Effect of spikelet position on starch proportion, granule distribution and related enzymes activity in wheat grain

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ABSTRACT

The starch proportion, starch granule distribution, and activities of enzymes involved in starch synthesis in different spikelet positions were examined during grain development in two high-yielding winter wheat cultivars. The results showed that grain number and weight per spikelet in different spatial position showed a single-peak curve from the base to the top in a wheat spike. Upper spikelets had the highest starch and amylose proportion followed by basal spikelets, whereas middle spikelets had the lowest. Starch and amylose absolute content was in opposition to their proportion. The volume of B- and A-type granule in grain of middle spikelets was remarkably higher and lower than those of basal and upper spikelets, respectively. However, no significant difference occurred in the number of A- and B-type granule in grains among different spikelet position. Compared with the basal and upper spikelets, the middle spikelets showed higher sucrose and ATP content and activities of starch biosynthetic enzymes, and subsequently higher starch absolute content. The results suggested that superior sucrose providing and degradation capacity and the high activities of enzymes involved in starch synthesis resulted in development of B-type starch granule in grain of middle spikelets.

Keywords: winter wheat (*Triticum aestivum* L.); spatial position; starch characteristic; grain development; starch synthesis

Starch, which constitutes 65–75% of the wheat (*Triticum aestivum* L.) grain weight, is an important determinant of the yield and processing properties of wheat (Hurkman et al. 2003). Starch is deposited in the endosperm as discrete semi-crystalline aggregates known as starch granule (Paul 1997). The endosperm of mature wheat grains contains two major classes of starch granules: large, elliptical A-type granules and small, spherical B-type granules (Soulaka and Morrison 1985). A-type granules and B-type granules have different physical, chemical, and functional properties (Hurkman et al. 2003). Edwards et al. (2008) reported that distributions involved in an increase in the sample volume of B-type granules and decrease in A-type granules were associated with increased rheology index values and higher flour yield. Based on differences in characteristics and properties, the ratio of A-type and B-type starch granules in wheat flour has an effect on the quality of traditional wheat-based products such as noodles (Black et al. 2000) and breads (Park et al. 2005).

A wheat spike is composed of a number of spikelets. Grain weight within a wheat spike is unevenly distributed depending upon the spatial position of kernel (Calderini and Reynolds 2000). Based on their flowering date and locations within a spike, the spikelets can be classified as superior

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or inferior (Stoddard 1999). Superior grains usually exhibit a faster rate of increase in dry weight than inferior grains in wheat (Liang et al. 2008). Low grain weight and slow grain filling of inferior grains were attributed to a limitation in carbohydrate supply (Jiang et al. 2003). However, other work suggested that there is no clear causative relationship between assimilate supply and grain development within a wheat spike (Zhang et al. 2000).

It was documented that enzymes involved in starch synthesis, including sucrose synthase (SuSy), adenosine diphosphate glucose pyrophosphorylase (AGPase), soluble starch synthase (SSS), granule-bound starch synthase (GBSS) and so on, play key roles in starch synthesis in wheat grain (Nakamura and Yuki 1992, Hurkman et al. 2003, Yan et al. 2010). The activities of ADPase and SSS (Liang et al. 2001) are associated with the inferior grain weight in rice, but it is not known whether a similar mechanism exists in grain within a wheat spike.

The purposes of the study were to investigate the starch content and granule size distribution in grains in different spikelets position of wheat, and to determine whether the variations in grain starch accumulation in wheat spike were attributed to their assimilate supply and sink activities. This information should aid the elucidation of the mechanism for regulating the spatial pattern of individual grain growth within a wheat spike.

MATERIAL AND METHODS

Experimental design. The field experiments were carried out at the Tai'an Experimental Station of Shandong Agricultural University (36°09'N, 117°09'E) in two growing seasons from October 2006 to June 2007 and from October 2007 to June 2008. Two high-yield wheat cultivars currently used in local wheat production, Gaocheng 8901 and Shannong 1391, were chosen in this study. The experiments were arranged in a randomized complete design with four replications.

Plant sampling. Spikes flowering on the same date were labeled with red thread at anthesis. From 7 days post anthesis (DPA), twenty labeled ears of each plot were sampled at 7-day intervals till maturity. These heads were frozen immediately in liquid nitrogen for at least 30 min and then stored at –40°C for enzyme assay. Additional heads were heated at 105°C for 30 min and then dried at 70°C for sucrose and starch determination. At maturity, wheat kernels were harvested for granules size distribution analysis. From basal 1st–5th spikelets, 6th–16th spikelets and 17th to top spikelets, respectively, the first and second basal grains on each spikelet were detached to assay in this study.

