



## Heterosis effects on photosynthesis of upland cotton (*Gossypium hirsutum*) hybrid cultivars

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### Abstract

The relationship between the environment and heterosis effects on the photosynthesis of upland cotton (*Gossypium hirsutum*) is unknown. No significant differences for net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), and transpiration rate ( $E$ ) were found between two studied cotton varieties Siza 3 and Zhongmiansuo 48. The  $P_N$  and  $g_s$  of Siza 3 demonstrated heterosis compared with its parents. The higher the effective photosynthetic radiation and  $CO_2$  concentration were, the more obvious the over better-parent heterosis (OH) of the  $P_N$  was. The photosynthesis parameters of Zhongmiansuo 48 in different environments did not display obvious OH, but the  $P_N$  of Siza 3 was higher than that of Zhongmiansuo 48. The performance index of the absorption ( $PI_{abs}$ ) of Siza 3 was higher than that of Zhongmiansuo 48. The  $PI_{abs}$  was significantly related to the OH of the  $P_N$ . The OH of the  $P_N$  of hybrid cotton can be predicted *via* the  $PI_{abs}$  under high temperature and light.

**Keywords:** environmental factor; fluorescence; light intensity; photosystem II.

### Introduction

Cotton is the main economic crop in China, and hybrids are widely planted due to their high yields (Xing 2007, Fu *et al.* 2015). As such, utilizing cotton heterosis has become an effective way to increase yields significantly, improve fiber quality, and enhance disease resistance in cotton (Zhu *et al.* 2011, Ge *et al.* 2016). Leaf gas-exchange trait such as photosynthetic rate is the key for the assimilation of photosynthates in plants and enhancement of physiological process may have positive impact on yield in cotton.

The heterosis effects on the net photosynthetic rate ( $P_N$ ) differ in different varieties and at different growth stages. Current scenario of climate change such as high temperature requires development of new cotton hybrids with improved leaf gas-exchange parameters. A study showed that cotton hybrid Xiangzhamian 3 had high  $P_N$  which was not affected by high intensity of light, temperature, and low humidity (Zeng *et al.* 2012). High temperature causes significant yield losses in cotton; some predictions suggested that cotton yields may decrease by 110 kg ha<sup>-1</sup> by every 1°C increment in temperature from

### Highlights

- The  $P_N$  and  $PI_{abs}$  of Siza 3 was higher than that of Zhongmiansuo 48
- The  $PI_{abs}$  was significantly related to the OH of the  $P_N$
- The OH of the  $P_N$  in hybrid cotton can be predicted by measuring the  $PI_{abs}$  under high-temperature and high-light conditions

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**Abbreviations:**  $C_i$  – intercellular  $CO_2$  concentration;  $C_{isat}$  –  $CO_2$ -saturation point;  $E$  – transpiration rate;  $F_0$  – original fluorescence;  $F_v/F_m$  – maximal photochemical efficiency;  $g_s$  – stomatal conductance;  $I$  – photosynthetically active radiation;  $I_c$  – light-compensation point;  $I_m$  – light-saturation point; NPQ – nonphotochemical quenching; OH – over better-parent heterosis;  $PI_{abs}$  – performance index of absorption;  $P_{max}$  – maximum photosynthetic rate;  $P_N$  – net photosynthetic rate;  $P_{Nmax}$  – light-saturated net photosynthetic rate;  $q_p$  – photochemical quenching coefficient;  $R_d$  – dark respiration rate; RH – relative humidity;  $R_p$  – photorespiration rate;  $T_{air}$  – air temperature;  $V_k$  – fluorescence intensity at the K-step; VPD – vapor pressure deficit;  $\Gamma$  –  $CO_2$ -compensation point.

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40°C in the field (Wang *et al.* 2017). High temperature is the most important environmental factor affecting plant growth and development (Wang *et al.* 2016). Under drought conditions, stomata are closed, and the CO<sub>2</sub> uptake is reduced, which affects the rate of photosynthesis and reduces growth and yield (Chaves *et al.* 2009, Liu *et al.* 2014). However, stomatal conductance ( $g_s$ ) is not always related to the  $P_N$ , although this relationship still needs clarification (von Caemmerer *et al.* 2004, Xu *et al.* 2010). However, relationships between the photosynthetic rate and CO<sub>2</sub> concentration in cotton have rarely been reported. Most of the photosynthesis performance indicators need to be measured with instruments such as the LI-6400 portable photosynthesis system analyzer (Zhao *et al.* 2017). Although this instrument is large, it provides a convenient means to perform measurements and is highly efficient. The Handy PEA instrument, which is used to measure chlorophyll (Chl) fluorescence, is small and portable and easy to operate. However, it has many different parameter settings, and complicated analysis of the data is needed (Wu *et al.* 2019). The relationship between the CO<sub>2</sub> response and photosynthetic light response, the relationship between photosynthetic rate and Chl fluorescence parameters, and the effects of heterosis on performance characteristics of these parameters have not been studied in cotton. Zhongmiansuo 48 and Siza 3 are two high-yielding hybrids widely used in China. However, it has not been reported whether there are effects of heterosis on photosynthesis performance. In this study, these two upland cotton hybrids and their parents were used to study parameters measured by the LI-6400 portable photosynthesis system analyzer, the effects of heterosis on Chl fluorescence parameters, and their relationships. The results highlighted the diurnal variation in the photosynthetic rate and the heterosis effects on photosynthesis under different light intensities and CO<sub>2</sub> concentrations. The purposes of this study were to explore the heterosis effects on photosynthesis in upland cotton leaves and their relationship with the environment and to provide a basis for the selection of improved photosynthesis of cotton varieties.

## Materials and methods

**Plant material and experimental site:** Two cotton (*Gossypium hirsutum* L.) cultivars and their parents were used in this study. The test materials included Siza 3 and its female parent (Siyang 211) and male parent (Siyang 280) as well as Zhongmiansuo 48 and its female parent (971300) and male parent (951188). The experiment was carried out at the Liuhe Experimental Station in Nanjing, Jiangsu, China. The seedlings were transplanted from a nutrient bowl (paper cup); the sowing date was 6 April 2012. The seedlings were film-transplanted on 6 May. Each material was planted in six rows, with a plant spacing of 30 cm. There were wide and narrow planting rows: the narrow row spacing was 50 cm, while the wide row spacing was 120 cm; the average row spacing was 85 cm. The soil type of the plots was a sandy loam. The basal fertilizer comprised a compound fertilizer (N:P:K = 1:1:1)

applied at 450 kg ha<sup>-1</sup> together with a compound microbial fertilizer applied at 2,250 kg ha<sup>-1</sup>. For topdressing, KCl was applied at 225 kg ha<sup>-1</sup>, compound fertilizer (N:P:K = 1:1:1) was applied at 150 kg ha<sup>-1</sup>, and urea was applied at 300 kg ha<sup>-1</sup>. Other cultural practices were consistent with conventional cultivation management practices in the field. Cotton growth conditions were recorded during measurements. During the boll opening stage, functional leaves of the main stem of the cotton plants were measured.

