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Starch granules size distribution in superior and inferior grains of wheat is related to enzyme activities and their gene expressions during grain filling

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ABSTRACT

Mature wheat endosperm contains A-, B-, C-type starch granules, and each class has unique physiochemical properties which determine the quality of starch. The dynamics of the starch granule size distribution, activities of starch synthases and expression of starch synthase encoding genes were studied in superior and inferior grains during grain filling. Compared with inferior grains, superior grains showed higher grain weight, contents of starch, amylose and amylopectin. The formation of A-, B-, C-type starch granules initiated at 4, 8, 20 DAF, respectively, and was well consistent with the temporally change patterns of starch synthase activities and relative gene expression levels. For instance, activities of soluble and granule-bound starch synthases (designated SSS and GBSS) peaked at 20 and 24 DAF. Genes encoding isoforms of starch synthases expressed at different grain filling periods. In addition, *SS I* was generally expressed over the grain filling stage; the *SS II* and *SS III* were expressed over the early and mid grain filling stage, and the *GBSS I* was expressed during the mid to late grain filling stage. In addition, the time-course changes in activities of starch synthases and expression of starch synthase encoding genes explained well the dynamics of the starch granule size distribution.

1. Introduction

The temporal and spatial patterns of starch synthesis in wheat kernels differ with their positions on a spike (Jiang et al., 2003). The basal flowerets located on the middle spikelets of wheat spikes usually flower earlier, take precedence in grain formation and filling, and obtain higher grain weight (the so-called superior grains). In contrast, distal flowerets on middle spikelets or those on the distal spikelets (inferior grains) are smaller (Langer and Hanif, 1973). The lower grain weights are reported to be related to the late development of the endosperm, less endosperm cells and low grain filling rate in the inferior grains (Gao et al., 1992; Ishimaru et al., 2003).

Starch accounts for two-thirds to three-quarters of wheat kernel dry weight (Hucl and Chibbar, 1996), and is distributed in different classes of granules in the endosperm. Grains of mature wheat, barley and their wild relatives contain at least two distinct starch granules according to their sizes, the large A-type and the small B-type, with a boundary diameter of 10 μm (Geera et al., 2006). A very small C-type starch granule has also been reported in these species (Bechtel and Wilson, 2003; Bechtel et al., 1990), and debated to be classified as a B-type because of the difficulty in plotting the boundary between them. In wheat endosperm, A-type granules constitute the majority of the starch by weight (Bechtel et al., 1990; Peng et al., 1999; Shinde et al., 2003), whereas B-type (including C-type) granules comprise up to 99% of granules in number (Raeker et al., 1998). Differences in starch granule composition (Peng et al., 1999; Shinde et al., 2003) and their molecular structure (Jane et al., 2003) are associated with the baking quality of wheat flour (Park et al., 2005).

Starch content and composition differ between the superior and inferior caryopses located at different positions in the wheat spike (Jiang et al., 2003). The low starch content and accumulation rate has been partially ascribed to the weak activities of enzymes for starch synthesis during grain filling in the inferior caryopses of wheat and rice (Jiang et al., 2003; Yang et al., 2004). In addition, the spatial expression pattern of starch synthase encoding genes might be involved in non-uniform starch synthesis in grains at different

Abbreviations: DAF, days after flowering; GBSS, granule-bound starch synthase; SSS, soluble starch synthase; *SS I*, starch synthase I; *SS II*, starch synthase II; *SS III*, starch synthase III; RT-PCR, reverse transcriptase-polymerase chain reaction.

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positions in the spike (Denyer et al., 1995; Hurkman et al., 2003). However, the difference in starch granule size distribution between the superior and the inferior grains has not been documented. In addition, little is known how the spatial differences in activities of starch synthases and related gene expression patterns contribute to the differential distribution patterns of starch granule size between the inferior and superior caryopses.

The objective of this paper was to compare the differences in changes of starch granule size distribution patterns during grain filling between the superior and inferior grain in wheat. It was also aimed to test the hypothesis that the differential starch granule size distribution patterns are related to the temporal and spatial patterns of activities of starch synthases and their encoding gene expressions.

2. Materials and methods

2.1. Plant material

Wheat (*Triticum aestivum* L., cv. Yangmai158) plants were grown in the experimental station of Jiangsu Academy of Agricultural Science, Nanjing, Jiangsu Province, PR China, in the growing season of 2004–2005. Uniform heads with the splikelets located in the mid part of the heads flowering on the same date were tagged from 4 days after flowering (DAF) with a four-day interval till harvest (grain moisture of 20–22%). The most basal grains from the basal 5–8 spikelets on the tagged spikes were detached as superior grains, whereas the most distal grains on the same spikelets were detached as inferior grains. The grains were immediately frozen in liquid nitrogen for at least 2 h and then stored at −80 °C until use.

