# Combined effect of irradiance and water regime on sorghum photosynthesis

Qingzhang XU** and M.B. KIRKHAM*

*Department of Agronomy, Kansas State University, Manhattan, Kansas 66506-5501, USA*

## Abstract

Grain sorghum [*Sorghum bicolor* (L.) Moench. cvs. TX430 and KS82] was grown in a Haynie very fine sandy loam (coarse-silty, mixed, superactive, calcareous, mesic Mollic Udifluvents) under constant 47 % shade or full irradiance in a greenhouse under two watering regimes to see the combined and individual effects of low irradiance (LI) and low water (LW) on the sorghum genotypes. Under the high-irradiance (HI) and high-water (HW) treatment (control) and the LI-HW treatment, TX430 grew taller than KS82. Both LI and LW reduced several times the fresh and dry masses. Under the control conditions, TX430 reached its maximum net photosynthetic rate ($P_{Nmax}$) of 28.93 μmol $m^{-2}$ $s^{-1}$ at a photosynthetic photon flux density (PPFD) of 1 707 μmol $m^{-2}$ $s^{-1}$, and KS82 reached its $P_{Nmax}$ of 28.32 μmol $m^{-2}$ $s^{-1}$ at a PPFD of 2 973 μmol $m^{-2}$ $s^{-1}$. The fact that TX430 had $P_{Nmax}$ under a lower PPFD than KS82 may relate to its taller growth under LI conditions. Hence genotypes of sorghum might be selected for low irradiance using curves relating $P_N$ to PPFD.

*Additional key words*: crop breeding programs; drought resistance; photosynthetic photon flux density (PPFD); *Sorghum bicolor*; stomatal resistance.

## Introduction

Air pollution in China affects crop-breeding programs. The country's great economic development of the past two decades has been accompanied by increases in air pollution due to burning of soft coal (Klebnikov 2000, 2001). At the Shandong Academy of Agricultural Sciences in Jinan, an inland city, many cloudy days occur because of pollution. Its cultivars yield less than those developed in sunny regions further east along the coast. A goal of the institute is to breed for high yielding crop cultivars under low-irradiance (LI) conditions. Air pollution is a problem in other countries. The region around New Delhi, India now has skies permanently darkened by smog. Environs of cities in agricultural states in the USA suffer from air pollution. Houston has surpassed Los Angeles as the city with the worst smog in the nation (Klebnikov 2002).

Some crops are grown under shade on purpose. A limited, special method of culture employed in growing cigar wrapper tobacco (*Nicotiana tabacum* L.) consists of covering the field with cheesecloth or slat shade. This shading maintains a higher soil and air moisture, which results in the production of a thin, elastic leaf suitable for cigar wrapper (McMurtrey and Barford 1971). Coffee producers have responded to concerns that growing coffee (*Coffea arabica* L.) causes rain-forest damage and are now raising it under a forest canopy, which, at the same time, improves the quality of coffee beans (Deutsch 2001).

Ecologists have long studied the different physiology of sun and shade leaves [Levitt 1980 (pp. 283-288), Fay and Knapp 1995, 1996, Lichtenthaler *et al.* 2000], but few studies have been done with economically important

*Received* 12 August 2002, *accepted* 11 November 2002.
*Author for correspondence; fax 785-532-6094, e-mail: mbk@ksu.edu
***Present address*: Monsanto Company, GG5B, 700 Chesterfield Parkway North, Chesterfield, MO 63198, USA.
*Abbreviations*: $C_a$, ambient atmospheric carbon dioxide concentration; $C_i$, internal carbon dioxide concentration in the leaf; HI, high irradiance; HW, high water; LI, low irradiance; LW, low water; $P_N$, net photosynthetic rate; $P_{Nmax}$, maximum photosynthetic rate; PPFD, photosynthetic photon flux density; $r_s$, stomatal resistance.
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crops. Crops have been grown experimentally under shade for different reasons – for example, to capture carbon dioxide for increased growth (Allen 1975); to determine the effects of shade on leaf anatomy, dry matter partitioning, and gas exchange in dense grass canopies (Allard *et al.* 1991a,b); to create different contents of saccharide reserves (Schussler and Westgate 1995); to determine the importance of tillering in grass production (Bahmani *et al.* 2000); or to study root and shoot growth under increasing sowing densities (Hébert *et al.* 2001).

