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Shading stress after heading enhances the remobilization of nonstructural carbohydrates in rice under different ecological conditions

Yuling Zeng[1,2], Qiuping Li[1,2], Hong Chen[1,2], Bo Li[1,2], Xiaoyuan Zhong[1,2], Zhenzhen Li[1,2], Hui Lu[1,2], Li Wang[1], Wanjun Ren[1,2], and Fei Deng[1,2*]

[1]Sichuan Agricultural University, College of Agronomy, Key Laboratory of Crop Ecophysiology and Farming System in Southwest China of Ministry of Agriculture, Chengdu 611130, China.
[2]Crop Ecophysiology and Cultivation, Key Laboratory of Sichuan Province, Chengdu 611130, China.
*Corresponding author (ddf273634096@163.com).

ABSTRACT

Low light levels during rice (*Oryza sativa* L.) growing seasons may lead to significant reductions in grain yield and quality. The effect of shading stress on remobilization of nonstructural carbohydrates (NSC) from the stem plus sheath (SPS) to the grain, a process crucial to rice grain formation, was investigated. Field shading experiments were conducted in Hanyuan and Wenjiang, China, using two rice varieties (Huanghuazhan and Guichao II) subjected to shading stress after heading. Stored NSC of 60.00-159.15 g m^{-2} were remobilized from SPS after heading, contributing 9.63%-23.63% to grain dry weight at maturity. Shading stress resulted in the 23.99%-50.30% and 31.65%-61.33% reduction in NSC content of SPS at 20 d after heading (DAH20) and maturity stages, respectively. This contributed to the 13.80%-89.00% increase in the remobilization of stored NSC from SPS to the grain (RASN) and 15.86%-73.30% increase in remobilization percentage of removed NSC from SPS to the grain (RPRN). The effect of shading stress on remobilization differed between sites and varieties. Owing to a greater sink capacity, shading stress led to a higher RASN and RPRN from heading to DAH20 for 'Huanghuazhan' in Hanyuan. To compensate for photosynthate deficiency under shading stress, rice is adapted to remobilize stored NSC during early grain filling stages. Consequently, grain yield loss caused by shading could be reduced by increasing the SPS remobilization ability (especially in low light areas). Increasing NSC accumulation in SPS before heading for later remobilization may be a feasible way to alleviate rice yield loss under adverse light conditions.

Key words: Nonstructural carbohydrates, *Oryza sativa*, remobilization characteristics, shading stress, yield.

INTRODUCTION

By the middle of this century, an estimated 40% of the world's population will face a food resource shortage (Ibarrola-Rivas et al., 2017). Rice (*Oryza sativa* L.), one of the three major food crops, is cultivated in more than 100 countries, with Asian countries accounting for 90% of the total crop yield (Fukagawa and Ziska, 2019). In China, rice is a staple for more than 60% of the population, and accounts for 40% of the total rice production globally (Wu et al., 2013); however, the cultivated area under rice has declined due to rapid development of cities and ongoing population growth. Consequently, there is an urgent need to improve rice production to meet future demands under these severe resource challenges (Bailey-Serres et al., 2019).

In recent years, increased environmental pollution and frequency of extreme climate events have seriously affected food production (Shao et al., 2019). In China and other countries, increased rainfall and decreased hours of sunshine

during the rice growth period are widespread (Chou et al., 2019; Ray et al., 2019), and heavy haze and aerosol pollution may further decrease solar irradiance by 28%-49% (Tie et al., 2016). The harsh light environment may seriously hinder the growth and development of this light-loving crop, resulting in decreased grain yield and quality (Liu et al., 2019). For example, if shading occurs before the heading stage, the number of effective panicles and spikelet differentiation may decrease (Deng et al., 2009; Wei et al., 2018), with a consequent decrease in grain filling capacity. If shading occurs after the heading stage, it may significantly decrease the spikelet filling rate and grain weight, with a consequent decrease in crop yield (Wang et al., 2015).

As rice crop yield is closely related to carbohydrates produced during photosynthesis, light energy utilization is key to rice production (Priyanka et al., 2019). Although rice plants show an increase in light capturing capacity under shading stress through an increase in chlorophyll content, there is a decrease in net photosynthetic rate, saturation irradiance, electron transport rate, along with a limited supply of electrons for photoreactive metabolism (Wang et al., 2015). Therefore, under shading stress, the production of rice photosynthates is limited, which consequently limits plant growth and development (Liu et al., 2019).

