Diffuse light weakens the dorsoventral asymmetry of photosynthesis in sorghum leaves

Xiaolin Wang [1, 2] , **Huifeng Yan** Corresp., [3] , **Yuanzheng Li** [4]

[1] Center of Cultivation and Nutrition, Tobacco Research Institute of Chinese Academy of Agricultural Sciences,, Qingdao, Shandong, China
[2] Institute of Agricultural Resources and Regional Planning of Chinese Academy of Agricultural Sciences, Beijing, China
[3] Center of Cultivation and Nutrition, Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao, Shandong, China
[4] School of Resources and Environment, Henan University of Economics and Law, Qingdao, Shandong, China

Corresponding Author: Huifeng Yan
Email address: yanhuifeng@caas.cn

Dorsoventral asymmetry of photosynthesis is important for light use patterns, but the asymmetry changes with environments. Photosynthetic asymmetry was have been intensively studied for their role in light use efficiency, but less is know about the impact of light properties on asymmetry. Aimed at the current changing fraction of diffuse light in sky radiation, this study investigated dorsoventral asymmetry of photosynthetic traits in direct, diffuse and self-transmitted light (residual light intercepted by one surface) respectively in an important food and energy crop, *Sorghum bicolor L*. An unique designed method was used to investigate the specific gas exchange of two surfaces in different light properties. We found that anatomical and morphological traits were obviously different in sorghum leaves, which might cause the photosynthetic asymmetry, the variation of photosynthetic rates and stomatal conductance was significant between two surfaces in direct and self-transmitted light, but insignificant in diffuse light. The abaxial stomatal sensitivity of single stoma was lower, while the higher stomatal density in abaxial surface compensated the weakness in stomatal sensitivity. Comparing to direct and self-transmitted light, diffuse light reduced the stomatal sensitivity, while the degree of decline was higher in adaxial surface, which caused weak dorsoventral asymmetry in photosynthesis. Taken together, the finding of this study suggested that stomatal sensitivity determined the degree of dorsoventral asymmetry in the susceptibility to light properties. The different stomatal sensitivity regulated photosynthetic variation, which was benefit to remain high photosynthetic rate in high and changing light. The findings provided new insight in to dorsoventral asymmetry and impact of diffuse light on photosynthesis in isobilateral leaves.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27267v1 | CC BY 4.0 Open Access | rec: 9 Oct 2018, publ: 9 Oct 2018

Author Cover Page:

Title:

Diffuse light weakens the dorsoventral asymmetry of photosynthesis in sorghum leaves

Xiaolin Wang[1,2], Huifeng Yan[1], Yuanzheng Li[3]

1 Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao, 266101, PR China. E-mail: aardvolf@163.com

2 Institute of Agricultural Resources and Regional Planning of Chinese Academy of Agricultural Sciences, Beijing, 100081, PR China. E-mail: yanhuifeng@caas.cn

3 School of Resources and Environment, Henan University of Economics and Law, Zhengzhou 450046, PR China. E-mail: yzli_pili@outlook.com

Corresponding author: Huifeng Yan
Tel: +86 532 88703786
Fax: +86 532 88702056
E-mail: yanhuifeng@caas.cn
Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Keyuanjing 11th Road, Laoshan district, Qingdao, 266101, PR China

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27267v1 | CC BY 4.0 Open Access | rec: 9 Oct 2018, publ: 9 Oct 2018

Abstract:

The dorsoventral asymmetry of photosynthesis is important for light-use patterns, but the asymmetry is environment dependent. Its role in photosynthetic regulation has been intensively studied, but little is known about the impacts of direct and diffuse light on the asymmetry. Because of the current changing fraction of diffuse light in sky radiation, this study investigated the dorsoventral asymmetry of photosynthetic traits under direct and diffuse light conditions in an important food and energy crop, *Sorghum bicolor L*. A unique method was used to investigate the specific gas exchange of each leaf surface. Anatomical and morphological traits were different between the two surfaces of sorghum leaves, which might result in photosynthetic asymmetry. The variations in photosynthetic rates and stomatal conductance were significant between the two surfaces both in direct and diffuse light, but the degree of dorsoventral asymmetry decreased in diffuse light. The abaxial stomatal sensitivity levels of single stoma were lower, while the greater stomatal density in the abaxial surface compensated for the weakness in stomatal sensitivity. Compared with direct light, diffuse light reduced the stomatal sensitivity, with the degree of decline being greater in the adaxial surface, which caused weak dorsoventral asymmetry in photosynthesis. Thus, stomatal sensitivity may determine the degree of dorsoventral asymmetry produced by direct and diffuse light. The different stomatal sensitivity levels regulated photosynthetic variation, which helped retain a high photosynthetic rate under high and changing light conditions. The findings provide new insights into dorsoventral asymmetry and the impact of diffuse light on photosynthesis in isobilateral leaves.

Introduction

The solar radiation reaching leaf surfaces is the primary driver of leaf photosynthesis (Mercado et al. 2009; Strada & Unger 2016). Alterations in sky conditions influence the properties of the incidental light illuminating plants (Cheng et al. 2015; Mercado et al. 2009). Under clear sky conditions, sunlight arrives in beams at the earth's surface and leaves are illuminated from a single direction(Urban et al. 2012; Williams et al. 2014), while under cloudy sky conditions, nearly all of the incoming light is diffuse, with clouds, haze or fog scattering the light before it reaches the plants (Brodersen et al. 2008). As the variations in atmospheric aerosols increase, more diffuse light reaches the earth's surface (Brient & Bony 2013; Mercado et al. 2009; Oliveira et al. 2011) as a result of an increasing diffuse light index (the fraction of diffuse light in total radiation, *DI*) in sky radiation. Does the increase in atmospheric aerosol change the photosynthetic traits of plants? This might directly depend on the photosynthetic responses of leaf light-use patterns to direct and diffuse light. Under field conditions, direct light illuminates the adaxial surfaces, while the abaxial surfaces receive diffuse light. Thus, the plant must acclimate, by altering structures and photosynthetic functions, to different light conditions. Photosynthetic asymmetry, the photosynthetic variation between the different surfaces, plays important roles in determining the leaf light-use efficiency of two surfaces and adaptation to field light conditions. In general, leaves of C_3 dicots show dorsoventral asymmetry in the palisade mesophyll tissue on the adaxial side and spongy mesophyll tissue on the abaxial side. These leaves are so-called bifacial leaves, and their photosynthetic structures and functions have been intensively studied (Terashima & Saeki 1983; Vogelmann 1993), revealing internal gradients in light intensity and photosynthetic activity within leaves (Gorton et al. 2010; Vogelmann & Björn 1984). In bifacial leaves, the structures and functions of the palisade tissue in the adaxial side and spongy tissue in the abaxial side are different (Gorton et al. 2010; Smith et al. 1997). In most terrestrial bifacial plants, more stomata are distributed on abaxial surfaces. When leaves were illuminated on the adaxial surfaces, the photosynthetic capacity was 30%–40% greater than that of the abaxial surfaces(Long et al. 1989). Thus, the photosynthetic activity levels on both sides of the bifacial leaves acclimated to light environments through specialization and cooperation.

In contrast to the dorsoventrally asymmetrical leaves of C_3 dicots, some C_4 monocotyledonous species have symmetrical leaves. Moss (1964) showed the leaves of maize and sugarcane exhibit no differentiation of palisade and spongy mesophyll tissues and both surfaces have similar photosynthetic responses to illumination. Therefore, the leaves were considered as having functional symmetry (Moss 1964). However, different photosynthetic responses to adaxial and abaxial illumination have been found in some C_4 monocotyledonous species (Driscoll et al. 2006; Long et al. 1989; Smith 2008; Soares-Cordeiro et al. 2011; Soares-Cordeiro et al. 2009). The isobilateral leaves have an obvious asymmetry in photosynthetic functions. Soares et al. (2008, 2009) reported that abaxial contributions to leaf integrated photosynthesis are greater than adaxial contributions, independent of whether the adaxial or abaxial surface was illuminated with the same light intensity, owing to the greater number of stomata on the abaxial surfaces (Soares-Cordeiro et al. 2009; Soares et al. 2008). A reason for differences in the net photosynthetic rates (P_n) of the two sides of isobilateral leaves is the greater stomatal density of the abaxial surfaces (Jiang et al. 2011; Soares et al. 2008). Variations in photochemical activities involving light capture, reaction center activities, and electron transfer rates could also result in photosynthetic asymmetry between two surfaces, but related reports are limited.

Plants can use diffuse light more efficiently than direct light on the scales of an individual leaf, stand, canopy or crown, and biome(Gu et al. 2003; Knohl & Baldocchi 2008; Mercado et al. 2009; Urban et al. 2012; Williams et al. 2014). Simulation models and eddy covariance methods revealed that the gross primary productivity of a canopy or individual plant tended to be greater under cloudy sky conditions, owing to the high proportion of diffuse light in the total irradiance (Mercado et al. 2009; Urban et al. 2012; Williams et al. 2014). A main possible mechanism whereby diffuse light stimulates the photosynthesis of individual leaves involves sufficient stomatal openness and high stomatal conductance, which are caused by light properties and thermal effects (Hughes et al. 2015; Li & Yang 2015; Reinhardt & Smith 2008; Reinhardt et al. 2010; Urban et al. 2012). Furthermore, Wang et al. (2008) reported that the response patterns of stomata were different under direct and diffuse lighting in leaves of *Helianthus annuus L* (Wang et al. 2008). Stomatal behavior plays dominant roles in photosynthetic variations when the non-stomatal limitation has not been met. Brodersen et al. (2008) reported that high light-grown leaves of *H. annuus* (C_3) and *Amaranthus retroflexus* (C_4) might be adapted to the greater efficiency of direct light compared with diffuse light, while shade-leaf structures did not appear to discriminate light properties based on their directionality (Brodersen et al. 2008). Light that reaches deep into the leaf can arrive at abaxial surfaces, forming up to 10%– 20% of the incidental light intensity (Earles et al. 2017). Self-transmitted light results in a large proportion of whole-leaf photosynthesis. The sensitivity of abaxial stomata to light is greater than that of adaxial stomata, and the abaxial stomata might be more sensitive to the self-transmitted light than to direct light in sun-grown leaves of *H. annuus L* (Wang et al. 2008). Thus, the light properties could change stomatal behaviors, resulting in changes in the photosynthetic levels of the two leaf surfaces.

Sorghum (*Sorghum bicolor* L.) is an important food and energy crop that is expected to alleviate food and energy insecurity. Therefore, it has attracted worldwide attention. The leaves of

C_4 monocotyledonous species are divided into two separate compartments by compact mesophyll tissue (Long et al. 1989; Morison & Lawson 2007; Smith 2008; Soares-Cordeiro et al. 2009), making these species appropriate materials for studying physiological traits in specific surfaces. Sorghum leaves contain two separate compartments (Jiang et al. 2011), and the microscopic morphology of the sorghum used in this study showed physical restrictions in the dorsoventral airspace (Fig. 1). Moreover, as a C_4 plant, the CO_2-fixation efficiency is greater than that of C_3 plants, which causes a stronger correlation between the photosynthetic rate and intercellular CO_2 concentration.

