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Morphological, anatomical, and physiological characteristics involved in development of the large culm trait in rice

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Abstract

The large culm trait is an important agronomical characteristic in crops. Although the effects of this trait on lodging resistance, yield production, and biomass accumulation have been widely examined, its significance in super rice breeding remains to be determined. In our current study, the morphological, anatomical, and physiological characteristics of three large culm rice (*Oryza sativa* L.) cultivars (JH186, JH126, and JH214) were compared to three common rice cultivars (Xiushui09, Xiushui63, and Zhenuo5). Overall, the large culm cultivars exhibited greater plant size, culm diameter, and flag leaf length and width, as well as lower tiller numbers, although variations were observed among the six rice cultivars. The large culm cultivars also exhibited larger leaf vascular bundles, more culm vascular bundles, more grains per panicle and blighted grains, lower seed-setting rates, and larger panicles. The transpiration rate was significantly higher in the large culm cultivars, whereas instantaneous water use efficiency was lower compared to the common cultivars. JH186 and JH214 also exhibited higher photosynthetic efficiency and apoplastic transport ability. These results indicate that a systematic modification of morphology, anatomy, and physiology was involved in the development of the large culm trait in rice, and cultivars with large culm characteristics are ideal candidates for super rice breeding.

Keywords: apoplastic transport; lodging resistance; photosynthesis; super rice breeding, rice (*Oryza sativa* L.)

Abbreviations: LED- light emitting diodes; PTS- 8-hydroxy-1,3,6-pyrenetrisulphonic acid; PPFD- photosynthetic photon flux density; WUE- water use efficiency; WVBA- whole vascular bundle area.

Introduction

As the staple food of most Asian people, rice (*Oryza sativa* L.) cultivation and genetic modification have been the subject of significant research and development efforts, particularly to improve production and grain quality. However, the yield potential of inbred and hybrid rice cultivars has apparently reached a plateau in the improvement of biomass and harvest indices (Khush, 1996; Khush and Peng, 1996). Several approaches have been proposed to breach the yield ceiling of rice cultivars. One approach is to find a new plant type with the ideal morphology, large panicles, photosynthetic efficiency, and lodging resistance (Khush and Peng, 1996; Xu et al., 2005). The large culm trait may potentially provide such a plant type and has, therefore, long attracted attention.

Lodging is one of the major factors limiting the yield potential of both inbred and hybrid rice cultivars and has received particular attention. Lodging can cause severe yield loss and poor grain quality because of reduced canopy photosynthesis, increased respiration, reduced translocation of nutrients and carbon for grain filling, and increased susceptibility to pests (Hitaka, 1969). Many studies have shown that the culm characteristics contributing to lodging resistance include basal internode length and thickness, plant height, culm wall thickness, and leaf sheath wrapping and thickness (Chang and Vergara, 1972; Hojyo, 1974; Matsuda et al., 1983), but the morphological and anatomical characteristics associated with the large culm trait in rice have not been systematically identified. Nevertheless, lodging resistance is positively correlated with the culm diameter and wall thickness of the basal internodes both in wheat (Li et al., 2000; Tripathi et al., 2003; Wang et al., 2006) and barley (Dunn and Briggs, 1989). Moreover, aside from the thick culm, the culm vascular bundle number in rice also contributes to lodging resistance (Xu et al. 1996; Duan et al. 2004). Zhu et al. (2008) have found that a large number of quantitative trait locus alleles affecting culm length, strength, and thickness in *indica*/*japonica* crosses of rice are related to lodging resistance. Kashiwagi et al. (2008) obtained similar results and suggested that increasing culm diameter in rice breeding programs can improve lodging resistance.

Aside from improving lodging resistance, a thick culm may also act as a carbohydrate store for high yield in rice (Hirose et al., 2006). Furthermore, morphological characteristics such as culm thickness, leaf size, leaf angle, and plant height at the heading stage have been considered important traits in breeding both super rice (Chen et al., 2005) and bioenergy crops (Ookawa et al., 2010). Cultivars with large culms, therefore, may be ideotypes for super rice breeding because the characteristics of semi-dwarfism, lodging resistance, and heavy panicles have been considered to be important traits for super rice breeding (Khush, 2000; Duan et al., 2004; Ma et al., 2004). However, very limited research has been done to identify these agronomic traits and to determine which gene or genes actually control such traits in rice. We investigated three rice cultivars (JH126, JH186, and JH214) that exhibit the large culm trait

Table 1. Morphological data for the common and large culm rice cultivars.