Rice yield depends not only on photosynthesis after the heading stage but also on the remobilization of carbohydrates stored before heading in the vegetative organs, particularly in the stem plus sheath (SPS) (Fu et al., 2011; Stella et al., 2016). These remobilized carbohydrates, also known as nonstructural carbohydrates (NSC), are soluble sugars and starch (Panda and Sarkar, 2014) which play an extremely important role in rice yield formation by contributing to C reserves and grain filling (Deng et al., 2016). For example, the SPS NSC concentration in rice can reach up to 4 t ha^{-1} (Fu et al., 2011). Normally, the rapid remobilization of NSC from the SPS to the grain (RASN) after heading contributes about 30% to the final grain weight (Pan et al., 2011; Panda and Sarkar, 2014). Although the filling rate of seeds is related to the NSC content of SPS before heading (Liang et al., 2017), the efficacy of RASN is also an important indicator of rice production potential (Slewinski, 2012).

Grain weight can be estimated by measuring the redistribution of NSC; however, the SPS NSC content is affected by environmental factors. For example, RASN increases under water stress, leading to increased grain filling and yield (Yang et al., 2001; Yang and Zhang, 2006). In contrast, under high temperature stress, the NSC remobilization from the SPS is inhibited (Zhen et al., 2019). There is evidence that shading conditions may reduce SPS DM accumulation and increase the output and conversion rate of stored photosynthetic products (Ren et al., 2003; Liu et al., 2019). However, how shading stress affects NSC accumulation and remobilization from the SPS under different ecological conditions has rarely been reported.

In our study, a shading stress field experiment using two rice varieties was carried out in Wenjiang and Hanyuan from 2018 to 2019. The main objectives of the present study were: (1) to investigate the accumulation of NSC before heading and the remobilization of NSC from the SPS under shading stress after heading; (2) to explore the contribution of SPS NSC to grain yield under shading stress after heading; and (3) to compare the difference of NSC remobilization under different ecological conditions. The present study provides a theoretical basis for agronomists to improve rice yield under abiotic stress.

MATERIALS AND METHODS

The field experiments were conducted in Hanyuan (29°29′ N, 102°37′ E) and Wenjiang (30°43′ N, 103°52′ E), Sichuan Province, PR China, in 2018 and 2019. Both sites are located in a subtropical monsoon humid climate. The climate data during the experimental period and the soil characteristics of the study sites were the same as reported by Li et al. (2020). The mean maximum and minimum temperatures and precipitation in Hanyuan were all lower than Wenjiang except for the maximum and minimum temperatures during shading period in 2018 and the maximum temperature after heading in 2019. The total radiation of Hanyuan in 2 yr was higher than Wenjiang; it was 2240.3 MJ m^{-2} (654.3 MJ m^{-2} during shading period) and 2285.6 MJ m^{-2} (564.4 MJ m^{-2} during shading period) in 2018 and 2019, respectively. In Wenjiang, it was recorded 1783.3 MJ m^{-2} (548.6 MJ m^{-2} during shading period) and 1678.3 MJ m^{-2} (502.9 MJ m^{-2} during shading period) in 2018 and 2019, respectively. The soil of the field in Hanyuan contained 25.50 g kg^{-1} organic matter, 2.20 g kg^{-1} total N, 1.62 g kg^{-1} total P, 28.14 g kg^{-1} total K, 182.00 mg kg^{-1} NaOH hydrolysable N, 112.40 mg kg^{-1} Olsen P and 296.60 mg kg^{-1} NH$_4$OAc extractable K in 2018; and 36.47 g kg^{-1}, 1.89 g kg^{-1}, 1.33 g kg^{-1}, 29.20 g kg^{-1}, 66.10 mg kg^{-1}, 94.50 mg kg^{-1}, and

214.30 mg kg^{-1} in 2019, respectively. Meanwhile, in Wenjiang the soil contained 28.50 g kg^{-1} organic matter, 1.50 g kg^{-1} total N, 0.94 g kg^{-1} total P, 17.50 g kg^{-1} total K, 137.20 mg kg^{-1} NaOH hydrolysable N, 50.82 mg kg^{-1} Olsen P and 143.10 mg kg^{-1} NH$_4$OAc extractable K in 2018; and 29.92 g kg^{-1}, 1.47 g kg^{-1}, 1.04 g kg^{-1}, 16.43 g kg^{-1}, 72.65 mg kg^{-1}, 43.83 mg kg^{-1}, and 164.10 mg kg^{-1} in 2019, respectively.

Using two conventional *indica* rice (*Oryza sativa* L.) cultivars, 'Huanghuazhan' and 'Guichao II', a two-factor split-plot design experiment with three replicates was conducted in paddy fields at Hanyuan and Wenjiang in 2018 and 2019. At each site, the shading treatment was located in the main plot, and the full sunlight plots constituted the control (CK). After the heading stage, the treatment plants were subjected to shading stress (SH) for 30 d. White cotton fabric with a weave aperture of 0.5 mm was used to create 2 m tall screens positioned above the rice. These screens were of sufficient size to maintain good ventilation while reducing radiation by approximately 53% and preventing lateral sunlight penetration (Li et al., 2020). There was nonsignificant difference in temperature and humidity between CK and SH plots. In Hanyuan, the shading treatment was conducted from 27 July to 25 August during both years, whereas in Wenjiang, shading was implemented from 30 July to 28 August 2018, and from 2 August to 31 August 2019.

