Zheng, Wang, Li, Huang, Tang and Tang (2018).
Seed Science and Technology, **46**, 3, 533-546.
https://doi.org/10.15258/sst.2018.46.3.10

Contributions of photosynthetic organs to the seed yield of hybrid rice: the effects of gibberellin application examined by carbon isotope technology

Huabin Zheng[1], Xiaomin Wang[2], Yunxia Li[1], Guangfu Huang[2], Qiyuan Tang[1]* and Jianwu Tang[3]

[1] Collaborative Innovation Center for Grain and Oil Crop in Southern Paddy Field, College of Agronomy, Hunan Agricultural University, Changsha, P.R. China, 410128
[2] College of Agronomy, Yunan University, Kunming, P.R. China, 650201
[3] The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543, USA
*Author for correspondence (E-mail: qytang@hunau.edu.cn)

(Submitted July 2018; Accepted October 2018; Published online December 2018)

Abstract

Changes in the structure and quality of a hybrid combination population have been observed after the application of gibberellins. Such changes would affect the accumulation and distribution of photosynthetic products, which would subsequently affect the yield during hybrid rice seed production. In this study, photosynthetic physiological characteristics and the distribution of photosynthetic products were evaluated in a field experiment. The transport of panicle photosynthetic products to grain was demonstrated using a ^{14}C isotope tracer technique. The contribution ratios of the panicle and leaf to yield in the hybrid rice seed production were 32.3 and 42.1%, respectively. Through isotope tracing technology, it was determined that about 90% of the photosynthetic products of the panicle and 50% of those of the leaf were delivered to the panicle. During the filling period, the contribution of panicle to yield was concentrated in the early period (0–10 days after pollination), and the contribution of leaf to yield was more significant in the late period (10 days after pollination to maturity). These results suggest that the panicle makes an important photosynthetic contribution (equivalent to that of the flag leaf) during the process of grain filling, especially at 0–5 days after the heading stage.

Keywords: ^{14}C labeling, contribution to yield, gibberellins, hybrid rice seed, panicle, photosynthetic product

Introduction

Compared with traditional rice cultivation, hybrid rice seed production is a form of out-crossing cultivation using male and female parents. It changes the pollination and breeding style of rice from self- to cross-pollination. The out-crossing frequency of hybrid rice seed production can be increased up to 30% using artificial processes (Mao and Virmani

2003), whereas the chance of out-crossing with inbred rice is less than 0.5% under natural conditions (Kim, 2003). One of the key technologies used in this artificial process is the spraying of gibberellins (GAs). However, fundamental changes in the structure and quality of hybrid combination populations have been observed after the application of GAs, including increased plant height of the female parent and an uplifted panicle layer and submerged leaf layer, which would affect the interception of solar radiation by the hybrid combination population. The reduction of these effects could alter the accumulation and effective distribution of photosynthetic products, affecting the yield of hybrid rice seed production. Thus, additional studies of the contribution of different photosynthetic organs to the seed yield of hybrid rice are urgently needed to encourage innovations in hybrid rice seed production techniques.

Leaf tissue is the main photosynthetic organ of rice. Numerous studies, have examined the characteristics and functions of leaf tissue, for example, photosynthetic characteristics (Wang *et al*., 2006; Huang *et al*., 2016), coordination among sources, sinks and translocation pathways (Shi *et al*., 2016), radia-tion use efficiency (Zhang *et al*., 2009), and leaf senescence (Yumiko *et al*., 2006; Lin and Nam, 2007). These detailed studies of leaf tissues have enabled effective rice breeding and high-yield cultivation. However, non-leaf organs, such as the stem and sheath (Nilsen, 1995; Zhang *et al*., 2015) and inflorescence and developing fruit (Weiss *et al*., 1988; Maydup *et al*., 2014), which have actual or potential photosynthetic capability (Guido and Hardy, 2003), have been much less extensively studied. For example, Zhang *et al.* (2011) reported that non-leaf organs (ear, peduncle and sheath) accounted for 73–81% of the total contribution of organs above the flag leaf node to wheat grain weight. Maydup *et al.* (2010) also reported that the contribution of ear photosynthesis to grain yield differed (from about 12 to 42%) depending on the experimental approach used. Araus *et al.* (1993) used carbon isotope technology to confirm that ≥ 59% of the materials in wheat grain came from photosynthesis in the ear tissue. Similar phenomena were also apparent in rice, but few studies have investigated the contribution ratio (CR) of non-leaf organs to yield.

Hybrid rice technology is effective in enhancing grain yield and farmers' income when it is used correctly (Peng, 2016). High yield, high quality, and low-cost hybrid rice seed is crucial for further promotion of hybrid rice (Peng, 2016). To acquire a high yield, the accumulation and effective distribution of photosynthetic products by green photosynthetic organs has become increasingly important. Consequently, the aim of this study was to determine the CR of different photosynthetic organs to yield and their photosynthetic physiological characteristics in hybrid rice seed production, which could provide the theoretical basis for further increasing the yield of hybrid rice seed production.

Materials and methods

Experimental site and hybrid combination

The field experiment was conducted in Liuyang County, Hunan Province, in 2015. The experimental hybrid combination was 'IIyou 416' ('R416' as male parent × 'II-32A' as female parent) and 'Jinyou 167' ('R167' as male parent × 'Jin23A' as female parent).