	Common cultivars			Large culm cultivars		
	Xiushui09	Xiushui63	Zhenuo5	JH186	JH126	JH214
Flag leaf length (cm)	25.2 ± 2.74c	22.8 ± 3.47c	23.3 ± 3.57c	48.0 ± 5.21a	36.3 ± 5.73b	48.0 ± 5.94a
Flag leaf width (cm)	2.00 ± 0.155c	1.48 ± 0.041e	1.80 ± 0.122d	2.36 ± 0.164a	2.18 ± 0.089b	2.44 ± 0.195a
Height (cm)	100.6 ± 6.1d	111.6 ± 5.9c	109.2 ± 4.9c	137.0 ± 9.2a	123.7 ± 5.3b	112.2 ± 3.8c
Number of tillers	13.3 ± 4.4a	15.7 ± 4.9a	10.1 ± 2.4b	9.8 ± 2.6b	9.4 ± 2.6b	10.3 ± 2.8b
Culm diameter (mm)	5.86 ± 0.24d	6.29 ± 0.25c	6.36 ± 0.26c	9.22 ± 0.41b	8.80 ± 0.44b	10.1 ± 0.70a

The values indicated are the means ± SD of six plants. One-way ANOVA was used to compare the mean values. Values (in each row) followed by the same letters are not significantly different at $p \leq 0.05$.

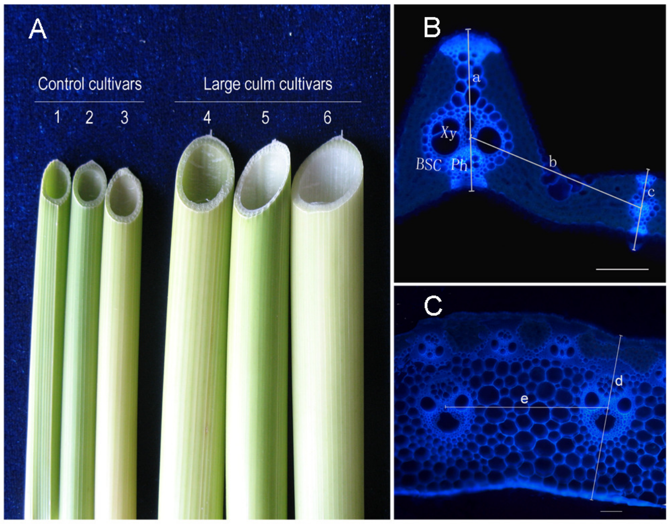

Fig 1. Morphological and anatomical characteristics in the culm and leaves of the common and large culm rice cultivars. A: Culm morphology, showing the different sizes of culm between the two rice types. 1. Xiushui09; 2. Xiushui63; 3. Zhenuo5; 4. JH186; 5. JH126; 6. JH214. B and C: Leaf anatomy of JH214 under UV epifluorescent illumination. Bars = 100 μm. Ph - phloem; Xy - xylem; BSC - bundle sheath cell. Different distances for measurement: a. Leaf large vascular thickness; b. Leaf interveinal distance; c. Leaf small vascular thickness; d. Culm wall thickness; e. Culm interveinal distance.

during their life cycle. Three common cultivars (Xiushui09, Xiushui63, and Zhenuo5), which are widely cultivated in southeast China, were used as controls for comparison. The plant and seed morphology, leaf and culm anatomy, yield characteristics, gas exchange properties, and apoplastic transport ability between the large culm lines and common rice cultivars were studied. The significance of these traits in large culm cultivars for super rice breeding is discussed.

Results

Morphological characteristics

The large culm cultivars (JH186, JH126, and JH214) showed significantly larger culm sizes (Fig. 1A), with ~50% larger culm diameters and a significantly higher biomass compared to the common cultivars (Table 1). The large culm rice exhibited significantly greater length and width of flag leaves than the common cultivars (Table 1). For example, the flag leaf length and width in JH186 were 1.9- and 1.2-fold higher than those in Xiushui09, respectively. The plant height of the large culm cultivars, particularly JH186 and JH126, was also higher than in the common cultivars (Table 1). In contrast, the large culm cultivars had markedly fewer tillers compared with the two common Xiushui09 and Xiushui63 cultivars (Table 1). The panicles were short, erect, and compact in the common cultivars (Xiushui09, Xiushui63, and Zhenuo5), whereas they were long, droopy, and loose in the large culm cultivars (Fig. 2A–F). Moreover, common cultivars had relatively short, thick seeds, but large culm cultivars had long, thin seeds (Fig. 2G-R).

