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

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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 (Φ_{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.* 1987, Huber *et al.* 1989,

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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 (Saliendra *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 CO_2 assimilation.

Materials and methods

Plants: Seeds of sorghum (*Sorghum bicolor* Moench.) hybrids C51 and C41 were sown in 4 000 cm^3 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 cm^3 of the solution per pot were supplied three times a week, and the plants were watered with tap water on each other days.

Fourty 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: P_N 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 CO_2 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, Φ_{PS2} was calculated as $(F'_m - F_s)/F'_m$, 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 $(F'_m - F_s)/(F'_m - F'_0)$, whereas q_N is defined as $(F_m - F'_m)/(F_m - F'_0)$, where F'_m, F_s, 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 (Ψ_{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, USA).

Results and discussion

High N fertilization increased leaf biomass production (values not shown) and P_N (Fig. 1*A*, Table 1). However, HN plants were predisposed to water stress due to increase in both transpiring area and water loss through transpiration (Fig. 1*C*, 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 effect 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 potencial, Ψ_{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.

	N	W	H	N×W	N×H	W×H	N×W×H
P_N [μmol m^{-2} s^{-1}]	***	***	NS	**	NS	NS	NS
g_s [mol m^{-2} s^{-1}]	***	***	NS	***	NS	NS	NS
E [mmol m^{-2} s^{-1}]	***	***	NS	**	NS	NS	NS
C_i [μmol mol^{-1}]	*	***	**	**	*	NS	NS
WUE [$μmol_{CO2}$ $mmol^{-1}_{H2O}$]	**	***	NS	*	NS	NS	NS
Ψ_{leaf} [MPa]	NS	***	NS	NS	NS	NS	NS