Experimental design

Two factorial experiments with a randomised design, including two fertilisation models and four doses of GAs, were conducted with three replications and a parental row ratio of 2:12. The total quantity of inorganic fertiliser was 120 kgN hm^{-2} and the $N:P_2O_5:K_2O$ ratio was 1:0.8:1. Urea for N, calcium superphosphate for P and potassium chloride for K were used throughout the experiments. Urea was applied twice in fertilisation model 1 (M1), 90% as basal fertiliser and 10% as panicle fertiliser. Fertilisation model 2 (M2) was applied with a three-way split of 50% as basal fertiliser, 30% as tillering fertiliser, and 20% as panicle fertiliser. The P and K fertilisers were applied as basal fertilisers before rice was transplanted. In addition, (Long Ping High-Tech Co., Ltd.) 920® was used a source of GAs throughout the experiments. Four doses were applied: 0 g hm^{-2} (D1), 150 g hm^{-2} (D2), 225 g hm^{-2} (D3), and 450 g hm^{-2} (D4).

The row and plant spacing of the male parent was 200 × 300 mm, 2–3 seeds per plant, and seeding density was 6×10^5 hm^{-2}. The row and plant spacing of the female parent was 133 × 167 mm, 3–4 seeds per plant, and seeding density was 42×10^5 hm^{-2}. The sowing time interval of 'IIyou 416' and 'Jinyou 167' is 26 days and 22 days, respectively. Pesticide and herbicide management followed the local practices.

Contributions of different photosynthetic organs to yield measurement

Five days after the full heading stage, 80 panicles on the main axis with a consistent flowering time and panicle type were marked. Twenty panicles were trimmed of all green leaves (A); 20 panicles were wrapped in tinfoil (B); 20 panicles were wrapped in tinfoil together with the stem and sheath (C); and 20 panicles were left untouched as a control (D). Micro-pores were punched by needle in the tinfoil to enable gas exchange. In the mature stage, the grain weight and filling ratio were determined after panicle harvesting. The calculation was as follows:

$$\text{Yield}\left(\frac{\text{Y, mg}}{\text{mg}}\right) = \text{total spikelet} \times \text{filling ratio} \times \text{grain weight} \times \text{panicle number} \times 10 \quad (1)$$

$$\text{CR of leaf to yield (\%)} = \left(\frac{Y_B + Y_C - Y_A}{Y_A + Y_B + Y_C}\right) \times 100 \quad (2)$$

$$\text{CR of panicle to yield (\%)} = \left(\frac{Y_A + Y_C - Y_B}{Y_A + Y_B + Y_C}\right) \times 100 \quad (3)$$

$$\text{CR of stem and sheath to yield (\%)} = \left(\frac{Y_A + Y_B - Y_C}{Y_A + Y_B + Y_C}\right) \times 100 \quad (4)$$

Distribution of photosynthetic products as measured by isotope tracing technology.

Three treatments were applied to determine the distribution of photosynthetic products by isotope tracing technology, including different photosynthetic organs five days after the heading stage, different sampling stages in the flag leaf, and different tagging times after the heading stage.

To feed the panicle and leaf, three applications of $^{14}CO_2$ were supplied. During 9:00–11:00, a home-made photosynthetic leaf chamber with a plastic membrane enclosed the panicle or leaf and was sealed with rubber dough while air was pumped in to the chamber. In the following stage, 0.75L $^{14}CO_2$ gas (5 mL L_{-1}) was injected into the chamber. The specific activity was 185 kBq L_{-1}. Thirty minutes after photosynthetic assimilation, the residual $^{14}CO_2$ was absorbed with an NaOH and $Ca(OH)_2$ mixture. The leaf chamber was then removed from the panicle and leaf. The $^{14}CO_2$-labeled plants were sampled at maturity. After trimming off the root, these plants were divided into four parts: leaf, panicle, stem and sheath, and other tissues. The samples were dried at 105°C for 20 minutes and then at 80°C for 48 hours, and were then ground to pass through a 0.3-mm sieve after weighing the samples. Samples were prepared by adopting the method of combustion in a Molotov cocktail. As an absorption liquid, 4 ml neovaricaine was used to absorb $^{14}CO_2$ in the Molotov cocktail. A pipette was used to transfer 3 ml neovaricaine from the Molotov cocktail to the test bottle. An additional 8 ml of scintillation solution was added to the test bottle. The scintillation solution contained glycol ether, naphthalene, PPO, and POPOP.

The radiometry of the samples in the test bottle was measured with a Wallac 1400DSA liquid scintillation counter (Perkin-Elmer, Waltham, MA, USA) and then converted to specific activity.

$$\text{Specific activity (Bq)} = \left(\frac{\Delta \text{ radiometry value} \times \text{sample weight}}{60} \right) \quad (5)$$

$$\text{The } ^{14}\text{C value (\%)} = \left(\frac{\text{specific activity in the different part}}{\text{total specific activity}} \right) \times 100 \quad (6)$$

Index of photosynthetic physiological characteristics

Chlorophyll was extracted with 80% acetone, and the content was determined with a spectrophotometer (Aron 1949). The fluorescence parameter was determined with an Imaging-PAM chlorophyll fluorometer (Walz, Effeltrich, Germany) according to the method described by Kumagai *et al.* (2007).