**Diurnal variation in the photosynthetic rate:** In 2012, an LI-6400 portable photosynthesis system analyzer (LI-COR, Inc., Lincoln, Nebraska, USA) was used to measure the gas-exchange parameters of the main stem functional leaves (each fourth leaf from the top) in Nanjing. Four plants were randomly selected for each material, and their leaves were marked for measurement. Measurements were performed every 2 h from 6:00–18:00 h. Each measurement was repeated three times under the same conditions [irradiance of 200 μmol(photon) m<sup>-2</sup> s<sup>-1</sup>; temperature of 26.7°C; relative humidity of 70.3%; and vapor pressure deficit of 1.03 kPa].

**Light-response and CO<sub>2</sub>-response curve:** In 2012, three typical Siza 3 plants and their parents and three typical Zhongmiansuo 48 plants and their parents were randomly selected in Nanjing. The LI-6400 portable photosynthesis system analyzer (LI-COR, Inc., Lincoln, Nebraska, USA) was used to measure the photosynthesis parameters of the functional leaves (each fourth leaf from the top) of the main stems. The light-response curves were measured under PPFD of 0, 50, 100, 150, 200, 250, 500, 800, 1,000, 1,200, 1,500, 1,800, and 2,000 μmol(photon) m<sup>-2</sup> s<sup>-1</sup>. The CO<sub>2</sub>-response curves were measured at the CO<sub>2</sub> concentration of 0, 50, 75, 100, 150, 200, 250, 300, 400, 600, 800, 1,000, 1,200, 1,400, 1,600, 1,800, and 2,000 μmol mol<sup>-1</sup> (temperature of 26.7°C, relative humidity of 70.3%, and vapor pressure deficit of 1.03 kPa).

**Chl fluorescence kinetic parameters:** In 2012, during the flocking season in the field at Nanjing, three typical Siza 3 plants and their parents and three typical Zhongmiansuo 48 plants and their parents were removed at 10:00, 12:00, and 14:00 h on sunny days, wetted, and then placed in a dark room for dark adaptation for 15–20 min. Chl fluorescence parameters were subsequently measured with actinic light intensity of 1,500 μmol(photon) m<sup>-2</sup> s<sup>-1</sup> using a plant efficiency analyzer (Handy PEA, Hansatech, UK).

**Statistical analysis:** Data analysis and plotting were performed using Excel XP and Origin 8.5 software. SPSS 19.0 was used to calculate the correlations between photosynthesis-related parameters and Chl fluorescence parameters.

**Light-response curve:** Cotton leaves were measured at various photosynthetic photon flux densities (PPFDs). A  $P_N$  response model was created, and Origin 8.5 data processing software was used to fit the data (Ye and Yu

2007). The following equation was used:

$$P_N = \alpha \frac{1 - \beta I}{1 + \gamma I} I - R_D$$

where  $\alpha$  is the initial slope of the light-response curve (dimensionless),  $\beta$  and  $\gamma$  are coefficients,  $P_N$  is the net photosynthetic rate,  $I$  is the photosynthetically active radiation, and  $R_D$  is the dark respiration rate.

**CO<sub>2</sub>-response curve:** The CO<sub>2</sub>-response curve of cotton leaves under the saturated light intensity was fitted using a photosynthesis CO<sub>2</sub>-response model and *Origin 8.5* data processing software (Ye and Yu 2009). The following equation was used:

$$P_N = a \frac{1 - bC_i}{1 + cC_i} C_i - R_p$$

where  $a$  is the initial carboxylation efficiency of the CO<sub>2</sub>-response curve,  $b$  and  $c$  are coefficients [ $\text{mol } \mu\text{mol}^{-1}$ ],  $P_N$  is the net photosynthetic rate,  $C_i$  is the intercellular CO<sub>2</sub> concentration, and  $R_p$  is the photorespiration rate.

**Over better-parent heterosis (OH):** The OH was calculated as:  $\text{OH} [\%] = [F_1 - \text{better (male parent, female parent)}] / \text{better (male parent, female parent)} \times 100$ .

## Results

**Net photosynthetic rate:** The  $P_N$  of Zhongmiansuo 48 and its parents exhibited double-peak curves, which were the highest at 10:00 h; thereafter they decreased substantially at 12:00 h, increased slightly at 14:00 h, and then decreased substantially again. The  $P_N$  of Zhongmiansuo 48 was slightly higher than that of its female parent, and there was no obvious OH; moreover, the  $P_N$  was significantly higher than that of the male parent (Fig. 1A). The diurnal variation in the  $P_N$  of Siza 3 and its female parent exhibited a double-peak curve, which was the highest at 10:00 h; then it decreased substantially at 12:00 h, increased slightly at 14:00 h, and then decreased substantially again. The  $P_N$  of the male parent exhibited a unimodal curve. From 10:00 to 12:00 h, the  $P_N$  of Siza 3 was higher than that of its parent, and its OH was obvious (Fig. 1B). The male parent of Siza 3 did not present a decrease in the  $P_N$  under stress and it was less affected by the environment. The OH of the  $P_N$  of Siza 3 was more obvious than that of Zhongmiansuo 48.

**Stomatal conductance:** At 10:00 and 14:00 h, there was no significant difference in  $g_s$  between Zhongmiansuo 48 and its female parent, but it was higher than that of the male parent. In addition, there was no significant difference in  $g_s$  at 12:00 h (Fig. 1C). The daily variation in  $g_s$  of Siza 3 and