Anatomical characteristics

Although the anatomical data varied among the six varieties of rice, the large culm cultivars had larger foliar vascular bundles, especially JH186 and JH214, compared to the common lines (Table 2). The area of whole leaf vascular bundles and phloem and xylem tissues was significantly higher in the large culm cultivars than in the common lines. Two large culm lines, JH186 and JH214, also possessed the highest bundle sheath cell area, leaf interveinal distance, and leaf thickness among the six rice cultivars. However, there was no difference in the ratio of each tissue area to the whole vascular bundle area (WVBA) between the two groups of rice cultivars even though the area of each vascular bundle was higher in the large culm cultivars than in the common lines. Similarly, there was no clear difference between the two rice groups in culm wall thickness and interveinal distance, although variations occurred between the individual cultivars (Table 2). In contrast, the large culm cultivars had significantly more culm vascular bundles than the common lines (Table 2). Hence, the results indicate that a relatively higher vascular bundle area may play an important role in the development of the large culm trait in rice.

Gas exchange and apoplastic transport

To understand whether the development of the large culm trait in rice influences physiological characteristics, gas exchange

Table 2. Anatomical characteristics of vascular bundles in the leaves and culms of the common and large culm rice cultivars.

	Common cultivars			Large culm cultivars		
	Xiushui09	Xiushui63	Zhenuo5	JH186	JH126	JH214
Leaf						
Whole vascular bundle area (WVBA) (μm^2)	22870 ±1 221c	22960 ± 2503c	20260 ± 2713d	29870 ± 4289a	26200 ± 2246b	28470 ± 5307ab
Bundle sheath cells area (BSCA) (μm^2)	10260 ± 1168b	9793 ± 1624b	7357 ± 1396c	11810 ± 2053a	10060 ± 1780b	11621 ± 2466ab
Percentage of BSCA to WVBA (%)	44.8 ± 4.6a	42.6 ± 4.8ab	36.2 ± 4.0c	39.4 ± 2.2b	38.2 ± 4.5bc	40.9 ± 4.1b
Phloem area (μm^2)	2444 ± 303c	2437 ± 467c	2395 ± 436c	3548 ± 463a	2773 ± 254b	3268 ± 828ab
Percentage of phloem area to WVBA (%)	10.7 ± 1.1b	10.6 ± 1.6b	11.8 ± 1.2a	12.0 ± 1.2a	10.6 ± 1.2b	11.4 ± 1.3a
Xylem area (μm^2)	7006 ± 667b	7291 ± 1238b	6904 ± 940b	10120 ± 940a	9475 ± 663.4a	9227 ± 2340a
Percentage of xylem area to WVBA (%)	30.7 ± 2.9c	31.7 ± 3.6c	34.3 ± 4.0ab	34.2 ± 3.0ab	36.3 ± 3.1a	32.2 ± 3.4bc
Ratio of xylem area:phloem area	2.91 ± 0.43b	3.04 ± 0.51b	2.93 ± 0.40b	2.88 ± 0.31b	3.46 ± 0.35a	2.86 ± 0.43b
Leaf interveinal distance (μm)	296.9 ± 22.3c	265.3 ± 12.1d	307.7 ± 11.6b	325.5 ± 40.3b	276.3 ± 25.8d	377.6 ± 25.5a
Leaf large vascular thickness (μm)	251.5 ± 11.4b	258.6 ± 21.3ab	218.0 ± 23.4c	276.2 ± 35.7a	251.9 ± 13.0ab	271.4 ± 27.1a
Leaf small vascular thickness (μm)	112.3 ± 12.0c	122.8 ± 14.6b	114.3 ± 8.54c	136.6 ± 15.8a	110.7 ± 11.1c	139.4 ± 14.6a
Culm						
Culm wall thickness (μm)	418.6 ± 31.4c	448.2 ± 28.4b	387.6 ± 40.0d	447.9 ± 21.0b	504.5 ± 22.8a	410 ± 48.4cd
Culm interveinal distance (μm)	373.2 ± 70.1bc	375.5 ± 60.5bc	331.5 ± 60.4c	420.7 ± 61.7a	383.2 ± 40.6b	414.3 ± 67.7ab
Number of culm vascular bundles	14.9 ± 2.4d	14.8 ± 1.9d	14.1 ± 1.2e	21.3 ± 2.5c	22.3 ± 1.6b	23.5 ± 1.8a