Data analysis

Descriptive statistics and a correlation analysis of the index were calculated using Statistix 8.0 and Microsoft Excel 2007. A one-way analysis of variance (ANOVA) and the least significant difference (LSD) test were used to assess differences among treatments.

Results

CR of photosynthetic organs to yield

The CR of leaf to yield was 32.3% (25–39%, table 1). Application of GAs increased the CR of leaf to yield. Compared with D1, GA application (D2–D4) increased the CR of leaf to yield by 30% on average (26–38%). Similarly, the CR of panicle to yield was 42.1%

Table 1. Variation in the contribution ratio of different green photosynthetic organs to hybrid rice seed yield.

Fertilisation model	Dosage of exogenous hormones	Contribution ratio (%)		
		Leaf	Panicle	Stem and sheath
'IIyou 416'				
M1	D1	32.12	17.51	50.37
	D2	39.67	29.65	30.68
	D3	34.27	38.25	27.48
	D4	34.35	36.51	29.14
M2	D1	24.59	48.00	27.41
	D2	38.66	48.64	12.71
	D3	37.70	62.30	0.00
	D4	36.85	53.18	9.96
LSD 0.05				
M		7.90^{ns}	9.75*	6.82**
D		11.18^{ns}	13.78^{ns}	9.64*
'Jinyou 167'				
M1	D1	24.75	41.54	33.71
	D2	28.93	46.43	24.64
	D3	33.16	39.29	27.54
	D4	32.33	41.45	26.22
M2	D1	28.20	46.24	25.57
	D2	38.12	46.09	15.80
	D3	28.05	38.91	33.03
	D4	35.72	38.89	25.39
LSD 0.05				
M		9.38^{ns}	4.90^{ns}	10.76^{ns}
D		13.26^{ns}	6.92^{ns}	15.22^{ns}

ns = not significant; *$P < 0.05$ and **$P < 0.01$.

(18–62%). The variation in the CR of panicle to yield when GAs were applied differed between 'IIyou 416' and 'Jinyou 167'. In addition, spraying GAs (D2–D4) decreased the CR of stem and sheath to yield by 35%, on average (3–72%).

Distribution of photosynthetic products among photosynthetic organs

^{14}C-labeling values in the leaf and panicle

The ^{14}C value in a ^{14}C-labeled panicle was significantly higher than that in a ^{14}C-labeled leaf (table 2); however, the ^{14}C value in the stem and sheath following the ^{14}C labeling of a leaf was higher than that following the ^{14}C labeling of a panicle. In addition, about 50% and more than 90% of the photosynthetic products in a leaf and panicle, respectively, were

Table 2. The ^{14}C value of different green photosynthetic organs to photosynthetic product in the hybrid rice seed production: ^{14}C-labelled test as hybrid combination 'IIyou 416' ('II-32A' × .R416').

^{14}C-labelled organs	Fertilisation model	Dosage of exogenous hormones	^{14}C value (%)			
			Leaf	Panicle	Stem	Other
Leaf	M1	D1	8.86	2.31	82.88	5.95
		D2	11.49	46.03	41.57	0.90
		D3	6.45	67.81	24.55	1.19
		D4	6.74	56.99	33.05	3.22
	M2	D1	10.99	22.59	60.77	5.64
		D2	11.01	29.65	58.88	0.47
		D3	12.83	51.61	31.58	3.99
		D4	9.73	79.94	10.14	0.20
	LSD 0.05					
	M		4.51^{ns}	34.87^{ns}	32.26^{ns}	3.79^{ns}
	D		6.37^{ns}	49.31^{ns}	46.04^{ns}	5.36^{ns}
Panicle	M1	D1	0.16	86.09	12.37	1.37
		D2	0.10	93.81	5.50	0.60
		D3	0.12	94.69	4.70	0.49
		D4	3.93	86.71	8.92	0.44
	M2	D1	0.25	84.67	12.06	3.01
		D2	0.08	97.96	1.87	0.09
		D3	0.17	96.53	3.13	0.16
		D4	0.10	98.53	1.22	0.16
	LSD 0.05					
	M		3.08^{ns}	8.96^{ns}	5.15ns	1.61^{ns}
	D		4.36^{ns}	12.68^{ns}	7.28*	2.28^{ns}

ns = not significant; *$P < 0.05$ and **$P < 0.01$.

delivered to the panicle. The average ^{14}C value of the panicle for the different GA doses (D2–D4) was 42.9% following ^{14}C labeling of a leaf and 9.3% following ^{14}C labeling of a panicle; both were higher than the corresponding values for the D1 treatment. The distribution of photosynthetic products in the stem and sheath fell from 71 to 21% (table 2) when different doses of GAs were sprayed in the ^{14}C-labeling leaf treatment. Similar results were also observed in the ^{14}C-labeling leaf treatment.

The effect of sampling stage in ^{14}C labeling of the flag leaf

The ^{14}C value of the panicle significantly increased from the milky ripeness stage to the mature stage, whereas the opposite pattern was observed in the stem (figure 1). The distribution of photosynthetic products from the flag leaf to the panicle increased as the growth process progressed.