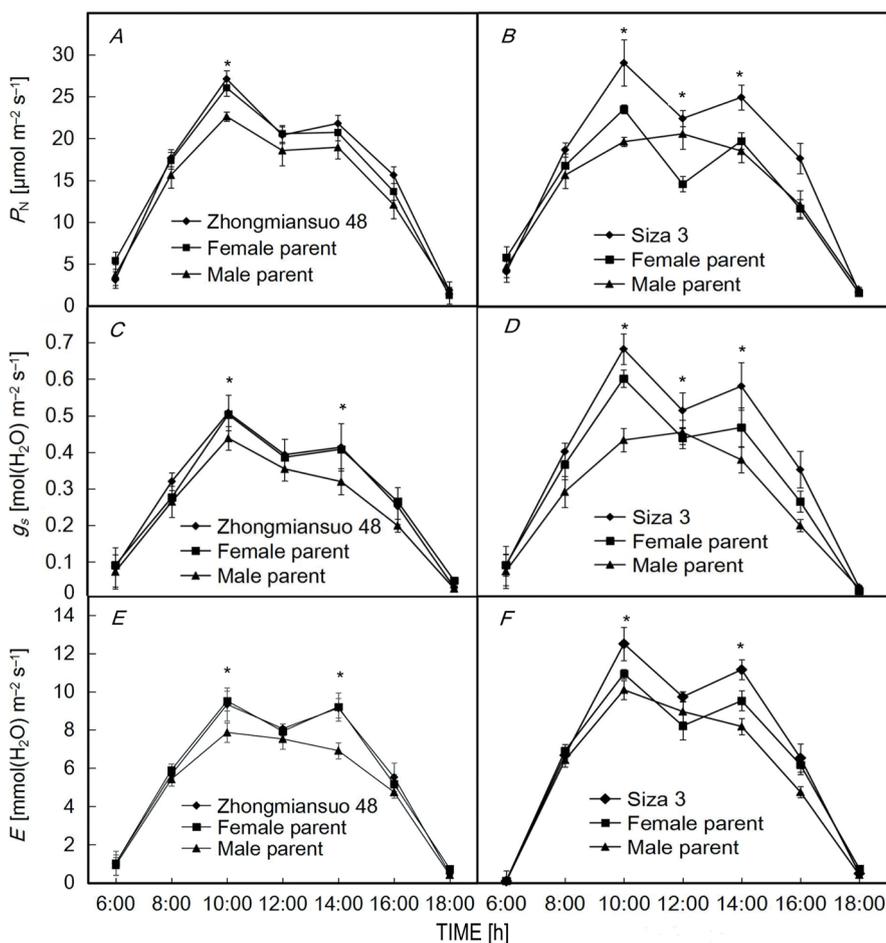


Fig. 1. Diurnal changes in gas-exchange parameters of Zhongmiansuo 48, Siza 3, and their parents. Net photosynthetic rate ( $P_N$ ) of Zhongmiansuo 48 and its parents (A) and Siza 3 and its parents (B). Stomatal conductance ( $g_s$ ) of Zhongmiansuo 48 and its parents (C) and Siza 3 and its parents (D). Transpiration rate (E) of Zhongmiansuo 48 and its parents (E) and Siza 3 and its parents (F). Values are means  $\pm$  SD,  $n = 3$ . \*The difference is significant at the 0.05 level.

its female parent exhibited a double-peak curve, while the daily variation in  $g_s$  of its male parent exhibited the single-peak curve. The  $g_s$  of Siza 3 was higher than that of its parents at both 10:00 and 14:00 h (Fig. 1D). Moreover, the results showed that, compared with that of Zhongmiansuo 48, the OH of Siza 3 in terms of  $g_s$  was more obvious. Overall, the daily trend of  $g_s$  was essentially consistent with that of the  $P_N$ .

**Transpiration rate:** The daily change in the  $E$  of Zhongmiansuo 48 and its parents exhibited a double-peak curve. Their  $E$  peaked at 10:00 h. There was no significant difference in  $E$  between Zhongmiansuo 48 and its female parent, but it was higher than that of the male parent. At 12:00 h, the  $E$  of Zhongmiansuo 48 was higher than that of its parent (Fig. 1E). The daily variation in the  $E$  of Siza 3 and its female parent exhibited a double-peak curve, while the  $E$  of its male parent exhibited a single-peak curve. The  $E$  of Siza 3 and its parents peaked at approximately 10:00 h, and the  $E$  of Siza 3 and its female parents did not significantly differ (Fig. 1F). The  $E$  of Zhongmiansuo 48 and Siza 3 were higher than those of their male parents but were not significantly different from those of their female parent.

**Diurnal changes in the OH of photosynthesis:** The  $P_N$ ,  $g_s$ , and  $E$  of Zhongmiansuo 48 and its parents did not demonstrate obvious OH (Fig. 2). However, the  $P_N$ ,  $g_s$ , and  $E$  of Siza 3 and its parents showed a clear OH. At approximately 16:00 h, the OH of the  $P_N$  and  $g_s$  peaked, which were 45.7 and 33.0% of Siza 3, respectively. At approximately 14:00 h, the OH of the  $E$  was 17.1%, which was the highest value of the day. These results show that this cultivar displayed obvious OH under conditions of high temperature and high light, and the OH was very obvious between 14:00–16:00 h (Fig. 2).

**Diurnal variation of environmental factors:** At 10:00 h [air temperature ( $T_{air}$ ) of 35.1°C; relative humidity (RH) of 56.5%; vapor pressure deficit (VPD) of 2.03 kPa], the  $P_N$ ,  $g_s$ , and  $E$  of each variety were the highest, indicating that the environment at that time was more suitable for photosynthesis of these six upland cotton varieties and their  $F_1$  hybrid progeny. At 12:00 h [ $T_{air}$  of 37.3°C; RH of 48.3%; VPD of 2.29 kPa; PPFD of 2,035  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ] (Fig. 3), the parameters of the  $P_N$ ,  $g_s$ , and  $E$  of the five varieties (lines), Zhongmiansuo 48 and its parents as well as Siza 3 and its female parent, were lower than those at 10:00 h. These results show that the environment at noon had an adverse effect on photosynthesis. At 14:00 h, although the maximum temperature reached 37.9°C, it was higher than that at 12:00 h. However, the photosynthesis of each variety (line) increased significantly compared to that at 12:00. This may be related to the increase in light intensity, RH, and VPD.

**Correlations between photosynthesis parameters and environmental factors:** Photosynthesis parameters are closely related to the environment. The  $P_N$ ,  $g_s$ , and  $E$  of Zhongmiansuo 48 and its parents and Siza 3 and its male

parent were extremely significantly positively correlated with PPFD (Table 1). The  $P_N$ ,  $g_s$ , and  $E$  of Zhongmiansuo 48 and Siza 3 and their respective parents were positively correlated with VPD. Furthermore, the  $P_N$ ,  $g_s$ , and  $E$  of Zhongmiansuo 48 and its parents and Siza 3 and its male parent were significantly positively correlated with  $T_{air}$ . However, the  $P_N$ ,  $g_s$ , and  $E$  of Zhongmiansuo 48 and Siza 3 and their respective parents were negatively correlated with RH. Taken together, these results show that high PPFD and  $T_{air}$  are beneficial for photosynthesis and that high RH is not beneficial for photosynthesis.