The values are the means ± SD. At least 20 vascular bundles of five different leaf or culm slices from different plants were measured for cross-section area determination of different tissues. One-way ANOVA was used to compare the mean values. Values (in each row) followed by the same letters are not significantly different at $p \leq 0.05$.

Table 3. Yield-related traits of the common and large culm rice cultivars.

	Common cultivars			Large culm cultivars		
	Xiushui09	Xiushui63	Zhenuo5	JH186	JH126	JH214
Spikes per plant	10.3 ± 2.9a	10.0 ± 1.7a	10.0 ± 2.7a	9.33 ± 2.5a	6.33 ± 2.3b	5.33 ± 0.58b
Grains per panicle	136.1 ± 29.8d	139.4 ± 17.1cd	151.3 ± 23.8c	205.4 ± 69.2b	222.6 ± 50.2b	293.5 ± 93.3a
Blighted grains	5.6 ± 3.4d	6.5 ± 5.7d	17.4 ± 9.3c	36.4 ± 19.7b	38.2 ± 25.4ab	55.9 ± 29.1a
Seed-setting rate (%)	96.0 ± 2.6a	95.6 ± 4.5a	89.7 ± 5.1b	84.9 ± 8.1c	85.4 ± 10.4bc	84.0 ± 11.3c
Spike length (cm)	14.4 ± 0.9e	15. 8 ± 0.7d	16.3 ± 0.9d	20.7 ± 1.6c	24.1 ± 1.3a	22.5 ± 1.6 b
Thousand-grain weight (g)	26.1 ± 0.0b	24.2 ± 0.1e	27.8 ± 0.1a	25.6 ± 0.1c	24.7 ± 0.1d	24.9 ± 0.1d
Yields (t ha^{-1})	8.26 ± 0.035d	7.58 ± 0.029f	9.47 ± 0.046b	11.02 ± 0.057a	7.84 ± 0.036e	8.75 ± 0.033c

The values are the means ± SD of 18–30 spikes from three plants. One-way ANOVA was used to compare the mean values. Values (in each row) followed by the same letters are not significantly different at $p \leq 0.05$.

properties and apoplastic transport ability were compared in the large culm cultivars and the common lines. As shown in Figure 3A, the rate of photosynthesis differed between the cultivars at different developmental stages. Although the photosynthetic rate was higher in Zhenuo5 compared to the other two common cultivars, the rate was markedly higher in the large culm cultivars (except in JH126) compared to the common cultivars during the three later developmental stages (heading, early grain filling, and later grain filling stages). To gain further insight into photosynthetic characteristics in these large culm cultivars, the light response curves of each rice line were measured (Fig. 4). Zhenuo5 and Xiushui09, as well as the two large culm cultivars, JH186 and JH214, exhibited relatively higher rates of photosynthesis (500–2000 μmol photons m^{-2} s^{-1} photosynthetic photon flux density, PPFD) compared to Xiushui63 and JH126. The transpiration rate was significantly higher in the large culm cultivars than in the common lines, particularly during the heading and early grain filling stages (Fig. 3B). In contrast, the instantaneous water use efficiency (WUE) was relatively lower in the large culm cultivars during the two early developmental stages compared to the common lines (Fig. 3C). JH126 possessed the lowest instantaneous WUE among the six rice cultivars. Interestingly, the cultivars that exhibited relatively higher photosynthetic rates among the six varieties also showed relatively higher yield production. For example, Zhenuo5, JH186, and JH214 exhibited higher photosynthetic rates during the examined developmental stages (Fig. 3A) and had 9.47, 11.02, and 8.75 t/ha yield productions, respectively (Table 3). The apoplastic transport ability in the rice cultivars was investigated using trisodium-8-hydroxy-1,3,6-pyrenetrisulphonic acid (PTS) treatment. PTS fluorescence showed no difference between the common cultivars at most of the time points of PTS treatment, whereas it was markedly different among the large culm cultivars (Fig. 5). JH214 exhibited the highest PTS fluorescence among the six rice cultivars at all time points of PTS treatment. JH186 and JH126 reached higher levels during the later stages of the treatment, although they exhibited obviously lower PTS fluorescence compared with the common cultivars during the early stages of the chemical treatment. These results suggest a higher capacity for apoplastic transport in the large culm cultivars.