Amylose and amylopectin determination. Amylose and amylopectin contents were determined with the dry grain according to a coupled spectrophotometer assay (He 1985).

Enzyme extraction and assays. The preparation procedure was performed to the methods of Smyth and Prescott (1989). The assay of SuSy and AGPase activity were carried out following the procedure of Smyth and Prescott (1989). The SSS and GBSS activities were determined according to the methods of Nakamura and Yuki (1992).

Starch isolation and purification. Starch was extracted from wheat grain according to the methods of Peng et al. (1999).

Particle size analysis. Particle size characteristics of starch were determined by using LS13320 laser diffraction particle size analyzer (Beckman Coulter, Brea, USA).

Statistical analyses. The data were analyzed for variance using the SPSS statistical analysis package (New York, USA). The experimental data from 2006 to 2007 were reported in this paper.

RESULTS

Spatial distribution of grain number and weight. The grain number (Figure 1a) and weight (Figure 1b) per spikelet showed a single-peak curve from the base to the top in a wheat spike. The 6th–16th spikelets had more grain number and higher grain weight, which indicated that middle spikelets have stronger capability of dry matter accumulation compared with basal and upper spikelets.

Starch accumulation. There were significant differences in starch and amylose proportion in grains differing in spikelet (Table 1). Upper spikelets had the highest starch and amylose proportion followed by basal spikelets, whereas middle spikelets had the lowest. Starch and amylose absolute content was in opposition to their proportion.

Starch granule size distribution. Upper spikelets and middle spikelets had the highest and the lowest volume of A-type starch granules, respectively (Table 2). Middle spikelets had the highest

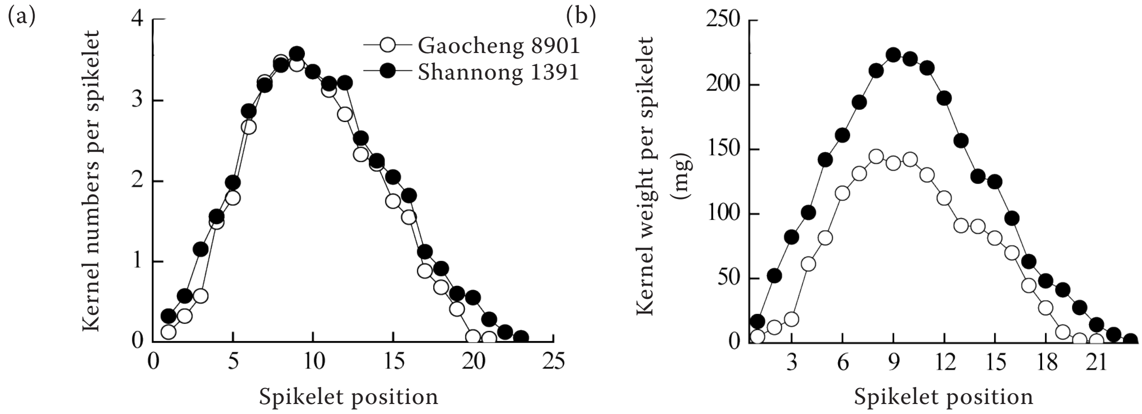

Figure 1. Kernel numbers per spikelet (a) and kernel weight per spikelet (b) of winter wheat

volume of B-type starch granules followed by basal spikelets, whereas upper spikelets had the lowest.

Proportions of B-type starch granules were in the ranges of about 99.9% of the total number, respectively, which showed that number of granules in wheat grain was made up of B-type starch granules. Spikelets exerted little effect on the alteration of percentage of A- and B-type starch granules.

Substrate content and enzyme activity. The sucrose contents in wheat grains decreased gradually with grain development (Figure 2a), and the sucrose contents in middle spikelets were higher than that in basal and upper spikelets from 7 to 21 DPA. ATP in wheat grains is an important source of energy. ATP contents were higher in middle spikelets than in basal and upper spikelets except for 7 DPA (Figure 2b).

SuSy catalyses the sucrose to UDP-glucose and fructose, which is thought to be the first step in the starch synthesis in wheat grains. SuSy activities showed single peak curves with the highest peak at 21 DPA (Figure 3a). The SuSy activity in middle spikelets was greatly higher than that in basal and upper spikelets during grain filling.