Here, we investigated the dorsoventral asymmetry of photosynthesis and anatomy in sorghum leaves, and the variations of stomatal behavior under direct, diffuse and self-transmitted light conditions. The objectives of our study were to reexamine the photosynthetic and anatomical dorsoventral asymmetry between adaxial and abaxial surfaces, and analyze the impacts of direct and diffuse light on photosynthetic asymmetry. We attempt to explain how the isobilateral leaves regulate photosynthesis under diverse direct and diffuse light conditions and tested the following hypotheses: (i) photosynthetic asymmetry is caused by different structures and light properties; and (ii) diffuse light weakens photosynthetic asymmetry by altering stomatal behavior. The findings are beneficial to understanding the responses of adaxial and abaxial surfaces of isobilateral leaves to direct and diffuse light conditions, and the photosynthetic regulation in amphistomatic leaves. Therefore, this study may clarify the impact of increasingly diffuse light on crop yields in the future.

Materials and Methods

Field sites and plant material

Experiments were carried out at an experiment field in Beijing (115.7°E–117.4°E, 39.4°N–41.6°N) from June to August in 2016. Beijing is located in a northern temperate zone with a semihumid, continental and monsoon climate. Sorghum was selected for investigation. The seeds were imbibed on wet paper for 1 d, and germinated seeds were sown in containers (30 cm ×2 0cm × 10 cm) filled with vermiculite. Plants were watered every 2 d. Two weeks later, 4-leaf seedlings were transplanted into plastic pots (15 cm in diameter, 20 cm in height) containing Hoagland's nutrient solution and grown in a site having simple rain shelters in the field. The nutrient solution was renewed every 3 d. The seedlings were used for gas-exchange measurements after 20 d.

Measurement of leaf optical properties

Leaf reflectance and transmittance on each surface of the sorghum leaves were measured using a bifurcated fiber optic cable and a leaf clip (PP Systems, USA). Leaves were attached and used for gas-exchange measurements across the spectrum of 310–1100 nm at 1-nm intervals. To calculate reflectance, leaf spectral radiance was divided by the radiance of a 99% reflective white reference standard (Spectralon, Labsphere, North Dutton, NH, USA) (Gamon & Surfus 1999). Leaf transmittance was measured using two straight-fiber optics and a custom-made device. One straight-fiber optic was used to irradiate the leaf from the adaxial side, and the other was used as a detector on the leaf abaxial side. Absorptance = 1− reflectance − transmittance.

Measurement of light properties

The waveform (over the wavelength of 400–800 nm) of the light source was measured using a fiber optic spectrometer equipped with a cosine corrector (AvaSpec-ULS 2048XL, Avantes, Netherland). Light intensity could be calculated from the waveform through the trapezoidal integration of the spectral curve (Wang et al. 2016). The *DI* over the wavelength range of 400 to 800 nm was measured using the same fiber optic spectrometer. In addition, a diffuser panel (Fotodiox Inc., USA) was used to intercept direct light during *DI* measurement. The intensities of direct and diffuse light used in the experiments were measured using a light sensor.

Measurement of gas exchange

Photosynthetic gas exchange rates were measured using an infrared gas analyzer (Li-6400, Li-Cor, Lincoln, NE, USA) equipped with a custom-made LED light source. The light source could provide direct light with an intensity range from 0 μmol m^{-2} s^{-1} to 1800 μmol m^{-2} s^{-1} by setting the voltage. The spectral compositions of sunlight and the LED we used are shown in Appendix A. A scatter plate (EVONIK Degussa, Germany), which possessed a transmissivity of over 85% was used to convert the direct light to diffuse light (See Appendix B). The spectral component of the LED light source was set according to the solar radiation provided during a normal sorghum growth season. To separate the stomatal sensitivities and gas exchange rates of two surfaces, a well-designed method was used. A transparent film (TF) that passed our optical testing was used to block the flux between the leaf surface and chamber in a gas-exchange analyzer system (LI-6400), allowing the gas exchange rates of the opposite surfaces to be measured (Fig. 2). Preliminary experiments showed that the TF had no obvious effects on the light intensity from the LED source (Appendix A) or the absorbance rates (310–1,100 nm) of leaves (Appendix C). Thus, the integrated and specific gas exchange rates of a leaf in direct, diffuse and self-transmitted light were measured, as follows:

(i) Adaxial-specific gas exchange rates in direct light and diffuse light: the TF was placed between the lower chamber and the abaxial surface, and direct and diffuse light were illuminated on the adaxial surface;

(ii) Abaxial-specific gas exchange in direct light and diffuse light: the TF was placed between the upper chamber and the adaxial surface, and direct and diffuse light were illuminated on the abaxial surface;

(iii) Adaxial-specific gas exchange in self-transmitted light: the TF was placed between the lower chamber and the abaxial surface, and direct and diffuse light were illuminated on the abaxial surface;

(iv) Abaxial-specific gas exchange in self-transmitted light: the TF was placed between the upper chamber and the adaxial surface, and direct and diffuse light were illuminated on the adaxial surface; and

(v) Gas exchange of two leaf surfaces (integrated gas exchange) in direct and diffuse light: direct and diffuse light were illuminated independently on the adaxial and abaxial surface without the TF.

In total, 24 plants were selected for the studies. The plants were measured in batches for either integrated or specific measurements. All measurements were performed on the youngest fully-expanded leaves. Measurements were conducted from 9:00 to 12:00 in a leaf chamber set at 30°C, with 45%–50% relative humidity, an ambient CO_2 concentration of 380 μmol mol^{-1} and an irradiance of 1,000 μmol m^{-2} s^{-1}. The ratios between the intercellular CO_2 concentration (C_i) and atmospheric (C_a) CO_2 concentration (C_i/C_a ratios) were presented as pooled data obtained in separate experiments. The light-response curves for the stomatal conductance of adaxial and abaxial surfaces in sorghum leaves were investigated under direct, diffuse and self-transmitted light, with the light intensity gradient of 1,200, 1,000, 800, 600, 400, 300, 250, 200, 150, 100, 50, 20 and 0 μmol m^{-2} s^{-1}. When the leaf was acclimated in setting light for 30 min, the CO_2 supply in the chamber was regulated by a CO_2 supply system equipped with an LI-6400 to maintain the C_i near 310 μL L^{-1}. The values were logged when P_n and stomatal conductance (G_s) reached a steady state and C_i reached 310 μL L^{-1}. The light response curves of G_s were obtained from the photosynthetic photon flux densities (PPFDs) of direct, diffuse and self-transmitted light versus G_s. The apparent quantum yield (AQY) was the initial slope of the regression curve of PPFD versus

P_n.

Stomatal densities (SDs) and sizes on leaf surfaces

The *SD* was determined according to the method "leaf imprints" [21,](Coupe et al. 2006). Once the leaves became fully expanded, a widely-used nail polish (the main constituents were nitrocellulose and ethyl acetate) was applied to dental imprints to obtain a replica of the leaf surfaces. The replicas were observed under a light microscope (Olympus BH-2; Olympus Optical Co. Ltd, Tokyo, Japan), and a digital camera was used to photograph the replicas. Samples were taken from the same area of the leaves that were used for the gas-exchange measurements. The numbers of stomata for each microphotograph were counted to calculate the *SD*s. We measured the long axis of each stoma to determine the size because the short axes of stomata change depending on the degree of opening. In total, 6 microphotographs from 10 marked leaves of 10 individual plants per treatment were selected for measurement.

Determination of stomatal sensitivity

The stomatal conductance increased as the light intensity increased. Stomatal conductance had a strong linear correlation with light intensity at low-light intensities (< 200 μmol m^{-2} s^{-1}). The regression line of stomatal conductance (y) versus light intensity (x) was:

$$y = \beta_1 x + b,$$

where β_1 represents the initial slope of the regression line at low-light intensities, and b represents the stomatal conductance of leaves in the dark. The value of β_1 could reflect the stomatal insensitivity to light intensity (Wang et al. 2008). However, the *SD* might be different between adaxial and abaxial surfaces. Thus, we used β_2 to investigate the unit of stomatal insensitivity to light:

$$\beta_2 = \beta_1/SD.$$

Transverse semi-thin leaf sections for optical microscopy

Leaf sections (2 × 2 mm) were immersed in a fixative consisting of 1% (v/v) par-formaldehyde and 3% (v/v) glutaraldehyde in a 0.1-mM sodium phosphate buffer. The sections were then washed, post-fixated and dehydrated (Soares-Cordeiro et al. 2009). Samples were embedded in Spur resin. Transverse semi-thin leaf sections (1 μm) were observed using a light microscope (Nikon-E800, Scientific Imaging Inc, USA).

The area of the sub-stomatal cavity (*ASC*) and the contact area between the bundle sheath and mesophyll cells (*CABM*) in transverse semi-thin leaf sections (Soares-Cordeiro et al. 2009; Wu et al. 2014) were determined. *CABM* was calculated according to the following specific steps: (i) assuming that mesophyll cells were spheroids, in accordance with Thain (Thain 1983), the total cell surface area per unit volume of tissue is equal to the total length of the cell profile perimeter in unit area of a tissue section; (ii) the lengths and the widths of cells can be easily measured, and the curvature correction factors (*F*) can be calculated using length/diameter ratios; and (iii) the contact area of the chloroplast facing the outer mesophyll cells was considered as

$$CABM = L_c / W \times F,$$

where L_c represents the lengths of chloroplasts facing the mesophyll cells and W represents the width of the leaf cross-section (Wu et al. 2014). The value of F in mesophyll cells of sorghum leaves was 1.45, and the values of W were calculated using the pictures of transverse semi-thin leaf sections with Image J-Fiji software.

Chlorophyll a (Chl a) fluorescence measurements

Chl a fluorescence was measured using a plant efficiency analyzer (Plant Efficiency Analyzer Hansatech, Norfolk, UK) on completely expanded leaves. Fully dark-adapted seedlings (12 h) were used to determine OJIP curves. The leaves were then exposed to a pulse of saturated light at an intensity of 3,500 μmol.m^{-2} s^{-1} and a wavelength of 650 nm for 5 s. The fluorescence transients were recorded from 10 μs to 1s (Strasser et al. 2004). The OJIP curves were obtained by plotting relative variable fluorescence intensity on a logarithmic time scale.

The degree of dorsoventral asymmetry

The asymmetry index (*ASI*) was used in our study to reflect the degree of dorsoventral asymmetry. The *ASI*s for morphological and anatomic structures, and gas-exchange parameters, were calculated as the ratio of the value of the abaxial side to that of the adaxial side. By definition, if a given parameter shows the same value for both sides, its *ASI* is 1.00. Thus, the greater deviation of *ASI* from 1.00 denotes a greater degree of *ASI*.

Statistical analysis

Data were subjected to separate statistical analyses using parametric tests. Differences between means were analyzed using Student's t-tests. Regression lines were obtained by the least squares method. Differences in the regression coefficient and in the intercept were detected using an analysis of covariance. The multiple comparison was assessed using Tukey's test. An analysis of the data was performed using the Statistical Package for Social Sciences (SPSS, Version 18.0, for Windows). Correlations of linear regressions were calculated using SigmaPlot (version 10.1).