Yield-related properties

Significant differences in yield-related traits were found between the large culm cultivars and the common lines (Table 3). The large culm cultivars exhibited significantly more grains per panicle, more blighted grains, and greater spike length, but markedly lower seed-setting rates. The number of spikes per plant was also lower in the large culm rice, particularly in JH126 and JH214. The large culm cultivars also had lower thousand-grain weight compared with the two common cultivars, Xiushui09 and Zhenuo5. The plant yields, however, varied significantly among the cultivars. The highest yield occurred in the large culm cultivar JH186, which was increased compared to Xiushui09 (by 33.4%) and Xiushui63 (by 45.0%). Although not all the large culm rice lines examined in this study showed higher yields compared to the common lines, the results indicate that these large culm cultivars had heavy panicles with high-yielding potentials.

Discussion

The large culm trait has been described as an important contributor to lodging resistance (Matsuda et al., 1983; Dunn

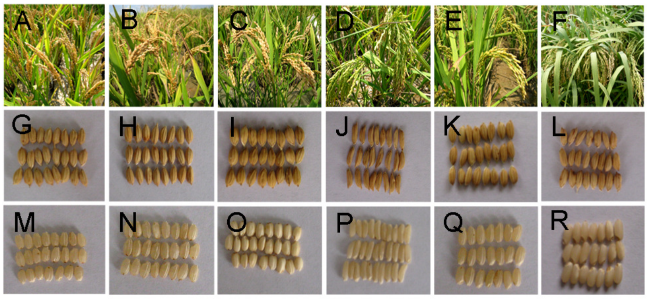

Fig 2. Morphology of the panicles and seeds of the common and large culm rice cultivars: A, G, M: Xiushui09; B, H, N: Xiushui63; C, I, O: Zhenuo5; D, J, P: JH186; E, K, Q: JH126; F, L, R: JH214.

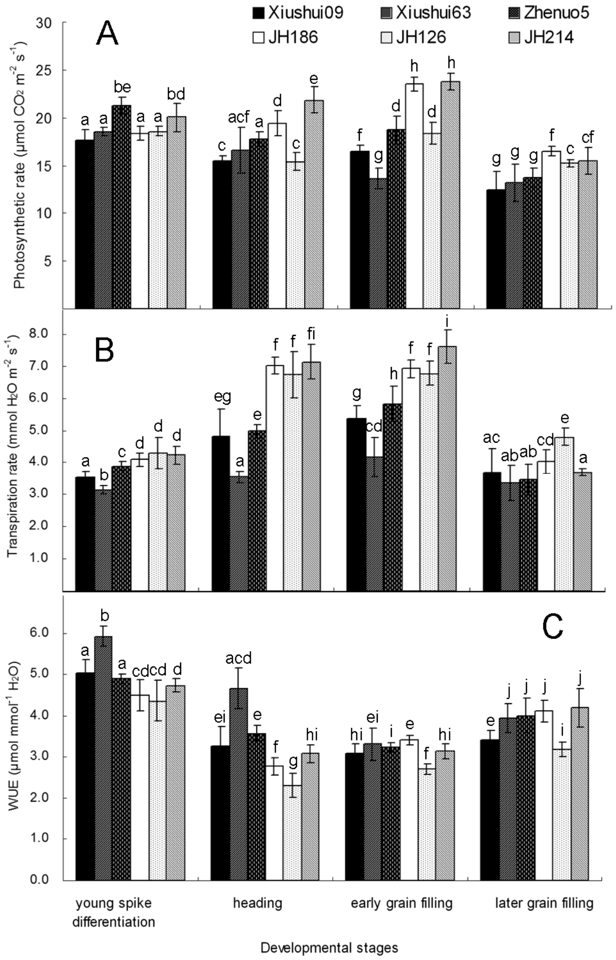

Fig 3. The photosynthetic rate, transpiration rate, and instantaneous water use efficiency (WUE) of the common and large culm rice cultivars during the different developmental stages. The values indicate the means ± SD of five plants. One-way ANOVA was used to compare the mean values. Markers (in each column) with the same letters are not significantly different at $p \leq 0.05$.

and Briggs, 1989; Tripathi et al., 2003; Wang et al., 2006; Kashiwagi et al., 2008). However, the potential application of the large culm trait in super rice breeding remains to be determined.