AGPase converts glucose-phosphate to ADP-glucose in the presence of ATP, and is regarded as the rate-limiting enzyme in starch biosynthesis. The changes of AGPase activities showed single peak curves with the highest peak at 21 DPA (Figure 3b). The AGPase activity in middle spikelets was much higher than that in basal and upper spikelets during grain filling.

SSS transfers a glucose residue from ADPG molecule to a starch primer molecule, and is responsible for amylopectin synthesis in wheat grain. The SSS activity in middle spikelets was much higher than that in basal and upper spikelets after 7 DPA (Figure 3c). GBSS has a similar function to SSS to elongate the starch molecular chain. However, GBSS is activated only when being bound in the starch granule. The GBSS activities exhibited single peak curves with peak at 21 DPA (Figure 3d). The SSS activity in middle spikelets was much higher after 7 DPA as compared with basal and upper

Table 1. Effect of spikelet position on starch accumulation in wheat grain

Cultivar	Spikelet position	ST proportion (%)	AM proportion (%)	ST absolute content (mg/grain)	AM absolute content (mg/grain)
Gaocheng 8901	US	61.9 ± 0.5[a]	16.2 ± 0.2[a]	23.4 ± 0.9[b]	6.1 ± 0.2[a]
	MS	59.8 ± 0.4[c]	14.8 ± 0.1[c]	27.2 ± 0.7[a]	6.7 ± 0.1[a]
	BS	60.7 ± 0.5[b]	15.4 ± 0.2[b]	25.6 ± 0.8[a]	6.5 ± 0.2[a]
Shannong 1391	US	63.4 ± 0.3[a]	17.6 ± 0.2[a]	32.2 ± 0.8[c]	8.9 ± 0.2[a]
	MS	62.2 ± 0.4[b]	16.9 ± 0.1[b]	40.9 ± 0.9[a]	11.1 ± 0.2[a]
	BS	62.9 ± 0.5[ab]	17.3 ± 0.2[a]	37.7 ± 1[b]	10.4 ± 0.2[a]

ST – starch; AM – amylose; US – upper spikelets; MS – middle spikelets; BS – basal spikelets. Means within cultivar followed by a different letter are significantly different at $P = 0.05$ level of probability

Table 2. Effect of spikelet position on the proportion by volume and number of starch granules in wheat grain

Cultivar	Spikelet position	Volume (%)		Number (%)	
		B starch granule	A starch granule	B starch granule	A starch granule
Gaocheng 8901	US	41.5 ± 0.3[c]	59.5 ± 0.3[a]	99.9[a]	0.1[a]
	MS	47.2 ± 0.4[a]	52.8 ± 0.4[c]	99.9[a]	0.1[a]
	BS	43.6 ± 0.4[b]	56.4 ± 0.4[b]	99.9[a]	0.1[a]
Shannong 1391	US	34.5 ± 0.3[c]	65.5 ± 0.3[a]	99.9[a]	0.1[a]
	MS	39.2 ± 0.4[a]	60.8 ± 0.4[c]	99.9[a]	0.1[a]
	BS	37.4 ± 0.3[b]	62.6 ± 0.3[b]	99.9[a]	0.1[a]

ST – starch; AM – amylose; US – upper spikelets; MS – middle spikelets; BS – basal spikelets. Means within cultivar followed by a different letter are significantly different at $P = 0.05$ level of probability

spikelets. These changes of SSS and GBSS activities indicated that the ability of starch synthesis in middle spikelets was noticeably higher than that in basal and upper spikelets.

DISCUSSION

There were differences in the floret development, carbohydrate supply and endogenous hormones and starch synthesis among grains within rice (Yang et al. 2003) and wheat (Calderini and Reynolds 2000) spike. Results from previous studies that examined whether the sucrose content was a limiting factor for starch synthesis in inferior grains were inconsistent. Jiang et al. (2003) suggested that superior grains had higher sucrose contents (substrate of starch synthesis) at early and middle grain filling, thus a greater ability for starch synthesis exhibited in superior grains compared to inferior ones.

