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Photosynthetic and physiological responses of foxtail millet (*Setaria italica* L.) to low-light stress during grain-filling stage

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Abstract

Two foxtail millet (*Setaria italica* L.) varieties were subjected to different shading intensity treatments during a grain-filling stage in a field experiment in order to clarify physiological mechanisms of low-light effects on the yield. Our results showed that the grain fresh mass per panicle, yield, photosynthetic pigment contents, net photosynthetic rate, stomatal conductance, effective quantum yield of PSII photochemistry, and electron transport rate decreased with the increase of shading intensity, whereas the intercellular CO_2 concentration increased in both varieties. In addition, shading changed a double-peak diurnal variation of photosynthesis to a one-peak curve. In conclusion, the lower yield of foxtail millet was caused mainly by a reduction of grain mass assimilated, a decline in chlorophyll content, and the low photosynthetic rate due to low light during the grain-filling stage. Reduced light energy absorption and conversion, restricted electron transfer, and reduced stomatal conductance might cause the decrease in photosynthesis.

Additional key words: agronomic characteristics; chlorophyll content; chlorophyll fluorescence; photosynthetic physiology; yield.

Introduction

Foxtail millet (*Setaria italica* L.), originating from Northern China, is one of the most important food crops in semi-arid regions, and is now planted all over the world (Jones and Liu 2009, Yang *et al*. 2012). It has many important attributes such as better adaptability to arid and barren lands than other crops, rapid growth, as well as good nutritional value (Sreenivasulu *et al*. 2004, Vetriventhan *et al*. 2012, Bai *et al*. 2013, Ning *et al.* 2016). In recent years, drought stress has seriously affected crop production. Foxtail millet is becoming more and more popular because of its better adaptability, stress resistance and the local consumer's appreciation for its food nutrition. Northern China, especially Shanxi Province is one of the most important production areas of foxtail millet in China. However, the later growth stages of foxtail millet in this area occur during the main rain season of the year; furthermore, continuously sunless and rainy weather during the grain-filling stage seriously influences the yield and quality of foxtail millet.

It is reported that shading during the reproductive growth period increases the abortive grain rate, thus reducing the number of grains of foxtail millet (Gu *et al*. 1989). According to several years of our research, the more days of rainy and sunless weather appearing during the

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Abbreviations: Car – carotenoid; Chl – chlorophyll; C_i – intercellular CO_2 concentration; CK – control; DAS – days after sowing; E – transpiration; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximum fluorescence yield of the dark-adapted state; FM – fresh mass; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; P_N – net photosynthetic rate; SP – saturation pulses; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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reproductive growth period, the lower the yield of foxtail millet. Studies on wheat (Li *et al*. 2010, Mu *et al*. 2010), maize (Jia *et al*. 2007, Lu *et al*. 2013) and rice (Liu *et al*. 2013) also suggest that insufficient light affected the physiological metabolism, decreased the crop yield and changed the nutritional quality of these crops. As the most important climate factor for plant growth, light impacts crop growth, morphology and various aspects of physiology and biochemistry (Estrada-Campuzano *et al*. 2008, Li *et al*. 2010, 2014), and finally crop yield and quality. However, low light affects not just the growth and development of plants, but also impacts plant photosynthesis, which is very important to plant productivity because light energy is used by plants to produce biological energy and carbon is fixed into carbohydrates (Dai *et al.* 2009, Deng *et al*. 2012). The impact of shading on crops depends on the crop species, shading period, and shading intensity.

Although some papers have reported the effects of shading on photosynthetic characteristics in wheat, maize and rice, little is focused on foxtail millet (Gu *et al*. 1989). In order to understand the related photosynthetic physiological mechanism of low light decreasing the yield of foxtail millet during grain-filling stage, we investigated the response of agronomic traits, yields, photosynthetic pigment content, photosynthetic rate, and chlorophyll fluorescence characteristics in two foxtail millet varieties to three shading intensities during grain-filling stage. The results of this study may help us determine what traits to look for when breeding foxtail millet for tolerance of low light conditions and what cultivation methods to recommend to get high yield and good quality foxtail millet.

Materials and methods

Experimental design: The experiment was conducted at the Experimental Farm of Shanxi Agricultural University, Taigu county (37°26'N, 112°32'E), Shanxi province, PR China in 2012. The soil is loam (carbonate brown soil) containing 18.1 g kg^{-1} organic matter, 0.93 g kg^{-1} total N, 71.2 mg kg^{-1} alkaline N, 50.1 mg kg^{-1} available P, and 94.9 mg kg^{-1} available K at 0–20 cm soil layer. Before sowing, 600 kg $ha^{-1}$of compound fertilizer (N:P:K = 10:4:6, humic acid was 25%) was applied to the soil. In this study, two foxtail millet varieties, Zhangza 5 (hybrid) and Jingu 21 (conventional), were used. Thinning combined with cultivating, soil banking and weed control were conducted 21 d after sowing (DAS). Seedlings were thinned to 300,000 plants ha^{-1}.

