

VIEWPOINT

Grain-filling problem in 'super' rice

Jianchang Yang¹ and Jianhua Zhang^{2,*}¹ Key Laboratory of Crop Genetics and Physiology of Jiangsu Province, Yangzhou University, Yangzhou, Jiangsu, China² Department of Biology, Hong Kong Baptist University, Hong Kong, China*Journal of Experimental Botany*, Vol. 61, No. 1, pp. 1–5, 2010

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Abstract

Modern rice (*Oryza sativa* L.) cultivars, especially the newly bred 'super' rice, have numerous spikelets on a panicle with a large yield capacity. However, these cultivars often fail to achieve their high yield potential due to poor grain-filling of later-flowering inferior spikelets (in contrast to the earlier-flowering superior spikelets). Conventional thinking to explain the poor grain-filling is the consequence of carbon limitation. Recent studies, however, have shown that carbohydrate supply should not be the major problem because they have adequate sucrose at their initial grain-filling stage. The low activities of key enzymes in carbon metabolism may contribute to the poor grain-filling. Proper field practices, such as moderate soil drying during mid- and late grain-filling stages, could solve some problems in poor grain-filling. Further studies are needed by molecular approaches to investigate the signal transport, the hormonal action, the gene expressions, and the biochemical processes in inferior spikelets.

Key words: Carbohydrate metabolism, grain-filling, inferior spikelets, soil drying, super rice.

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. Providing 35–60% of the dietary calories, rice is consumed by more than 3 billion people (Fageria, 2007). In the last half-century, world rice production has dramatically increased, primarily as the result of genetic improvement: increasing harvest index by the use of the semi-dwarf gene during the First Green Revolution, and the use of heterosis by producing hybrids that started in the 1970s (Zhang, 2007). However, the increase in rice yield has declined since the mid-1980s, as reflected by the sharp drop in the rate of annual yield increase from 2.7% in the 1980s to 1.1% in the 1990s (Horie *et al.*, 2005). Rapid population growth and economic development are growing pressures for an increase in food production (Zhang, 2007).

In rice and other cereal crops, grain yield can be defined as the product of yield sink capacity and filling efficiency (Kato and Takeda, 1996). To increase yield further and to break the yield ceiling, breeding efforts have expanded the yield sink capacity (the maximum size of sink organs to be harvested) mainly by increasing the number of spikelets per panicle (Kato *et al.*, 2007). As a result, cultivars with large panicles or extra-heavy panicle types with numerous spikelets per panicle have become available, such as the New Plant Type of the International Rice Research Institute (Peng *et al.*, 1999), and hybrid rice and 'super' rice or 'super' hybrid rice in China (Cheng *et al.*, 2007; Peng *et al.*, 2008). These cultivars, however, frequently do not exhibit their high yield potential due to their poor grain-filling, as in a slow grain-filling rate and many unfilled grains (Peng *et al.*, 1999; Yang *et al.*, 2002; Ao *et al.*, 2008).

The degree and rate of grain-filling in rice spikelets differ largely with their positions on a panicle. In general, earlier-flowering superior spikelets, usually located on apical primary branches, fill fast and produce larger and heavier grains. While later-flowering inferior spikelets, usually located on proximal secondary branches, are either sterile or fill slowly and poorly to produce grains unsuitable for human consumption (Mohapatra *et al.*, 1993; Yang *et al.*, 2000, 2006). The slow grain-filling problem in inferior spikelets is more aggravated in the newly bred 'super' rice cultivars, although they generally show a yield potential of 8–20% more than other conventional rice cultivars (Cheng *et al.*, 2007; Zhang, 2007). For example, 12 'super' rice cultivars in the lower Yangtze River basin were investigated in 2006 and 2007 and it was found that the average grain weight and filling proportion of inferior spikelets were 20.9% and 20.7%, respectively, lower than those of superior spikelets, while, for three conventional cultivars, on average, it was only 10.5% and 6.3%, respectively (Table 1). The mechanism underlying the poor grain-filling in inferior spikelets remains unknown.