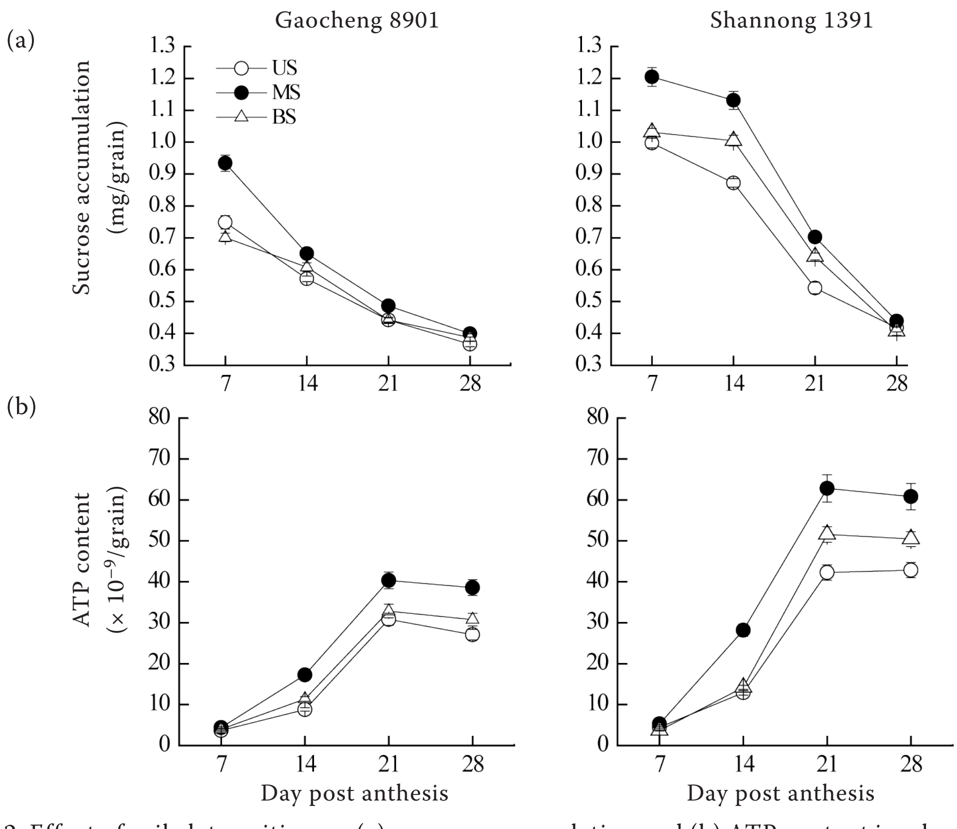

Figure 2. Effect of spikelet position on (a) sucrose accumulation and (b) ATP content in wheat grain. US – upper spikelets; MS – middle spikelets; BS – basal spikelets