Results

Leaf morphology and structure

Both sides of leaves had stomata, and the *SD* of the abaxial surfaces were 1.5 times greater than those of the adaxial surfaces ($P < 0.001$), while the adaxial stomata lengths were approximately the same as the abaxial values (Table 1).

Transverse sections (Fig. 1) observed by light microscopy showed that the sorghum leaves were not differentiated into palisade and spongy tissues. However, the long axes of the adaxial mesophyll cells were longer than those of the abaxial mesophyll cells. There were many bulliform cells in the adaxial epidermis but none in the abaxial epidermis. The vascular bundles were surrounded by parenchymal cells, and the paths from bundle sheath cells to both surfaces were nearly the same. The sub-stomatal cavities of the adaxial and abaxial mesophyll cells were separated by compact mesophyll tissues (Fig. 1). Further analyses showed that the *ASC* and *CABM* of the abaxial surfaces were significantly greater than those of the adaxial surfaces ($P < 0.01$) (Table 1). Thus, the photosynthetic structures were asymmetric between the two surfaces of sorghum leaves.

Leaf optical properties

Leaf reflectance properties were similar on the adaxial and abaxial surfaces in sorghum over the wavelength range of 310–1,100 nm, whether the measurement was made on the adaxial or abaxial surface (Fig. 3). The absorbance rates of abaxial surfaces were a little greater than those of the adaxial surfaces over the wavelength range of 350–450 nm (Fig. 3). Consequently, there was no difference in the absorbance levels between the two surfaces of sorghum leaves over most of the investigated wavelengths.

Photochemical activities of the two sorghum leaf surfaces

The Chl a fluorescence in sorghum leaves demonstrated a typical transient polyphasic rise that included phases O, J, I, and P (Fig. 4). There was no obvious difference in the relative variable fluorescence intensity (V_t) between the two surfaces in the O-phase. The V_t was greater in the abaxial surfaces than in the adaxial surfaces, especially at = 0.3, 2, and 30 ms (Fig. 4). Thus, the PSII photochemistry of the adaxial surfaces was greater than that of the abaxial surfaces in sorghum leaves.

Dorsoventral asymmetry of photosynthesis in sorghum leaves in direct and diffuse light

The integrated P_n and G_s of adaxial surfaces illuminated with direct light were the greatest measured (Fig. 5A, B). The integrated P_n of the adaxial surface illuminated by direct light was significantly greater than that of the illuminated abaxial surface ($ASI = 2.83$), while the ASI of the integrated P_n in diffuse light dropped to 1.69 (Fig. 5A). In addition, the G_s changed as the P_n varied, and the ASI of the integrated G_s dropped from 2.23 in direct light to 1.67 in diffuse light (Fig. 5B). The C_i/C_a remained nearly constant under direct and diffuse light conditions, and the G_s was not affected by the C_i (Fig. 5C).

Effects of light properties on the stomatal sensitivity of sorghum leaves

The specific G_s increased as the intensities (PPFDs) of direct, diffuse, and self-transmitted light increased ($P < 0.001$) (Fig. 6). The specific G_s response to PPFD in sorghum leaves was not the same in adaxial and abaxial surfaces. In the direct light, the adaxial-specific G_s was greater than the abaxial-specific G_s at most light intensities (Fig. 6A), and the initial slope of the regression reflected that the stomatal sensitivity, as indicated by β_1, was significantly greater in the adaxial surfaces than in the abaxial surfaces ($P < 0.0001$) (Table 2). In the diffuse light, there was no obvious variation in the specific G_s (Fig. 6B), and the β_1 was not significant between the two surfaces (Table 2). However, in self-transmitted light, the adaxial-specific G_s was much lower than the abaxial-specific G_s (Fig. 6C), and the β_1 of the abaxial surfaces was 3.5 times greater than that of the adaxial surfaces ($P < 0.01$). The sensitivity of unit stoma, as indicated by β_2, of the adaxial surfaces was more than 2 times greater than that of the abaxial surfaces in direct light, while only 1.5 times greater in diffuse light (Table 2). Thus, light properties could change the variation of G_s and the stomatal sensitivities between the two sorghum leaf surfaces. The adaxial-specific AQY was significantly greater than the abaxial-specific AQY in both direct and diffuse light (Fig. 6D, E), but was significantly lower in self-transmitted light. The values of the specific AQYs in both surfaces were slightly greater in the direct light, but the ASI of AQY was nearly the same in direct and diffuse light. The *ASI* in self-transmitted light was very different from the values in direct and diffuse light (Fig. 6F).

Discussion

Morphology determined the photosynthetic asymmetry in sorghum leaves

There were significant morphological differences between the two surfaces that might cause photosynthetic asymmetry. Field environments change rapidly, and photosynthetic regulation needs to adapt to diverse light conditions because leaf photosynthesis is optimized through evolutionary fine tuning (Blankenship 1992). Leaf morphology and structure affect the photosynthetic capacity through *SD*, stomatal size and movement (Smith et al. 1998; Soares-Cordeiro et al. 2009), as well as mesophyll tissue (Earles et al. 2017; Oguchi et al. 2005) and vascular bundle (Oguchi et al. 2005; Soares et al. 2008) structures. Photosynthesis in C_4 species requires the collaboration of mesophyll cells and vascular bundle sheath cells, and the rate of gas exchange between these two types of cells plays a crucial role in photosynthesis (Sowinski et al. 2008). Thus, *ASC* and *CABM* are very important in determining P_n. The greater *SD*, *ASC* and *CABM* values in the abaxial leaf side compared with the adaxial side demonstrated that the gas-supply potential was greater in the former than the latter (Table 1). The photosynthetic capacities of C_4 isobilateral leaves exposed to direct light tends to be greater in abaxial surfaces, owing to the optimal tissue morphology and high *SD* (Driscoll et al. 2006; Long et al. 1989; Soares-Cordeiro et al. 2011; Soares et al. 2008). Therefore, the CO_2-supply capacity is lower in the adaxial sides of most C_4 isobilateral leaves, which seemed to conflict with greater integrated P_n from illuminated adaxial surfaces.

In addition to CO_2-supply capacity, light-use capacity, which involves light absorptance and photochemical activity, is also a key trait in determining the P_n. Leaf optical properties reflect the proportion of the light incident into leaves, which may be affected by surface properties, pigment content, and leaf morphology (Gorton et al. 2010). However, our data demonstrated that leaf absorptance and reflectance profiles were almost the same on the adaxial and abaxial surfaces in sorghum over most of the 310–1,100-nm wavelength range (Fig. 3). This result was consistent with other studies on isobilateral leaves (Brodersen & Vogelmann 2010; Delucia et al. 1991; Gorton et al. 2010; Soares et al. 2008). Therefore, the light energy absorbed by the leaf was nearly the same whether illuminated on the adaxial or abaxial surface. Chl a fluorescence reflects the primary photochemical reaction of PSII and the redox state of the photosynthetic apparatus; therefore, OJIP curves are usually efficient for investigating the potential of photochemistry (Strasser et al. 2004). Here, the OJIP curves showed that the light capture and primary photochemical activity were greater in the adaxial surfaces of sorghum leaves (Fig. 4). Thus, the photosynthetic activity of the adaxial side was greater, but it was not morphologically acclimated to the lower CO_2-supply capacity. In fact, the light environments of the two surfaces were more complex whether illuminated from the adaxial or abaxial side. For example, the light that illuminated the abaxial surface was rarely direct light but usually diffuse and self-transmitted light. In our experiments, the integrated P_n of illuminated adaxial leaf surfaces were greater than those of illuminated abaxial leaf surfaces (Fig. 5A), which was consistent with previous reports (Driscoll et al. 2006; Long et al. 1989; Soares-Cordeiro et al. 2011; Soares et al. 2008). The greater integrated P_n of illuminated adaxial leaf surfaces might be caused by the much greater abaxial-specific G_s in self-transmitted light (Fig. 6C), because the adaxial-specific G_s in self-transmitted light was very low when the abaxial surface was illuminated. However, the P_n of the illuminated adaxial surface was only slightly greater than that of the abaxial surface in diffuse light (Fig. 5A). Thus, the apparent conflicts with photosynthetic asymmetry in previous conclusions (Cheng et al. 2015; Earles et al. 2017; Long et al. 1989; Soares-Cordeiro et al. 2009; Urban et al. 2012; Wang et al. 2008) might be caused undistinguished light properties.

Dorsoventral asymmetry of photosynthesis in sorghum leaves changed with direct or diffuse light.

In bifacial leaves, there is a pronounced division of the physiological functions of photosynthesis between palisade and spongy cells (Gorton et al. 2010; Smith et al. 1997; Vogelmann et al. 1996). The palisade tissues function in light capture and photosynthetic reactions, and the spongy tissues function as channels for gas exchange. In our investigation, both the adaxial and abaxial sides of sorghum leaves have chlorophyll-containing mesophyll cells and gas channels, such as stomata and substomatal cavities (Fig. 1). In direct light, the integrated P_n of illuminated adaxial surfaces was more than two times greater than that of illuminated adaxial surfaces (*ASI* = 2.83) (Fig. 5A), which is consistent with other bifacial species (Moss 1964; Soares-Cordeiro et al. 2009). The integrated G_s could be considered as the sum of the specific G_s in incident (direct or diffuse) and self-transmitted light. Therefore, the greater adaxial-specific G_s in direct light (Fig. 6A) and abaxial-specific G_s in self-transmitted light (Fig. 6C) were the reasons for the greater integrated G_s of illuminated adaxial surfaces. In contrast, the abaxial-specific G_s was lower in direct light (Fig. 6A), and the adaxial specific G_s was less than 30% of the abaxial-specific G_s in self-transmitted light (Fig.6C), which caused the lower integrated P_n and G_s of abaxial surfaces illuminated with direct light. However, the *ASI* of P_n and G_s greatly decreased in diffuse light compared with in direct light (Fig. 5A, B). The specific G_s showed no variation between the two surfaces in diffuse light (Fig 6B), in spite of the different *SD*s (Table 1). In addition, the specific *AQY* in diffuse light was a little lower than in direct light (Fig. 6D, E) for both surfaces, which might explain the lower integrated P_n in diffuse light compared with in direct light. However, the difference in the specific *AQY* between the two surfaces was not changed in direct or diffuse light

(Fig. 6D, E), indicating that the asymmetry in photochemical processes was not changed in diffuse light. Therefore, diffuse light could weaken the dorsoventral asymmetry of photosynthesis by decreasing the asymmetric degree of the specific G_s in sorghum leaves.