The experimental design was a split-plot with three replicates, with varieties in the main plots and shading in the sub-plots, and the size of each plot was 3 × 5 m. Black polyethylene screens with different light transmittance covered the top of the foxtail millet canopy for 15 d during grain-filling period (from 30th August to 13th September) to provide three shading treatments, which blocked about 30% (T1), 60% (T2) or 85% (T3) of the full radiation above the canopy. These treatments simulated the light radiation of a partly cloudy, cloudy, very cloudy or rainy period of two weeks. The radiation values in partly cloudy and rainy day are 550 ± 260 μmol(photon) m^{-2} s^{-1} and 120 ± 90 μmol(photon) m^{-2} s^{-1}, respectively. No shading was set as the control (CK). The screens were more than 250 cm above the ground to ensure good ventilation and were large enough to fully cover the corresponding shaded plots.

Microclimate measurements: The light radiation, canopy temperature, and CO_2 concentrations were recorded at the average height of foxtail millet using *CI-340* portable photosynthesis system (*CID Bio-Science*, *Inc.*, USA). These parameters (means ± SE, n = 3) were recorded at 10:00 h on the last day of shading during grain-filling stage. Effects of shading treatments on the microclimate of the foxtail millet populations were as follows:

Variety	Treatment	Light intensity [μmol(photon) m^{-2} s^{-1}]	Temperature [°C]	CO_2 concentration [μmol mol^{-1}]
Zhangza 5	CK	1063.3 ± 10.0	28.3 ± 0.6	353.0 ± 4.0
	T1	719.7 ± 21.2	27.7 ± 0.8	353.7 ± 8.4
	T2	545.0 ± 11.5	27.4 ± 0.9	354.7 ± 2.4
	T3	264.3 ± 13.3	27.1 ± 0.6	354.3 ± 3.2
Jingu 21	CK	1060.7 ± 15.6	28.1 ± 0.5	354.0 ± 3.8
	T1	713.0 ± 13.5	27.8 ± 0.6	354.7 ± 1.9
	T2	548.3 ± 9.7	27.3 ± 0.5	352.7 ± 4.0
	T3	259.3 ± 15.6	27.5 ± 0.6	354.4 ± 6.2

Sampling: Each plot was divided into two parts, one for sampling and major physiological measurements while another for grain yield determination. Uniform plants were tagged for sampling and measurements of single leaf photosynthetic pigments content, photosynthesis, and chlorophyll fluorescence parameters. The penultimate leaf on the main stem of foxtail millet was sampled between 10:00–11:00 h on the 15th d of shading during grain-filling stage for the following physiological tests.

Photosynthetic pigments and gas exchange: Photosynthetic pigments were extracted from leaf discs with 80% (v/v) acetone and assayed spectrophotometrically using extinction coefficients according to Porra *et al.* (1989). The diurnal variation of net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentrations (C_i) were measured simultaneously by *CI-340* portable photosynthesis system (*CID Bio-Science*, *Inc*., USA) every 2 h from 07:00 to 17:00 h.

Chlorophyll fluorescence parameters were measured by the *PAM-2500* measurement system (*Heinz Walz*, Effeltrich, Germany), using the automated Induction and Recovery Curve routine provided by the *PAM-2500* software. Prior to measurements, treated leaves were placed in the leaf disk chamber for 30 min, and a fluorescence induced curve was determined in ''Slow Kinetics mode''. Firstly, minimal fluorescence yield of the dark-adapted state (F_0) was established and subsequently maximal fluorescence yield of the dark-adapted state (F_m) was determined by the Saturation Pulse method. Secondly, actinic illumination was started and saturation pulses (SP) were given every 20 s, with the pulses serving for fluorescence analysis.

Maximal quantum yield of PSII photochemistry (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_0)/F_m$. Other PSII energy dissipation parameters were estimated by the *Dual PAM* software. Apparent electron transfer efficiency in PSII in light was calculated according to ETR(II) = PAR × 0.84 × 0.5 × Φ_{PSII}, and was used to measure electron transfer of carbon fixation resulted from photochemical reactions. Effective quantum yield of PSII photochemistry (Φ_{PSII}) was evaluated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$ (Kramer *et al*. 2004).

Agronomic characteristics and yield: Fifteen tagged stems were harvested at maturity to measure panicle length, panicle diameter, and plant height in each plot. The middle part of panicle was measured with a caliper for panicle diameter. Both panicle length and plant height were recorded using a metric ruler. Plant height was measured from the top of panicle to the first node closest to the ground. Panicles in 2 m^2 (2 × 1 m) were harvested in each plot. Panicles were cupped by hand, grains were threshed down, cleaned and dried on lab bench for 2–3 weeks, and samples were weighed.

Data analysis: The data were processed with *Microsoft Excel 2003* to obtain averages and graphs. Data of gas-exchange parameters for each variety were analyzed using the Data Processing System (*DPS 7.05*) program package according to a two-factor randomized complete block design to compare different times and light conditions. All the other data were subjected to the split-plot design analysis of variance (*ANOVA*), and the *Duncan*'s Multiple Range Test at the 5% probability level was used to determine the significance of differences between treatments using the data processing system (*DPS 7.05*).