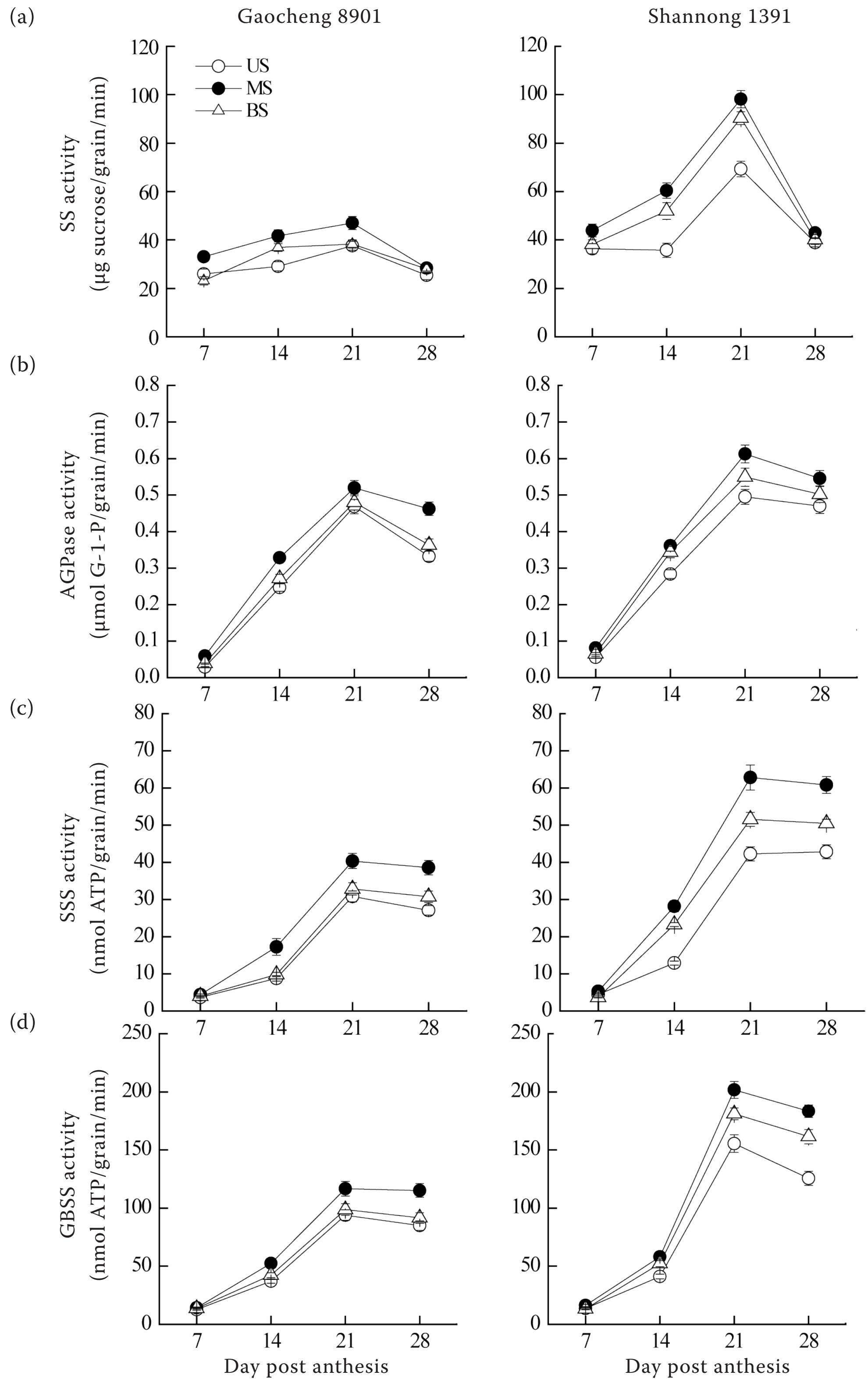

Figure 3. Effect of spikelet position on (a) sucrose synthase (SS) activity; (b) adnosine diphosphate glucose pyrophosphorylase (AGPase) activity; (c) soluble starch synthase (SSS) activity, and (d) granule bounded starch synthase (GBSS) activity in wheat grain. US – upper spikelets; MS – middle spikelets; BS – basal spikelets

However, Zhang et al. (2000) found that the source supply was satisfied with grain filling, while the sink size and ability of assimilation conversion were the main limiting factors for starch accumulation in wheat grains. SuSy is the key enzyme to catalyse the cleavage of sucrose in wheat grain. In this study, the sucrose content and SuSy activities in grain of middle spikelets were significantly higher than that of basal and upper spikelets during grain filling, which indicates that low starch accumulation in grains of basal and upper spikelets were attributed to a limitation in carbohydrate supply, and middle spikelets had strong substrate utilization compared with basal and upper spikelets.

Grain filling is mainly a process of starch biosynthesis and accumulation. It is generally accepted that grain filling rate in cereals is mainly determined by sink strength. The sink strength can be described as the product of sink size and sink activity. Sink size is a physiological restraint that includes cell number and cell size (Jenner 1991, Liang et al. 2001). Sink activity is mostly determined by the activities of enzymes involved in carbohydrate utilization and storage (Smyth and Prescott 1989). In this study, starch accumulation was much higher in grain of middle spikelets, which may be correlated to their growth preference. Integrating the characterization of enzyme activities, we could consider that there was a difference in conversion ability of sucrose to starch resulting in starch accumulation difference of grains among the different spikelets in wheat, and the physiological mechanism of higher starch accumulation in grain of middle spikelets was owing to the stronger sink activities, especially during mid an late grain filling.

The volume of A- or B-type starch granule varied with spikelet position in this study. Middle spikelets had significantly higher proportion of B-type starch granules compared with basal and upper spikelets. This study also showed that number of B- and A-type starch granules in grains had no significant difference among upper, middle and basal spikelets, which indicates that it is more conducive to the development of small starch granules in grain of middle spikelets. The reason may be high substrate supply and degradation capacity and high activities of enzymes involved in starch synthesis made for development of B-type granules as compared with basal and upper spikelets.

Starch granule size distribution had a close association with its component proportion. Various studies reported that amylose proportion was higher in the large starch granules than in the small granules. And some reported the A-type granules have a 4–10% higher amylose proportion than the B-type granules (Peng et al. 1999), while Ames et al. (1999) found only minor differences (2–3%) in amylose proportion. Raeker et al. (1998) reported that the starch proportion was negatively correlated with volume percentage of granule < 5 and 9.9 μm. Upper spikelets had the highest amylose proportion followed by basal spikelets, whereas middle spikelets had the lowest. Integrating with starch granule size distribution, this study suggested that amylose proportion in grain was positively correlated to volume percentage of A-type granule (R = 0.9823; P = 0.00047).

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