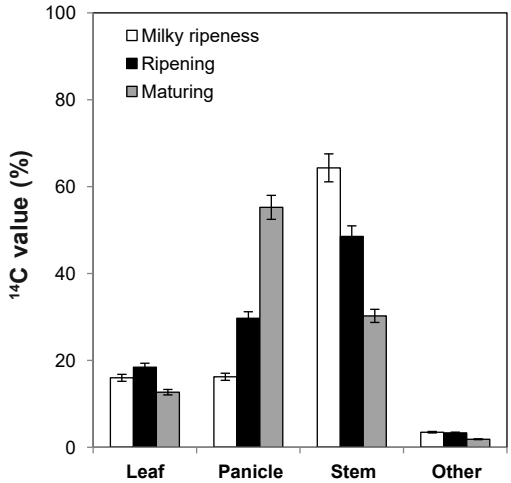

Figure 1. The ^{14}C value of different green photosynthetic organs to photosynthetic product in hybrid rice seed production: ^{14}C-labelled test as hybrid combination 'Jinyou 167' ('Jin-23A' × 'R167')

The effect of tagging time after the heading stage

The ^{14}C value in the panicle following ^{14}C labeling of the flag leaf increased with tagging time after the heading stage, from 49.3% in the heading stage to 97.4% at 10 days after the heading stage (figure 2A). However, the ^{14}C value in the panicle following ^{14}C labeling of the panicle decreased with tagging time after the heading stage, from 93.4% in the heading stage to 73.2% at 10 days after the heading stage (figure 2B).

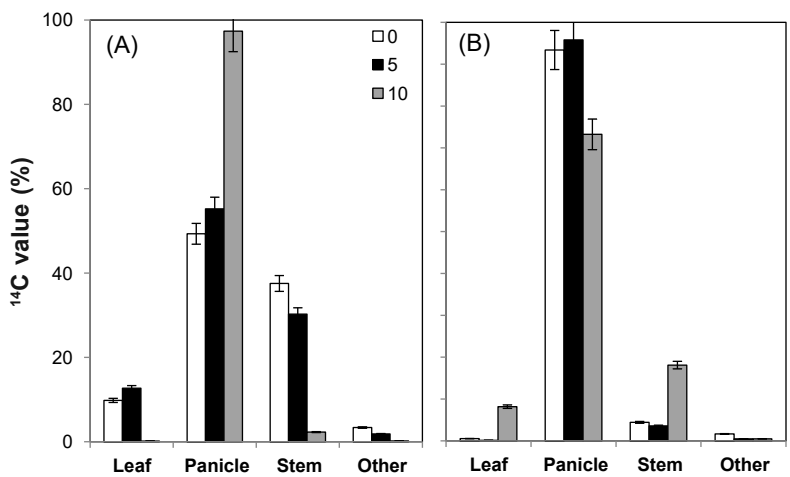

Figure 2. The ^{14}C value of different green photosynthetic organs (**A**) flag leaf; (**B**) panicle, to photosynthetic product in hybrid rice seed production: ^{14}C-labelled test as hybrid combination 'Jinyou 167' ('Jin-23A' × 'R167') in different growth and development stage. 0, 5 and 10 represent 0, 5 and 10 days after the heading stage, respectively.

Photosynthetic physiological characteristics in the non-leaf organs

There was a significant difference in the photosynthetic physiological characteristics between the leaf and non-leaf organs. The chlorophyll content of the average leaf was 10 times greater than that of a panicle (table 3). The non-photochemical quenching (NPQ) value of non-leaf organs, especially the panicle, was higher than that of a leaf; however, the quenching coefficient (qL) of non-leaf organs was lower than that of leaf organs (tables 4 and 5).

Table 3. Variation in the chlorophyll content of different green organs in different fertilisation models and dosage of exogenous hormones (mg g_{-1}).

Fertilisation model	Exogenous hormones	Leaf			Panicle			Stem and sheath		
		FL	FL+ 10	FL+ 20	FL	FL+ 10	FL+ 20	FL	FL+ 10	FL+ 20
'IIyou 416'										
M1	D1	5.29	2.09	2.96	0.15	0.19	0.29	9.52	1.38	1.17
	D2	3.62	1.71	2.54	0.16	0.15	0.16	9.13	1.36	0.90
	D3	4.03	3.01	1.94	0.16	0.07	0.16	10.44	1.37	1.18
	D4	4.06	1.82	1.76	0.14	0.16	0.09	6.42	1.29	0.98
	Mean	**4.25**	**2.16**	**2.30**	**0.15**	**0.14**	**0.18**	**8.88**	**1.35**	**1.06**
M2	D1	4.44	1.9	3.76	0.15	0.21	0.3	6.71	1.45	1.19
	D2	1.89	1.56	1.41	0.15	0.18	0.09	8.59	0.95	1.29
	D3	2.96	1.25	1.26	0.15	0.21	0.17	10.27	1.51	1.10
	D4	4.18	1.51	1.06	0.16	0.22	0.19	10.52	1.28	0.90
	Mean	**3.37**	**1.56**	**1.87**	**0.15**	**0.21**	**0.19**	**9.02**	**1.30**	**1.12**
'Jinyou 167'										
M1	D1	2.07	2.89	1.16	0.15	0.08	0.11	0.56	0.67	0.96
	D2	2.46	4.11	0.52	0.21	0.09	0.04	0.34	0.85	0.88
	D3	2.11	3.22	0.64	0.28	0.07	0.03	0.48	0.78	0.62
	D4	2.29	2.89	0.58	0.25	0.08	0.05	0.27	0.83	0.85
	Mean	**2.23**	**3.28**	**0.72**	**0.22**	**0.08**	**0.06**	**0.41**	**0.78**	**0.83**
M2	D1	2.44	2.79	0.7	0.19	0.12	0.03	0.52	0.74	0.99
	D2	2.75	2.30	0.89	0.24	0.09	0.04	0.52	0.78	0.94
	D3	2.78	3.33	1.04	0.22	0.14	0.03	0.41	0.79	0.72
	D4	1.66	1.91	0.99	0.23	0.07	0.05	0.35	0.86	0.74
	Mean	**2.41**	**2.58**	**0.9**	**0.22**	**0.11**	**0.04**	**0.45**	**0.79**	**0.85**