Results

Effects of shading during grain-filling stage on the agronomic characteristics and yield of foxtail millet: As shown in Table 1, shading treatments during grain-filling stage had no significant effects on plant height of both Zhangza 5 (hybrid variety) and Jingu 21 (conventional variety), but decreased their yields significantly (P<0.05). Panicle diameter, panicle length, panicle fresh mass (FM), and grain FM per panicle declined variously as shading intensity increased. Compared with each control, T2 reduced the panicle diameter of Jingu 21 by 13% and that of Zhangza 5 was declined by 13% in the T3 treatment. For panicle length of Zhangza 5, there was no significant difference between each treatment. However, T3 showed significant differences from the control for panicle length of Jingu 21. The effects of shading treatments on panicle FM and grain FM per panicle in the two foxtail millet varieties were similar. It was observed that panicle FM and grain FM per panicle in two varieties were significantly reduced in T1 treatment. But there were no differences between T1 and T2 treatments in Zhangza 5, nor between T2 and T3 treatments in Jingu 2. There were differences between varieties in plant height and yield with Zhangza 5 greater than Jingu 21. In general, shading impact on foxtail millet yield was mainly caused by the decrease of grain mass.

Effects of shading during grain-filling stage on the photosynthetic pigment content in leaves of foxtail millet: Total chlorophyll (Chl), Chl *a*, Chl *b*, and carotenoid (Car) contents, and Chl *a*/*b* in leaves of foxtail millet declined with the increasing of shading intensity (Table 2). The photosynthetic pigment contents in Zhangza 5 were significantly higher than in Jingu 21. There were differences (P<0.05) between each shading treatment for each variety. Although both Zhangza 5 and Jingu 21 showed similar trends, the decreasing degree of photosynthetic pigment content in each variety was not the same. Compared with the control, Chl *a* of Zhangza 5 was reduced by 32, 46, and 57% from low shading to high shading intensity treatment, respectively (Table 2). Chl *b* of Zhangza 5 was reduced by 19, 30, and 39%, respectively. Carotenoid content of Zhangza 5 declined by 21, 37, and 50%, respectively. Chl *a* of Jingu 21 also declined more quickly than Chl *b*. Overall, Chl (*a*+*b*), Chl *a*, Chl *b*, and Car contents of Zhangza 5 were higher than that of Jingu 21 except Chl *a*/*b*.

Table 1. Effects of shading during grain-filling stage on the agronomic characteristics and yield of foxtail millet. CK refers to the "no shading" treatment (control); T1, T2, and T3 refer to shading of 30, 60, and 85% of the incident solar radiation, respectively. Data are means of three replicates. For each variety, *different letters* in each row indicate significant differences at P=0.05 as analyzed by the *Duncan*'s multiple range tests. FM – fresh mass.

Variety	Treatment	Plant height [cm]	Panicle diameter [mm]	Panicle length [cm]	Panicle FM [g]	Grain FM per panicle [g]	Yield [kg ha^{-1}]
Zhangza 5	CK	160.7^{a}	39.8^{a}	29.6^{a}	42.9^{a}	35.9^{a}	7676.0^{a}
	T1	160.6^{a}	39.2^{a}	28.8^{a}	28.5^{b}	21.4^{b}	4370.0^{b}
	T2	155.6^{a}	36.4^{ab}	28.4^{a}	26.0^{b}	17.1^{b}	3401.5^{c}
	T3	153.5^{a}	34.5^{b}	28.0^{a}	17.5^{c}	12.2^{c}	1383.0^{d}
Jingu 21	CK	186.8^{a}	37.4^{a}	28.2^{a}	29.0^{a}	23.8^{a}	6833.0^{a}
	T1	190.2^{a}	36.5^{a}	27.3^{a}	24.0^{b}	19.4^{b}	2481.5^{b}
	T2	180.2^{a}	32.7^{b}	27.0^{ab}	16.5^{c}	13.4^{c}	1885.0^{c}
	T3	179.3^{a}	30.3^{b}	25.6^{b}	13.4^{c}	10.7^{c}	1154.0^{d}

Table 2. Effects of shading during grain-filling stage on the photosynthetic pigment content in leaves of foxtail millet. CK refers to the "no shading" treatment (control); T1, T2, and T3 refer to shading of 30, 60, and 85% of the incident solar radiation, respectively. Data are means of three replicates. For each variety, *different letters* in each row indicate significant differences at P=0.05 as analyzed by the *Duncan*'s multiple range tests. Car – carotenoid; Chl – chlorophyll; FM – fresh mass.