**The light-response curve and OH:** The higher the light intensity was, the higher the  $P_N$  of each plant variety was. Under low-light intensities, the photosynthetic rate of the different varieties was not significantly different. The higher the light intensity, the more obvious the difference in photosynthetic rate between the different varieties. The

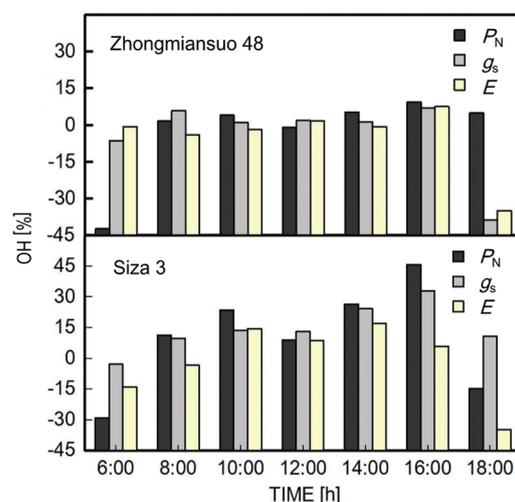


Fig. 2. Diurnal variations of the trend of the over better-parent heterosis (OH) in two cotton cultivars.  $E$  – transpiration rate;  $g_s$  – stomatal conductance;  $P_N$  – net photosynthesis rate.

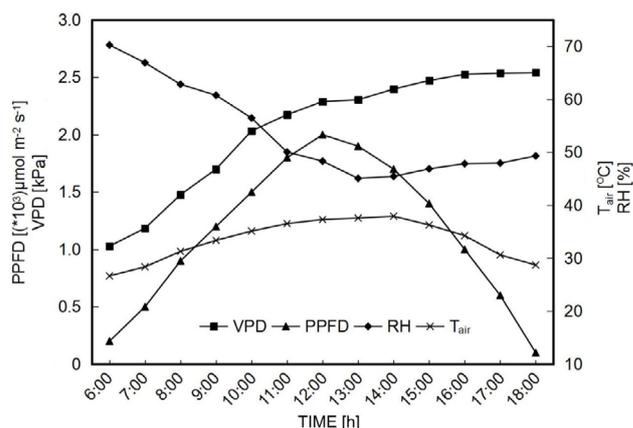


Fig. 3. Diurnal variation of environmental factors. PPFD – photosynthetic photon flux density; VPD – vapor pressure deficit;  $T_{air}$  – air temperature; RH – relative humidity.

light-saturation points ( $I_m$ ) of the different parents and  $F_1$  hybrids were significantly different. Siza 3 had the highest  $I_m$ , and its male parent had the lowest  $I_m$ . When the light intensity was lower than  $500 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ , the  $P_N$  of Zhongmiansuo 48 and its parents were essentially the same. When the light intensity was higher than  $500 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ , the difference between the  $P_N$  of Zhongmiansuo 48 and that of its parents increased. Zhongmiansuo 48 demonstrated the highest  $P_N$ , followed by its female parent, whereas the male parent presented the lowest  $P_N$ . However, the increase in the  $P_N$  was not significant. The apparent quantum efficiency,  $I_m$ , light-saturated net photosynthetic rate ( $P_{N\text{max}}$ ), light-compensation point ( $I_c$ ), and  $R_D$  of Zhongmiansuo 48 were higher than those of its parents, but there was no obvious OH. When the light intensity was lower than  $250 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ , the  $P_N$  of Siza 3 and its parents was essentially the same. When the light intensity was higher than  $500 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ , the  $P_N$  significantly differed between Siza 3 and its parents. Siza 3 had the highest  $P_N$ , followed by its female parent, and the male parent had the lowest  $P_N$ . Siza 3 displayed obvious heterosis. As the light intensity increased, the OH increased. When the light intensity reached  $1,500 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ , the OH peaked by 33.3%. These results show that the photosynthesis of this hybrid is related to light intensity. The higher the light intensity, the stronger the heterosis (Fig. 4, Table 2). The OH of Siza 3 was obvious, and the OH of apparent quantum efficiency and the  $P_{N\text{max}}$  were 34.7 and 18.6%, respectively, for this variety. However, the  $I_c$  displayed a negative OH (-18.5%). When the light intensity exceeded  $1,500 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ , the  $P_N$  of

each variety did not increase significantly.

The apparent quantum efficiency,  $I_m$ , and  $P_{N\text{max}}$  of Zhongmiansuo 48 were lower than those of Siza 3, indicating that photosynthesis of Siza 3 is more efficient than that of Zhongmiansuo 48 under different light intensities (Fig. 4). The  $I_c$  of Zhongmiansuo 48 was higher than that of Siza 3, indicating that the ability to use weak light is lower in the former than in Siza 3. Furthermore, the dark respiration rate of Zhongmiansuo 48 was essentially the same as that of Siza 3.

#### The effects and OH of different PPFs on $g_s$ of two hybrids:

Under different light intensities, the  $g_s$  of the male parent of Zhongmiansuo 48 was higher than that of Zhongmiansuo 48 and its female parent, and there was no heterosis. The  $g_s$  of Siza 3 and its parents increased with increasing light intensity. When the light intensity was lower than  $300 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ , there was no significant difference in  $g_s$  between the three varieties. However, when the light intensity was higher than  $300 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ , the  $g_s$  of Siza 3 was higher than that of its parent, and it presented a very significant OH. The OH gradually increased with increasing light intensity. When the light intensity reached  $2,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ , the OH peaked (20.2%). This shows that with increasing light intensity, the OH of the  $g_s$  of Siza 3 also gradually increased. The higher the light intensity, the more obvious the OH (Fig. 5).

**CO<sub>2</sub>-response curve and OH:** The higher the CO<sub>2</sub> concentration was, the higher the  $P_N$  of each plant variety was. When the CO<sub>2</sub> concentration was higher than 900

Table 1. Correlations between photosynthesis parameters of the different cotton varieties and environmental factors. \*Correlation is significant at the 0.05 level. \*\*Correlation is significant at the 0.01 level.  $E$  – transpiration rate;  $g_s$  – stomatal conductance;  $P_N$  – net photosynthetic rate; PPF – photosynthetic photon flux density; RH – relative humidity;  $T_{\text{air}}$  – air temperature; VPD – vapor pressure deficit.