Values are means (n = 6). FL, FL + 10 and FL + 20 are full heading stage, 10 days after full-heading stage and 20 days after full-heading stage, respectively.

Table 4. Variation in NPQ and qL of different green organs in different fertilisation models and dosage of exogenous hormones during hybrid rice seed production (the cultivar 'IIyou 416').

Fertilisation model	Exogenous hormones	Leaf			Panicle			Stem and sheath		
		FL	FL+ 10	FL+ 20	FL	FL+ 10	FL+ 20	FL	FL+ 10	FL+ 20
NPQ										
M1	D1	0.13	0.16	0.15	0.24	0.23	0.13	0.13	0.15	0.11
	D2	0.13	0.20	0.15	0.23	0.19	0.15	0.12	0.13	0.10
	D3	0.19	0.14	0.11	0.22	0.19	0.09	0.15	0.17	0.09
	D4	0.20	0.12	0.11	0.22	0.20	0.17	0.15	0.17	0.19
	Mean	**0.16**	**0.15**	**0.13**	**0.23**	**0.20**	**0.13**	**0.14**	**0.15**	**0.12**
M2	D1	0.14	0.18	0.12	0.21	0.22	0.10	0.14	0.12	0.20
	D2	0.18	0.09	0.18	0.19	0.17	0.18	0.17	0.11	0.20
	D3	0.14	0.10	0.14	0.18	0.17	0.17	0.17	0.18	0.18
	D4	0.17	0.09	0.13	0.21	0.17	0.23	0.18	0.18	0.20
	Mean	**0.16**	**0.12**	**0.14**	**0.20**	**0.18**	**0.17**	**0.16**	**0.15**	**0.20**
qL										
M1	D1	0.53	0.64	0.37	0.39	0.43	0.58	0.40	0.54	0.57
	D2	0.46	0.63	0.61	0.31	0.34	0.40	0.41	0.50	0.45
	D3	0.57	0.66	0.59	0.37	0.31	0.49	0.44	0.57	0.45
	D4	0.68	0.64	0.59	0.30	0.40	0.23	0.43	0.54	0.58
	Mean	**0.56**	**0.64**	**0.54**	**0.34**	**0.37**	**0.42**	**0.42**	**0.54**	**0.51**
M2	D1	0.51	0.68	0.51	0.33	0.36	0.22	0.40	0.47	0.51
	D2	0.56	0.52	0.56	0.20	0.33	0.41	0.42	0.43	0.46
	D3	0.55	0.60	0.64	0.30	0.44	0.45	0.43	0.56	0.47
	D4	0.50	0.84	0.60	0.28	0.35	0.37	0.44	0.58	0.52
	Mean	**0.53**	**0.66**	**0.58**	**0.28**	**0.37**	**0.36**	**0.42**	**0.51**	**0.49**

Values are means (n = 6). FL, FL + 10 and FL + 20 are full heading stage, 10 days after full-heading stage and 20 days after full-heading stage, respectively.

Discussion

In this study, we found that the CRs of leaf and panicle to yield were 32.3% and 42.1%, respectively. However, the CR of panicle to yield varied widely from 17 to 62%. Previous studies also found that contributions to grain filling varied, with the rate of deposition of assimilation products to grains ranging from approximately 10 to 76% (Gebbing and Schnyder, 2001; Tambussi *et al*., 2007; Aranjuelo *et al*., 2011). The variability of these

Table 5. Variation of NPQ and qL of different green organs in different fertilisation model and dosage of exogenous hormones (the cultivar 'Jinyou 167').