Variety	Treatment	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Car [mg g^{-1}(FM)]	Chl (*a*+*b*) [mg g^{-1}(FM)]	Chl *a*/*b*
Zhangza 5	CK	3.83^{a}	1.18^{a}	0.52^{a}	5.01^{a}	3.26^{a}
	T1	2.61^{b}	0.95^{b}	0.41^{b}	3.56^{b}	2.82^{ab}
	T2	2.07^{c}	0.83^{c}	0.33^{c}	2.89^{c}	2.58^{b}
	T3	1.63^{d}	0.72^{c}	0.26^{d}	2.35^{d}	2.59^{b}
Jingu 21	CK	2.18^{a}	0.59^{a}	0.41^{a}	2.76^{a}	3.70^{a}
	T1	1.75^{b}	0.52^{ab}	0.33^{b}	2.28^{b}	3.37^{ab}
	T2	1.31^{c}	0.42^{bc}	0.24^{c}	1.73^{c}	3.08^{b}
	T3	0.93^{d}	0.33^{c}	0.20^{c}	1.27^{d}	2.88^{b}

Effects of shading during grain-filling stage on the gas-exchange parameters in leaves of foxtail millet: The diurnal variation curve of photosynthesis in Zhangza 5 and Jingu 21 was a double-peak curve in the condition of full sunlight, and the peak values appeared at about 11:00 and 15:00 h, respectively (Fig. 1). The photosynthesis had a significant midday depression phenomenon for both varieties in the full sunlight condition. However, the effects of each shading treatment on the diurnal variation curve of photosynthesis in the two varieties were different. For Zhangza 5, the diurnal variation curve of photosynthesis in shading for 30 and 60% was a double-peak curve, time of the peak was the same as the control, and the photosynthetic rate of corresponding shading treatment was lower than the control. Meanwhile, the conditions of shading for 85% resulted in a single-peak curve with a single-peak value appearing at about 15:00 h. For Jingu 21, the diurnal variation of photosynthesis in each shading treatment was a single-peak curve; however, time of the peak was delayed from 11:00 to 15:00 h with increasing shading intensity. All of these showed that shading may change the midday depression phenomenon of the foxtail millet.

For both varieties, shading treatment decreased transpiration in a shading intensity-dependent manner (Fig. 2). Diurnal change of transpiration (E) in each shading treatment including the control showed a typical single peak curve, which was different from the double-peak curve of photosynthetic rate, and the peak of E appeared at about 11:00 h.

As shown in Fig. 3, the trend of stomatal conductance (g_s) is similar to E, with one difference, the peak time of g_s appeared at about 09:00 h.

The diurnal variation of intercellular CO_2 concentrations (C_i) in both varieties showed a trend of decreasing after increasing; the values were high at 07:00 and 17:00 h, while the lowest values appeared at 11:00 or 15:00 h (Fig. 4). However, there are some differences between shading treatment for each variety. It seemed that C_i was enhanced with increasing shading intensity.

Effects of shading during grain-filling stage on the chlorophyll fluorescence parameters in leaves of foxtail millet: F_0 and F_m were significantly higher in Zhangza 5 than in Jingu 21, however, there were no differences in F_v/F_m, ETR, and Φ_{PSII} between the two varieties, respectively. In both varieties, F_m, F_v/F_m, ETR, and Φ_{PSII} declined variously as intensity of shading increased, but

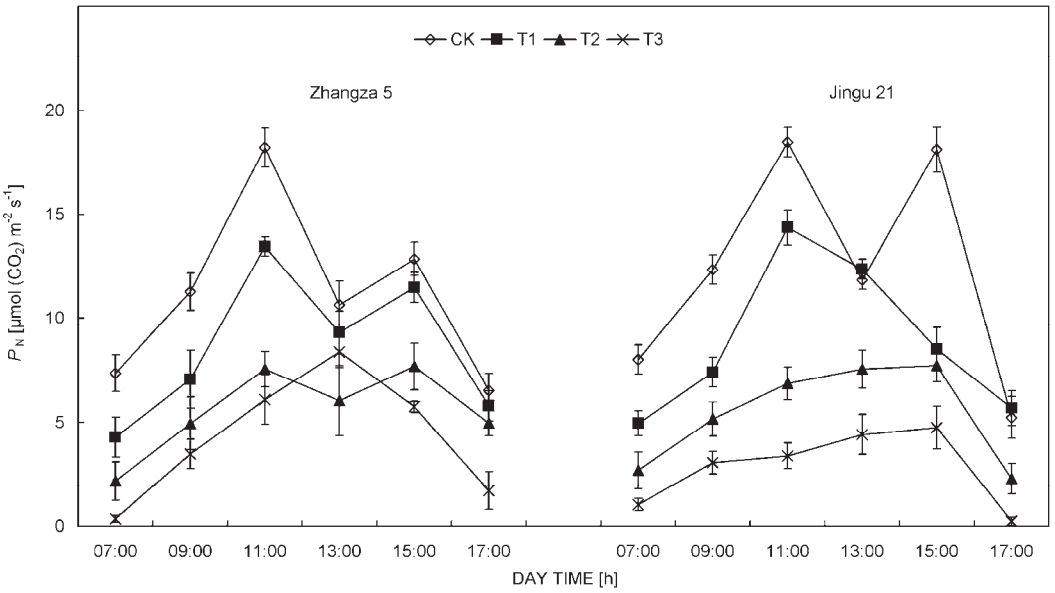

Fig. 1. Effects of shading during grain-filling stage on the diurnal variation of net photosynthetic rate in leaves of foxtail millet. Each data represents the mean of three replications and the vertical bars represent the standard deviation. The abscissa in the figure represents the measurement time in one day. P_N – net photosynthetic rate.