Stomata, as a major pathway of the gas exchange between the atmosphere and the plant, respond to many environmental variables, such as light, CO_2 concentration, humidity, and water availability (Comstock 2002; Medlyn et al. 2001; Xu & Zhou 2008; Yu et al. 2004). Because the C_i was kept nearly constant during all of the experiments, the G_s was not affected by the C_i in the light-response curves. Light intensity is the most important ecological factor in stomata opening, even though stomata opening has been observed in the darkness in some species (Shimazaki et al. 2007). To investigate *in vivo* responses of stomata to light, independent of other factors, this study precisely controlled the other environmental factors, and the specific G_s displayed a substantial correlation with PPFD (Fig. 6). Although the *SD* was significantly greater in abaxial surfaces than in adaxial surfaces (Table 1), the abaxial-specific G_s was lower than the adaxial-specific G_s (Fig. 6A, B). The *SD* had a lower correlation with different specific G_s values. Stomatal sensitivity, as indicated by β_1, in leaf surfaces has an important role in G_s (Goh et al. 2002; Wang et al. 2008), and the differences in the specific β_1 between adaxial and abaxial surfaces were consistent with variations of G_s versus illuminating light (Table 2). The results in self-transmitted light were consistent with previous conclusions (Wang et al. 2008) (Fig. 6C), but the specific β_1 was greater in adaxial surfaces in direct light (Fig. 6A) and nearly the same in the two surfaces in diffuse light (Fig. 6B). Many *in vitro* investigations indicated that the stomatal sensitivity to light is greater in the abaxial surface than adaxial surface (Goh et al. 2002; Wang et al. 2008). The different conclusion might be caused by the distribution of the stomatal apparatus (stomatal size and *SD*) on the two surfaces and the incident light, which might have considerable impacts on β_1. In our investigation, the stomata in both leaf surfaces were similar in size (Table 1), and β_1 had strong correlations with *SD*s of adaxial (Fig. 7A) and abaxial (Fig. 7B) surfaces from different sorghum leaves. Hence, although our results showed no direct effect of *SD* on specific G_s, the specific β_1 could be affected by the *SD*.

Although the specific β_1 showed a positive correlation with *SD*, the specific β_1 was still lower on the abaxial surface, which had the greater *SD* (Fig. 7; Tables 1 and 2). Variations in β_2 indicated that single stomatal sensitivities were greater on the adaxial than abaxial surfaces both in direct and diffuse light (Table 2). The greater *SD* could partly compensate for the low specific β_2 of the abaxial surface but not the low specific β_1. However, in diffuse light, the adaxial-specific β_2 decreased, while the abaxial-specific β_2 showed no significant variation, indicating that the diffuse light decreased the degree of difference of the specific β_2. This might be a reason for the weakened asymmetry of G_s in the diffuse light. Stomatal movement should be important for specific β_2 since the stomatal sizes were nearly the same (Table 1). Changes in the turgor pressure and proton potential of guard cells and mesophyll cells causes the movement of guard cells, as well as stomatal movement (Wang et al. 2008). The bulliform cells with a high content of water were observed in adaxial surfaces but not abaxial surfaces(Fig. 1), and these cells have a significant role in the curling of isobilateral leaves because of their high sensitivity to turgor pressure (Smith 2008). We hypothesized that the bulliform cells could increase the response of single stoma to light. Our OJIP curves demonstrated that the photochemical activities of epidermal cells in adaxial surfaces were greater than those in abaxial surfaces (Fig. 4), which means that the plasma membrane H^+-ATPases accumulated more rapidly in adaxial epidermal cells, driving stomatal movement. Thus, our results provide evidence for a greater adaxial-specific β_2 in sorghum leaves.

In self-transmitted light, the β_2 in the abaxial surface was significantly greater than in the adaxial surface, which was in contrast with the values in direct and diffuse light (Table 2). The spectral composition of self-transmitted light differs greatly from those of direct and diffuse light. When the leaf was illuminated with polychromatic light, mesophyll cells of the illuminated side selectively absorbed red and blue light, while the remaining light that arrived at the opposite side consisted mostly of green light. Green light might be more efficient for stomata opening (Sun et al. 1998; Terashima et al. 2009; Wang et al. 2008). Under field conditions, the level of diffuse light is far less than that of direct light, and the abaxial surfaces of sorghum leaves mainly use self-transmitted light for photosynthesis (Wang et al. 2012). Wang et al. (2008) showed that the self-transmitted and leaf-transmitted light were more efficient in opening the abaxial stomata of sunflower leaves than direct light, which was explained by the mostly green light in transmitted light (Falcioni et al. 2017; Wang et al. 2008). Consequently, discrepancies in the specific β_1 and β_2 in self-transmitted light might be caused by variations in the spectral composition of incident light. Estimations of leaf stomatal sensitivities should take leaf surface traits and the light spectrum into consideration.

Regulation of dorsoventral asymmetry in sorghum leaves

The photosynthesis of bifacial leaves occurs mostly in palisade tissues, and the photosynthetic rate depends on the thickness of mesophyll tissues, in particular palisade tissues, because an increase in thickness increases light retention and absorption (Delaney & Dobrenz 1974; Smith et al. 1997). The leaf thickness and specific leaf weight are highly correlated with photosynthetic capacity (John et al. 2017; Poorter et al. 2009). For an isobilateral leaf, both sides with palisade tissues or isodiametric cells, which increase light retention and absorption. Sorghum is native to xerothermic areas, and the high light of midday may cause photoinhibition in leaves (Jiang et al. 2011). In isobilateral leaves, shaded adaxial mesophyll tissues decrease the probability of photoinhibition occurring in the abaxial side, and the greater photosynthetic rate of the abaxial side in a higher level of self-transmitted light could compensate for the loss of CO_2 assimilation owing to stomata closure on the adaxial surface when exposed to extremely high radiation. Thus, the whole isobilateral leaf can maintain a high level of CO_2 assimilation under excessive light, which has been reported previously (Jiang et al. 2011; Long et al. 1989; Smith 2008).

In open field conditions, the adaxial stomata are exposed to more direct radiation, while the abaxial stomata are shaded by the adaxial side and receive light transmitted through the mesophyll and scattered from the surroundings(Wang et al. 2008; Williams et al. 2014). Under clear sky conditions, 80%–90% visible light can be absorbed by leaves, with 10% being reflected. The remaining 5% is transmitted (Gorton et al. 2010). Thus, the diffuse light surrounding abaxial surfaces is much less than the direct light illuminating the adaxial surfaces (Wang et al. 2016). Here, the greatest P_n occurred in adaxial surfaces illuminated by direct light and the lowest P_n occurred in abaxial surfaces illuminated by direct light (Fig. 5A). Thus, we hypothesize that the adaxial surfaces might mainly use direct light, while the abaxial surfaces might mainly use self-transmitted light, but not diffuse light, for photosynthesis under clear sky conditions. However, under cloudy sky conditions, the diffuse light surrounding adaxial and abaxial surfaces were approximately equal, although the light could be affected by planting density and cloud cover. The degree of asymmetry of P_n showed decreased in diffuse light, which was consistent with the cloudy sky conditions.

Conclusions

Dorsoventral asymmetry in morphology and anatomy resulted in different photosynthetic capacities. The variations in photochemical traits and *SD* affect the stomatal sensitivity in sorghum leaves. The variation of G_s between the two surfaces was not correlated with *SD* but with the specific stomatal sensitivity. In direct light, the integrated P_n was significantly different between illuminated adaxial and abaxial surfaces, but diffuse light weakened the photosynthetic asymmetry because there was no significance difference in the specific G_s between the two surfaces. The photosynthetic asymmetry in sorghum leaves adapts to high light radiation and the changing light properties of different sky conditions. This research showed that the size, shape, and arrangement of epidermal and mesophyll cells had direct effects on P_n and G_s under direct and diffuse light conditions, which could targeted in plant breeding to increase productivity in the increasingly diffuse light of the future.

Appendices:

Appendix A: The spectra composition of sunlight, LED, and LED light penetrated a transparent film (TF+LED) over the wavelength of 400 – 800 nm

Appendix B: The diffuse index (*DI*) of the direct light (closed circles and solid line) and diffuse light (closed triangles and dotted line) in the experiments.

Appendix C: Effects of covering transparent film (TF) on the reflectance and absorbance rates (310-1100 nm) of adaxial surfaces (A) and abaxial surfaces (B) in sorghum leaves.

Acknowledgements

We thank Pro. C D Jiang for the assistance with the experiments in the Institute of Botany (CAS), and International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript.

References

Blankenship RE. 1992. Origin and early evolution of photosynthesis. *Photosynth Res* 33:91-111. 10.1007/bf00039173

Brient F, and Bony S. 2013. Interpretation of the positive low-cloud feedback predicted by a climate model under global warming. *Climate Dynamics* 40:2415-2431. 10.1007/s00382-

011-1279-7

Brodersen CR, and Vogelmann TC. 2010. Do changes in light direction affect absorption profiles in leaves? *Functional Plant Biology* 37:403-412. 10.1071/fp09262

Brodersen CR, Vogelmann TC, Williams WE, and Gorton HL. 2008. A new paradigm in leaf-level photosynthesis: direct and diffuse lights are not equal. *Plant Cell and Environment* 31:159-164. 10.1111/j.1365-3040.2007.01751.x

Cheng SJ, Bohrer G, Steiner AL, Hollinger DY, Suyker A, Phillips RP, and Nadelhoffer KJ. 2015. Variations in the influence of diffuse light on gross primary productivity in temperate ecosystems. *Agricultural and Forest Meteorology* 201:98-110. https://doi.org/10.1016/j.agrformet.2014.11.002

Comstock JP. 2002. Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. *Journal of Experimental Botany* 53:195-200. 10.1093/jexbot/53.367. 195

Coupe SA, Palmer BG, Lake JA, Overy SA, Oxborough K, Woodward FI, Gray JE, and Quick WP. 2006. Systemic signalling of environmental cues in Arabidopsis leaves. *Journal of Experimental Botany* 57:329-341. 10.1093/jxb/erj033

Delaney RH, and Dobrenz AK. 1974. Morphological and anatomical features of Alfafa leaves as related to CO2 exhange. *Crop Science* 14:444-447.

Delucia EH, Shenoi HD, Naidu SL, and Day TA. 1991. Photosynthetic symmetry of sun and shade leaves of different orientations. *Oecologia* 87:51-57.