Fertilisation model	Exogenous hormones	Leaf			Panicle			Stem and Sheath		
		FL	FL+ 10	FL+ 20	FL	FL+ 10	FL+ 20	FL	FL+ 10	FL+ 20
NPQ										
M1	D1	0.08	0.06	0.11	0.17	0.13	0.25	0.14	0.06	0.13
	D2	0.10	0.07	0.11	0.18	0.21	0.24	0.12	0.07	0.15
	D3	0.10	0.12	0.10	0.21	0.24	0.20	0.11	0.15	0.22
	D4	0.10	0.09	0.13	0.25	0.22	0.17	0.11	0.11	0.18
	Mean	**0.09**	**0.09**	**0.11**	**0.20**	**0.20**	**0.22**	**0.12**	**0.10**	**0.17**
M2	D1	0.10	0.08	0.09	0.23	0.34	0.20	0.18	0.10	0.17
	D2	0.18	0.09	0.10	0.21	0.17	0.21	0.13	0.10	0.23
	D3	0.15	0.12	0.10	0.29	0.24	0.27	0.20	0.10	0.15
	D4	0.13	0.12	0.11	0.28	0.20	0.20	0.17	0.12	0.14
	Mean	**0.14**	**0.10**	**0.10**	**0.25**	**0.24**	**0.22**	**0.17**	**0.10**	**0.17**
qL										
M1	D1	0.54	0.52	0.64	0.39	0.46	0.52	0.39	0.34	0.53
	D2	0.50	0.49	0.65	0.52	0.39	0.51	0.36	0.34	0.34
	D3	0.52	0.61	0.57	0.55	0.56	0.36	0.36	0.41	0.58
	D4	0.60	0.64	0.70	0.38	0.39	0.28	0.34	0.39	0.50
	Mean	**0.54**	**0.57**	**0.64**	**0.46**	**0.45**	**0.42**	**0.36**	**0.37**	**0.49**
M2	D1	0.49	0.46	0.60	0.45	0.42	0.31	0.43	0.35	0.54
	D2	0.60	0.56	0.66	0.55	0.48	0.45	0.41	0.38	0.48
	D3	0.53	0.51	0.61	0.61	0.51	0.53	0.44	0.38	0.42
	D4	0.56	0.57	0.56	0.62	0.60	0.41	0.38	0.50	0.43
	Mean	**0.54**	**0.52**	**0.61**	**0.56**	**0.50**	**0.43**	**0.41**	**0.40**	**0.47**

Values are means (n = 6). FL, FL + 10 and FL + 20 are full heading stage, 10 days after full-heading stage and 20 days after full-heading stage, respectively.

results may reflect the variation of the contribution of ear photosynthesis to grain yield, which is affected by genetic diversity and growing conditions (Sanchez-Bragado *et al*., 2014a, b). Another possibility is deficiencies in the methods used (Sanchez-Bragado *et al*., 2014). In the present experiment, the effect of fertilisation and exogenous hormones regime for 'IIyou 416' was significant. The CR of panicle to yield in M2 was 70% higher than that in M1. The growing conditions for the cultivars were also different, with the growing seasons of 'Jinyou 167' and 'IIyou 416' being spring and autumn, respectively.

The CR of non-leaf organs (panicle, stem and sheath) to yield was 67.0% (table 1). Zhang *et al.* (2011) also indicated that non-leaf organs (ear, peduncle, and sheath) accounted for 73–81% of the total contribution of organs above the flag leaf node to wheat grain weight. However, dry matter accumulated before the heading stage by leaf photosynthesis was stored in the stem and sheath. In this study, we did not differentiate between dry matter accumulated before and after the heading stage in the stem and sheath. This could indirectly increase the CR of the stem and sheath to yield, and then the CR of the non-leaf organs to yield. Therefore, our results suggest that the CR of the stem and sheath to yield was overestimated.

The effects of GAs are not limited to stimulating growth. They have many other roles, including delaying leaf senescence and abscission, and breaking dormancy in seeds (Öpik *et al.*, 2005). In this study, GA application increased the CR of leaf to yield by an average of 28.4% with respect to the control (table 1), which indicated that applying different doses of GAs would delay leaf senescence and further increase the quantity of photosynthetic products transferred from the leaf to the panicle. Similarly, we speculated that this would also apply in the stem and sheath. The CR of the stem and sheath to yield decreased by an average of 14%. The isotope tracing study provided indirect evidence that the ^{14}C value in the panicle increased with the quantity of GAs applied, but the opposite result was found for the stem and sheath, in which the ^{14}C value decreased with the quantity of GAs applied. Consequently, spraying GAs could enable the photosynthetic products in the leaf and/or stem and sheath to be transferred more effectively to the panicle.

Through the ^{14}C-labeling test of different green organs, we also found that about 90% of the photosynthetic products of the panicle and about half the photosynthetic products of the leaf were available for filling the panicle. Through $\delta^{13}C$ analysis, Sanchez-Bragado *et al.* (2014a) showed that the contribution of the ear represented about 70% of the total assimilates contributing to grain filing, while the role of the flag leaf blade was significantly smaller, with a contribution of just 10%. Other studies using ^{13}C labeling have shown that only small amounts of the soluble sugars derived from the photosynthetic products in the leaf transfer to the ear, with the remainder allocated as structural carbon compounds and starch, and then respired (Aranjuelo *et al*., 2011). In summary, this study found that the carbon synthesised in the panicle was used for grain filling. Pervious studies also found that the main photosynthetic organs contributing to grain filling were the flag leaf blade and the ear (e.g., Tambussi *et al*., 2007; Maydup *et al*., 2010). The variation in ^{14}C values at different sampling stages during grain filling has also provided indirect evidence. When sampling in the milky ripeness stage, 16% of the total photosynthetic products in the flag leaf were transferred to the panicle, whereas this figure was 55% when sampling was conducted at the mature stage. A ^{14}C-labeling test with different tagging times after the heading stage also indicated that more than 90% of the photosynthetic product of panicle photosynthesis was used for grain filling at 0–5 days after the heading stage; however, in the same period, only 50% of carbon assimilated by the flag leaf transferred to the grain. Consequently, these results suggested that the panicle made an important photosynthetic contribution during the process of grain filling (equivalent to that of the flag leaf), especially at 0–5 days after the heading stage.