In this study we compared growth and gas exchange of two genotypes of grain sorghum grown under constant shade or full light in a greenhouse. We chose sorghum because it is a major crop in semi-arid regions (Haussmann *et al.* 2000), including our state of Kansas, the leading producer of grain sorghum. Because in these areas drought limits growth, we grew the plants under two watering regimes to see the combined and individual effects of LI and LW on the sorghum genotypes.

## Materials and methods

Two genotypes of grain sorghum [*Sorghum bicolor* (L.) Moench.], TX430 and KS82, were grown under greenhouse conditions in Manhattan, Kansas, USA (39°08'N; 96°37'W; 314 m ASL). They are pure, inbred lines (Dr. George Liang, personal communication, Oct. 24, 2000). KS82 was developed in Manhattan, Kansas, by U.S.D.A. breeder Alfred J. Casady, and TX430 was developed at Texas A&M. The two genotypes were chosen for their difference in drought resistance. TX430 is thought to be more drought resistant than KS82 (Dr. George Liang, personal communication, 18 Jan. 2000).

Seeds were planted in black plastic pots (17 cm diameter; 18 cm height; 5 drainage holes per pot), filled with a Haynie very fine sandy loam, on Sept. 20, 1993 (day 0), 10 seeds per pot, and emergence began on September 23. Pots were thinned to 8 plants per pot on September 27. The plants were fertilized at a rate of 22.5 g(urea) $m^{-2}$ two times during the experiment (just before planting and 30 d after planting, day 30).

The experiment consisted of the two genotypes grown under four treatments with eight replications for a total of 64 pots. Half of the plants were well watered [called the "high-water" (HW) treatment] and half of the plants were water stressed [called the "low-water" (LW) treatment]. Soil-water measurements are described in the paragraph after next. Half of the plants grew under 47 % shade (*Hummert International*, Earth City, Missouri 63045, USA; catalog number *27-8060-PC-1*, 47 % shade cloth) and half of the plants grew under the natural light. A wooden frame, 2.2 m tall and 2.3 m wide, was built over which the shade cloth was placed, and pots were placed under the frame. Under each radiation treatment, all pots were completely randomized. The plants that grew under HW and HI were called the controls.

Photosynthetic photon flux density (PPFD) was measured outside of the shade cloth and under the shade cloth 16 times between days 14 and 57 by using model *LI-190SA* Quantum Sensor and model *LI-250* Light Meter (*Li-Cor*, Lincoln, Nebraska, USA). Daylight savings time ended October 31 (day 41). Measurements of PPFD, including those after October 31, were made between 09:45 and 14:40 CDT. Ten measurements were made on each day under each irradiation regime. On days 15, 20, and 25, diurnal change in PPFD was measured, but only the data for day 15 will be presented. Ten measurements were made under each irradiation regime hourly between 06:00 and 18:00.

The amounts of water given to the plants under the four treatments are given in Table 1. The aim was to keep the soil with the HW plants near field capacity. The surface soil (0-4 cm) with LW plants was allowed to dry until there was no measurable water before re-watering. Field capacity of the Haynie very fine sandy loam is about 0.33 $m^3$ $m^{-3}$ (Song *et al.* 1999). At –1.5 MPa, the soil has a water content of 0.049 kg $kg^{-1}$ (Song *et al.* 2000). Water content of the soil was measured at the 4-cm and 10-cm depths using a hand-held meter (Soil Moisture Meter, *Li-Cor*, Lincoln, Nebraska, USA). The meter reads from 0 (dry) to 10 (wet). The probe was calibrated against known amounts of water in soil. Approximate readings were 1, 3, 6, 8, 9, and 10 for water contents of 0.08, 0.15, 0.17, 0.23, 0.28, and 0.36 kg $kg^{-1}$, respectively. Each pot was measured at the two depths on each measurement day, and each value reported is the average of the eight measurements (8 replications per treatment).