Thirty-day-old healthy seedlings with uniform growth were selected and transplanted by hand at a spacing of 33.3 × 20.0 cm with two plants per hill. The dimensions of each subplot were 3.0 × 10.0 m at Wenjiang in both years, and 4.0 × 4.0 m in 2018 and 3.0 × 8.0 m in 2019 at Hanyuan. For field fertilization treatment, 180 kg N ha^{-1} as urea, 90 kg P$_2$O$_5$ ha^{-1} as single superphosphate, and 180 kg K$_2$O ha^{-1} as potassium chloride were used. The P$_2$O$_5$ was applied at the start of the experiment and K$_2$O was applied in an equal-split at the start of the experiment and at the beginning of the panicle initiation stage. Urea was split-applied as follows: 42% at transplanting, 18% at tillering stage, 20% at panicle initiation, and 20% at spikelet differentiation stage. Herbicides and pesticides were used to control weeds, insects, and diseases throughout the growth period. Water management measures were implemented according to our previous study (Wang et al., 2015).

The tiller (or panicle) number of rice plants on 60 hills in each plot was counted and three representative hills of the 60 were sampled at three stages: heading, 20 d after heading (DAH20), and maturity (Zhang et al., 2013). The stem plus sheath (SPS) (for all three stages) and grains (at maturity) were separated from each plant, dried at 105 °C for 1 h, and oven dried at 75 °C to constant mass. The dried samples were weighed, and the SPS material was milled and sifted through a 0.5 mm screen. The stored nonstructural carbohydrates (NSC) content of the SPS material at the three stages was determined by anthrone colorimetry, following Yoshida et al. (1976) with modifications. 0.1 g dried samples were extracted with 5 mL 80% ethanol in 80 °C water bath for 30 min, centrifuged (4000 rpm) for 10 min, the previous steps were repeated three times, the supernatant was collected and fixed volume to measure the content of soluble sugar with anthrone reagent at 620 nm wavelength. The rest precipitate was used for starch evaluation; 2 mL distillate water was added to the precipitate and stored in 80 °C water bath to evaporate the ethanol. And then the precipitate was gelatinized in the boiling water bath for 15 min. After cool down to room temperature, the sample was extracted with 9.2 and 4.6 mol L^{-1} perchloric acid and centrifuged (4000 rpm) for 10 min, respectively. The supernatant was collected to determine the starch content with anthrone reagent at 620 nm wavelength. The NSC content was calculated as soluble sugar content plus starch content.

The remobilization of stored NSC from SPS to the grain (RASN), remobilization percentage of removed NSC from SPS to the grain (RPRN), and contribution of RASN to the grain (CRASN) were calculated as described by Yang et al. (2001) and Pan et al. (2011). The specific formulas were as follows:

$$RASN \ (g \ m^{-2}) = NSC \ in \ SPS \ at \ t1 - NSC \ in \ SPS \ at \ t2;$$
$$RPRN \ (\%) = (RASN/NSC \ in \ SPS \ at \ t1) \times 100;$$
$$CRASN \ (\%) = (RASN/Dry \ kernel \ mass \ of \ grain \ at \ maturity) \times 100$$

where, t represents heading, DAH20, or maturity, t1 and t2 represents the previous and current stages, respectively.

ANOVA was performed to assess the effect of shading stress in rice stem plus sheath characteristics using the SPSS version 18.0 (IBM, Armonk, New York, USA). Means of treatments were compared between year, site, light treatment and rice variety according to Fisher's protected least significance differences (LSD) test at the 5% level. Graphs were drawn with GraphPad Prism 5.0 (GraphPad Software, San Diego, California, USA).

RESULTS

Effect of shading stress in rice stem plus sheath characteristics

The effects of year, site, variety, shading stress, and their interaction on SPS dry weight (DWS), grain dry weight (DWG), and SPS NSC characteristics are shown in Table 1. Except for the RPRN, year and site had a significant effect on the DWS, DWG, and SPS NSC characteristics. Both rice variety and light treatment significantly affected stored NSC content, RASN, RPRN, and CRASN. There were nonsignificant interactions between year and light treatment, and among year, site, rice variety, and light treatment and the measured parameters (dry weight and NSC characteristics). However, except for CRASN, the interaction Year × Site had a marked influence on dry weight and NSC characteristics. Stored NSC content was significantly influenced by the interaction Year × Variety, whereas DWG was markedly affected by the interaction Site × Light treatment. Moreover, DWS, stored NSC content, and RPRN were affected by the interaction Site × Variety. The interaction Variety × Light treatment had a significant effect on stored NSC content and RPRN. The DWS, RPRN, and CRASN were significantly affected by the interaction Year × Site × Light treatment, whereas the interaction Year × Site × Variety had a significant effect on stored NSC content, RASN, and CRASN. Furthermore, stored NSC content and RPRN were affected by the interaction Year × Variety × Light treatment, and that of Site × Variety × Light treatment, respectively.