		PPFD	VPD	$T_{\text{air}}$	RH
$P_N$	Siza 3	0.907**	0.246	0.877**	-0.342
	Female parent	0.805*	0.055	0.75	-0.148
	Male parent	0.956**	0.187	0.882**	-0.316
	Zhongmiansuo 48	0.894**	0.240	0.859*	-0.326
	Female parent	0.899**	0.135	0.829*	-0.234
	Male parent	0.914**	0.192	0.857*	-0.294
$g_s$	Siza 3	0.915**	0.231	0.875**	-0.330
	Female parent	0.889**	0.152	0.825*	-0.246
	Male parent	0.965**	0.205	0.878**	-0.329
	Zhongmiansuo 48	0.901**	0.168	0.840*	-0.264
	Female parent	0.919**	0.249	0.877**	-0.341
	Male parent	0.901**	0.163	0.824*	-0.251
$E$	Siza 3	0.928**	0.339	0.914**	-0.434
	Female parent	0.902**	0.321	0.892**	-0.409
	Male parent	0.936**	0.273	0.884**	-0.371
	Zhongmiansuo 48	0.947**	0.303	0.925**	-0.417
	Female parent	0.936**	0.290	0.911**	-0.402
	Male parent	0.954**	0.259	0.902**	-0.369

$\mu\text{mol mol}^{-1}$ , the  $P_N$  of Zhongmiansuo 48 displayed no obvious OH. When the  $\text{CO}_2$  concentration was lower than  $750 \mu\text{mol mol}^{-1}$ , the  $P_N$  of Zhongmiansuo 48 was higher than that of its parents, but the differences were not significant. The intercellular  $\text{CO}_2$  concentration ( $C_i$ ),  $P_{N\text{max}}$ ,  $\text{CO}_2$ -compensation point ( $\Gamma$ ), and  $R_p$  of Zhongmiansuo 48 displayed negative OH, and the OH of initial carboxylation efficiency reached only 9.3% (Fig. 6; Table 1S, supplement). At saturated light intensity, the higher the  $\text{CO}_2$  concentration, the more obvious the OH of the  $P_N$  of Siza 3. The initial carboxylation efficiency of Siza 3 was significantly higher than that of its parent, and its OH was obvious. The  $\text{CO}_2$ -concentration saturation

point of Siza 3 exhibited no obvious OH, and the  $\Gamma$  point and  $R_p$  were significantly lower than those of its parents, which resulted in a negative OH (Fig. 6, Table 1S). In the  $\text{CO}_2$ -response curve, the OH of the initial carboxylation efficiency and the maximum  $P_N$  of Siza 3 were 26.0 and 16.6%, respectively, which were higher than those of Zhongmiansuo 48. The  $C_i$ ,  $\Gamma$ , and  $R_p$  of Siza 3 displayed negative OH, which was essentially the same as that of Zhongmiansuo 48. The  $\Gamma$  and  $R_p$  of Zhongmiansuo 48 and Siza 3 displayed negative OH, indicating that, compared to their parents, these two hybrids had lower  $\Gamma$  and photorespiration rates at relatively low  $\text{CO}_2$  concentrations and displayed heterosis effects.

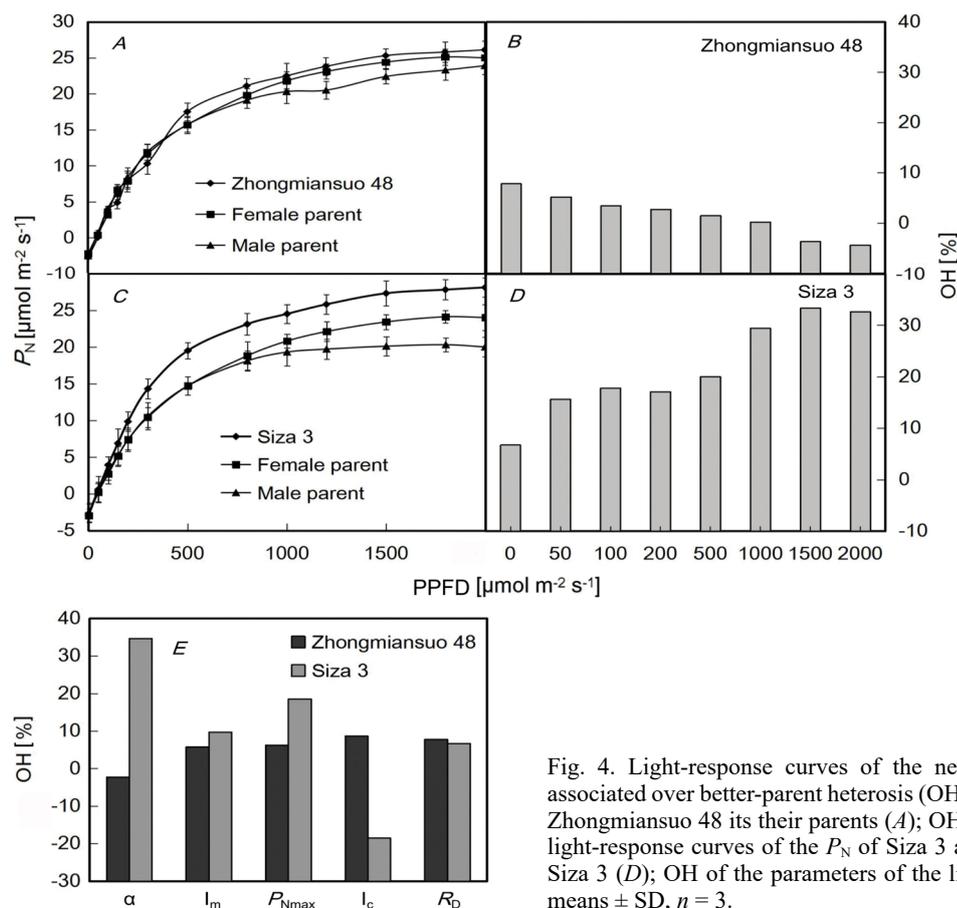


Fig. 4. Light-response curves of the net photosynthetic rate ( $P_N$ ) and the associated over better-parent heterosis (OH). Light-response curves of the  $P_N$  of Zhongmiansuo 48 and its parents (A); OH of the  $P_N$  of Zhongmiansuo 48 (B); light-response curves of the  $P_N$  of Siza 3 and its parents (C); OH of the  $P_N$  of Siza 3 (D); OH of the parameters of the light-response curves (E). Values are means  $\pm$  SD,  $n = 3$ .

Table 2. Parameters of the light-response curves.  $\alpha$  – apparent quantum yield;  $I_m$  – light-saturation point;  $P_{N\text{max}}$  – maximum net photosynthetic rate;  $I_c$  – light-compensation point;  $R_D$  – dark respiration rate. Values are means  $\pm$  SD ( $n = 3$ ). Different lowercase letters denote significant differences at  $P \leq 0.05$ .