In today's crop production systems with their high yield outputs, improvement in grain-filling has become more challenging than ever (Zahedi and Jenner, 2003; Kato *et al.*, 2007). The questions to be answered are: what is the regulation mechanism that limits grain-filling in inferior spikelets and how could grain-filling in inferior spikelets be enhanced and, consequently, the yield be increased, in rice, especially in 'super' rice. Research related to these questions is the focus of this review. The discussions here will cover (i) assimilate supply in relation to grain-filling; (ii) the enzymatic activity and molecular mechanisms involved in grain-filling; and (iii) post-anthesis moderate soil-drying enhances grain-filling.

* To whom correspondence should be addressed. E-mail: jzhang@hkbu.edu.hk

Table 1. Spikelet weight and filling proportion of 12 ‘super’ rice cultivars in comparison with three conventional rice cultivars

Data are averages of 2006 and 2007 in the lower Yangtze River basin. Superior spikelets were sampled from the top three primary branches in the panicle while the inferior spikelets were spikelets from the secondary branches at the lower part of the panicle. Filling proportion (%)=filled grains (determined under 1.06 specific weight salt solution)/total spikelets×100 (data are not published).

Cultivars	Grain weight (mg)			Filled grains (%)		
	Whole panicle	Superior	Inferior	Whole panicle	Superior	Inferior
Super rice						
Liangyoupeijiu	25.2	28.7	21.6	85.1	92.5	77.7
Xinliangyou 6	27.5	31.2	23.5	83.2	93.1	73.3
Ilyou7954	27.4	30.9	23.6	84.9	94.3	75.5
Ilyou 084	27.1	30.4	23.4	85.6	95.2	76.0
Ilyouhan 1	26.6	30.6	22.7	84.8	97.2	72.4
Ilyouming 86	27.2	30.5	23.8	87.3	97.5	77.6
Ill you 98	27.0	30.3	23.5	83.9	93.8	74.0
Fengyou 299	26.2	30.2	21.8	82.5	93.6	71.4
DYou 527	28.2	31.9	24.3	81.8	96.9	66.7
Wujing 15	28.87	31.8	24.9	86.4	96.5	76.3
Ninjing 1	26.7	29.4	23.8	85.1	94.3	75.9
Huaidao 9	27.2	31.5	23.9	87.5	97.9	77.1
Average	27.1	30.6	23.4	84.8	95.2	74.5
Conventional rice						
Shanyou 63 CK	27.4	29.2	25.6	92.4	96.6	88.2
Yangdao 6 (CK)	27.2	28.6	25.8	92.1	94.9	89.3
Yangfujing 8 CK	26.7	28.1	25.3	95.8	98.2	93.4
Average	27.1	28.6	25.6	94.1	96.6	90.3

Assimilate supply in relation to grain-filling

The slow grain-filling rate and low grain weight of inferior spikelets have often been attributed to a limitation in carbohydrate supply (Sikder and Gupta, 1976; Murty and Murty, 1982; Zhu *et al.*, 1988). However, it was found that the later-flowering inferior spikelets of an inbred cultivar, Yangdao 4, and a ‘super’ rice cultivar, Liangyoupeijiu (both have numerous spikelets on a panicle), actually have more soluble carbohydrate and sucrose than earlier-flowering superior spikelets at the early grain-filling stage (Fig. 1). Similar observations have also been made by Mohapatra *et al.* (1993) and Tao *et al.* (2003) who reported that inferior spikelets accumulated higher concentrations of soluble assimilates than superior spikelets during the grain-filling period. These results suggest that assimilate supply is not the main factor that leads to poor grain-filling and that there are other, unknown, factors resulting in slow or aborted grain-filling in inferior spikelets.