Stem plus sheath and grain biomass accumulation

The DWS at the heading stage was markedly higher than that at DAH20, indicating vigorous remobilization of stored DM from these organs between the heading and DAH20 stages (Table 2). The DWS was significantly greater at Hanyuan than at Wenjiang in both years. Nonsignificant difference in DWS was observed at the heading and DAH20 stages at either site between the years. However, shading stress at the Hanyuan site significantly decreased the DWS of 'Guichao II' and 'Huanghuazhan' at the DAH20 stage in 2019 and 'Guichao II' at the DAH20 stage in 2018. Shading stress also markedly decreased the DWS at the maturity stage, leading to a 16.99%-22.19% and 0.93%-31.00% reduction at Wenjiang and Hanyuan, respectively. Furthermore, the average reduction in DWS caused by shading stress was greater for 'Guichao II' than for 'Huanghuazhan' at the DAH20 and maturity stages at both sites. Moreover, the DWG varied between sites and years (Figure 1). Shading stress led to a 6.96%-14.29% and 13.80%-22.52% reduction in DWG at the maturity stage at Wenjiang and Hanyuan, respectively. 'Huanghuazhan' and 'Guichao II' decreased by 11.20%-20.92% and 6.96%-22.52% respectively in 2 yr compared with the control. These results suggested that shading stress markedly increased the remobilization of stored DM after the heading stage. However, the effect of shading stress on DWS and DWG differed between rice varieties.

Table 1. Effects of year, site, variety, light treatments, and their interaction on various rice stem plus sheath characteristics.

Variation source	DWS	DWG	NSC	RASN	RPRN	CRASN
Y (year)	11.35**	22.47**	680.11**	7.02**	0.139	23.85**
S (site)	376.92**	427.31**	645.25**	57.99**	1.32	9.47**
V (variety)	1.34	0.426	652.92**	8.44**	24.44**	7.73**
L (light treatment)	35.85**	55.70**	1895.64**	47.52**	328.54**	94.21**
Y × S	41.55**	22.79**	687.51**	4.99*	66.06**	0.209
Y × V	0.573	0.047	56.28**	0.297	0.024	2.01
Y × L	3.48	0.159	0.002	0.041	0.708	0.183
S × V	6.43*	0.024	4.98*	0.007	5.99*	0.316
S × L	1.81	11.28**	0.647	0.975	0.167	0.134
V × L	0.744	0.007	35.55**	2.78	8.67**	2.32
Y × S × V	2.12	0.004	171.78**	7.95**	0.010	7.43*
Y × S × L	8.03**	1.35	0.436	1.73	10.12**	5.31*
Y × V × L	3.87	0.020	14.39**	0.049	0.004	0.104
S × V × L	1.82	0.009	2.69	0.835	9.56**	1.57
Y × S × V × L	0.105	0.275	0.518	1.99	3.98	1.41

DWS: Dry weight of stem plus sheath; DWG: dry weight of grain; NSC: stored nonstructural carbohydrate content of stem plus sheath; RASN: remobilization amount of stored NSC; RPRN: redistribution percentage of remobilized NSC; CRASN: contribution of RASN from stem plus sheath to grain.
*, **Significant at the 0.05 and 0.01 probability levels, respectively.

Table 2. Dry matter weight of the stem plus sheath (DWS) of rice harvested at heading, 20 d after heading (DAH20), and maturity stages from plants grown under control (CK) and shade (applied after the heading stage) at Hanyuan and Wenjiang in 2018 and 2019.

Site	Variety	Treatment	Heading		DAH20		Maturity	
			2018	2019	2018	2019	2018	2019
					g m⁻²			
Wenjiang	HHZ	CK	576.00b	600.60b	484.80bc	453.60d	494.85cd	405.15f
		Shading	617.25b	604.65b	480.15bc	422.10d	403.65e	336.30g
	GC	CK	638.55b	643.20b	494.70bc	439.50d	563.10abc	459.00e
		Shading	533.85b	619.20b	430.50c	424.35d	438.15de	366.45fg
		Mean	591.45B	616.9B5	472.50B	434.85B	474.90B	391.80B
Hanyuan	HHZ	CK	817.20a	922.20a	565.20ab	773.70a	594.75a	871.50a
		Shading	823.65a	892.65a	538.65ab	613.95c	589.20a	601.35c
	GC	CK	809.85a	907.50a	603.15a	695.55b	574.20ab	695.10b
		Shading	840.00a	881.70a	502.65bc	589.65c	507.00bcd	539.55d
		Mean	822.75A	901.05A	552.45A	668.25A	565.65A	676.95A

HHZ: 'Huanghuazhan'; GC: 'Guichao II'.
Different uppercase and lowercase letters indicate significant differences between sites and light treatments at the 5% level, respectively.