Development of the large culm trait in rice is associated with a modification in morphology and anatomy

Culm-related characteristics including wider culm diameter, greater wall thickness, and fewer tillers are significantly related to crop physiology and yield production by increasing plant lodging resistance (Hitaka, 1969; Xu et al., 1996; Tripathi et al., 2003; Duan et al., 2004). In our current study, the three large culm cultivars not only had markedly wider culm diameters, greater plant height, and greater flag leaf width and length, but also fewer tillers compared with the common lines (Fig. 1, Table 1), implying that higher lodging resistance had developed in these rice cultivars. Moreover, considering the bigger leaf vascular bundles and higher number of culm vascular bundles in the large culm cultivars (Table 2), the results reported here indicate that a systematic modification in morphology and anatomy was involved in the development of the large culm trait in rice, although the gene/genes controlling the large culm trait remain unknown. This modification can contribute to high lodging resistance and thus, higher yield potential.

The large culm trait influences photosynthetic characteristics and apoplastic transport

The vascular system (including xylem, phloem, and the bundle sheath) is the most important architectural component in plant tissues, is responsible for the transport of water and assimilates (Steudle and Frensch, 1996; Hose et al., 2001; Cholewa and Griffith, 2004), and functions as an apoplastic barrier for plant stress tolerance (Steudle, 2000; Saijo et al., 2001; Equiza and Tognetti, 2002; Chen et al., 2006). The vascular bundle size (He and Zhang, 2003) and the density of bundle sheath cells (Ogle, 2003) are strongly correlated with photosynthesis and transpiration. In our current study, although there were differences between developmental stages, relatively higher photosynthesis and transpiration rates and lower instantaneous WUE occurred in the large culm rice cultivars (Fig. 3). This suggests that the large culm trait characterized by modifications in the vascular system influences the gas exchange properties in rice plants. This may also contribute to a potentially higher yield because a simple relationship between a higher photosynthetic rate and a higher yield was observed among the six rice cultivars (Fig. 3 and 4; Table 3). Of course, the relatively higher transpiration and lower instantaneous WUE indicate more water consumption in the large culm rice, but water supply is not a major limiting factor for rice cultivation.

As described previously (Steudle and Frensch, 1996; Steudle, 2000), apoplastic transport ability is significantly associated with the vascular system structure, which has important roles in a diverse range of processes including intercellular signaling, plant-microbe interactions, and water and nutrient transport (Sattelmacher, 2001; Baker et al., 2005). Therefore, it would be of interest to know whether the large culm trait influences apoplastic transport in rice plants, because the development of this agronomic trait strongly alters the leaf vascular bundle size (Table 2). Based on the use of the PTS apoplastic tracer (Gong et al., 2006), we observed development of higher apoplastic transport ability in the large culm cultivars of rice, implying that water and nutrients can be mobilized more rapidly in the large culm cultivars and thus may contribute, at least partially, to high-yield production. However, whether a greater ability for apoplastic transport can consequently lead to high-yield production remains unclear because of the significant variations among cultivars. This is an interesting issue that needs additional experiments for further validation.