Enzymatic activity and molecular mechanisms involved in grain-filling

Starch in rice grains contributes 80–90% of the final dry weight of an unpolished grain (Yoshida, 1972; Duan and

Sun, 2005). Grain-filling is actually a process of starch accumulation and it has been reported that there are 33 major enzymes involved in the metabolism of carbohydrates in developing rice endosperm (Nakamura *et al.*, 1989). Among them, however, five enzymes are considered to play a key role in this process (Nakamura *et al.*, 1989; Kato, 1995; Yang *et al.*, 2001). They are sucrose synthase (SuSase, EC 2.4.1.13), invertase (β -fructofuranosidase, EC 3.2.2.26), adenosine diphosphate-glucose pyrophosphorylase (AGPase, EC 2.7.7.27), starch synthase (StSase, EC 2.4.1.21), and starch branching enzyme (SBE, EC 2.4.1.18). It has been reported that the sodium azide (NaN_3)-induced rice mutant, SA 419, which accumulates dry matter faster and ripens earlier than its wild-type cultivar, Tainung 67, exhibits higher activities of AGPase, soluble StSase, SBE, and starch debranching enzyme (EC 2.4.1.41) in grains at the early grain-filling stage (Jeng *et al.*, 2003). It has been observed that the activities of AGPase, StSase, and SBE were markedly lower in inferior spikelets than in superior spikelets during the active grain-filling period (from 5% to 95% of the final grain weight) (Yang *et al.*, 2001). These results suggest that some key enzymes play a regulatory role and limit grain-filling in inferior spikelets.

Recently, studies on the expression of genes related to carbohydrate metabolism in developing grains provide us with a deeper understanding of the role of the same enzymes in the grain-filling of rice (Duan and Sun, 2005; Ishimaru *et al.*, 2005; Jeng *et al.*, 2007; Wang *et al.*, 2008). It is proposed that three putative cell-wall invertase genes, *OsCIN1*, *OsSIN2*, and *OsCIN 3*, play complementary/synergistic roles in assimilate unloading during the grain-filling stage (Wang *et al.*, 2008). Ishimaru *et al.* (2005) observed that the gene expressions of vacuolar invertase (*INV3*), SuSase (*RSus3*), and AGPase (*AGPL-1* and *AGPS2*) were much higher in superior spikelets than in inferior ones at the early and/or mid-grain-filling stage(s). Very recently, a cell-wall invertase encoded by the rice *GIFI* (grain incomplete filling 1) gene has been found to play a key role in carbon deposition during early grain-filling and an overexpression of *GIFI* can increase grain-filling and final grain weight (Wang *et al.*, 2008). These new molecular methods could provide an opportunity to improve the grain-filling of inferior spikelets.

Post-anthesis moderate soil-drying enhances grain-filling

Grain-filling in cereals depends on carbon from two sources: current assimilates and assimilates redistributed from reserve pools in vegetative tissues either pre- or post-anthesis (Kobata *et al.*, 1992; Schnyder, 1993; Samonte *et al.*, 2001). The contribution of reserved assimilates in culms and leaf sheaths of rice plants is estimated at around 30% of the final yield depending on cultivar and environmental conditions (Gebbing and Schnyder, 1999; Takai