2.2. Starch isolation

The isolation procedure of starch was according to Bechtel et al. (1990) with minor modifications. Briefly, the caryopses were cut at the end of the embryo to remove the embryo using a clean razor blade. The endosperm was then carefully squeezed out of the caryopsis. The endosperm was mixed with buffer solution (25 mM Tricine, 5 mM magnesium acetate, 50 mM potassium acetate, pH 7.5) at 4 °C and milled in a micro-blender. The slurry was centrifuged, and the pellets were collected and were suspended in ethanol. The suspension was filtered through a nylon screen with a pore size of 53 μm and was washed with excess ethanol. The starch sample was then collected by centrifugation, and resuspended in 0.1 M aqueous NaCl solution containing 10% toluene and stirred for 1 h using a magnetic stirrer at a high speed to remove protein. This step was repeated until the toluene layer became clear and no protein was present. The starch was re-purified by washing three times with water and twice with ethanol and then dried at 30 °C for 48 h. During the whole procedure, each centrifugation was done at 3000*g* (10 min, 4 °C) and the incubations were conducted at 20 °C.

2.3. Starch composition

Amylose and amylopectin contents in wheat grains were determined with a coupled spectrophotometer assay, depending on Jiang et al.'s (2003) description with little modification. Fifty milligrams of standard amylose or amlopectin were stirred with 0.5 ml of absolute alcohol and 4.5 ml of 1 M NaOH for 20 min at 85 °C and then diluted to a volume of 50 ml with distilled water. Standard amylose solutions, 0.25, 0.5, 0.75, 1, 1.25 and 1.5 ml were diluted, respectively, with 20 ml distilled water and adjusted to pH 3.5 with 1 M acetum, and then 0.5 ml of I_2–KI reagent was added to the solution, which was diluted with distilled water to a final volume of 50 ml. Standard amylopectin solution, 0.5, 1.25, 2, 2.75, 3.5 and 4.25 ml were diluted, respectively, and carried out under the same reaction condition as above. After blending for 20 min, the mixtures were scanned with a Heλios Gamma spectrophotometer (Thermo Spectronic, Cambridge, UK) between 400 and 960 nm. The absorption peaks of standard amylose reacting with I_2–KI reagent were 630 and 460 nm, whereas those of amylopectin were 740 and 550 nm. The standard equation of amylose or amylopectin was achieved through calculating the relationship of the composition versus the absorption values. Hundred milligrams of milled wheat grains were prepared for analysis and 2 ml of solution, which was

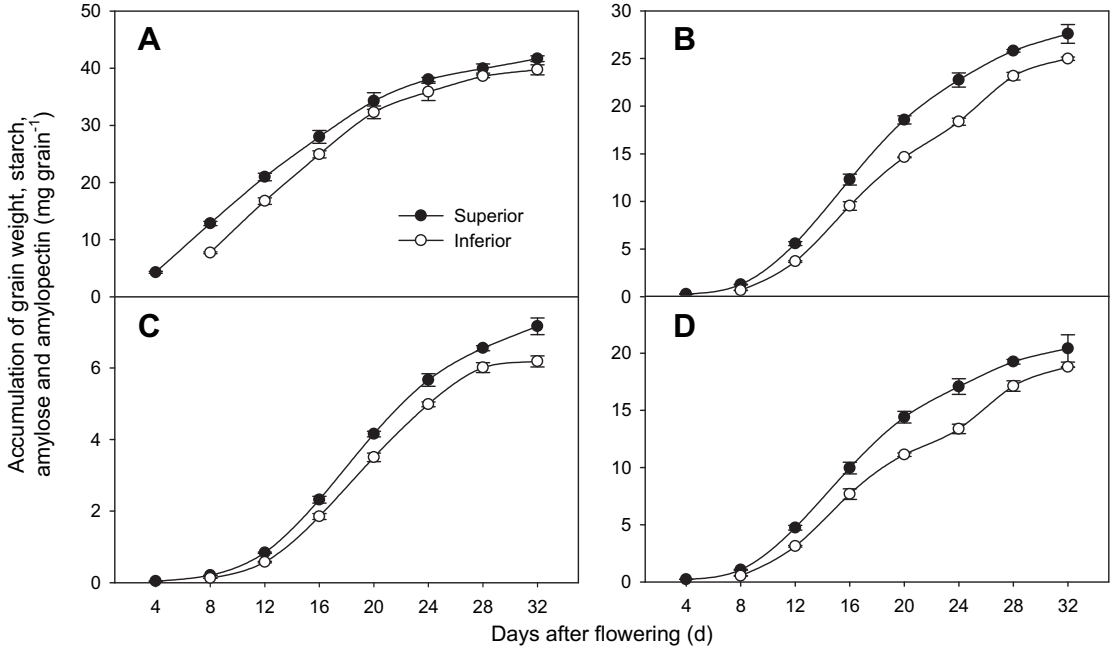

Fig. 1. Accumulation of grain weight (A) starch (B), amylose (C) and amylopectin (D) in superior and inferior grains of wheat during grain filling.