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Changes in Enzyme Activities Involved in Starch Synthesis and Hormone Concentrations in Superior and Inferior Spikelets and Their Association with Grain Filling of Super Rice

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Abstract: The changes in activities of key enzymes involved in sucrose-to-starch conversion and concentrations of hormones in superior and inferior spikelets of super rice were investigated and their association with grain filling was analyzed. Four super rice cultivars, Liangyoupeijiu, IIyou 084, Huaidao 9 and Wujing 15, and two high-yielding and elite check cultivars, Shanyou 63 and Yangfujing 8, were used. The activities of sucrose synthase (SuSase), adenosine diphosphoglucose pyrophosphorylase (AGPase), starch synthase (StSase) and starch branching enzyme (SBE), and the concentrations of zeatin + zeatin riboside (Z + ZR), indole-3-acetic acid (IAA) and abscisic acid (ABA) in superior and inferior spikelets were determined during the grain filling period and their relationships with grain filling rate were analyzed. Maximum grain filling rate, the time reaching the maximum grain-filling rate, mean grain filling rate and brown rice weight for superior spikelets showed a slight difference between the super and check rice cultivars, but were significantly lower in the super rice than in the check rice for inferior spikelets. Changes of enzyme activities and hormone concentrations in grains exhibited single peak curves during the grain filling period. The peak values and the mean activities of SuSase, AGPase, StSase and SBE were lower in inferior spikelets than in superior ones, as well as the peak values and the mean concentrations of Z + ZR and IAA. However, the peak value and the mean concentration of ABA were significantly higher in inferior spikelets than in superior ones and greater in the super rice than in the check rice. The grain filling rate was positively and significantly correlated with the activities of SuSase, AGPase and StSase and the concentrations of Z + ZR and IAA. The results suggested that the low activities of SuSase, AGPase and StSase and the low concentrations of Z + ZR and IAA might be important physiological reasons for the slow grain filling rate and light grain weight of inferior spikelets in super rice.

Key words: super rice; superior spikelet; inferior spikelet; enzyme activity; hormone concentration; grain filling

Rice yield can be defined as the product of yield sink capacity and filling efficiency (Kato and Takeda, 1996). To further increase the yield and improve yield capacity, breeding efforts have expanded yield sink capacity and the maximum size of sink organs to be harvested, mainly by increasing the number of spikelets per panicle (Kato et al, 2007), such as the new plant type rice in the International Rice Research Institute (IRRI) (Peng et al, 1999), intersubspecific hybrid rice and super hybrid rice or super rice in China (Cheng et al, 2007; Peng et al, 2008). These cultivars with large panicles, however, do not frequently exhibit their high yield potential in the production of large-scale popularization due to their poor grain filling, which is mainly due to the slow grain filling rate and many unfilled grains of the later-flowering inferior spikelets (Ao et al, 2008; Zhang et al, 2009; Yang and Zhang, 2010).

The degree and the rate of grain-filling and grain weight of rice spikelets differ largely within 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, whereas later-flowering inferior spikelets, usually located on proximal secondary branches, are either sterile or fill slowly and poorly to produce grains (Mohapatra et al, 1993; Yang et al, 2000). The difference of grain filling

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in superior and inferior spikelets is more aggravation in the newly bred super rice cultivars with large panicles. There are many explanations for the poor filling of inferior spikelets, including assimilate supply, hormone levels, enzyme activities and gene expression (Nakamura and Yuki, 1992; Liang et al, 2001; Yang et al, 2002), but the mechanism underlying the poor grain-filling in inferior spikelets remains unknown.