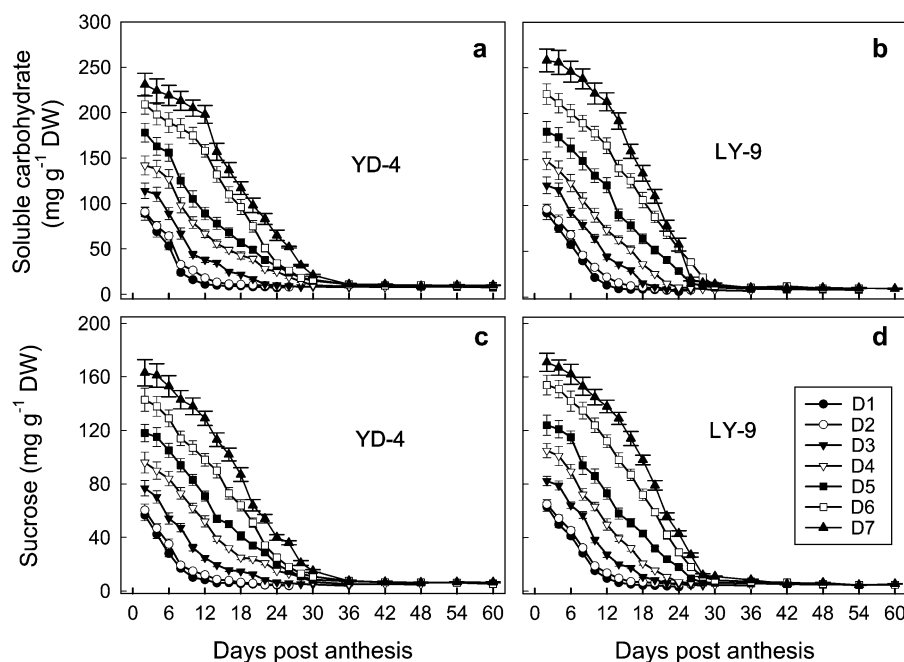


Fig. 1. Concentrations of soluble carbohydrate (a, b) and sucrose (c, d) in rice spikelets. The indica inbred cultivar YD-4 (a, c) and indica ‘super’ rice cultivar LY-9 (b, d) were field-grown. D1, D2, D3, D4, D5, D6, and D7 are the spikelets that flowered on the first, second, third, fourth, fifth, sixth, and seventh days, respectively, on a panicle. Vertical bars represent \pm SE of the mean ($n=4$) where these exceed the size of the symbol. The figure is reproduced from Yang *et al.* (2006).

et al., 2005). Remobilization and transfer of the stored assimilates in vegetative tissues to the grain in monocarpic plants such as rice and wheat require the initiation of whole-plant senescence. Delayed whole-plant senescence can lead to poorly filled grains and unused carbohydrates in straws. Slow grain-filling can often be associated with a delay in whole-plant senescence (Zhu *et al.*, 1997; Mi *et al.*, 2002; Gong *et al.*, 2005). Currently, there are at least three common causes of whole-plant senescence being unfavourably delayed. They include the over-use of nitrogen fertilizers (Peng *et al.*, 2006), the adoption of lodging-resistant cultivars that stay ‘green’ for too long (Zhu *et al.*, 1997; Yuan, 1998), and the introduction of hybrid rice (Yang *et al.*, 2002; Yuan, 2003). Their senescence is defined as unfavourably delayed because the gain from the extended grain-filling period is less than the loss due to slow grain-filling and unused assimilates left in the straw. It was found that a controlled soil drying or a moderate soil drying (so that the plant can rehydrate overnight and photosynthesis is not severely inhibited) during the mid- and late grain-filling stages can greatly promote whole-plant senescence and assimilate remobilization. The gain from a better use of pre-stored assimilates outweighs the possible loss due to a shortened grain-filling period, leading to an increased grain-filling rate and increased grain yield (reviewed in Yang and Zhang, 2006). In recent years, this work has been extended into rice production in Jiangsu, China, and it has effectively enhanced grain-filling and harvest index in the field of commercial crop production (Yang *et al.*, 2007), which has

proved that proper field practice can solve some slow grain-filling problems.

Concluding remarks

Poor grain-filling of the later-flowering inferior spikelets of modern rice cultivars with numerous spikelets per panicle, especially for ‘super’ rice, is a serious problem and frequently limits yield potential. Carbohydrates may not be the major limiting factor to the development of inferior spikelets. Low activities and/or gene expressions of enzymes involved in sucrose-to-starch conversion, SuSase, cell wall invertase, StSase, AGPase, and SBE, in inferior spikelets contribute to their poor grain-filling. Molecular approaches and proper field practices, such as a controlled or moderate soil drying during the mid- and late grain-filling stages, could solve some problems in poor grain-filling.

‘Super’ rice has a huge capacity to produce photosynthetic assimilates which provides us with a unique opportunity to convert them into grain yield. Modifications over the assimilate translocation and deposit processes should have the potential to make full use of the source capacity. Further studies are essential and may focus on (i) investigating how environmental factors, such as soil moisture and mineral nutrients, affect the filling rates of both superior and inferior spikelets; (ii) investigating how the factors inside spikelets, including hormones and mRNA transcriptions, protein expressions, and activities of key enzymes involved in sucrose-to-starch conversion, regulate

the filling process of superior and inferior spikelets, and (iii) investigating how the factors within the plant but outside the spikelets, such as root activity, root-sourced signals and their transportation, and sink–source relationships, regulate the filling of superior and inferior spikelets of ‘super’ rice.

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