Figure 1. Dry matter weight of mature rice grains (DWG) harvested from plants grown under light and shade (applied after the heading stage) at Hanyuan (A) and Wenjiang (B).
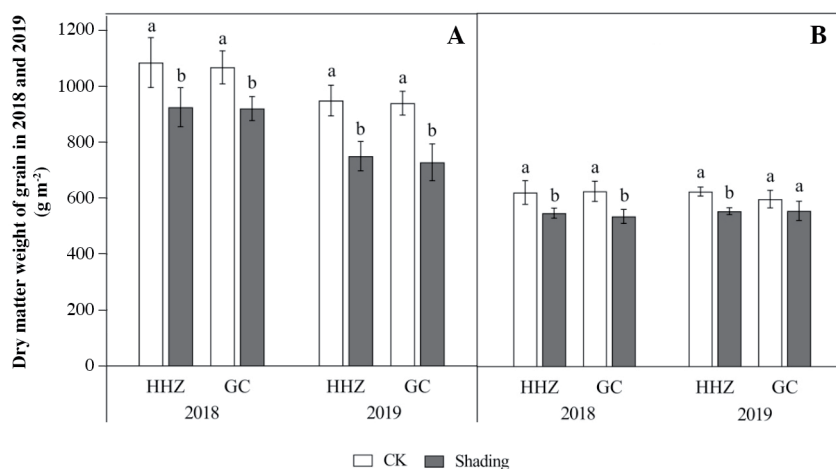
CK: Control; HHZ: 'Huanghuazhan'; GC: 'Guichao II'.
Different lowercase letters indicate significant differences between light treatments at the 5% level.

Stored nonstructural carbohydrate content

Stored NSC contributed approximately one-fifth of the DWS at the heading stage (Table 3). The SPS stored NSC content varied between years and sites. In 2018, the SPS-stored NSC content at the heading and DAH20 stages at Hanyuan was significantly lower than that at Wenjiang, whereas in 2019, the SPS-stored NSC content at the heading stage at both sites, as well as at the DAH20 and maturity stages at Hanyuan were higher. Generally, the SPS-stored NSC content gradually decreased after the heading stage, and shading stress increased the reduction of SPS-stored NSC content, both at the DAH20 and maturity stages, indicating remobilization of NSC from the SPS, especially under shading stress. Shading stress significantly decreased the SPS-stored NSC content after the heading stage, leading to a 23.99%-50.30% and 31.65%-61.33% reduction at the DAH20 and maturity stages at both sites in 2018 and 2019, respectively. However, the effect of shading stress on the SPS-stored NSC content differed between rice varieties. Shading led to a greater decrease in SPS-stored NSC content in 'Guichao II' than in 'Huanghuazhan' at the DAH20 at Wenjiang and Hanyuan, and maturity stages at Hanyuan, respectively, and the decrease in SPS-stored NSC content was greater in 'Huanghuazhan' than in 'Guichao II' at the maturity stage at Wenjiang.

Table 3. Nonstructural carbohydrate content in the stem plus sheath of rice harvested at heading, 20 d after heading (DAH20), and maturity stages from plants grown under control (CK) and shade (applied after the heading stage) at Hanyuan and Wenjiang in 2018 and 2019.

Site	Variety	Treatment	Heading		DAH20		Maturity	
			2018	2019	2018	2019	2018	2019
			mg g^{-1}					
Wenjiang	HHZ	CK	208.23ab	205.88c	218.30a	185.17a	121.22a	104.25b
		Shading	210.59a	205.18c	165.92b	132.90d	52.11d	54.25f
	GC	CK	203.33b	240.84a	140.98c	150.15c	86.71b	88.84c
		Shading	207.59ab	245.54a	94.55e	89.96f	55.39d	60.72e
		Mean	207.44A	224.36A	154.94A	139.55A	78.86A	77.02A
Hanyuan	HHZ	CK	194.27c	230.96b	128.42d	170.62b	91.99b	122.92a
		Shading	192.62c	229.07b	63.83f	122.94e	43.65e	80.22d
	GC	CK	202.94b	211.47c	72.81f	135.74d	66.98c	106.31b
		Shading	205.08ab	207.46c	40.58g	92.45f	25.90f	60.52e
		Mean	198.73B	219.74A	76.41B	130.44A	57.13A	92.49A

HHZ: 'Huanghuazhan'; GC: 'Guichao II'.
Different uppercase and lowercase letters indicate significant differences between sites and light treatments at the 5% level, respectively.