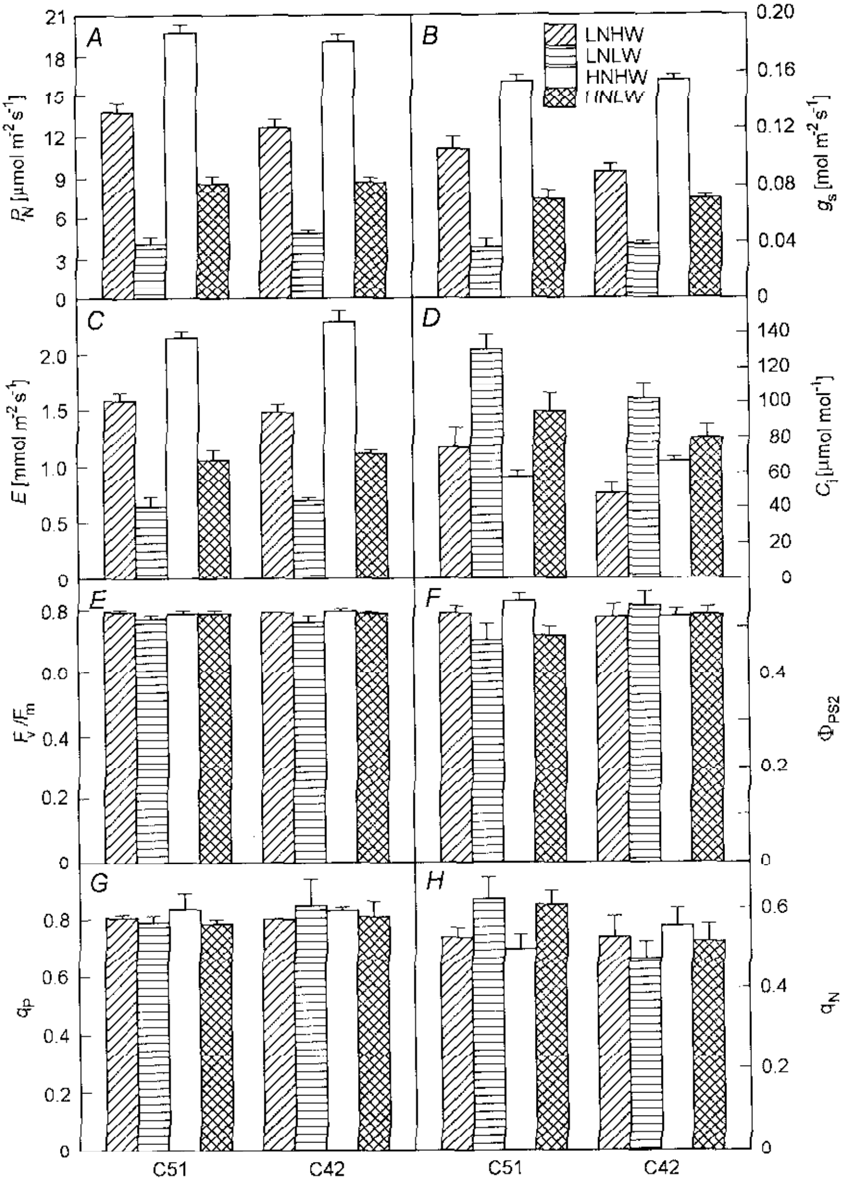

Fig. 1. Net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*), transpiration rate, *E* (*C*), intercellular CO_2 concentration, C_i (*D*), and chlorophyll *a* fluorescence parameters, F_v/F_m characterizing photochemical efficiency of open PS2 reaction centres of dark-adapted leaf (*E*), quantum efficiency of photosystem 2 electron transport of light-adapted leaf, Φ_{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 5 replicates for *A-D* and of 3 replicates for *E-H*.

Water stress reduced Ψ_{leaf} in both hybrids irrespective of N regime (Fig. 2*A*, Table 1). When plants experienced no water stress, the effect of nitrogen on P_N, g_s, and E

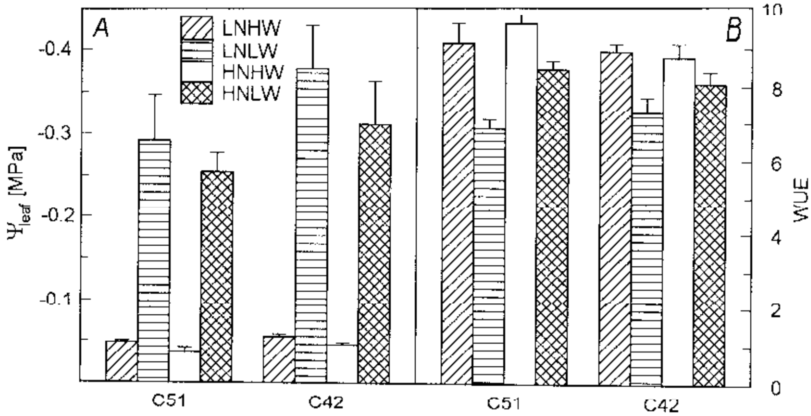

Fig. 2. Leaf water potential, Ψ_{leaf} (*A*) and water use efficiency, WUE (*B*) of sorghum hybrids C51 and C42 grown under two nitrogen concentrations (LN: low nitrogen; HN: high nitrogen) and two water regimes (LW: low water; HW: high water), measured 7 d after the beginning of water stress. Means±SE of 5 replicates.

was positive in both hybrids (Fig. 1*A*,*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 P_N, g_s, and E was dependent on N supply. A linear relationship between P_N and g_s was observed for both hybrids (Fig. 3). While water stress reduced g_s and P_N, increasing N supply led to an increase of g_s and P_N. It might seem that this relationship between g_s and P_N represents the contribution of g_s on photosynthesis and, therefore, accounts for the reduction of P_N under water stress.

C_i was significatively affected by N supply, water stress, and their interactions (Fig. 1*D*, Table 1). HN caused a decrease in the response of C_i to water stress. The

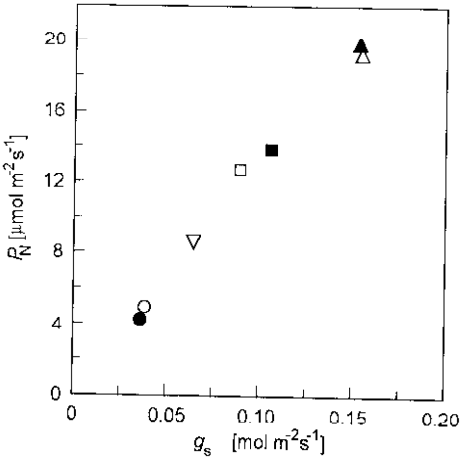

Fig. 3. Relationship between net photosynthetic rate, P_N and stomatal conductance, g_s of sorghum hybrids C51 (*closed symbols*) and C42 (*open symbols*) grown under two nitrogen concentrations and two water regimes (□: low nitrogen-high water; ○: low nitrogen-low water; Δ: high nitrogen-high water; ∇: high nitrogen-low water), determined 7 d after the beginning of water stress. Standard errors of the mean are shown in Fig. 1.

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 significatively lower at LN supply independent of the hybrid (Fig. 2*B*, 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 accompained by small alterations of Chl fluorescence parameters (Fig. 1*E-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. 1*G,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. 1*E*), 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 Φ_{PS2}, but there was significant interaction ($0.01<p<0.05$) between water regimes and hybrids (not shown). Thus, stressing plants of hybrid C42 did not substantially decrease the potential photochemical activity of PS2 (Fig. 1*F*). 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 photorespiration. However, Lal and Edwards (1996) provide evidence that in other C_4 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 photorespiration. 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.

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