Differences of Starch Granule Distribution in Grains from Different Spikelet Positions in Winter Wheat

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Abstract

Wheat starch development is a complex process and is markedly difference by changes in spikelet spatial position. The present study deals with endosperm starch granule distribution and spatial position during filling development. The study was conducted with pure starch isolated from wheat (Triticum aestivum L.), Jimai20 and Shannong1391, at 7–35 days after anthesis (DAA). The results showed that grain number, spikelet weight and grain weight per spikelet in different spatial position showed parabolic changes. Upper spikelets had highest starch and amylose content followed by basal spikelets, then middle spikelets. The paper also suggested the volume percents of B-type and A-type granule in grain of middle spikelets were remarkably higher and lower than those of basal and upper spikelets, respectively. However, no significant difference occurred in the number percents of the two type granule. The ratio of amylase to amylopectin was positively correlated with the volume proportion of 22.8–42.8 μm, but was negatively related to the volume proportion of <9.9 μm. The results indicated that the formation and distribution of starch granules were affected significantly by spikelet position, and grains at upper and basal spikelet had the potential of increasing grain weight through increasing the volume of B-type granules.

Introduction

Wheat (Triticum aestivum L.) is one of the most important food crops [1]. The starch is an important part of wheat endosperm, and it accounts for 65–75% of
the final dry weight of the grain and serves as a multifunctional ingredient for the food industry [2]. The relative proportion and structure of starch constituents, amylose and amylopectin, greatly influence its end-use. Structurally, Amylose is an almost linear \( \alpha-1,4 \) glucan molecule that comprises 25–30% of wheat grain starch. Amylopectin is a much larger glucan polymer that is highly branched and comprises 70–75% of wheat grain starch [6], amylose to amylopectin normally occur in a ratio of \( \approx 1:3 \), by weight [7].

Starch concentration increased approximately 10% from 12 to 15 days after anthesis (DAA) in all genotypes, as well as maximum starch accumulation, occurred from 7 to 14 DAA [3]. Starch, however, usually presents as granules in the endosperm of wheat grain. Briefly, granule size distribution in wheat starch is an important factor that affecting the end-use quality [8]. It is widely acknowledged that the starch is deposited in two types of granules, B-type granules (diameter \(<9.9 \mu m\)) and A-type granules (diameter \(>9.9 \mu m\)) in mature wheat grains [9, 10, 11]. Starch deposition in endosperm occurs until maturation [12]. The A-type granules start to form in the amyloplast at about 4–5 DAA and continue to form until the end of the endosperm cell division phase [4]. The diameter of these early synthesized granules stops increasing at 19 DAA but the volume continues to increase [5]. On the other hand, the formation of B-starch granules begins about 12–14 DAA and continues to enlarge until 21 DAA [13]. A-granules contain 30–36% amylose while B-granules contain 24–27% amylase [14, 15]. Also, different size starch granules have different physical, chemical and functional properties [16, 17]. These differences result in the two starch granule types being used differently in industrial food and non-food applications. For instance, starch has several roles in the bread-making process, and starch granule size affects a range of properties [18, 19]. Using 98 hard red winter wheat cultivars and 99 hard red spring wheat cultivars as materials, Park et al. [8] reported that, parameters of B-granules showed many significant correlations with wheat and flour properties.