Remobilization of stored nonstructural carbohydrates

RASN after the heading stage varied between sites, with 60.00-129.90 and 104.10-159.15 g m^{-2} stored NSC remobilized at Wenjiang and Hanyuan, respectively (Table 4). Even though RASN from DAH20 to maturity was markedly lower in 2018, RASN after heading in Hanyuan was significantly improved primarily due to the increase in RASN from heading to DAH20. Nonsignificant difference in RASN from DAH20 to maturity was observed between the control and shading stress treatments. However, increases of 27.99%-286.17% and 25.87%-58.60% in RASN from the heading to the DAH20 stages were recorded under shading stress in Wenjiang and Hanyuan, respectively. Finally, the RASN of Wenjiang and Hanyuan increases by 13.80%-89.00% and 26.16%-47.17% respectively in the mature stage, indicating that shading significantly improved RASN after the heading stage. Furthermore, the increase in RASN of 'Huanghuazhan' (27.81%-89.00%) after the heading stage was higher than that of 'Guichao II' (13.80%-27.48%) under shading stress, suggesting that the effect of shading stress depended on rice variety.

Table 4. Remobilization of stored nonstructural carbohydrates from the stem plus sheath (RASN) in rice harvested at heading, 20 d after heading (DAH20), and maturity stages from plants grown under control (CK) and shade (applied after the heading stage) at Hanyuan and Wenjiang in 2018 and 2019.

Site	Variety	Treatment	Heading-DAH20		DAH20-Maturity		Heading-Maturity	
			2018	2019	2018	2019	2018	2019
			g m^{-2}					
Wenjiang	HHZ	CK	14.10e	39.60e	45.90a	41.85a	60.00e	81.45d
		Shading	54.45d	68.10d	58.95a	37.80a	113.40bcd	105.90c
	GC	CK	55.80d	88.95c	21.15b	25.20b	76.80de	114.15bc
		Shading	75.15cd	113.85ab	16.50b	16.05c	91.50cde	129.90b
		Mean	49.95B	77.70B	35.70A	30.30A	85.50B	107.85B
Hanyuan	HHZ	CK	89.40c	81.15cd	14.70b	24.60b	104.10bcd	105.90c
		Shading	124.20b	128.70a	8.85b	27.30b	133.05ab	155.85a
	GC	CK	120.60b	97.50bc	5.55b	20.55bc	126.15abc	117.90c
		Shading	151.80a	128.40a	7.20b	21.90bc	159.15a	150.30a
		Mean	121.50A	108.90A	9.15B	23.55A	130.65A	132.45A

HHZ: 'Huanghuazhan'; GC: 'Guichao II'.
Different uppercase and lowercase letters indicate significant differences between sites and light treatments at the 5% level, respectively.

Remobilization percentage of stored nonstructural carbohydrates

RPRN after heading reached 48.61%-85.38% at Wenjiang and 49.62%-92.36% at Hanyuan (Table 5). RPRN was lower at Hanyuan than at Wenjiang from DAH20 to maturity stages but higher from heading to DAH20 stages, which, in 2018, resulted in an 11.50% increase in RPRN after the heading stage. However, RPRN after the heading stage was significantly lower at Hanyuan in 2019, primarily due to the reduction in RPRN from DAH20 to maturity stages. With the exception of the RPRN of 'Guichao II' at Wenjiang in 2019 (from DAH20 to maturity stages), 'Huanghuazhan' at Hanyuan in 2018, and 'Guichao II' at Wenjiang in 2018 (from heading to DAH20 stages), shading stress significantly increased RPRN from heading to DAH20 stages and from DAH20 to maturity stages in both years, leading to a 15.86%-73.30% and 21.29%-53.55% increase in RPRN after the heading stage at Wenjiang and Hanyuan, respectively. However, the effectiveness of shading stress on RPRN differed between rice varieties. Shading stress resulted in a greater increase in RPRN of 'Huanghuazhan' than of 'Guichao II' after the heading stage in both years, due to the obvious improvement in RPRN from heading to DAH20 stages at both sites and that from DAH20 to maturity stages at Hanyuan. These results suggest that the increased remobilization of stored NSC from the SPS is a rice plant adaptation to shady conditions.

Contribution of remobilized nonstructural carbohydrates

The CRASN contributed 9.92%-23.63% and 9.63-20.92% of DWG at Wenjiang and Hanyuan, respectively (Table 6). Nonsignificant differences in CRASN from DAH20 to maturity stages were observed between the control and shading

Table 5. Redistribution percentage of remobilized nonstructural carbohydrates from the stem plus sheath (RPRN) in rice harvested at heading, 20 d after heading (DAH20), and maturity stages from plants grown under control (CK) and shade (applied after the heading stage) in Hanyuan and Wenjiang in 2018 and 2019.