Cultivar	$\alpha$	$I_m$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	$P_{N\text{max}}$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	$I_c$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	$R_D$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]
Zhongmiansuo 48	$0.0701 \pm 0.0043^a$	$2,072.8 \pm 34.3^a$	$25.97 \pm 2.35^a$	$44.94 \pm 2.35^a$	$2.94 \pm 0.17^a$
Female parent	$0.0717 \pm 0.0034^a$	$1,958.7 \pm 52.3^a$	$24.25 \pm 2.95^a$	$37.66 \pm 2.46^a$	$2.53 \pm 0.14^a$
Male parent	$0.0779 \pm 0.0045^a$	$1,883.4 \pm 56.7^a$	$21.95 \pm 2.18^a$	$36.76 \pm 2.24^a$	$2.79 \pm 0.17^a$
Siza 3	$0.0964 \pm 0.0035^a$	$2,290.7 \pm 40.9^a$	$28.06 \pm 2.19^a$	$38.75 \pm 2.47^b$	$3.43 \pm 0.13^a$
Female parent	$0.0667 \pm 0.0030^b$	$2,086.9 \pm 62.8^b$	$23.67 \pm 2.25^b$	$47.55 \pm 2.39^a$	$2.94 \pm 0.17^b$
Male parent	$0.0715 \pm 0.0045^b$	$1,575.9 \pm 46.9^c$	$20.30 \pm 1.83^b$	$43.82 \pm 2.52^a$	$2.89 \pm 0.24^b$

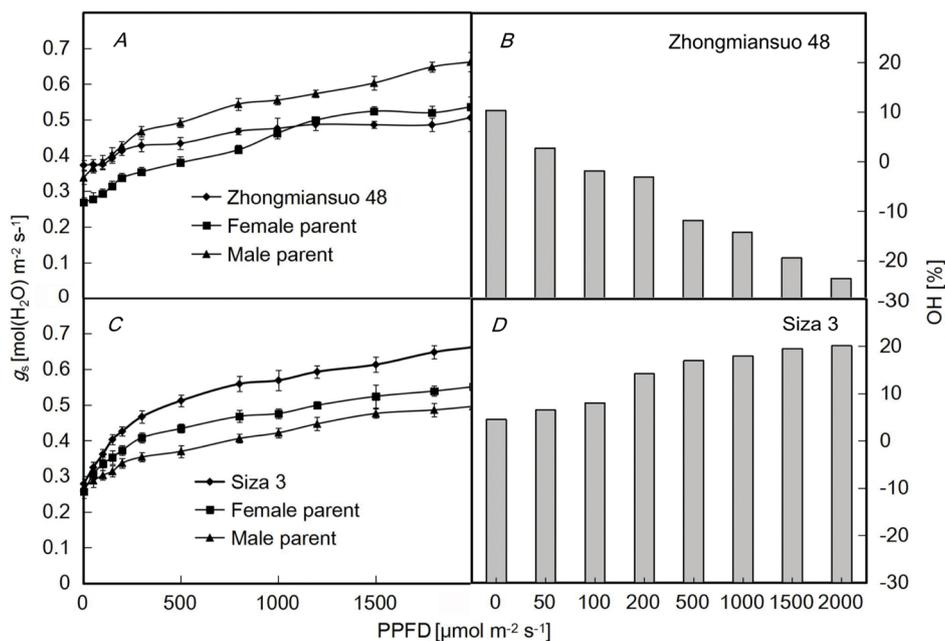


Fig. 5. Light-response curves of stomatal conductance ( $g_s$ ) and the associated over better-parent heterosis (OH). Light-response curves of the  $g_s$  of Zhongmiansuo 48 and its parents (A); OH of the  $g_s$  of Zhongmiansuo 48 (B); light-response curves of the  $g_s$  of Siza 3 and its parents (C); OH of the  $g_s$  of Siza 3 (D). Values are means  $\pm$  SD,  $n = 3$ .

#### CO<sub>2</sub>-response curves of the $g_s$ and the associated OH:

The higher the CO<sub>2</sub> concentration, the lower the  $g_s$  of each variety. The  $g_s$  of Zhongmiansuo 48 and its parents increased when the CO<sub>2</sub> concentration was lower than 200  $\mu\text{mol mol}^{-1}$ . However, when the CO<sub>2</sub> concentration was higher than 200  $\mu\text{mol mol}^{-1}$ , the  $g_s$  decreased. The change in  $g_s$  of Siza 3 and its parents was similar to that of Zhongmiansuo 48. There was no significant difference in  $g_s$  between Zhongmiansuo 48 and its parents under different CO<sub>2</sub> concentrations (Fig. 1S, *supplement*). When the CO<sub>2</sub> concentration was higher than 200  $\mu\text{mol mol}^{-1}$ , the  $g_s$  of Siza 3 was not significantly different from that of its male parent but was higher than that of its female parent. The higher the CO<sub>2</sub> concentration, the greater the OH of the  $g_s$  of Siza 3 (Fig. 1S).

**Changes in the  $F_0$  and OH:** Original fluorescence ( $F_0$ ) is a measure of the fluorescence intensity when the PSII reaction centers of the dark-adapted photosynthetic apparatus are fully opened. The increase in thermal dissipation of the PSII antenna leads to a decrease in  $F_0$ , and the destruction or reversible inactivation of the PSII reaction center causes an increase in  $F_0$  (Biber 2012). The state of the reaction center can be inferred from changes in  $F_0$  (Georgieva and Yordanov 1993). At 10:00 h, there was no significant difference in  $F_0$  between Zhongmiansuo 48 and its parents. At 12:00 h, the  $F_0$  tended to increase from 10:00 h, and the  $F_0$  of Zhongmiansuo 48 was lower than that of its parent. At 14:00 h, the  $F_0$  of the parents decreased slightly, and that of Zhongmiansuo 48 remained essentially unchanged. The  $F_0$  of Zhongmiansuo 48 at the three time points did not change significantly (Fig. 2SA, *supplement*). Moreover, at 10:00 h, the difference in  $F_0$  between Siza 3 and its parent was not significant. At 12:00 h, the  $F_0$  of Siza 3 and its female parent increased significantly, but that of its male parent did not change significantly. At 14:00 h, the  $F_0$  of Siza 3 and its female parent was lower than that

at 12:00 h, but the change in the Siza 3 male parent was not significant (Fig. 2SB). Together, these results show that the  $F_0$  of different varieties is not significantly different under nonstress conditions, that the  $F_0$  increases under stress conditions at 12:00 h, and that there is a significant difference between varieties.