Driscoll SP, Prins A, Olmos E, Kunert KJ, and Foyer CH. 2006. Specification of adaxial and abaxial stomata, epidermal structure and photosynthesis to CO2 enrichment in maize leaves. *Journal of Experimental Botany* 57:381-390. 10.1093/jxb/erj030

Earles JM, Théroux-Rancourt G, Gilbert ME, McElrone AJ, and Brodersen CR. 2017. Excess Diffuse Light Absorption in Upper Mesophyll Limits CO(2) Drawdown and Depresses Photosynthesis. *Plant Physiology* 174:1082-1096. 10.1104/pp.17.00223

Falcioni R, Moriwaki T, Bonato CM, de Souza LA, Nanni MR, and Antunes WC. 2017. Distinct growth light and gibberellin regimes alter leaf anatomy and reveal their influence on leaf optical properties. *Environmental and Experimental Botany* 140:86-95. https://doi.org/10.1016/j.envexpbot.2017.06.001

Gamon JA, and Surfus JS. 1999. Assessing leaf pigment content and activity with a reflectometer. *New Phytologist* 143:105-117. 10.1046/j.1469-8137.1999.00424.x

Goh CH, Hedrich R, and Nam HG. 2002. Evidence for the functional organization of chloroplasts in adaxial guard cells of Vicia faba leaves by single cell analysis. *Plant Science* 162:965-972. 10.1016/s0168-9452(02)00047-x

Gorton HL, Brodersen CR, Williams WE, and Vogelmann TC. 2010. Measurement of the Optical Properties of Leaves Under Diffuse Light. *Photochemistry and Photobiology* 86:1076-1083. 10.1111/j.1751-1097.2010.00761.x

Gu LH, Baldocchi DD, Wofsy SC, Munger JW, Michalsky JJ, Urbanski SP, and Boden TA. 2003. Response of a deciduous forest to the Mount Pinatubo eruption: Enhanced photosynthesis. *Science* 299:2035-2038. 10.1126/science.1078366

Hughes NM, Carpenter KL, Cook DK, Keidel TS, Miller CN, Neal JL, Sanchez A, and Smith WK. 2015. Effects of cumulus clouds on microclimate and shoot-level photosynthetic gas exchange in Picea engelmannii and Abies lasiocarpa at treeline, Medicine Bow Mountains, Wyoming, USA. *Agricultural and Forest Meteorology* 201:26-37. 10.1016/j.agrformet.2014.10.012

Jiang C-D, Wang X, Gao H-Y, Shi L, and Chow WS. 2011. Systemic Regulation of Leaf Anatomical Structure, Photosynthetic Performance, and High-Light Tolerance in Sorghum. *Plant Physiology* 155:1416-1424. 10.1104/pp.111.172213

John GP, Scoffoni C, Buckley TN, Villar R, Poorter H, and Sack L. 2017. The anatomical and compositional basis of leaf mass per area. *Ecology Letters* 20:412-425. 10.1111/ ele.12739

Knohl A, and Baldocchi DD. 2008. Effects of diffuse radiation on canopy gas exchange processes in a forest ecosystem. *Journal of Geophysical Research: Biogeosciences* 113:n/a-n/a. 10.1029/2007JG000663

Li T, and Yang Q. 2015. Advantages of diffuse light for horticultural production and perspectives for further research. *Frontiers in plant science* 6:704-704. 10.3389/fpls.2015.00704

Long SP, Farage PK, Bolharnordenkampf HR, and Rohrhofer U. 1989. Separating the contribution of the upper and lower mesophyll to photosynthesis in Zea mays L. leaves. *Planta* 177:207-216. 10.1007/bf00392809

Medlyn BE, Barton CVM, Broadmeadow MSJ, Ceulemans R, De Angelis P, Forstreuter M, Freeman M, Jackson SB, Kellomäki S, Laitat E, Rey A, Roberntz P, Sigurdsson BD, Strassemeyer J, Wang K, Curtis PS, and Jarvis PG. 2001. Stomatal conductance of forest species after long-term exposure to elevated CO2 concentration: a synthesis. *New Phytologist* 149:247-264. 10.1046/j.1469-8137.2001.00028.x

Mercado LM, Bellouin N, Sitch S, Boucher O, Huntingford C, Wild M, and Cox PM. 2009. Impact of changes in diffuse radiation on the global land carbon sink. *Nature* 458:1014-U1087. 10.1038/nature07949

Morison JIL, and Lawson T. 2007. Does lateral gas diffusion in leaves matter? *Plant Cell and Environment* 30:1072-1085. 10.1111/j.1365-3040.2007.01685.x

Moss DN. 1964. Optimum lighting of leaves. *Crop Sci* 4:131-136.

Oguchi R, Hikosaka K, and Hirose T. 2005. Leaf anatomy as a constraint for photosynthetic acclimation: differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. *Plant Cell and Environment* 28:916-927. 10.1111/j.1365-3040.2005.01344.x

Oliveira PJC, Davin EL, Levis S, and Seneviratne SI. 2011. Vegetation-mediated impacts of trends in global radiation on land hydrology: a global sensitivity study. *Global Change Biology* 17:3453-3467. 10.1111/j.1365-2486.2011.02506.x

Poorter H, Niinemets Ü, Poorter L, Wright IJ, and Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* 182:565-588. 10.1111/j.1469-8137.2009.02830.x

Reinhardt K, and Smith WK. 2008. Impacts of cloud immersion on microclimate, photosynthesis and water relations of Abies fraseri (Pursh.) Poiret in a temperate mountain cloud forest. *Oecologia* 158:229-238. 10.1007/s00442-008-1128-5

Reinhardt K, Smith WK, and Carter GA. 2010. Clouds and cloud immersion alter photosynthetic light quality in a temperate mountain cloud forest. *Botany-Botanique* 88:462-470. 10.1139/b10-008

Shimazaki K-i, Doi M, Assmann SM, and Kinoshita T. 2007. Light regulation of stomatal movement. *Annu Rev Plant Biol* 58:219-247.

Smith WK. 2008. C-4 leaf curling coupling incident light, stomatal and photosynthetic asymmetries. *New Phytologist* 177:5-8.

Smith WK, Bell DT, and Shepherd KA. 1998. Associations between leaf structure, orientation, and sunlight exposure in five Western Australian communities. *American Journal of Botany* 85:56-63. 10.2307/2446554

Smith WK, Vogelmann TC, DeLucia EH, Bell DT, and Shepherd KA. 1997. Leaf form and photosynthesis. *Bioscience* 47:785-793. 10.2307/1313100

Soares-Cordeiro AS, Driscoll SP, Arrabaca MC, and Foyer CH. 2011. Dorsoventral variations in dark chilling effects on photosynthesis and stomatal function in Paspalum dilatatum leaves. *Journal of Experimental Botany* 62:687-699. 10.1093/jxb/erq302

Soares-Cordeiro AS, Driscoll SP, Pellny TK, Olmos E, Arrabaca MC, and Foyer CH. 2009. Variations in the dorso-ventral organization of leaf structure and Kranz anatomy coordinate the control of photosynthesis and associated signalling at the whole leaf level in monocotyledonous species. *Plant Cell and Environment* 32:1833-1844. 10.1111/j.1365-3040.2009.02043.x

Soares AS, Driscoll SP, Olmos E, Harbinson J, Arrabaca MC, and Foyer CH. 2008. Adaxial/abaxial specification in the regulation of photosynthesis and stomatal opening with respect to light orientation and growth with CO2 enrichment in the C4 species Paspalum dilatatum. *New Phytologist* 177:186-198. 10.1111/j.1469-8137.2007.02218.x

Sowinski P, Szczepanik J, and Minchin PEH. 2008. On the mechanism of C(4) photosynthesis intermediate exchange between Kranz mesophyll and bundle sheath cells in grasses. *Journal of Experimental Botany* 59:1137-1147. 10.1093/jxb/ern054

Strada S, and Unger N. 2016. Potential sensitivity of photosynthesis and isoprene emission to direct radiative effects of atmospheric aerosol pollution. *Atmos Chem Phys* 16:4213-4234. 10.5194/acp-16-4213-2016

Strasser RJ, Tsimilli-Michael M, and Srivastava A. 2004. Analysis of the Chlorophyll a Fluorescence Transient. In: Papageorgiou GC, and Govindjee, eds. *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. Dordrecht: Springer Netherlands, 321-362.

Sun J, Nishio JN, and Vogelmann TC. 1998. Green Light Drives CO2 Fixation Deep within Leaves1. *Plant and Cell Physiology* 39:1020-1026. 10.1093/oxfordjournals.pcp.a029298

Terashima I, Fujita T, Inoue T, Chow WS, and Oguchi R. 2009. Green Light Drives Leaf Photosynthesis More Efficiently than Red Light in Strong White Light: Revisiting the Enigmatic Question of Why Leaves are Green. *Plant and Cell Physiology* 50:684-697.

10.1093/pcp/pcp034

Terashima I, and Saeki T. 1983. Light Environment within a Leaf I. Optical Properties of Paradermal Sections of Camellia Leaves with Special Reference to Differences in the Optical Properties of Palisade and Spongy Tissues. *Plant and Cell Physiology* 24:1493-1501. 10.1093/oxfordjournals.pcp.a076672

Thain JF. 1983. Curvature correction factors in the measurement of cell-surface areas in plant tissues. *Journal of Experimental Botany* 34:87-94. 10.1093/jxb/34.1.87

Urban O, Klem K, Ac A, Havrankova K, Holisova P, Navratil M, Zitova M, Kozlova K, Pokorny R, Sprtova M, Tomaskova I, Spunda V, and Grace J. 2012. Impact of clear and cloudy sky conditions on the vertical distribution of photosynthetic CO2 uptake within a spruce canopy. *Functional Ecology* 26:46-55. 10.1111/j.1365-2435.2011.01934.x

Vogelmann TC. 1993. Plant tissue optics. *Annu Rev Plant Physiol Plant Mol Biol* 44:233-251.

Vogelmann TC, and Björn LO. 1984. Measurement of light gradients and spectral regime in plant tissue with a fiber optic probe. *Physiologia Plantarum* 60:361-368. doi:10.1111/j.1399-3054.1984.tb06076.x

Vogelmann TC, Bornman JF, and Yates DJ. 1996. Focusing of light by leaf epidermal cells. *Physiologia Plantarum* 98:43-56.

Wang X, Zhou H, Han F, Li Y, and Hu D. 2016. Dynamic spatial patterns of leaf traits affect total respiration on the crown scale. *Scientific Reports* 6:26675. 10.1038/srep26675. https://www.nature.com/articles/srep26675#supplementary-information

Wang XL, Li ZQ, Jiang CD, Shi L, Xing Q, and Liu LA. 2012. Effects of Diffuse and Direct Light on Photosynthetic Function in Sorghum Leaf. *Acta Agronomica Sinica* 2012:1452-1459. (In Chinese with English Abstract)

Wang Y, Noguchi K, and Terashima I. 2008. Distinct light responses of the adaxial and abaxial stomata in intact leaves of Helianthus annuus L. *Plant Cell and Environment* 31:1307-1316. 10.1111/j.1365-3040.2008.01843.x

Williams M, Rastetter EB, Van der Pol L, and Shaver GR. 2014. Arctic canopy photosynthetic efficiency enhanced under diffuse light, linked to a reduction in the fraction of the canopy in deep shade. *New Phytologist* 202:1266-1276. 10.1111/nph.12750

Wu B-J, Chow WS, Liu Y-J, Shi L, and Jiang C-D. 2014. Effects of stomatal development on stomatal conductance and on stomatal limitation of photosynthesis in Syringa oblata and Euonymus japonicus Thunb. *Plant Science* 229:23-31. 10.1016/j.plantsci. 2014.08.009

Xu Z, and Zhou G. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany* 59:3317-3325. 10.1093/jxb/ern185

Yu Q, Zhang Y, Liu Y, and Shi P. 2004. Simulation of the Stomatal Conductance of Winter Wheat in Response to Light, Temperature and CO2 Changes. *Annals of Botany* 93:435-441. 10.1093/aob/mch023

Figure Legends:

Fig. 1 Microscopic photographs of cross-sections in sorghum leaf. a: motor cell; b: stomatal cavity at the adaxial side of leaf; c: stomatal cavity at the abaxial side of leaf; d: bundle sheath cells; e: mesophyll cells; x: xylem; p: phloem; bar = 20 μm.