Height of plants was measured 20 times between days 7 and 63 by measuring one plant in each pot. Plants were harvested on day 63 (November 22) and fresh mass, dry mass, and leaf area were determined. The experiment was terminated before reproductive structures emerged. Measurements of net photosynthetic rate ($P_N$), photosynthetically active radiation (PPFD), and internal $CO_2$ concentration ($C_i$) in the leaf were taken with a portable photosynthetic system (*LI-6200*, *Li-Cor*, Lincoln, Nebraska, USA). Stomatal resistance ($r_s$) also was measured with the system, which is calculated, using a linear-flow law, from the gradient of the water-vapor pressure and transpiration rate (Jarvis 1971, Slavík 1971). During the experiment, four tests were done on days 42 and 44 (Test 1), 57 (Test 2), 58 (Test 3), and 59 (Test 4). They were:

Test 1: Measurements one day before watering and one day after watering; all plants watered on day 43 (Table 1).
Test 2: Measurements of relationship between $P_N$ *vs.* $C_i$.

Test 3: Diurnal measurements.
Test 4: Measurements of relationship between $P_N$ and PPFD.

Because the *LI-6200* is a closed gas exchange system (Jarvis *et al.* 1971), different concentrations of $CO_2$ for Test 2 could be obtained by opening the *LI-6200* chamber, placing the leaf inside, and blowing into the sensor chamber. The $CO_2$ concentration in the chamber was recorded on the read-out, and when a high concentration was reached in the chamber, the chamber was closed and then readings were taken until the $CO_2$ concentration had fallen to about zero. For Test 3 on day 58 (November 17), diurnal change in photosynthesis was measured by taking measurements throughout the day from 08:37 to 17:01. For Test 4 on day 59 (November 18), when the relationship between $P_N$ and PPFD was determined, pots were removed from their natural growth conditions and placed under a high-pressure sodium lamp. Different PPFD's were obtained by placing pots at different distances from the lamp.

## Results and discussion

**Environmental conditions and growth**: The average PPFD during the experiment with no shade cloth was 314.5 µmol $m^{-2}$ $s^{-1}$; with shade cloth, the average was 95.1 µmol $m^{-2}$ $s^{-1}$ (average of values in Fig. 1*A*). The ratio was 0.30, which is lower than the 0.47 value given by the manufacturer. The dips in the curve for the non-shade conditions in Fig. 1 indicate cloudy days.

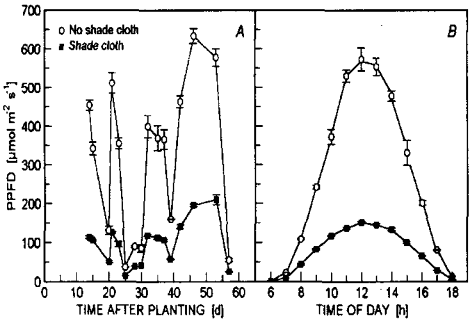


Fig. 1. Photosynthetic photon flux density under shade cloth and natural greenhouse irradiation conditions throughout the experimental period at midday (*A*) and diurnal changes 15 d after planting (*B*). *Vertical bars* show standard error (*n* varied between 10 and 18 measurements for the left figure; *n* = 10 for the right figure).

HW pots had soil moisture readings between 7 and 10 at both depths (4 and 10 cm) throughout the experiment (Fig. 2). Soil water content in LW pots varied with amount of water added (Table 1).

Under both the control and LI conditions, TX430 grew taller than KS82 (Fig. 3*A,C*). Under LW, the height of the two genotypes generally was similar (Fig. 3*B,D*). Either the LW stress or the LI stress reduced fresh mass, dry mass, and leaf area of both genotypes (Table 2). The genotypes grown under the combined stresses had the lowest fresh and dry masses and leaf areas.