**Changes in the  $PI_{\text{abs}}$  and OH:** The  $PI_{\text{abs}}$  is a photochemical performance index of PSII and accurately reflects the state of the plant photosynthetic apparatus. The  $PI_{\text{abs}}$  is more sensitive to certain stresses than the maximal photochemical efficiency ( $F_v/F_m$ ) and reflects the impact of stress on the photosynthetic apparatus (Li *et al.* 2005). There was no significant difference in the  $PI_{\text{abs}}$  between Zhongmiansuo 48 and its parents at 10:00 h. Moreover, at 12:00 h, the  $PI_{\text{abs}}$  of all three of these varieties declined. The  $PI_{\text{abs}}$  of Zhongmiansuo 48 did not significantly differ compared with that of the Zhongmiansuo 48 female parent but was lower than that of the male parent, without obvious heterosis. At 14:00 h, the  $PI_{\text{abs}}$  of Zhongmiansuo 48 was higher than that of its parents (Fig. 2SC). There was no significant difference in  $PI_{\text{abs}}$  between Siza 3 and its parents at 10:00 h. However, at 12:00 h, the  $PI_{\text{abs}}$  of Siza 3 and its female parent decreased significantly, the  $PI_{\text{abs}}$  of the female parent was lower than that of Siza 3, and the  $PI_{\text{abs}}$  of the male parent decreased slightly. At 14:00 h, the  $PI_{\text{abs}}$  of Siza 3 was higher than that of its parents (Fig. 2SD). The  $PI_{\text{abs}}$  of Siza 3 was always higher than the values of Zhongmiansuo 48, indicating that Siza 3 has a higher electron transport ability. Overall, these results indicate that the  $PI_{\text{abs}}$  of cotton leaves decreased under stress, and photosynthesis was essentially the same comparing the different varieties at 10:00 h. At 12:00 h, the  $PI_{\text{abs}}$  of Siza 3 and Zhongmiansuo 48 displayed no OH. At 14:00 h, they displayed the OH, indicating that the leaf functions of the two hybrids recovered faster than did those of their parents after the stress was released. Under

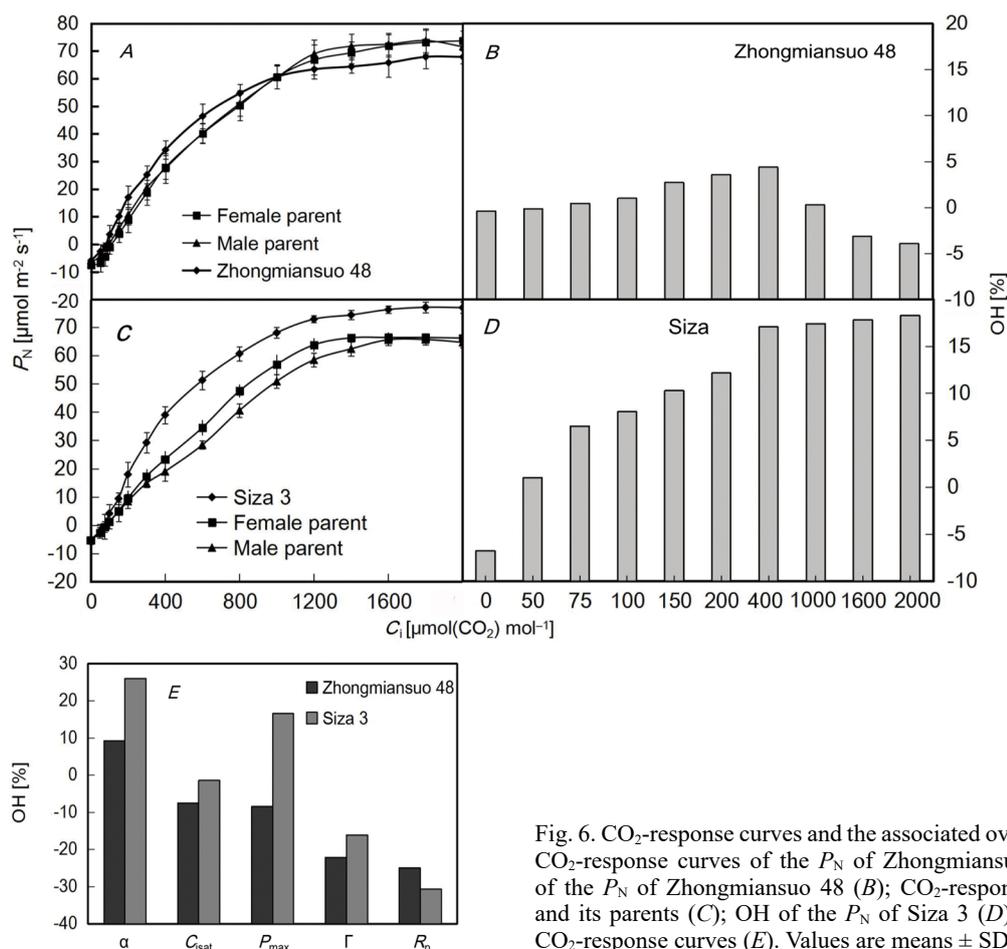


Fig. 6. CO<sub>2</sub>-response curves and the associated over better-parent heterosis (OH). CO<sub>2</sub>-response curves of the  $P_N$  of Zhongmiansuo 48 and its parents (A); OH of the  $P_N$  of Zhongmiansuo 48 (B); CO<sub>2</sub>-response curves of the  $P_N$  of Siza 3 and its parents (C); OH of the  $P_N$  of Siza 3 (D); OH of the parameters of the CO<sub>2</sub>-response curves (E). Values are means  $\pm$  SD,  $n = 3$ .

nonstress conditions, there was no significant difference in  $PI_{\text{abs}}$  between the six varieties, but the differences in the  $PI_{\text{abs}}$  between varieties could be identified under stress conditions.

#### Chl fluorescence intensity at the K-step ( $V_k$ ) and OH:

Environmental stress leads to the emergence of the K-step on the O–J–I–P curve. The  $V_k$  can reflect the degree of damage of the oxygen-evolving complex (Li *et al.* 2005). At 10:00 h, the  $V_k$  values of Zhongmiansuo 48 and its parents were not significantly different. At 12:00 h, the  $V_k$  of the parents increased, and the  $V_k$  of Zhongmiansuo 48 was lower than that of its parents. At 14:00 h, the  $V_k$  of the parents slightly decreased, whereas that of Zhongmiansuo 48 remained essentially unchanged. The change in  $V_k$  of Zhongmiansuo 48 at the three time points was not significant (Fig. 2SE). At 10:00 h, there was no significant difference in the  $V_k$  between Siza 3 and its parents. However, at 12:00 h, the  $V_k$  of Siza 3 and its female parent increased significantly, but the change in  $V_k$  in the male parent was not significant. At 14:00 h, the  $V_k$  of Siza 3 and its female parent began to decrease, but the change in  $V_k$  in the male parent of Siza 3 was not significant (Fig. 2SF). Together, these results show that, under stress, the  $V_k$  of the different varieties is essentially the same and that the  $V_k$  of different varieties differs under stress conditions.