Fig. 2 Design of measuring the integrated and specific gas exchanges of a leaf in direct, diffuse and self-transmitted light.

Fig. 3 Changes in leaf reflectance (closed symbols) and absorbance (open symbols) rate over the wavelength of 310 - 1100 nm in sorghum. The light is oriented either to adaxial surfaces (square and rhombus) or to the abaxial surfaces (circle and triangle).

Fig. 4 The rise kinetics of chlorophyll a fluorescence transient (V_t: relative variable fluorescence plotted on logarithmic time scale) in adaxial (black circles) and abaxial (white circles) surfaces of sorghum leaves.

Fig. 5 Variations of integrated gas exchanges of illuminating adaxial (black bars) and abaxial (white bars) leaf surfaces respectively in direct and diffuse light. A for photosynthetic rate (P_n), B for stomatal conductance (G_s) and C for ratio between the intercellular and the atmospheric CO_2 concentrations (C_i/C_a). Values in parentheses are the ratios of abaxial to adaxial values (*ASI*) of P_n, G_s, and C_i/C_a in direct and diffuse light. Data are the mean ± SE of 24 plants. Bars superscripted by different letters are significantly different, based on one-way ANOVA ("*" for P=0.05, "**" for P = 0.01), NA, no significant between adaxial and abaxial values.

Fig. 6 Light responses of the specific stomatal conductance (G_s) in direct (A), diffuse (B) and self-transmitted (C) light and apparent quantum yield (*AQY*) of the adaxial (black circles) or abaxial (white circles) surfaces. Values in parentheses are the ratios of abaxial to adaxial values (*ASI*) of *AQY*. Data are the mean ± SE of 24 plants. Bars superscripted by different letters are significantly different, based on one-way ANOVA ("*" for P=0.05, "**" for P = 0.01), between adaxial and abaxial values.

Fig. 7 The correlations of stomatal sensitivity (β_1) in direct (circles), diffuse (triangles), and self-transmitted (squares) light with *SD* in adaxial (A) and abaxial (B) surface of sorghum leaves. The regression lines are as follows: y = 0.0028 x + 0.0251 ($R^2 = 0.864$, P = 0.003) for adaxial β_1 versus the direct light, y = 0.0027 x + 0.084 ($R^2 = 0.971$, P < 0.0001) for adaxial β_1 versus the diffuse light, y = 0.0026 x – 0.0181 ($R^2 = 0.841$, P =0.032) for adaxial β_1 versus the self-transmitted light, y = 0.0010 x - 0.0305 ($R^2 = 0.699$, P < 0.0001) for abaxial β_1 versus the direct light, y = 0.0012 x – 0.0212 ($R^2 = 0.756$, P < 0.002) for abaxial β_1 versus the diffuse light, y = 0.0025 x - 0.0182 ($R^2 = 0.901$, P < 0.0001) for abaxial β_1 versus the self-transmitted light. N = 90.

Table 1(on next page)

Variations of stomatal density (SD), long axis of stomatal aperture, area of sub-stomatal cavity (ASC), contact area between bundle sheath and mesophyll cells (CABM) between the adaxial and abaxial sides in sorghum.

All the parameters were calculated from samples with number of N in tables.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27267v1 | CC BY 4.0 Open Access | rec: 9 Oct 2018, publ: 9 Oct 2018

	SD (number of stomata mm^{-2})	Long axis of stomatal aperture (μm)	*ASC* (μm^2)	*CABM*（m^2 m^{-2}）
Adaxial	78.6±2.10	31.2±2.31	76.7±7.05	2.12±0.20
Abaxial	126.5±3.30	30.3±2.18	262.3±23.15	2.53±0.30
N	108	120	20	20
P-value	<0.001	0.056	<0.01	<0.01
Ratio(adaxial/abaxial)	0.62	1.02	0.29	0.84

Table 2(on next page)

The slope (β_1), Y-intercept (*b*) of regression lines, and the unit stomatal insensitivity to light intensity of adaxial and abaxial surface in direct, diffuse, and self-transmitted light.

The determination ratio (R^2), and P-value of ANOVA of regression equation $y = \beta_1 x + b$ are shown in the table. N=24. The statistical analysis ($P < 0.01$) compares data for β_1 and β_2 separately, the different letters representing significant differences of six cases from three light properties and two surfaces.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27267v1 | CC BY 4.0 Open Access | rec: 9 Oct 2018, publ: 9 Oct 2018

Light property	Leaf surface	β_1	β_2 (10^{-3})	b	P value	R^2
Direct light	Adaxial	0.187b	2.384a	29.41	<0.0001	0.973
	Abaxial	0.144d	1.134d	32.37	<0.0001	0.970
Diffuse light	Adaxial	0.158c	2.008b	35.62	<0.0001	0.972
	Abaxial	0.174bc	1.377d	32.52	<0.0001	0.970
Self-transmitted light	Adaxial	0.063e	0.800e	27.38	0.0004	0.974
	Abaxial	0.230a	1.814c	38.16	<0.0001	0.935

Figure 1(on next page)

Microscopic photographs of cross-sections in sorghum leaf.

a: motor cell; b: stomatal cavity at the adaxial side of leaf; c: stomatal cavity at the abaxial side of leaf; d: bundle sheath cells; e: mesophyll cells; x: xylem; p: phloem; bar = 20 µm.

Figure 1

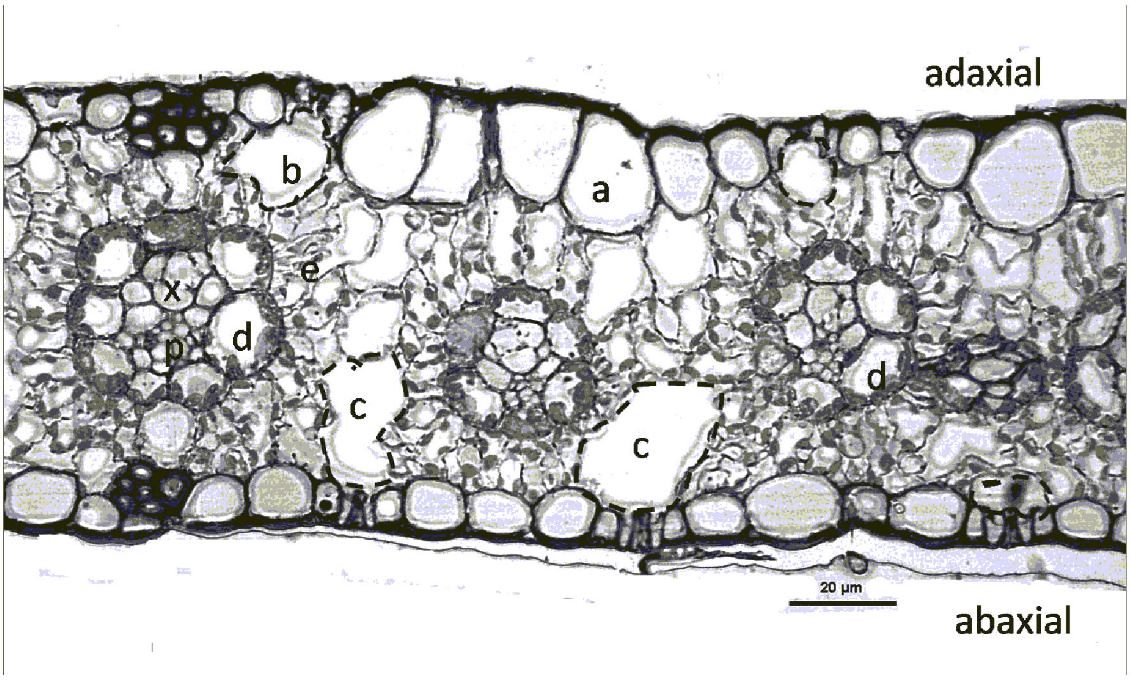


Figure 2(on next page)

Design of measuring the integrate and specific gas exchanges of a leaf in direct, diffuse and self-transmitted light.

Figure 2

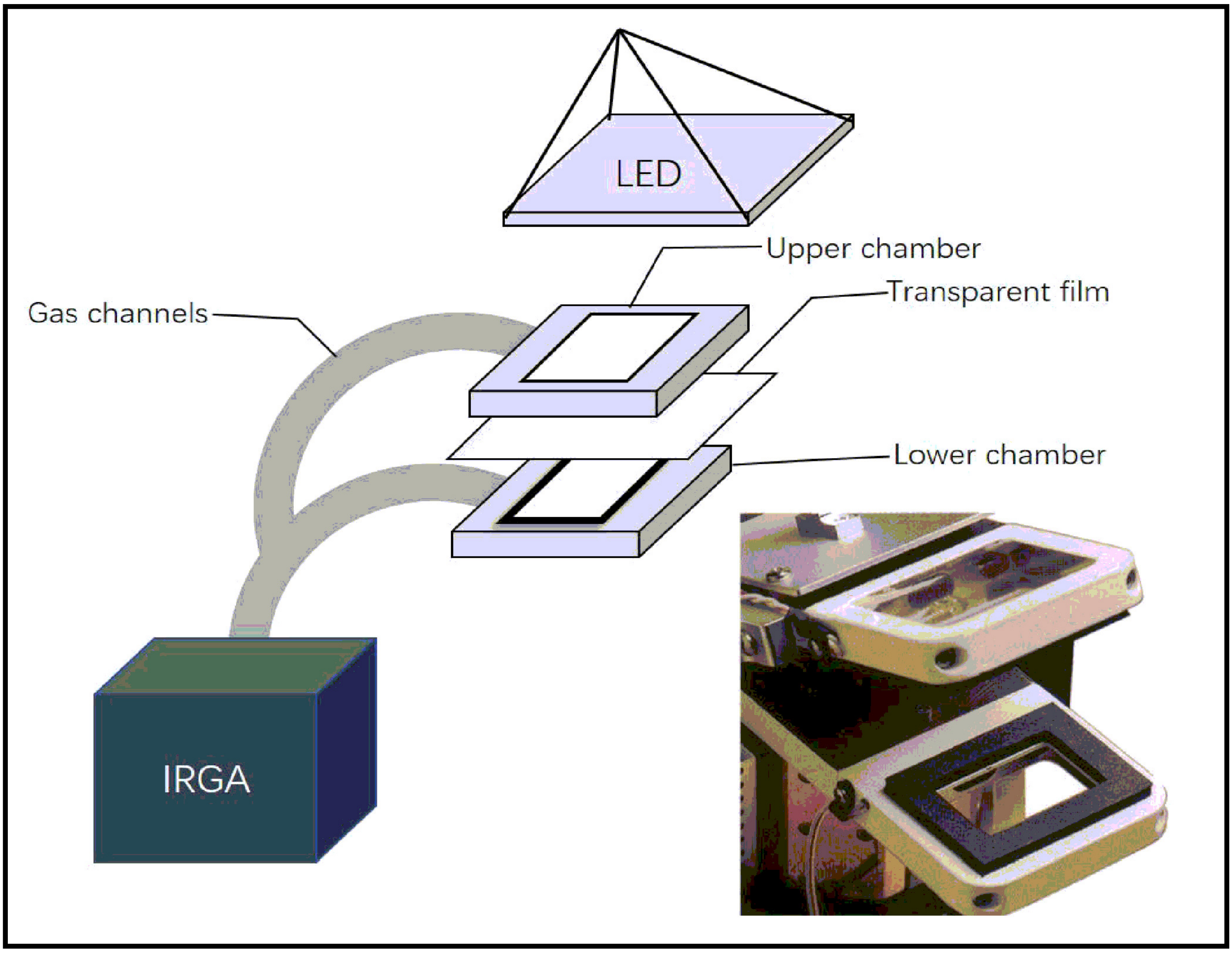

LED
Upper chamber
Transparent film
Gas channels
Lower chamber
IRGA

Figure 3(on next page)

Changes in leaf reflectance (closed symbols) and absorbance (open symbols) rate over the wavelength of 310 - 1100 nm in sorghum.