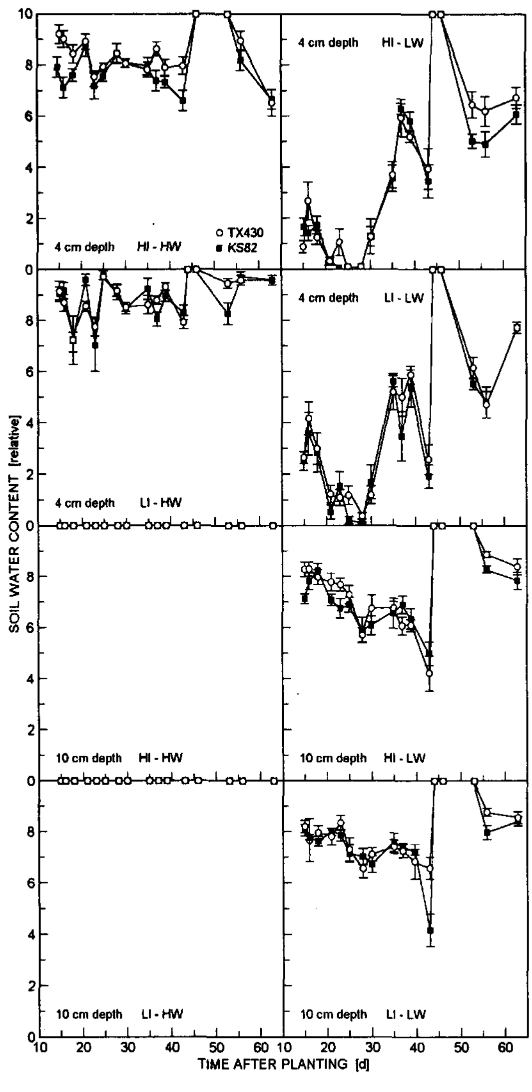


Fig. 2. Water content of soil with two genotypes of sorghum, KS82 (■) and TX430 (O), grown under four treatments. *Vertical bars* show standard error (*n* = 8).

**Effect of watering (Test 1)**: One day after watering all plants on day 43, $P_N$ of the plants grown under the LW-HI treatment increased (Table 3). The $P_N$ of KS82 increased from 8.37 to 13.42 µmol $m^{-2}$ $s^{-1}$, and the $P_N$ of TX430 increased from 4.36 to 12.48 µmol $m^{-2}$ $s^{-1}$. Similarly, the $r_s$ decreased. However, plants grown under LI-LW did not respond to the watering. Their $P_N$ remained low, and their $r_s$ remained high.

**$P_N$ *vs.* $C_i$ curves (Test 2)**: Under the control conditions, the $P_N$ of TX430 was higher than that of KS82 for all $C_i$ above about 15 μmol mol$^{-1}$ (Fig. 4*A*). With no stress, TX430 had $P_{Nmax}$ of 37 μmol m$^{-2}$ s$^{-1}$, and KS82 had a $P_{Nmax}$ of 29 μmol m$^{-2}$ s$^{-1}$. The ability of TX430 to maintain a higher $P_N$ under the control conditions may be one reason it was taller than KS82 (Fig. 3*A*). Under the other three treatments, KS82 usually had a higher $P_N$ as $C_i$ increased. Ambient atmospheric $CO_2$ concentration ($C_a$) was about 340 μmol mol$^{-1}$. While inflection points of the curves were hard to pinpoint (end of straight-line relation between $P_N$ and $C_i$), especially under the LW conditions (Fig. 4*B,D*), all curves tended to have the inflection point at $C_i \approx C_a$. This finding agrees with the theoretical analysis of Farquhar and Sharkey (1982, p. 333), who said that the largest possible $P_N$ occurs at $C_i \approx C_a$. Under the HW treat-ments (Fig. 4*A,C*), the curves went through zero, typical for $C_4$ plants (Raschke 1986, p. 92).

