

**Leaf water potential** ($\Psi_{leaf}$) was determined on the same leaf used for $P_N$ and Chl fluorescence measurements by using a Scholander pressure bomb (model 3005, Soilmoisture Equipment Corp., Santa Barbara, CA, U.S.A.).

**Results and discussion**

High N fertilization increased leaf biomass production (values not shown) and $P_N$ (Fig. 1A, Table 1). However, HN plants were predisposed to water stress due to increases in both transpiring area and water loss through transpiration (Fig. 1C, Table 1). Therefore small amounts of water were added to the HN plants to achieve a gradual decline in dehydration comparable to that of LN plants.

Table 1. Three-way analysis of variance of the effects of nitrogen concentration (N), water stress (W), and hybrids (H) on net photosynthetic rate, $P_N$, stomatal conductance, $g_s$, transpiration rate, $E$, intercellular CO$_2$ concentration, $C_i$, instantaneous water use efficiency, WUE, and leaf water potential, $\Psi_{leaf}$ of the sorghum hybrids C51 and C42. Actual values are in Fig. 1. *: 0.01<p<0.05; **: 0.001<p<0.01; ***: p<0.001; NS: not significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>W</th>
<th>H</th>
<th>N×W</th>
<th>N×H</th>
<th>W×H</th>
<th>N×W×H</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_N$ [umol m$^{-2}$ s$^{-1}$]</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>$g_s$ [mol m$^{-2}$ s$^{-1}$]</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>$E$ [mmol m$^{-2}$ s$^{-1}$]</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>$C_i$ [umol mol$^{-1}$]</td>
<td>*</td>
<td>***</td>
<td>**</td>
<td>**</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>WUE [umol CO$_2$ m$^{-2}$ s$^{-1}$]</td>
<td>**</td>
<td>***</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>$\Psi_{leaf}$ [MPa]</td>
<td>NS</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
Fig. 1 Net photosynthetic rate, $P_N$ (A), stomatal conductance, $g_s$ (B), transpiration rate, $E$ (C), intercellular CO$_2$ concentration, $C_i$ (D), and chlorophyll $a$ fluorescence parameters, $F_v/F_m$, characterizing photochemical efficiency of open PS2 reaction centres of dark-adapted leaf (E), quantum efficiency of photosystem 2 electron transport of light-adapted leaf, $\Phi_{PS2}$ (F), photochemical ($q_p$) and total nonphotochemical ($q_N$) quenching coefficients (G and H) of sorghum hybrids C51 and C42 grown under two nitrogen concentrations (LN: low nitrogen, HN: high nitrogen) and two water regimes (LW: low water; HW: high water), determined 7 d after the beginning of water stress. Means±SE of 3 replicates for A-D and of 2 replicates for E-H.
Water stress reduced Ψ Leaf in both hybrids irrespective of N regime (Fig. 2A, Table 1). When plants experienced no water stress, the effect of nitrogen on PN, gs, and E was positive in both hybrids (Fig. 1A, B, C, Table 1). In LW plants the promotive effect of N diminished in both hybrids compared to HW plants, being still significantly higher in HNLW plants than in LNLW plants. There was significant interaction between N and water regimes (see Table 1), indicating that the amplitude of the effect of water stress on PN, gs, and E was dependent on N supply. A linear relationship between PN and gs was observed for both hybrids (Fig. 3). While water stress reduced gs and PN, increasing N supply led to an increase of gs and PN. It might seem that this relationship between gs and PN reflects the contribution of gs to photosynthesis and, therefore, accounts for the reduction of PN under water stress.

Cj was significantly affected by N supply, water stress, and their interactions (Fig. 1D, Table 1). HN caused a decrease in the response of Cj to water stress. The
decline in $g_s$ together with $P_N$ is often taken to indicate that water stress affects photosynthesis via stomatal limitation. Stomatal closure without a change in mesophyll capacity will inevitably lower $C_i$. My results and those of Matthews and Boyer (1984) and Wise et al. (1990) show that although water stress causes a decrease in $g_s$ and $P_N$, the stomatal closure does not result in any decline in $C_i$. Although precise determination of stomatal limitation requires a $CO_2$ response curve (Farquhar and Sharkey 1982), the increase in $C_i$ suggests that stomatal closure can not fully account for the decline in $P_N$ in sorghum under water stress. This is further supported by the fact that photosynthesis in sorghum leaves does not fully recover from water stress (Loreto et al. 1995). The effect of water stress was greater on $P_N$ than $E$ at LN compared to HN. As a result, instantaneous WUE was significantly lower at LN supply independent of the hybrid (Fig. 2B, Table 1).

Chl fluorescence of PS2 plays an important role in the response of leaf photosynthesis to environmental stresses (Baker 1991), thus revealing stress response mechanisms. The inhibition of photosynthesis by water stress was accompanied by small alterations of Chl fluorescence parameters (Fig. 1E-H). Differences in photon utilization by PS2 between hybrids C51 and C42 were observed in the development of photoprotection by thermal deactivation. The main factors (N, water regimes, and hybrid) did not affect $q_N$ or $q_P$ (not shown). However, there was significant interaction (0.01<p<0.05) between N and water regimes, and between water regimes and hybrid. Thus, water induced an increase in $q_N$, and at the same time a small decrease in $q_P$ in C51 but not in C42 (Fig. 1G,H). Such thermal dissipation can occur within the light-harvesting antenna complexes of PS2 (see Ruban and Horton 1995), removing excess excitation energy before it reaches the reaction centres, and thus photoprotecting PS2 from over-reduction and potential damage. Water stress caused a small decrease (0.01<p<0.05) in $F_v/F_m$ in both hybrids (Fig. 1E), and there was no significant interaction between the main factors (not shown). Under water stress in this experiment, $q_P$ and $F_v/F_m$ did not vary much. Certainly, under more severe water stress they would be more affected.

The main factors did not affect $\Phi_{ps2}$, but there was significant interaction (0.01<p<0.05) between water regimes and hybrids (not shown). Thus, stress plants of both hybrids did not substantially decrease the potential photochemical activity of PS2 (Fig. 1F). Loreto et al. (1995) point out that the residual electron transport present when photosynthesis of sorghum leaves is reduced by water stress is used in processes other than photosynthesis. However, Lal and Edwards (1996) provide evidence that in other C4 species water stress reduces $P_N$ through a limitation of the supply of $CO_2$ to ribulose-1,5-bisphosphate carboxylase resulting in an increase in photosynthesis. Thus PS2 in C42 may tolerate higher water deficit. Such high tolerance of PS2 was observed in wheat plants (He et al. 1995). In wheat, the loss in PS2 activity under severe water stress was associated with the decrease in contents of major PS2 polypeptides due to increased degradation.

In conclusion, the gas exchange measurements and some Chl fluorescence parameters suggest that sorghum hybrids C51 and C42 differ in their photochemical response to water stress. The loss in photosynthetic performance in water stressed
plants of hybrid C51 at both N supplies largely results from increased wasteful energy dissipation.

References