The light is oriented either to adaxial surfaces (square and rhombus) or to the abaxial surfaces (circle and triangle).

Figure 3

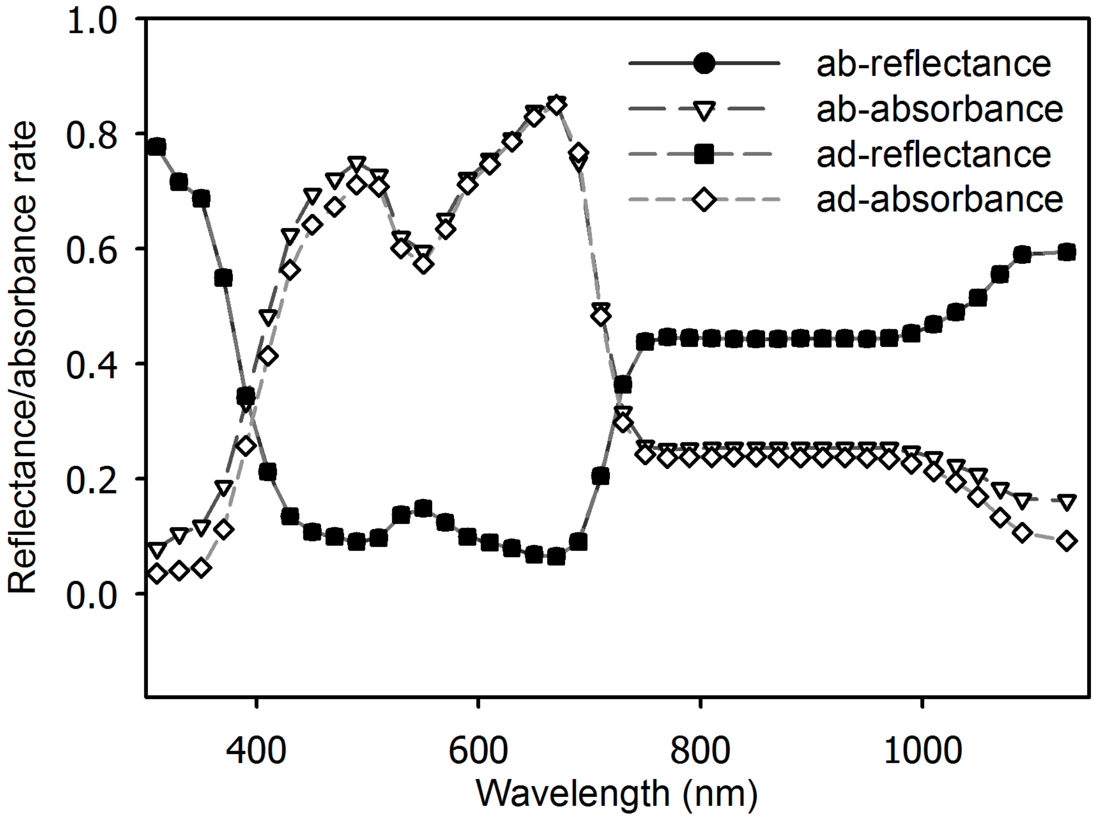
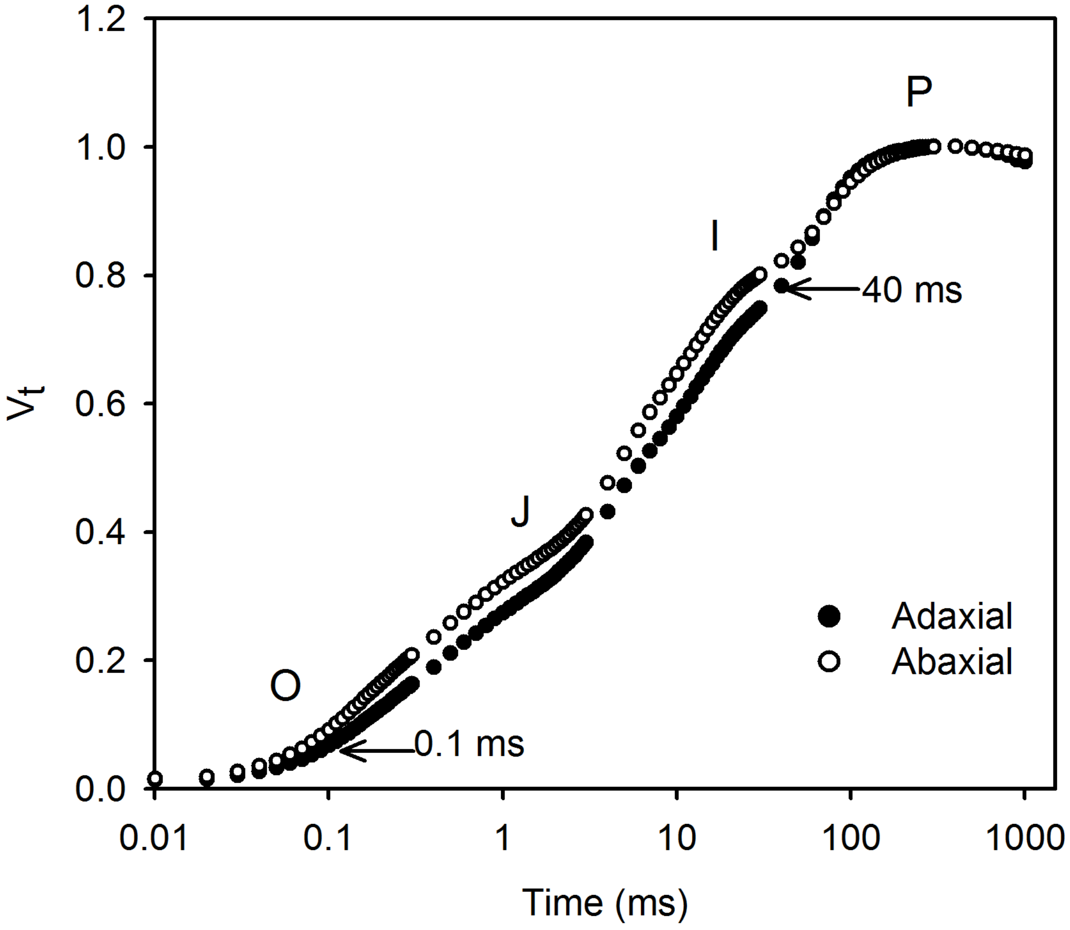

1.0
0.8
0.6
0.4
0.2
0.0
Reflectance/absorbance rate
400
600
800
1000
Wavelength (nm)
ab-reflectance
ab-absorbance
ad-reflectance
ad-absorbance

Figure 4(on next page)

The rise kinetics of chlorophyll a fluorescence transient (V_t: plotted on logarithmic time scale) in adaxial (black circles) and abaxial (white circles) surfaces of sorghum leaves.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27267v1 | CC BY 4.0 Open Access | rec: 9 Oct 2018, publ: 9 Oct 2018

Figure 4



PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27267v1 | CC BY 4.0 Open Access | rec: 9 Oct 2018, publ: 9 Oct 2018

Figure 5(on next page)

Variations of integrated gas exchanges of illuminating adaxial (black bars) and abaxial (white bars) leaf surfaces respectively in direct and diffuse light.

A for photosynthetic rate (P_n), B for stomatal conductance (G_s) and C for ratio between the intercellular and the atmospheric CO_2 concentrations (C_i/C_a). Values in parentheses are the ratios of abaxial to adaxial values (*ASI*) of P_n, G_s, and C_i/C_a in direct and diffuse light. Data are the mean ± SE of 24 plants. Bars superscripted by different letters are significantly different, based on one-way ANOVA ("*" for P=0.05, "**" for P = 0.01), NA, no significant between adaxial and abaxial values.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27267v1 | CC BY 4.0 Open Access | rec: 9 Oct 2018, publ: 9 Oct 2018