Table 1. Watering regime.

| Time after planting [d] | Water added [cm$^3$] | | | |
|---|---|---|---|---|
| | HI-HW | LI-HW | HI-LW | LI-LW |
| 7 (Sept. 27) | 250 | 250 | 0 | 0 |
| 13 | 250 | 125 | 0 | 0 |
| 18 | 250 | 250 | 0 | 0 |
| 23 | 250 | 250 | 0 | 0 |
| 28 | 125 | 0 | 50 | 50 |
| 30 | 125 | 0 | 50 | 0 |
| 32 | 250 | 250 | 125 | 125 |
| 35 | 250 | 0 | 0 | 0 |
| 36 | 125 | 0 | 0 | 0 |
| 37 | 250 | 125 | 125 | 125 |
| 38 | 250 | 0 | 125 | 0 |
| 39 | 0 | 0 | 0 | 0 |
| 43 | 250 | 250 | 600 | 250 |
| 45 | 600 | 250 | 125 | 125 |
| 46 | 0 | 600 | 600 | 250 |
| 47 | 1000 | 0 | 0 | 0 |
| 48 | 1000 | 0 | 0 | 0 |
| 49 | 1000 | 0 | 0 | 0 |
| 50 | 1000 | 0 | 0 | 0 |
| 51 | 1000 | 0 | 0 | 0 |
| 52 | 1000 | 0 | 0 | 0 |
| 53 | 0 | 250 | 0 | 0 |

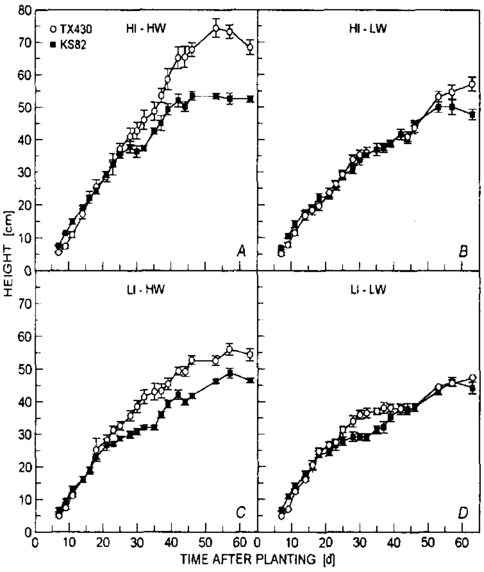


Fig. 3. Heights of two genotypes of sorghum, KS82 (■) and TX430 (O), grown under four treatments. *Vertical bars* show standard error ($n$ = 8).

**Diurnal changes (Test 3)**: For the controls, the average $P_N$ (all diurnal values averaged together) of TX430 and KS82 were 9.6 and 8.5 μmol m$^{-2}$ s$^{-1}$, respectively (Fig. 5*A*), and the average $r_s$ values were 3.7 and 4.9 s cm$^{-1}$, respectively (Fig. 6*A*). The average $P_N$ of TX430

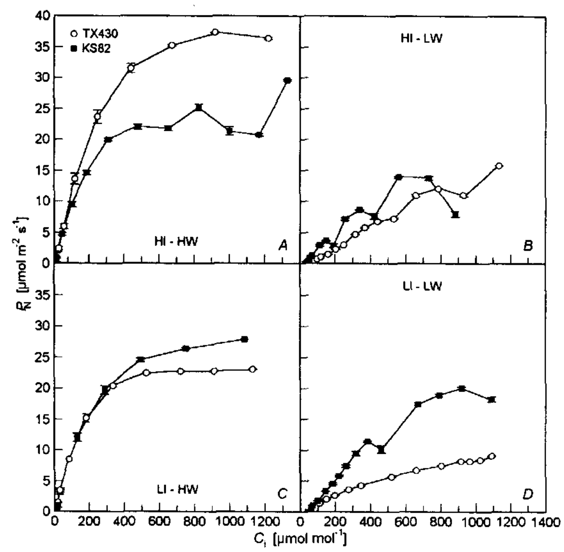


Fig. 4. Net photosynthetic rate ($P_N$) *versus* intercellular carbon dioxide concentration ($C_i$) for two genotypes of sorghum, KS82 (■) and TX430 (O), 57 d after planting and grown under four treatments. *Vertical bars* show standard error ($n$ = 3).