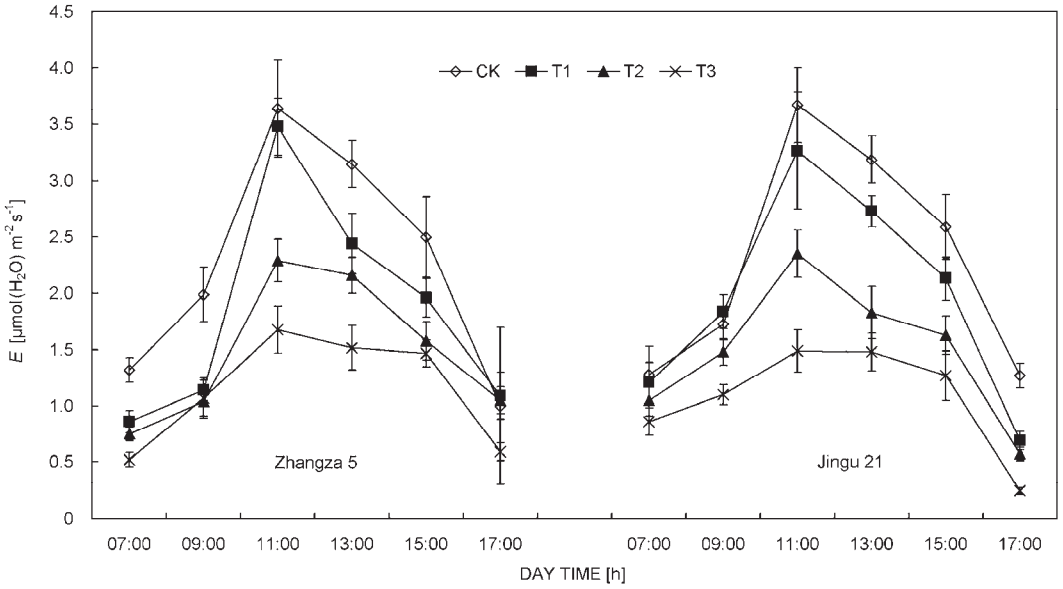

Fig. 2. Effects of shading during grain-filling stage on the diurnal variation of transpiration rate in leaves of foxtail millet. Each data represent the mean of three replications and the vertical bars represent the standard deviation. The abscissa in the figure represents the measurement time in one day. E – transpiration.

the changes of F_0 showed the opposite trend (Table 3).

For Zhangza 5, F_0 in shading was much higher than CK (P<0.05), but there were no differences between shading treatments. F_m in T3 was significantly lower than in T1, but the differences between T2, T1, and CK were not significant. Compared with the control, F_v/F_m decreased by 2.6, 3.9, and 5.5%, ETR declined by 15.8, 39.3, and 66.7%, and Φ_{PSII} decreased by 13.9, 32.1, and 51.4%, for T1, T2, and T3, respectively. There were significant differences between the two treatments for ETR and Φ_{PSII}. Although the differences of F_v/F_m between T1 and T2, T2 and T3 were not significant, respectively, significant differences existed among T3, T1, and CK.

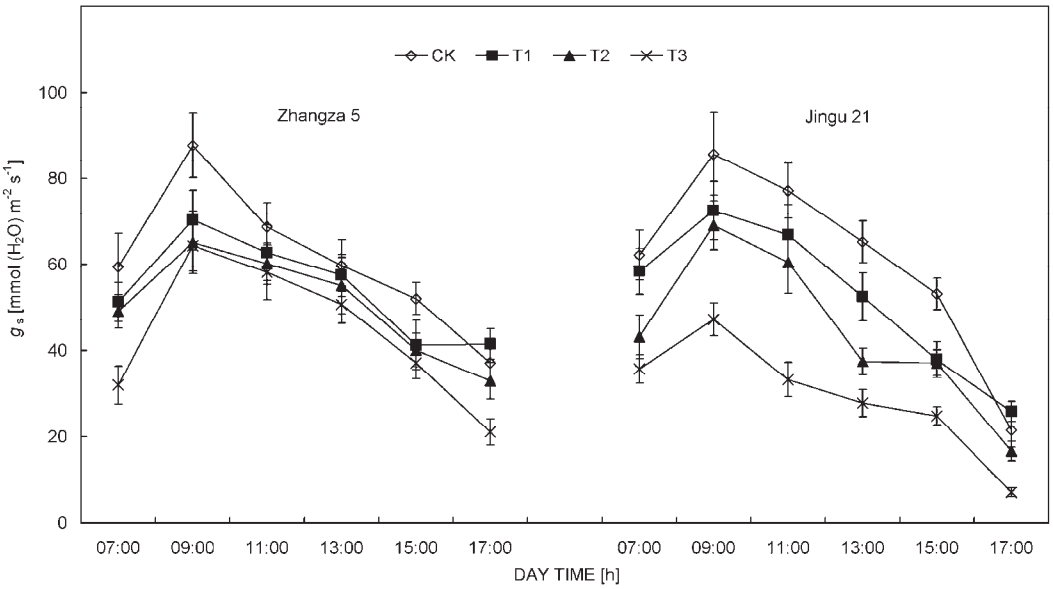

Fig. 3. Effects of shading during grain-filling stage on the diurnal variation of stomatal conductance in leaves of foxtail millet. Each data represent the mean of three replications and the vertical bars represent the standard deviation. The abscissa in the figure represents the measurement time in one day. g_s – stomatal conductance.