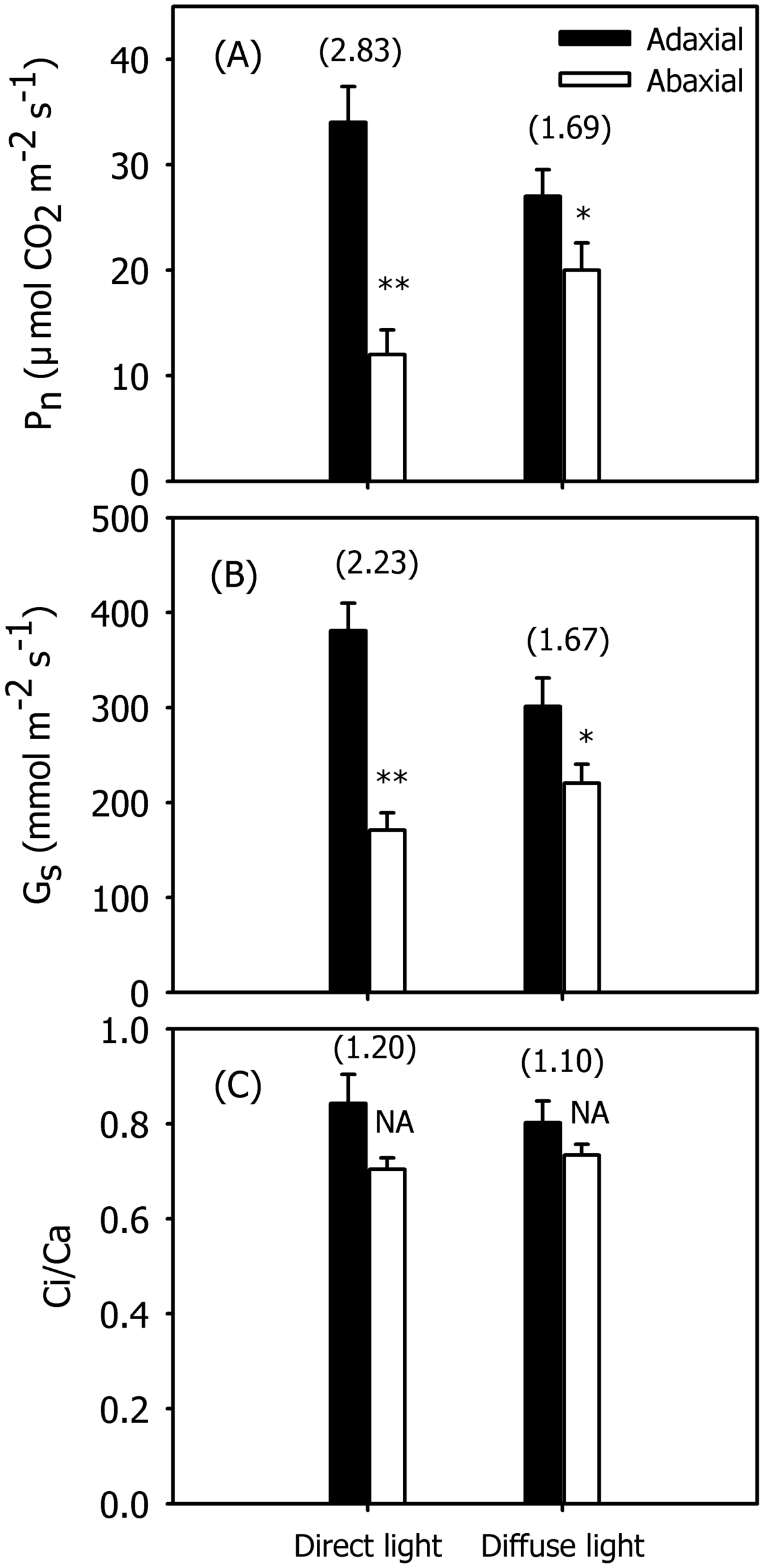

(A)
P_n (µmol CO_2 m^{-2} s^{-1})
0
10
20
30
40
Adaxial
Abaxial
(2.83)
**
(1.69)
*
(B)
G_s (mmol m^{-2} s^{-1})
0
100
200
300
400
500
(2.23)
**
(1.67)
*
(C)
Ci/Ca
0.0
0.2
0.4
0.6
0.8
1.0
(1.20)
NA
(1.10)
NA
Direct light
Diffuse light

Figure 6(on next page)

Light responses of the specific stomatal conductance (G_s) in direct (A), diffuse (B) and self-transmitted (C) light and apparent quantum yield (*AQY*) of the adaxial (black circles) or abaxial (white circles) surfaces.

Values in parentheses are the ratios of abaxial to adaxial values (*ASI*) of *AQY*. Data are the mean ± SE of 24 plants. Bars superscripted by different letters are significantly different, based on one-way ANOVA ("*" for P=0.05, "**" for P = 0.01), between adaxial and abaxial values.

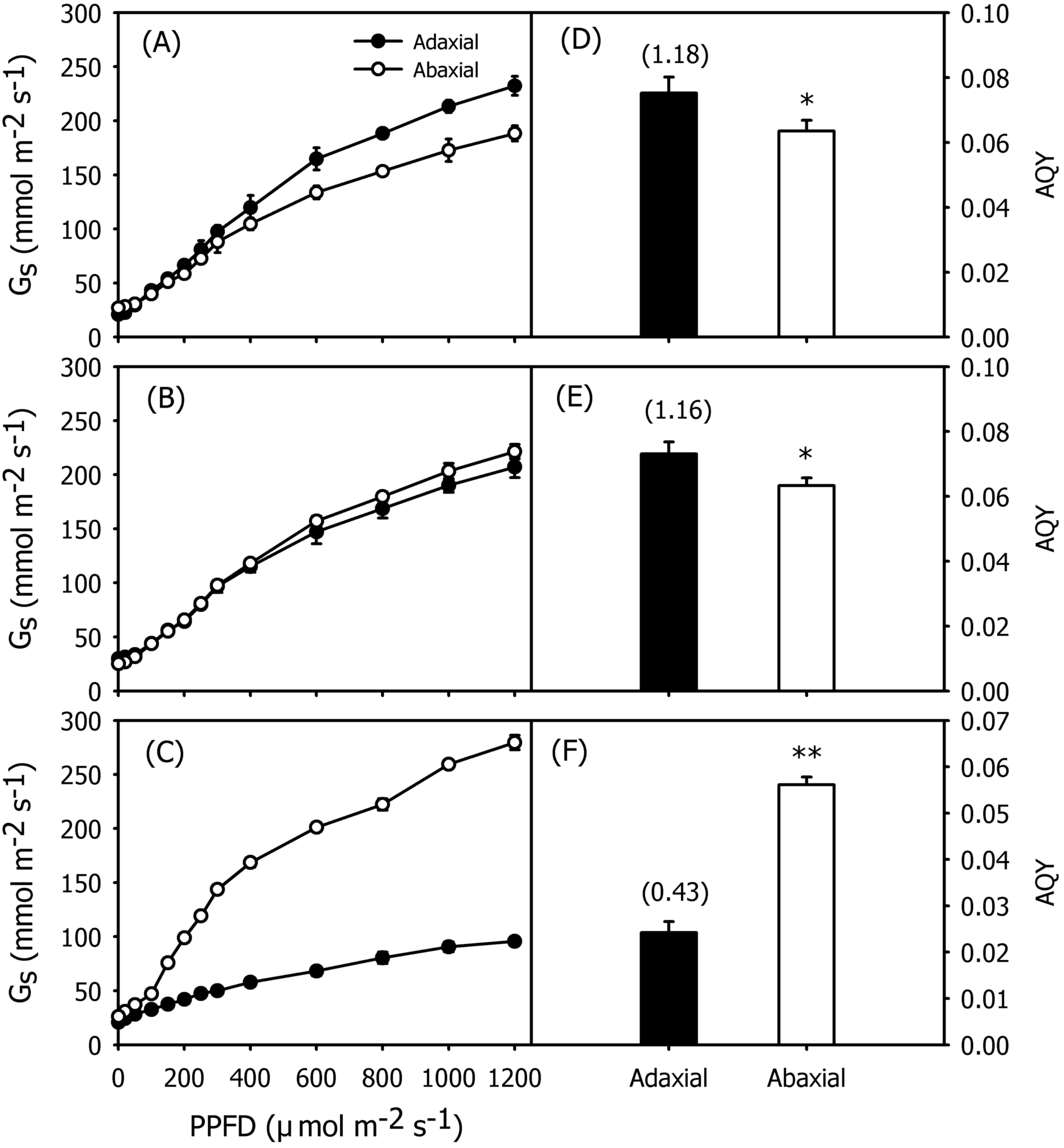
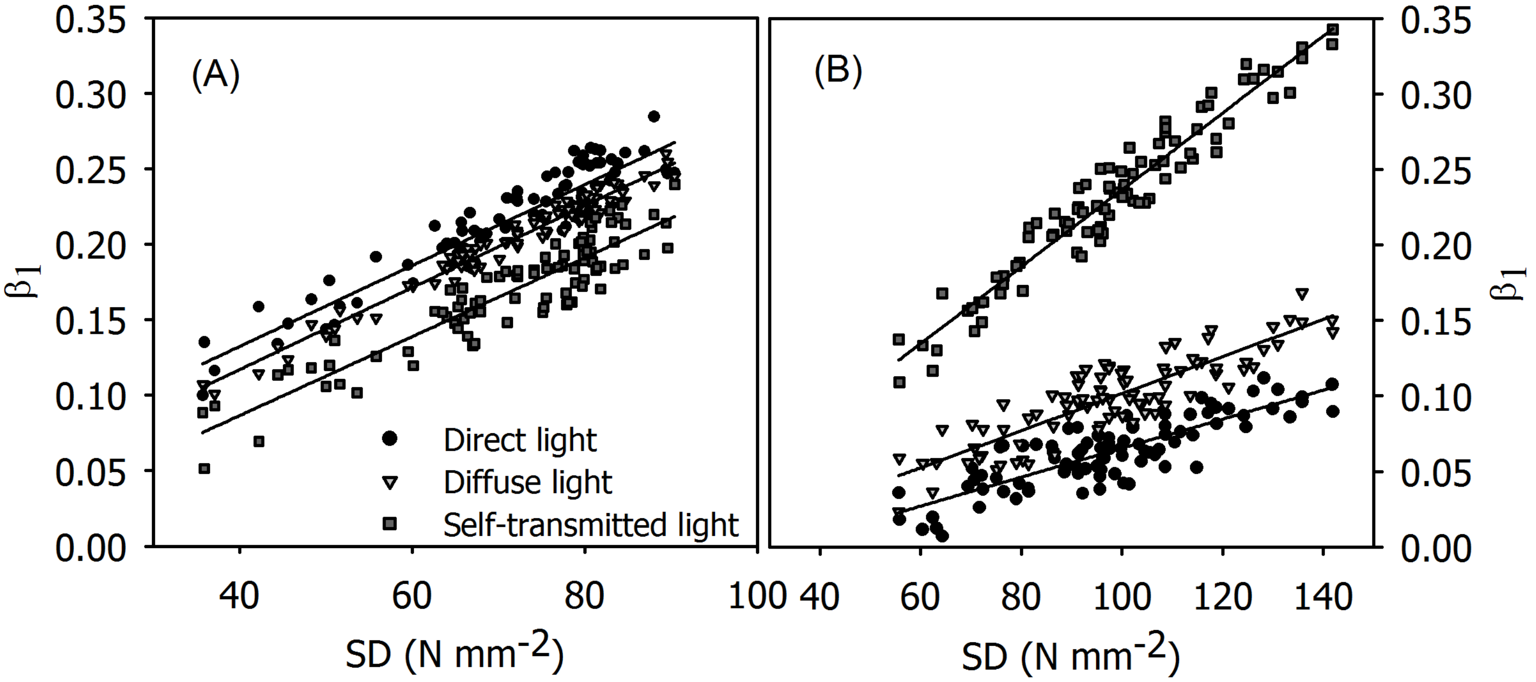

(A)
(B)
(C)
(D)
(E)
(F)
Adaxial
Abaxial
Gs (mmol m-2 s-1)
PPFD (μ mol m-2 s-1)
AQY
0
50
100
150
200
250
300
0
200
400
600
800
1000
1200
0.00
0.02
0.04
0.06
0.08
0.10
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
(1.18)
(1.16)
(0.43)
*
*
**

Figure 7(on next page)

The correlations of stomatal sensitivity (β_1) in direct (circles), diffuse (triangles), and self-transmitted (squares) light with *SD* in adaxial (A) and abaxial (B) surface of sorghum leaves.

The regression lines are as follows: y = 0.0028 x + 0.0251 (R^2 = 0.864, P = 0.003) for adaxial β_1 versus the direct light, y = 0.0027 x + 0.084 (R^2 = 0.971, P < 0.0001) for adaxial β_1 versus the diffuse light, y = 0.0026 x – 0.0181 (R^2 = 0.841, P =0.032) for adaxial β_1 versus the self-transmitted light, y = 0.0010 x - 0.0305 (R^2 = 0.699, P < 0.0001) for abaxial β_1 versus the direct light, y = 0.0012 x – 0.0212 (R^2 = 0.756, P < 0.002) for abaxial β_1 versus the diffuse light, y = 0.0025 x - 0.0182 (R^2 = 0.901, P < 0.0001) for abaxial β_1 versus the self-transmitted light. N = 90.

Figure 7



PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27267v1 | CC BY 4.0 Open Access | rec: 9 Oct 2018, publ: 9 Oct 2018