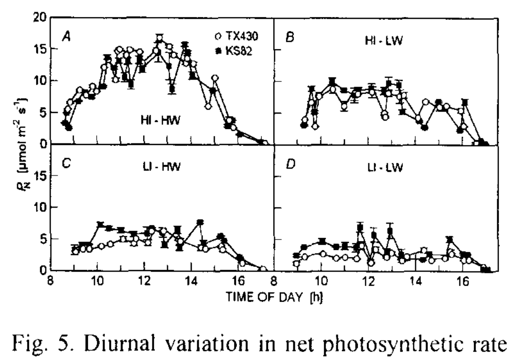


Fig. 5. Diurnal variation in net photosynthetic rate ($P_N$) of two genotypes of sorghum, KS82 (■) and TX430 (O), 58 d after planting and grown under four treatments. *Vertical bars* show standard error ($n$ = 3).

and KS82 under the LI, HW treatment were 3.5 and 4.5 μmol m$^{-2}$ s$^{-1}$, respectively (Fig. 5*C*). $r_s$ values for TX430 and KS82 under the LI-HW treatment were similar and averaged about 9 s cm$^{-1}$ (Fig. 6*C*). The average $P_N$ values of TX430 and KS82 under the HI-LW treatment were 5.9 and 6.4 μmol m$^{-2}$ s$^{-1}$, respectively (Fig. 5*B*). The $r_s$ values for TX430 and KS82 under the HI-LW treatment were similar and averaged about 9 s cm$^{-1}$ (Fig. 6*B*). Under the LI-LW conditions, the average $P_N$ of TX430 and KS82 were 2.3 and 3.4 μmol m$^{-2}$ s$^{-1}$, respectively (Fig. 5*D*), and the average $r_s$ values were 12 and 11 s cm$^{-1}$, respectively (Fig. 6*D*).

**Effect of irradiance (Test 4)**: Under the control conditions, TX430 reached its $P_{Nmax}$ of 28.93 μmol m$^{-2}$ s$^{-1}$ at a PPFD of 1 707 μmol m$^{-2}$ s$^{-1}$, and KS82 reached its $P_{Nmax}$ of 28.32 μmol m$^{-2}$ s$^{-1}$ at a PPFD of 2 973 μmol m$^{-2}$ s$^{-1}$ (Fig. 7*A*). The fact that TX430 had a $P_{Nmax}$ under a lower irradiance than KS82 may relate to its better height growth under low PPFD conditions (Fig. 3*A,C*). Photosynthesis of puka (*Meryta sinclairii* Seemann), a shade tolerant New Zealand native forest tree, was saturated at a low irradiance (at about 200 μmol m$^{-2}$ s$^{-1}$) (Kelliher *et al.* 2000). Puka's shade tolerance may be related to its ability to photosynthesize at a maximum rate under low irradiance. Similarly, because TX430 had a $P_{Nmax}$ at a lower irradiance than KS82, it may be able to grow better under LI. These results suggest it might be possible to select plants for low irradiance using curves relting $P_N$ to PPFD.

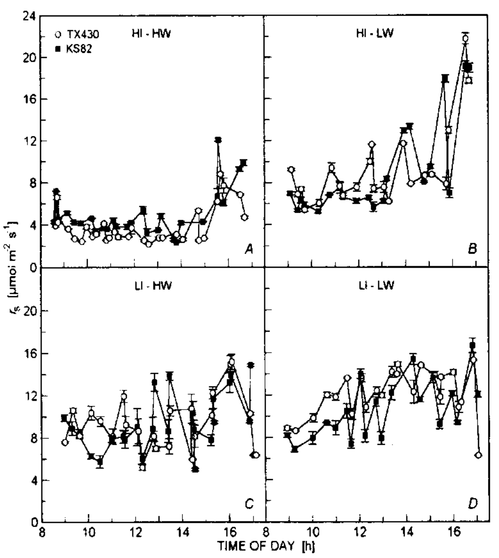


Fig. 6. Diurnal variation in stomatal resistance ($r_s$) of two genotypes of sorghum, KS82 (■) and TX430 (O), 58 d after planting and grown under four treatments. *Vertical bars* show standard error ($n = 3$).