However, the high CR of panicle to yield and its photosynthetic physiological characteristics did not match. For example, the chlorophyll content, soluble protein content, and PEP carboxylase activity in the non-leaf organs were significantly lower than those in the leaf, and there was less light for photochemical reactions in the panicle. The CR of panicle to yield should be about one-third the CR of leaf to yield when considering the photosynthetic physiological characteristics in the leaf. Several explanations for this phenomenon can be suggested. One is concerned with the distribution of the photosynthetic products of the leaf organs and the feature of the leaf's light interception and leaf respiration. Previous studies have reported that the flag and the lower leaves provide assimilation products for the growth and development of the reproductive sink. Shoot photosynthesis may affect the quantity of fertile florets and kernels, and then their potential size (Slafer and Savin, 1994). In addition, the flag leaf has a key role in redistributing nitrogen to the grain (Bahrani and Joo, 2010). During the experiment, we also found that the effective leaf area was substantially reduced because the panicle layer in the female parent was higher than the leaf layer. To ensure that pollination proceeded smoothly, the third upper leaf was especially short. Another explanation is based on the morphological structure of assimilatory cells and their photosynthesis in the panicle. Jia *et al*. (2015) reported that ear photosynthesis involved a certain amount of C_4 pathway enzymes and suggested that the high enzyme activity of the C_4 pathway and the increased capacity of assimilation product transport were the reasons for the increasing drought tolerance of ears. Our results also indicated that about 90% of photosynthetic products was used for grain filling. On the other hand, the unique structure of chloroplasts in the panicle was similar to that of the bundle sheath cell of C_4 plants. Li *et al*. (2002) determined the stomatal frequency of non-leaf organs among four crops and suggested that non-leaf organs might use a form of photosynthesis rather than being categorised within existing C_3 and C_4 types. Consequently, it is possible that the panicles need less of this enzyme, which is by far the most abundant protein in leaves, because it works more efficiently in the panicle than in the leaf.

Conclusions

The main purpose of this study was to determine the CR of panicle to yield and its photosynthetic physiological characteristics in hybrid rice seed production. The median CR of panicle to yield in hybrid rice seed production was 36%. Isotope tracing technology was used to determine that about 90% of the photosynthetic products of the panicle and 50% of the photosynthetic products of the leaf were delivered to the panicle. In the entire filling period, the contribution of panicle to yield was concentrated in the early period (0–10 days after pollination), whereas the contribution of leaf to yield was more significant in the late period (10 days after pollination to maturity). Compared with variations in female plant density, the application of GAs was a more important factor for significantly increasing the out-crossing rate and increasing seed yield in hybrid rice seed production.

Acknowledgements

We are thankful to anonymous reviewers and editors for their helpful comments and suggestions. This research was part of the project for the National Natural Science Foundation of China (No. 31271666), "12th 5-year plan" Agro-Scientific Research in the Public Interest (Grant No. 201303002) and the Earmarked Fund for China Agriculture Research System (Grant No. CARS-01-26).

References

Aranjuelo, I., Cabrera-Bosquet, L., Morcuende, R., Avice, J.C., Nogués, S., Araus, J.L., Martínez-Carrasco, R. and Pérez, P. (2011). Does ear C sink strength contribute to overcoming photosynthetic acclimation of wheat plants exposed to elevated CO_2? *Journal of Experimental Botany*, **62**, 3957-3969.

Araus, J.L., Brown H.R., Febrero, A., Bort, J. and Serret, M.D. (1993). Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO_2 to differences in grain mass in durum wheat. *Plant Cell and Environment*, **16**, 383-392.

Aron, D.I. (1949). Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, **24**, 1-15.

Bahrani, A. and Joo, M.H. (2010). Flag leaf role in N accumulation and remobilization as affected by nitrogen in a bread and durum wheat cultivars. *American-Eurasian Journal of Agricultural and Environmental Science*, **8**, 728-735.

Gebbing, T. and Schnyder, H. (2001). ^{13}C labelling kinetics of sucrose in glumes indicates significant refixation of respiratory CO_2 in the wheat ear. Aust. *Journal of Plant Physiology*, **28**, 1047-1053.

Guido, A. and Hardy, P. (2003). Non-foliar photosynthesis-a strategy of additional carbon acquisition. *Flora*, **198**, 81-97.

Huang, M., Shan, S.L., Zhou, X.F., Chen, J.N., Cao, F.B., Jiang, L.G. and Zou, Y.B. (2016). Leaf photosynthetic performance related to higher radiation use efficiency and grain yield in hybrid rice. *Field Crops Research*, **193**, 87-93.

Jia, S., Lv, J., Jiang, S., Liang, T., Liu, C. and Jing, Z. (2015). Response of wheat ear photosynthesis and photosynthate carbon distribution to water deficit. *Photosynthetica*, **53**, 95-109.