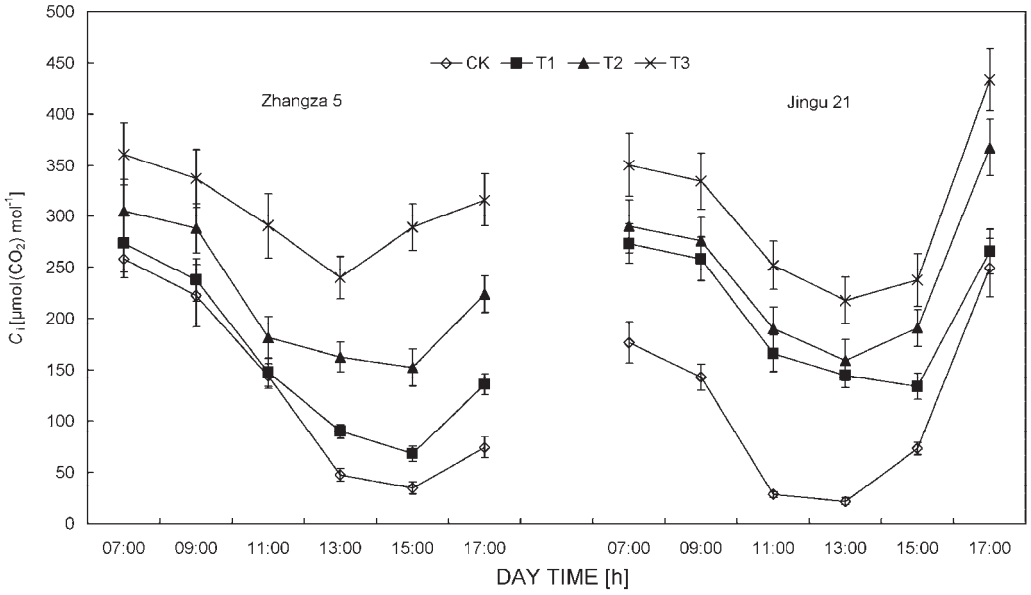

Fig. 4. Effects of shading during grain-filling stage on the diurnal variation of intercellular CO_2 concentration in leaves of foxtail millet. Each data represent the mean of three replications and the vertical bars represent the standard deviation. The abscissa in the figure represents the measurement time in one day. *C*i – intercellular CO_2 concentration.

For Jingu 21, compared to CK, T2 dramatically enhanced F_0 (P<0.05), however there were no significant differences between T1 and CK or T2 and T1. F_m was not significantly reduced by shading treatment (P>0.05). F_v/F_m in T2 was much lower than the control and significantly higher than that in T3. The trend of ETR and Φ_{PSII} in Jingu 21 was similar to Zhangza 5, and the values progressively declined by 25, 33, 70%, and 11, 36, 46% from shading of 30 to 85%, respectively. There were significant differences between the two treatments for ETR and Φ_{PSII}.

As shown in Table 4, the yield of foxtail millet in shading treatments during grain-filling stage had the most significant positive correlation with P_N, F_v/F_m, and ETR (P<0.01), and significant positive correlation with Chl (a+b), F_m, and Φ_{PSII} (P<0.05). P_N had significant positive correlation with F_0 and the most significant correlation with F_v/F_m, ETR, and Φ_{PSII}. There was significant positive correlation between Chl (a+b) and F_v/F_m (P<0.05), and the most significant correlation between Chl (a+b) and F_m (P<0.01).