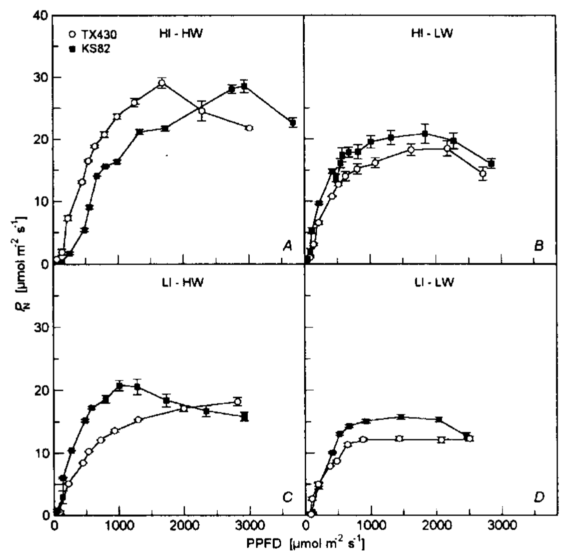


Fig. 7. Net photosynthetic rate ($P_N$) *versus* photosynthetic photon flux density (PPFD) for two genotypes of sorghum, KS82 (■) and TX430 (O), 59 d after planting and grown under four treatments. *Vertical bars* show standard error ($n = 3$).

Table 2. Fresh mass, dry mass, and leaf area [per pot] of two genotypes of sorghum at harvest 63 d after planting and grown under two irradiances and two watering regimes. Means ± standard errors are shown ($n = 8$).

| | HI-HW | | HI-LW | | LI-HW | | LI-LW | |
|---|---|---|---|---|---|---|---|---|
| | KS82 | TX430 | KS82 | TX430 | KS82 | TX430 | KS82 | TX430 |
| Fresh mass [g] | 56.29±1.68 | 63.06±0.95 | 11.89±0.45 | 11.92±1.03 | 8.79±0.56 | 7.84±0.35 | 3.25±0.23 | 3.03±0.14 |
| Dry mass [g] | 9.08±0.29 | 8.02±0.34 | 2.08±0.13 | 1.92±0.13 | 1.00±0.04 | 1.06±0.09 | 0.50±0.03 | 0.53±0.05 |
| Leaf area [cm$^3$] | 1127.7±36.5 | 1426.0±51.4 | 313.0±11.9 | 390.4±20.4 | 239.2±15.7 | 238.6±12.2 | 119.0±6.4 | 101.6±4.9 |

Table 3. Net photosynthetic rate ($P_N$) [µmol $m^{-2}$ $s^{-1}$] and stomatal resistance ($r_s$) [s $cm^{-1}$] of two genotypes of sorghum grown under two irradiances and two watering regimes. Means ± standard error are shown with number of measurements in parentheses.

| | | $P_N$ KS82 | TX430 | $r_s$ KS82 | TX430 |
|---|---|---|---|---|---|
| 1 d before watering (day 42) | HI-HW | 18.77±1.12 (33) | 14.84±0.84 (33) | 2.97±0.18 (33) | 3.49±0.20 (33) |
| | HI-LW | 8.37±0.90 (24) | 4.36±0.87 (24) | 7.26±0.63 (24) | 11.94±1.64 (24) |
| | LI-HW | 5.37±0.74 (12) | 4.93±0.59 (12) | 7.35±0.70 (12) | 7.87±0.49 (12) |
| | LI-LW | 5.01±0.70 (12) | 5.24±0.77 (12) | 8.28±0.77 (12) | 7.39±0.30 (12) |
| 1 d after watering (day 44) | HI-HW | 15.37±1.38 (9) | 12.75±1.58 (12) | 2.98±0.24 (9) | 2.70±0.22 (12) |
| | HI-LW | 13.42±0.68 (15) | 12.48±1.08 (12) | 3.54±0.23 (15) | 4.11±0.41 (12) |
| | LI-HW | 5.96±0.33 (18) | 5.26±0.46 (18) | 7.11±0.29 (18) | 8.01±0.57 (18) |
| | LI-LW | 5.01±0.36 (18) | 5.19±0.37 (18) | 7.17±0.86 (18) | 7.29±0.65 (18) |

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