Site	Variety	Treatment	Heading-DAH20		DAH20-Maturity		Heading-Maturity	
			2018	2019	2018	2019	2018	2019
					%			
Wenjiang	HHZ	CK	11.29f	32.02f	42.47b	49.62b	48.61d	65.88d
		Shading	40.90e	54.72cd	73.15a	67.45a	84.24ab	85.23a
	GC	CK	43.89e	57.36c	30.12bcd	38.08c	60.85c	73.69c
		Shading	63.38c	74.87a	40.49b	41.03bc	78.11b	85.38a
		Mean	40.15B	54.74A	46.56A	49.05A	67.95A	77.55A
Hanyuan	HHZ	CK	56.37d	37.98e	21.13de	18.61d	65.57c	49.62f
		Shading	78.35b	62.78b	23.62cde	36.04c	83.73ab	76.19c
	GC	CK	72.85b	50.80d	12.18e	21.73d	76.15b	61.50e
		Shading	88.15a	70.23a	35.58bc	40.03c	92.36a	82.16b
		Mean	79.78A	55.45A	23.13B	29.10B	79.45A	67.37B

HHZ: 'Huanghuazhan'; GC: 'Guichao II'.
Different uppercase and lowercase letters indicate significant differences between sites and light treatments at the 5% level, respectively.

Table 6. Contribution of remobilization amount of stored nonstructural carbohydrates to the grain from the stem plus sheath (CRASN) in rice harvested at heading, 20 d after heading (DAH20), and maturity stages from plants grown under control (CK) and shade (applied after the heading stage) at Hanyuan and Wenjiang in 2018 and 2019.

Site	Variety	Treatment	Heading-DAH20		DAH20-Maturity		Heading-Maturity	
			2018	2019	2018	2019	2018	2019
					%			
Wenjiang	HHZ	CK	2.34d	6.41f	7.58b	6.73a	9.92c	13.14c
		Shading	10.02c	12.38cd	10.78a	6.89a	20.80a	19.26b
	GC	CK	8.97c	15.01bc	3.40c	4.27b	12.36bc	19.28b
		Shading	14.13ab	20.68a	3.10cd	2.95bcd	17.23ab	23.63a
		Mean	8.89A	13.62A	6.22A	5.21A	15.08A	18.83A
Hanyuan	HHZ	CK	8.28c	8.53ef	1.36cd	2.60cd	9.63c	11.13c
		Shading	13.44ab	17.26b	0.93cd	3.66bc	14.37bc	20.92ab
	GC	CK	11.18bc	10.38de	0.51d	2.19d	11.69c	12.57c
		Shading	16.47a	17.74ab	0.79cd	3.02bcd	17.26ab	20.76ab
		Mean	12.34A	13.48A	0.90B	2.87B	13.24A	16.35A

HHZ: 'Huanghuazhan'; GC: 'Guichao II'.
Different uppercase and lowercase letters indicate significant differences between sites and light treatments at the 5% level, respectively.

treatments at both sites and in both years. However, with the exception of 'Guichao II' (Wenjiang) and 'Huanghuazhan' (Hanyuan) in 2018, shading stress significantly increased CRASN from heading to maturity stages, which contributed to 22.56%-109.68% and 47.65%-87.96% increases in CRASN after heading stage at Wenjiang and Hanyuan, respectively. Shading stress led to a greater increase in CRASN of 'Huanghuazhan' than that of 'Guichao II' after heading stage by increasing CRASN from heading to DAH20 stages. These results suggest that the effect of shading stress on CRASN is primarily dependent on the growth duration from heading to DAH20, and on the rice variety.

DISCUSSION

Shading stress caused by environmental pollution and extreme weather has become a serious problem in rice production and often results in the reduction of both rice yield and quality (Tie et al., 2016; Chou et al., 2019). A previous study demonstrated the effect of shading stress on photosynthesis in rice (Wang et al., 2015); however, during the reproductive stage, rice yield formation depends not only on the transport of photosynthates from leaves to the reproductive organs but also on the redistribution and transport of carbohydrates stored before heading (Fu et al., 2011). In the present study, the effect of shading on the remobilization of stored NSC from the SPS was examined in detail.