## Discussion

**Differences in photosynthesis parameters and OH:** Due to different environments, the photosynthesis parameters of all varieties displayed obvious dynamic changes at different time points under different light intensities and CO<sub>2</sub> concentrations during the day. The  $P_N$  of each variety was the highest at 10:00 h, indicating that various environmental parameters at this time were more suitable for photosynthesis of the leaves. Furthermore, the  $P_N$  was lower at 12:00 h than that at 10:00 h, indicating that environmental factors at this time were not conducive to photosynthesis. When the male parent of Siza 3 was under stress at 12:00 h, the  $P_N$  did not decrease, indicating that the  $P_N$  of this variety was relatively stable. Therefore, there are differences in the diurnal variation in the  $P_N$  between varieties. The higher the light intensity and CO<sub>2</sub> concentration, the higher the photosynthetic rate. During the day, the change in the  $P_N$  of Zhongmiansuo 48 was essentially the same as that of its female parent, and there was no obvious OH. However, the  $P_N$  of Siza 3 displayed obvious OH under different conditions. Under low-light intensities, the  $P_N$  of Siza 3 exhibited no obvious heterosis. The higher the light intensity, the stronger the heterosis on the  $P_N$ . Therefore, during cotton growth, the sunnier the days are, the more OH of the  $P_N$  in Siza 3. The  $R_p$  and

$R_D$  affect the  $P_N$ . The  $R_D$  of Siza 3 was higher than that of its parent, and the  $R_P$  was also lower, indicating a strong negative OH. Leaf transpiration and the advantages of the  $E$  and  $g_s$  of the hybrids may be a major reason for the superiority of the  $P_N$  (Zeng *et al.* 2012). The  $P_N$ ,  $E$ , and  $g_s$  of the two hybrids and their parents were essentially the same during the day. The  $P_N$ ,  $g_s$ , and  $E$  of Zhongmiansuo 48 and its parents did not display obvious OH. Moreover, the  $P_N$ ,  $g_s$ , and  $E$  of Siza 3 and its parents showed obvious OH. The apparent heterosis of the  $P_N$  of Siza 3 may be related to the heterosis on the yield. The  $P_N$  of Xiangzamian 3 displayed an OH throughout the day, which is consistent with the findings for Siza 3, but the  $P_N$  of Xiangzamian 3 was stronger under high-temperature and high-light conditions (Zeng *et al.* 2012). These results indicate that there is heterosis on the photosynthesis of upland cotton hybrids, and the heterosis differs for different combinations.

The higher the light intensity, the higher the  $P_N$ . When the light intensity was higher than 500  $\mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$ , the  $P_N$  of each variety significantly differed. The difference in the photosynthetic rate of the different varieties occurred mainly under conditions of high-light intensity, and there was no significant difference in the photosynthetic rate between the different varieties under low-light intensity.

#### Effects of CO<sub>2</sub> concentration on the OH effects on the photosynthetic rate:

With the increase in CO<sub>2</sub> concentration, the superiority of the  $P_N$  of Siza 3 gradually increased. Under low CO<sub>2</sub> concentrations, the effects of changes in CO<sub>2</sub> concentration on the superiority of the  $P_N$  were obvious. In addition, under low CO<sub>2</sub> concentrations, the superior  $g_s$  of both Zhongmiansuo 48 and Siza 3 remained stable. When the CO<sub>2</sub> concentration exceeded 1,000  $\mu\text{mol mol}^{-1}$ , the advantageous and superior  $g_s$  of Siza 3 increased with increasing CO<sub>2</sub> concentration. The superior  $g_s$  of Zhongmiansuo 48 exhibited a unimodal curve when the CO<sub>2</sub> concentration ranged from 200–2,000  $\mu\text{mol mol}^{-1}$ , showing both positive and negative superiority. The heterosis on the  $g_s$  of the two hybrids was not obvious, indicating that  $g_s$  plays a small role in the changes in CO<sub>2</sub> concentration on the photosynthetic rate. The higher the CO<sub>2</sub> concentration, the more obvious the superiority of the  $P_N$  of Siza 3. These results indicate that there is a significant difference in the photosynthetic rates of the different cotton varieties at this CO<sub>2</sub> concentration. The CO<sub>2</sub> concentration in the atmosphere is generally 400 ppm (Khan and Rizvi 2020). At this CO<sub>2</sub> concentration, the  $P_N$  of Siza 3 displays a clear OH, and the  $P_N$  of Zhongmiansuo 48 displays no obvious OH.

#### Relationship between Chl fluorescence parameters and OH:

Photochemical quenching ( $q_P$ ), nonphotochemical quenching (NPQ), potential PSII activity ( $F_v/F_0$ ), and  $F_v/F_m$  are Chl fluorescence parameters often used for measuring heterosis (Banks 2017, Sonobe and Wang 2018). In this experiment, the  $F_0$  tended to increase under stress conditions and displayed a negative OH, which is consistent with previous results (Zhang *et al.* 2006). The  $V_k$  also tended to increase under stress conditions, but

the negative OH was lower. The correlation coefficients between the OH of both  $F_0$  and  $V_k$  and  $P_N$  are  $-0.622$  and  $-0.613$ , respectively. The  $PI_{\text{abs}}$  tended to decrease and displayed a negative OH, but its correlation with  $P_N$  reached 0.845. Under high temperature and high light, the OH of the  $PI_{\text{abs}}$  was highly correlated with the  $P_N$ . The OH of the  $P_N$  of hybrid cotton can be predicted by measuring the  $PI_{\text{abs}}$  under high temperature and high light.

The  $P_N$  was the highest at 10:00 h, indicating that the environmental conditions were more suitable for photosynthesis of leaves at that time than at other times, and the fluorescence parameters of the different varieties did not differ significantly at this time. At 12:00 h, the  $P_N$  significantly decreased, indicating that the temperature and light at this time were stressful, which had a suppressive effect on photosynthesis. At 12:00 h, the  $F_0$  increased, the  $PI_{\text{abs}}$  decreased significantly, the electron transport rate decreased, the  $V_k$  increased, and the oxygen-evolving complex was damaged to some extent. This shows that under stress conditions, the decrease in the  $P_N$  and the changes in the  $F_0$ ,  $PI_{\text{abs}}$ , and  $V_k$  occurred simultaneously. Since determining fluorescence parameters is more convenient than using an *LI-6400* instrument, the change in fluorescence parameters in cotton leaves during the day should be measured. Changes in key parameters such as the  $F_0$ ,  $V_k$ , and  $PI_{\text{abs}}$  during stress conditions are more suitable for rapid evaluation of photosynthetic traits and their superiority in hybrid cotton.

**Conclusion:** The photosynthesis parameters of Zhongmiansuo 48 in the different environments did not display obvious OH, but the  $P_N$  of Siza 3 was higher than that of Zhongmiansuo 48. In addition, the  $PI_{\text{abs}}$  of Siza 3 was higher than that of Zhongmiansuo 48, indicating that Siza 3 has a higher electron transport ability. The  $PI_{\text{abs}}$  was significantly related to the OH of the  $P_N$ . Overall, the OH of the  $P_N$  of hybrid cotton can be predicted by measuring the  $PI_{\text{abs}}$  under high-temperature and high-light conditions.

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