Starch in rice grains contributes to about 90% of the final dry weight of unpolished grain (Murata and Matsushima, 1975). In fact, the process of grain filling in rice is the process of starch accumulation. Photosynthates are transported from source organs (leaves, culms and sheaths) to grains in the form of sucrose and formed starch by a series of enzymatic reactions (Nakamura et al, 1989; Nakamura and Yuki, 1992). The key enzymes for sucrose-to-starch conversion in grains, such as sucrose synthase (SuSase), adenosine diphosphate glucose pyrophosphorylase phosphorylase (AGPase), starch synthase (StSase) and starch branching enzyme (SBE), play important roles in this process (Kato, 1995; Ahmadi and Baker, 2001). Meanwhile, endogenous hormones in grains, such as zeatin + zeatin riboside (Z + ZR), 3-indole acetic acid (IAA) and abscisic acid (ABA), probably regulate sugar metabolism and starch synthesis (Yang et al, 2002, 2003a, 2006). However, changes in the activities of the enzymes involved in sucrose-to-starch conversion and the concentrations of endogenous hormones in superior and inferior spikelets of super rice and their association with grain filling are little studied. Therefore, this study systematically investigated the changes in the activities of key enzymes involved in sucrose-to-starch conversion and the concentrations of hormones in superior and inferior spikelets and their association with grain filling of super rice. The objective of this study was to further reveal the mechanism of grain filling in super rice and provide a theoretical basis for the development of super rice.

MATERIALS AND METHODS

Rice materials and cultivation

Tested super rice cultivars were Liangyoupeijiu (indica-inclined two-line hybrid), IIyou 084 (indica-inclined three-line hybrid), Huaidao 9 (japonica) and Wujing 15 (japonica), and two high-yielding and elite check cultivars, Shanyou 63 (indica hybrid) and Yangfujing 8 (japonica).

The experiment was conducted at a farm belonging to Yangzhou University, Yangzhou, China during the rice growing season of 2010. Wheat was grown in the last season, and the soil was a sandy loam with 15.7 g/kg organic matter, 64.7 mg/kg alkali hydrolysable N, 20.4 mg/kg available P and 120.0 mg/kg available K in 0−20 cm soil layer (air-dried sample). Seedlings were raised in the seedbed with sowing date on May 11 and transplanted on June 7 at a hill spacing of 0.20 m × 0.20 m with one seedling per hill for Shanyou 63, Liangyoupeijiu and IIyou 084 and two seedlings per hill for Yangfujing 8, Huaidao 9 and Wujing 15. Plot dimension was in 5.0 m × 4.0 m. Each cultivar had three plots as repetitions in a complete randomized block design. N (90 kg/hm^2 in form of urea), P (30 kg/hm^2 in form of single superphosphate) and K (140 kg/hm^2 in form of KCl) were incorporated and applied before transplanting. N in the form of urea was also applied as topdressing at the early-tillering (7 d after transplanting, 40 kg/hm^2) and spikelet-differentiating stages (25 d before heading, 60 kg/hm^2). The total N application was 190 kg/hm^2 in line with the local high-yielding practice. Other field managements were according to the local high-yielding cultivation.

Sampling and measurement

Sampling and measuring grain filling rate

Two hundred panicles that headed on the same day were chosen and tagged in each plot. The flowering date and the position of each spikelet on the tagged panicles were recorded. Fifteen tagged panicles from each plot were sampled every 5 d from flowering to 45 d after flowering to measure grain weight, the activities of key enzymes involved in the sucrose-starch metabolic pathway, and hormone concentrations in superior and inferior spikelets. Superior spikelets that flowered on the first or second day on a panicle and inferior ones that flowered on the last 2 d on a panicle were separated from the sampled panicles. About two-thirds sampled grains were frozen in liquid nitrogen for 1 min and then stored at -80 °C for determining hormone concentrations and the activities of key enzymes involved in the sucrose-starch metabolic pathway, and the other one-third sampled grains were dried at 70 °C to a constant weight for 72 h, then dehulled and weighed. The process of grain filling were fitted by the Richards' growth equation (Richards, 1959) as described by Zhu et al (1988): $W = A / (1 + Be^{-Kt})^{1/N}$. Where, W is the grain weight (mg), A is the final grain weight (mg), t is the time after flowering (d), B, K and N are coefficients determined by the