Dry matter accumulation after the heading stage is particularly important for rice yield formation, and it is estimated that approximately 60% of grain assimilates are produced by photosynthesis during this stage (Wu et al., 2018). Under shading stress (or decreased solar radiation), however, plant photosynthesis is inhibited (Dhruw and Guhey, 2018; Chen et al., 2019), resulting in insufficient photosynthate supply from foliage for grain production. Consequently, rice plants show increased remobilization of stored DM from the SPS to the grain as a compensation mechanism (Ren et al., 2003; Deng et al., 2009). In the present study, shading stress significantly decreased the DWS at the DAH20 and maturity stages, suggesting stronger remobilization of DM from the SPS (Table 2). However, even though more DM was redistributed from the SPS to the grain under shading stress, it was not sufficient to make up for shading damage, leading to a 6.96%-22.52% reduction in DWG at the maturity stage (Figure 1). These results are consistent with those of previous studies that have reported a decrease in DM production in rice plants and in the proportion of DM allocated to grain caused by shading stress, which led to insufficient grain filling (Wang et al., 2015; Dhruw and Guhey, 2018).

Crop yield is closely related to the amount of assimilates produced by photosynthesis and depends on the direct accumulation of DM after the heading stage and the remobilization of stored NSC in vegetative organs (especially in the SPS) (Panda and Sarkar, 2014; Deng et al., 2016). In rice, NSC are important photosynthetic products stored in the SPS, which play a significant role in rice grain filling and yield formation (Stella et al., 2016). Generally, redistribution of NSC during the rice grain filling stage can contribute up to 30% of grain yield (Pan et al., 2011; Panda and Sarkar, 2014). In the present study, the NSC content of the SPS gradually decreased after the heading stage, resulting in 60.00-159.15 g m^{-2} of NSC stored in the SPS being remobilized to the grains, which contributed 9.63%-23.63% of the DWG at the maturity stage (Tables 3, 4, and 6).

In addition to genetic factors, environmental conditions also have a significant impact on the remobilization of stored NSC in rice (Zhen et al., 2019). Rice under CO_2 enrichment and water stress conditions during the filling period show enhanced remobilization of SPS-stored NSC after the heading stage (Yang et al., 2001; Morita and Nakano, 2011). In the present study, shading stress significantly decreased the SPS-stored NSC content at the DAH20 and maturity stages, leading to a 13.80%-89.00% and 15.86%-73.30% increase in RASN and RPRN, respectively, from heading to maturity stages, which contributed 14.37%-23.63% of the DWG at the maturity stage. These results are consistent with those of Okawa et al. (2003), who demonstrated that rice plants show increased remobilization of stored NSC from the SPS to make up for grain filling limitations caused by the reduction of assimilate sources. Moreover, nonsignificant difference in RASN from DAH20 to maturity stages was observed between the shade-stressed and control plants. Shading stress appeared to enhance the remobilization of stored NSC from the SPS, primarily during the heading to DAH20 period (Table 4), indicating the importance of remobilization of stored NSC during early grain filling stage in rice (Nagata et al., 2001). In general, owing to a greater sink capacity, shading stress led to a greater increase in RASN and RPRN from heading to DAH20. This increase was higher for 'Huanghuazhan' than for 'Guichao II' and higher at Hanyuan than at Wenjiang such that a higher grain yield from 'Huanghuazhan' at Hanyuan was observed. This finding is similar to that of Yoshinaga et al. (2013), who reported that rice NSC remobilization capacity increases with sink capacity. In addition,

that rice plants at Wenjiang (low light areas), showed higher CRASN and lesser yield loss than that at Hanyuan (high light areas) (Li et al., 2020). Shading led to a 13.13% and 9.02% decrease in grain yield at Wenjiang and a 14.20% and 21.74% decrease at Hanyuan in 2018 and 2019, respectively. This is consistent with Yang and Zhang (2006), who showed that when photosynthesis decreases, the remobilization ability of NSC from the SPS increases, partially alleviating the yield loss caused by shading. It appears that the grain yield loss caused by shading can be reduced by increasing the remobilization ability of the SPS (especially in low light areas), which might due to the interaction effect between the study sites and the light treatments.

CONCLUSIONS

In conclusion, we found that the remobilization of stored nonstructural carbohydrates (NSC) from the stem plus sheath (SPS) contributed significantly to rice grain yield. Shading stress after the heading stage significantly increased NSC remobilization from the SPS. The SPS-stored NSC content at the 20 d after heading (DAH20) and maturity stages decreased significantly, leading to significant increases in the remobilization of NSC from SPS to the grain (RASN), remobilization percentage of redistributed NSC from SPS to the grain and the contribution of RASN to the grain after the heading stage (especially up to DAH20). Furthermore, the effect of shading stress on the NSC characteristics of the SPS varied between the sites and rice varieties. The NSC remobilization ability was higher at Hanyuan than at Wenjiang, and shading stress had a greater influence on NSC remobilization of 'Huanghuazhan' than of 'Guichao II'. It appears that the grain yield loss caused by shading can be reduced by increasing the remobilization ability of the SPS, and it is particularly important for low light areas. Our study indicates that increased accumulation of DM before the heading stage and NSC remobilization from the SPS during the early stages of grain filling are adaptive strategies of rice plants that could reduce yield loss under shading stress.

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