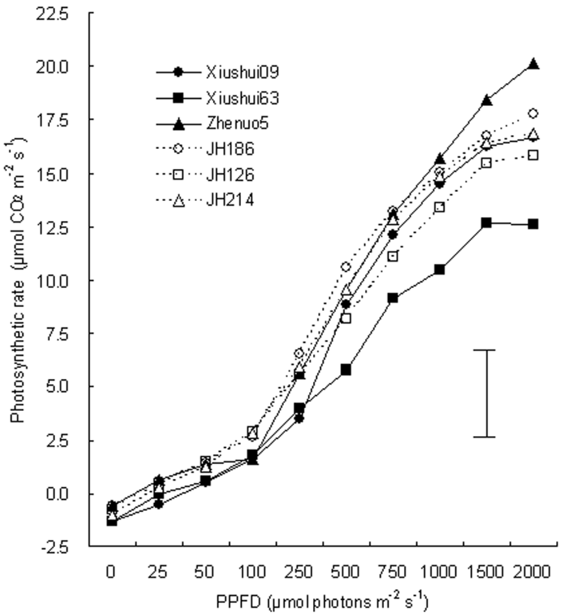

Fig. 4. The light response curves of photosynthesis in the common and large culm rice cultivars. Ten to fifteen flag leaves from different plants were measured to generate the curve for each cultivar. PPFD, photosynthetic photon flux density.

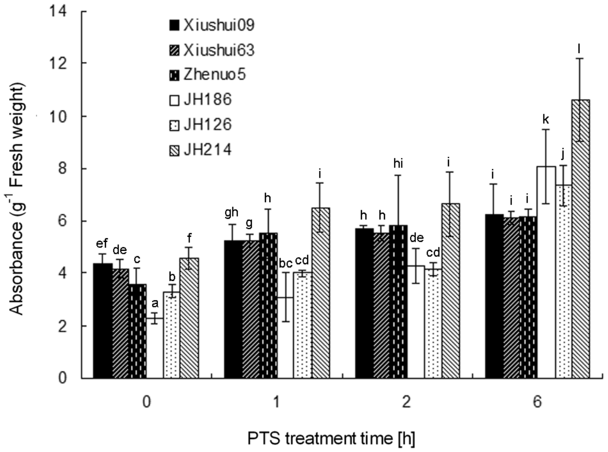

Fig. 5. PTS fluorescence in the common and large culm rice cultivars. The means ± SD from five independent experiments (each with three replicates). One-way ANOVA was used to compare the mean values. Markers (in each column) with the same letters are not significantly different at $p \leq 0.05$. PTS, 8-hydroxy-1,3,6-pyrenetrisulphonic acid.

Large culm trait rice cultivars are ideal candidates for super rice breeding

The yield potential of rice cultivars has reached a plateau in improving the biomass and harvest indices. Improvement in high-yield rice is now focused on increasing photosynthetic efficiency (Khush, 1996; Khush and Peng, 1996; Amther, 2007).

One important approach is to find a new plant type with ideal morphology, large panicles, high photosynthetic efficiency, and strong lodging resistance (Khush and Peng, 1996). Our current study shows that the three rice cultivars with the large culm trait exhibit large panicles and strong lodging resistance, highlighting their potential usefulness in agricultural practice. Furthermore, the large culm cultivars fulfill the requirement for semi-dwarfism in super rice breeding. In fact, their morphological characteristics, including stem thickness, leaf size, leaf angle, neck stem vascular bundle abundance, and plant height during the heading stage are important indices in super rice breeding (Chen et al., 2005; Xu et al., 2005). Characteristics such as semi-dwarfism, strong lodging resistance, and large panicles are considered the most important traits in super rice breeding (Khush, 2000; Duan et al., 2004; Ma et al., 2004). Therefore, the two large culm cultivars, JH186 and JH214, which also showed a relatively high photosynthetic rate (Fig. 3 and 4), are ideal candidates for super rice breeding.

There are still some obstacles for using these cultivars. For instance, JH186 and JH214 can provide a relatively higher yield. However, their blighted grains, seed-setting rate, and thousand-grain weight (Table 3) remain unsatisfactory for agricultural production. Panicle length is strongly negatively correlated with the grain insertion density, grain quality, and seed-setting ability because excessive panicle length is not favorable for erect positioning and thus disadvantageous for photosynthesis (Xu et al., 2005). Therefore, the lower seed-setting rate and higher blighted grains per spike in the large culm cultivars may be due to the longer panicle length, which is common in the large culm cultivars (Table 3). Although relatively higher yield production was observed in the large culm cultivars (JH186 and JH214), this type of rice exhibited lower spikes per plant because of fewer tillers. Recently, high water-soluble carbohydrate (WSC) wheat lines were found that have fewer and heavier individual stems with a lower proportion of structural biomass (Dreccer et al., 2009). These lines had similar or higher yields compared to normal lines because the changes appeared to converge to increase the WSC level in the stems and make more WSC available to the spike as well, so that the high WSC lines had more grains per spike (Dreccer et al., 2009). A similar mechanism may also occur in rice. The morphology of the loose panicles in the large culm cultivars (Fig. 2) is also not ideal because compact-panicle rice cultivars have been shown to be more favorable for high-yield potential relative to loose-panicle cultivars (Chen et al., 2001; Chen et al., 2005; Cheng et al., 2007). Further experiments in improving panicle morphology and other yield-related properties, such as the seed-setting rate and thousand-grain weight in these large culm cultivars should be conducted. Identification of genes that control the large culm trait will assist in creating useful lines.