Discussion

Agronomic characteristics and yields: The final results of different light intensities on crops should be reflected by agronomic characteristics (Cui *et al*. 2012) and grain yields (Lu *et al*. 2013). Many studies have shown that shading reduces grain mass (Gu *et al*. 1989, Mu *et al*. 2010). The present study also shows that shading treatments during grain-filling stage significantly decrease both the yields of hybrid variety (Zhangza 5) and conventional variety (Jingu 21). The more intense the shading was, the larger the yield reduction was. This was coincident with the previous research, the abortive grain rate increased with the increase of shading intensity during the reproductive growth period of foxtail millet (Gu *et al*. 1989), thus supporting the foxtail production proverb, "full filling grain is in sun, abortive grain is in rain". The response of agronomic characteristics such as plant height to shading differs with crop species, shading intensity, and shading stage. Cui *et al.* (2012) found that the plant height of summer maize decreased significantly with a shading degree of 60%, although there were differences at different shading stages. However, sorghum had a taller plant height because of mutual shading (Li *et al*. 2014). Winter wheat height was also found to be taller under applied shading between jointing and maturity (Li *et al*. 2010). Compared to the unshaded control, the increment in the wheat internodes in 92, 85, and 77% of full radiation treatments were larger in YM158 (shading tolerant) than YM 11 (shading-sensitive) by 2, 5, and 6% in YM158, 1, 2, and 4% in YM 11, respectively (Li *et al*. 2010). Our results show that shading during the grain-filling stage does not reduce the plant height of Zhangza 5, but decreases the plant height of Jingu 21 slightly (P>0.05). The plant height of foxtail millet reaches its peak at the grain-filling stage, so shading during this time would not have much effect. It appears that sensitivity of the agronomic characteristics in response to shading during the grain-filling stage is grain FM per panicle = panicle FM > panicle diameter > panicle length > plant height (Table 1). Yield of graminaceous crops is determined by grain mass per panicle and panicle number per area. In this paper, the crop density of under each shading treatment was the same, so, the decreased yield of foxtail millet due to low light during the grain-filling stage was mainly caused by a reduction of grain mass assimilated.

Content of photosynthetic pigments: It is well-known that plants use photosynthetic pigments to capture light energy for photosynthesis, and light is one of the most important factors affecting the content and the distribution of photosynthetic pigments. Jia *et al*. (2010) suggest that the net photosynthetic rate in ear leaves of summer maize declined rapidly, partly because of the decreased

Table 3. Effects of shading during grain-filling stage on the chlorophyll fluorescence parameters in leaves of foxtail millet. CK refers to the "no shading" treatment (control); T1, T2, and T3 refer to shading of 30, 60, and 85% of the incident solar radiation, respectively. Data are means of three replicates. For each variety, *different letters* in each row indicate significant differences at P=0.05 as analyzed by the *Duncan*'s multiple range tests. ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximum fluorescence yield of the dark-adapted state; F_v/F_m – maximum quantum yield of PSII photochemistry; Φ_{PSII} – effective quantum yield of PSII photochemistry.

Variety	Treatment	F_0	F_m	F_v/F_m	ETR	Φ_{PSII}
Zhangza 5	CK	0.212^{b}	1.000^{a}	0.788^{a}	135.0^{a}	0.504^{a}
	T1	0.232^{a}	0.998^{a}	0.767^{b}	113.7^{b}	0.434^{b}
	T2	0.235^{a}	0.969^{ab}	0.757^{bc}	82.0^{c}	0.342^{c}
	T3	0.236^{a}	0.925^{b}	0.745^{c}	45.0^{d}	0.245^{d}
Jingu 21	CK	0.204^{c}	0.949^{a}	0.785^{a}	140.0^{a}	0.549^{a}
	T1	0.208^{bc}	0.904^{a}	0.770^{ab}	105.3^{b}	0.491^{b}
	T2	0.214^{b}	0.893^{a}	0.760^{b}	93.7^{c}	0.349^{c}
	T3	0.241^{a}	0.894^{a}	0.729^{c}	42.7^{d}	0.296^{d}

Table 4. Correlative coefficient between yield and photosynthetic physiological indexes. Data with * indicate significant correlation at P=0.05, and data with ** indicate significant correlation at P=0.01. Chl (a+b) – chlorophyll (a+b); ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximum fluorescence yield of the dark-adapted state; F_v/F_m – maximum quantum yield of PS photochemistry; P_N – net photosynthetic rate; Φ_{PSII} – effective quantum yield of PSII photochemistry.

	Yield	P_N	Chl (a+b)	F_0	F_m	F_v/F_m	ETR	Φ_{PSII}
Yield	1	0.9331^{**}	0.830^{*}	0.561	0.756^{*}	0.877^{**}	0.872^{**}	0.809^{*}
P_N		1	0.661	0.793^{*}	0.524	0.939^{**}	0.937^{**}	0.936^{**}
Chl (a+b)			1	0.267	0.908^{**}	0.731^{*}	0.663	0.544

chlorophyll content after shading. Shading showed little effect on the flag leaf chlorophyll content of shading-resistant winter wheat varieties, but decreased the chlorophyll content of shading-sensitive varieties during the early grain-filling stage (Mu *et al*. 2008). However, shading significantly increases the chlorophyll content at late grain-filling stage for all varieties (Mu *et al*. 2008). In this experiment, not only did total chlorophyll, Chl *a*, Chl *b*, and carotenoid content decrease, but also Chl *a*/*b* in leaves of the two foxtail millet varieties was reduced dramatically when accompanied by increasing shading intensity at the grain-filling stage (Table 2). Because light is the main factor affecting the formation of chlorophyll, low light may be disadvantageous to the biosynthesis of chlorophyll. Nevertheless, the ability of Chl *b* to absorb scattered light is greater than Chl *a*. The decrease of Chl *a*/*b* in shading may be the adaptation of foxtail millet to low light conditions. This strategy is similar to the previous study (Dai *et al*. 2009).