Kim, S.S. (2003). *Hybrid Rice Seed Production using Herbicide Resistant Photoperiod Sensitive Genetic Male Sterility in Rice*, MS thesis, Yeungnam University, Korea.

Kumagai, E., Araki, T. and Kubota, F. (2007). Effects of nitrogen supply restriction on gas exchange and photosystem 2 function in flag leaves of a traditional low-yield cultivar and a recently improved high-yield cultivar of rice (*Oryza sativa* L.). *Photosynthetica*, **45**, 489-495.

Li, H.B., Bai, KZ, Hu, Y.X., Kuang, T.Y. and Lin, J.X. (2002). Stomatal frequency on some non-leaf organs of four crop species and their significance in photosynthesis. *Acta Phytoecologica Sinica*, **26**, 351-354. [In Chinese.]

Mao, C.X. and Virmani, S.S. (2003). Opportunities for and challenges to improving hybrid rice seed yield and seed purity. Hybrid rice for food security, poverty alleviation, and environmental protection. In: *Proceedings of the 4th International Symposium on Hybrid Rice. Hanoi, Vietnam*, Los Baños, Laguna, Philippines.

Maydup, M.L., Antonietta, M., Guiamet, J.J., Graciano, C., López, J.R. and Tambussi, E.A. (2010). The contribution of ear photosynthesis to grain filling in bread wheat (*Triticum aestivum* L.). *Field Crops Research*, **119**, 48-58.

Maydup, M.L., Antonietta, M., Graciano, C., Guiamet, J.J. and Tambussi, E.A. (2014). The contribution of the awns of bread wheat (*Triticum aestivum* L.) to grain filling: Responses to water deficit and the effects of awns on ear temperature and hydraulic conductance. *Field Crops Research*, **167**, 102-111.

Peng, S.B. (2016). Dilemma and way-out of hybrid rice during the transition period in China. *Acta Agrnomica Sinica*, **42**, 313-319. [In Chinese.]

Press, M.C. (1999). The functional significance of leaf structure: a search for generalizations. *New Phytologist*, **143**, 213-219.

Sanchez-Bragado, R., Molero, G., Reynolds, M.P. and Arausl, J.L. (2014a). Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ δ13C. *Journal of Experimental Botany*, **65**, 5401-5413.

Sanchez-Bragado, R., Elazab, A., Zhou, B., Serret, M.D., Bort, J., NietoTaladriz, M.T. and Araus, J.L. (2014b). Contribution of the ear and the flag leaf to grain flling in durum wheat inferred from the carbon isotope signature: genotypic and growing conditions effects. *Journal Integrative Plant Biology*, **56**, 444-454.

Shi, W., Xiao, G., Struik, P.C., Jagadish, K.S.V. and Yin, X.Y. (2016). Quantifying source-sink relationships of rice under high night-time temperature combined with two nitrogen levels. *Field Crops Research*, **202**, 36-46.

Slafer, G.A. and Savin, R. (1994). Post-anthesis green area duration in semidwarf and standard-height wheat cultivar as affected by sink strength. *Australian Journal of Agricultural Research*, **45**, 1337-1346.

Tambussi, E.A., Bort, J., Guiamet, J.J., Nogués, S. and Araus, J.L. (2007). The photosynthetic role of ears in C3 cereals: metabolism, water use efficiency and contribution to grain yield. *Critical Reviews in Plant Sciences*, **26**, 1-16.

Wang, Q., Zhang, Q., Fan, D. and Lu, C. (2006). Photosynthetic light and CO_2 utilization and C4 traits of two novel super-rice hybrids. *Journal of Plant Physiology*, **163**, 529-537.

Weiss, D., Schonfeld, M. and Halevy, A.H. (1988). Photosynthetic activities in the Petunia corolla. *Plant Physiology*, **87**, 666-670.

Xiao, C.L., Liu, A.M., Zhang, H.Q., Xiao, X. and She, X.Q. (2010). Progress and developing direction of hybrid rice production technology in China. *Hybrid Rice*, **(suppl-1)**, 46-50. [In Chinese.]

Yumiko, S.O., Tamizi, S., Daisaku, Y., Taichiro, O. and Tadashi, H. (2006). The effect of planting pattern on the rate of photosynthesis and related processes during ripening in rice plants. *Field Crops Research*, **96**, 113-124.

Zhang, Y.B., Tang, Q.Y., Zou, B.Y., Li, D.Q., Qin, J.Q., Yang, S.H., Chen, L.J., Xia, B. and Peng, S.B. (2009). Yield potential and radiation use efficiency of "super" hybrid rice grown under subtropical conditions. *Field Crops Research*, **114**, 91-98.

Zhang, Y.H., Yang, Y.M., Cao, L., Hao, Y.F., Huang, J., Li, J.P., Yao, D.X. and Wang, Z.M. (2015). Effect of high temperature onphotosynthetic capability and antioxidant enzyme activity of flag leaf and non-leaf organs in wheat. *Acta Agrnomica Sinica*, **41**, 136-144. [In Chinese.]

Zhang, Y.P., Zhang, Y.H., Wang, Z.M. and Wang, Z.J. (2011). Characteristics of canopy structure and contributions of non-leaf organs to yield in winter wheat under different irrigated conditions. *Field Crops Research*, **123**, 187-195.