Materials and methods

Plant materials and growth conditions

Rice seeds were obtained from the Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, China. One *japonica* line (JH126) and two *indica* restore lines (JH186 and JH214) that present the large culm trait during development were used. The progeny from these three rice lines are known to fully inherit the agronomic large culm trait of their parents. Three *japonica* lines widely cultivated in southeast China (Xiushui09, Xiushui63, and Zhenuo5) were used as the comparison cultivars. All plants were cultivated in the experimental field at the institute during the regular growing season with common cultivation conditions. The rates of photosynthesis and transpiration were measured using an Li-6400 portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA) with the red-blue light source around 9:00 AM on August 13, and September 2, 12, and 28, 2008 when the plants were in their young spike differentiation, heading, early grain filling, and later grain filling stages, respectively. The light response curves for photosynthesis were generated on September 8–12, 2009. The seeds of the control and large culm cultivars were harvested in 2009. Grain yield (t/ha) was estimated from the trial plots. Ten plants were taken from the experimental field before harvest and were used to calculate tiller number per plant, grains per panicle, blighted grains, seed-setting rate, and thousand-grain weight. Thousand-grain weight was determined after the fresh grains were dried to a constant weight at 60 °C. The flag leaves and the culms near the spikes were fixed with FAA solution (5% (v/v) formalin, 5% (v/v) acetic acid, 50% (v/v) ethanol) immediately after harvesting to facilitate anatomical determination.

Morphological and anatomical measurements

Plant height and flag leaf length were measured when the plants were at their grain filling stage. The first basal internode sections were cut off and photographed for culm morphology analyses. Anatomical determinations were carried out as described by Duan et al. (2008). Leaf and culm sections (~0.5 × 0.3 cm) from the large culm and control plants, fixed for at least 2 h in FAA solution, and then hand-sectioned using a razor blade. Transverse sections (~20 – 40 µm thick) were directly examined under UV epifluorescent illumination using an inverted microscope and fluorescent microscope DM IRB (Leica, Germany). Photographs were taken using a Leica light microscope and a DFC300 FX camera (Leica, Germany). A minimum of 20 vascular bundles from six different leaves were analyzed using an image analyzer (Pro Version 2.2; Leica Qwin) to determine the cross-sectional area of different tissues.

Determination of gas exchange

Gas exchange measurements were carried out using an open-gas portable photosynthesis system (LI-6400, LI-COR) as described by Chen et al. (2010). Ten to fifteen plants were used to measure the light response curves for each line. The formula WEU = P_n / T_r was used to calculate instantaneous WUE, where P_n represents the photosynthetic rate, and T_r represents the transpiration rate.

Determination of apoplastic transport ability

Apoplastic transport ability was estimated using a fluorescent, membrane-impermeant dye, trisodium salt of 8-hydroxy -1,3,6-pyrenetrisulphonic acid (PTS), based on the method described by Yeo et al. (1999). Seedlings growing during the regular growth season in the experimental fields were cut off from the basal end of shoots on September 18, 2009 when the plants were at the grain filling growth stage. The plants were transferred to plastic pots containing 30 mg L^{-1} PTS solution and subjected to normal growth light conditions for 1 to 6 hrs. The flag leaves from the plants were harvested for analysis. Leaf tissues were extracted and submerged in distilled water for 2 h at 90 °C (Yeo et al., 1999). PTS fluorescence was measured at $\lambda_{excitation}$ = 403 nm and $\lambda_{emission}$ = 510 nm using a fluorescence spectrometer (Perkin-Elmer LS-50B; Beaconsfield, Buckinghamshire, UK). A minimum of five plants was used for measurements.

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