Diurnal variation of photosynthesis: Photosynthesis contributes about 90% of the whole dry biomass to crops (Makino 2011), and it has close relationship with the crop yield. Some studies show that the diurnal variation of photosynthesis in foxtail millet is a double-peak curve (Liao and Wang 1999, Zhong *et al*. 2008, Fan *et al.* 2011). However, Yang *et al*. (2004) considered that the diurnal variation of photosynthesis in the new full expanded leaf of foxtail millet was the one-peak curve at booting stage. Our experiment showed that the diurnal variation curve of photosynthesis in Zhangza 5 and Jingu 21 were double-peak in the condition of full sunlight (Fig. 1). It is likely that the diurnal variation of photosynthesis is affected by plant species and various environmental factors. This is also supported by our current study which showed that P_N of hybrid foxtail millet appeared to have a one-peak curve in 85% shading. More interestingly, P_N of conventional foxtail millet was a one-peak curve in all shading treatments, and the time of the peak was delayed from 11:00 to 15:00 h with increasing shading intensity (Fig. 1).

Gas-exchange parameters: Liao and Wang (1999) observed that the diurnal variation curve of transpiration in the flag leaf of foxtail millet was a double-peak curve, but the trend of stomatal resistance is the opposite. A similar result was reported in the diurnal change of stomatal conductance in the studies of Yang *et al*. (2004) and Zhong *et al*. (2008). From these previous studies, it may be suggested that the diurnal variation trend of E is similar to P_N and g_s. However, based on the present experiment, the diurnal variation curves of E and g_s were one-peak curves, and not the same as the P_N curve. This indicates that the midday depression phenomenon of foxtail millet may not just be the result of the decline in g_s, which is supported by the opinion of Liao and Wang (1999). Although both the diurnal variation curve of E and g_s were one-peak curves, the peak times were not consistent. So, in addition to stomata, other factors including light intensity (Fig. 2), air temperature (Table 1), air humidity, and leaf blade surface vapor pressure may contribute to E. Combined with C_i and the peak times of P_N and g_s, it is suggested that the decline in P_N in the midday may be caused by both stomatal limitation and nonstomatal factors.

Chlorophyll fluorescence parameters: Compared to leaves in the sun, the leaves in the shade exhibited a lower photosynthetic capacity (Li *et al*. 2014). Detection of chlorophyll fluorescence dynamics is a rapid and noninvasive probe for researching plant photosynthetic functions, and has been widely applied to study the effects of different types of environmental stresses (van Kooten and Snel 1990, Cai and Xu 2002, Schreiber 2004, Dai *et al*. 2009, Li *et al.* 2010, Fu *et al*. 2012, Yuan *et al*. 2013, 2014). F_0 is the total fluorescence yield of which primary electron acceptor (Q_A) was oxidized completely when PSII reaction center was fully opened. The reduction in chlorophyll content will result in the decline of F_0, while PSII reaction center reversible inactivation or destruction will cause F_0 to increase (Schnettger *et al*. 1994, Mu *et al*. 2008). The change of F_0 depends on the factor that plays the leading role (Xu and Wu 1996). In our study, shading decreased chlorophyll content (Table 2), but enhanced F_0 value in leaves of foxtail millet (Table 3), suggesting that the PSII reaction center was reversibly inactivated or destructed in shading. F_v/F_m is the light quantum efficiency value when the PSII center is fully opened and is often used to assess the PSII original light conversion efficiency of plant leaves, reflecting the capacity of solar energy use in PSII. Φ_{PSII} measures the actual light energy conversion efficiency when a leaf is in the light. Simultaneously, ETR means apparent electron transfer efficiency in light, and is used to measure the transfer of carbon fixation resulted by photochemical reactions. For the change of F_v/F_m, Φ_{PSII}, and ETR affected by shading, the results varied with different treatments and crop species (Jia *et al*. 2007, 2010; Mu *et al*. 2008, Dai *et al*. 2009, Li *et al*. 2010, Cui *et al*. 2013). Our study indicates that F_v/F_m, PSII, and ETR decrease dramatically in shading. It was partly in agreement with Jia *et al*. (2007, 2010) that F_v/F_m and PSII were reduced. Findings from the present study may suggest that shading causes the reversible inactivation in the PSII reaction center, low efficiency of light energy conversion and electron transfer in foxtail millet at grain-filling stage. It may help us determine the traits with high chlorophyll concentration, PSIIreaction center activity, and net photosynthetic rate to look for when breeding foxtail millet for low light tolerance.

Conclusion: Light intensity levels significantly affected the growth of foxtail millet during the grain-filling stage. The inhibition of low light on yield in foxtail millet was mainly caused by the reduction in grain assimilated, the reduction in chlorophyll content, and the low photosynthetic rate. Although Chl *a*/*b* was reduced to adapt the

low light-intensity stress, the ability to absorb and convert light energy was limited, the PSII reaction center may be damaged, and electron transfer was restricted. Both stomatal limitation and nonstomatal factors lead to decreased photosynthesis. Shading can also change the double-peak diurnal variation of photosynthesis in foxtail millet to a one-peak curve.

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