Furthermore, a wheat panicle is composed of a number of spikelets. Grain weight within a wheat spike is unevenly distributed depending upon the spatial position of kernel [20, 21]. Based on their flowering date and locations within a spike, the spikelets can be classified as superior or inferior [22, 23]. Superior grains usually exhibit a faster rate of increase in dry weight than inferior grains in wheat [24]. Low grain weight and slow grain filling of inferior grains have been attributed to a limitation in carbohydrate supply [25]. There are many explanations to the poor filling of inferior spikelets, including carbon limitation [26, 27, 28], sink capacity limitation [29], unbalance in hormonal levels [30, 31], low activities and/or gene expression of enzymes involved in sucrose-to-starch conversion [32, 33, 34], and assimilate transportation impediment [35, 36]. Recent studies have shown that low physiological activities if sink (grain) at the initial grain filling and low conversion efficiency from sucrose to starch during the active grain filling period contribute to the poor filling of inferior spikelets [37]. However, knowledge about how starch content and granule size distribution in different spikelets position is limited.
In this investigation, starch extracted from two winter wheat cultivars in different spikelets position was studied in order to realize the starch content and granule size distribution in different spikelets position of wheat, and the relationship between them. This information should contribute to the understanding of the mechanism for regulating the spatial pattern of individual grain growth within a wheat spike.

Materials and Methods

1.1. Experimental design

The field experiments were carried out in two growing seasons from 10th October 2010 to June 2011 and from 10th October 2011 to June 2012 at Tai’an Experimental Station of Shandong Agricultural University, Tai’an, China (36°09’ N, 117°09’ E). Two winter wheat (*Triticum aestivum* L.) cultivars currently used in local wheat production, Jimai20 and Shannong1391, were chosen in this study. The soil was a sandy loam and maize (*Zea mays* L.) was the previous crop. The organic matter concentration was 13.5 g kg⁻¹, the total nitrogen was 0.89 g kg⁻¹, and the available nitrogen, phosphorus and potassium concentrations in the soil were 77.8, 26.6 and 75.2 mg kg⁻¹ in the 0–20 cm soil layer, respectively. In addition, basal fertilizer was applied at the rate of 120 kg N ha⁻¹, 175 kg P₂O₅ ha⁻¹, 150 kg K₂O ha⁻¹ before planting. Another 120 kg N ha⁻¹ was top-dressed at the jointing stage (GS31) [38]. The experiments were arranged in a randomized complete design with four replications for each cultivar. The plot size was 3 m × 3 m with 10 rows (0.25 m between rows) with the seeds density of 180 plants m⁻², and the yield was harvested on 16th June 2011 and 14th June 2012. Pests, diseases, and weeds were controlled by appropriate chemical applications during crop cycle. Other cultural practices followed were according to the precise high-yielding cultivation system of Yu [39]. The mean temperatures and sunshine durations from anthesis to maturity in the two growing seasons were shown in Fig. 1.

1.2. Sampling

At anthesis, 100 spikes in each regimen flowering on the same date of the double central rows were labeled with red thread in order to maintain uniformity. At maturity, wheat kernels were harvested for starch granules size analysis. The spike was divided into three parts as follow: the basal spikelets (basal the first to fifth spikelets), middle spikelets (from sixth to sixteenth spikelets), the upper spikelets (seventeenth to top spikelets), respectively. Only the first and second grains in each spikelet were used. In addition, the first and second basal grains on each spikelet were detached to assay in this study.
1.3. Starch isolation and purification

Starch was extracted from the wheat cultivars according to the methods of Malouf and Hoseney [40] and Peng et al. [14, 15], with some modifications as follows: wheat kernels (2 g) were covered with 30 ml double distilled water at 4 °C for 24 h. The softened seeds were degermed and ground with a pestle and mortar in double-distilled water until essentially all starch granules had been released. The slurry was filtered through a 74 μm screen and centrifuged at 1700 g for 10 min to sediment the crude starch. The crude starch was purified three times using 5 ml of 2 M NaCl, 2 mg NaOH g⁻¹, 20 mg SDS g⁻¹ and double-distilled water. To remove water, the starch was washed once with acetone, air-dried at room temperature (18 °C) and stored at −20 °C.

Fig. 1. Mean daily temperature and sunshine durations from anthesis to maturity of wheat.

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1.4. Particle size analysis

The particle size characteristics of the starch were determined using a LS13320 laser diffraction particle size analyser (Beckman Coulter, USA). About 50 mg of starch was weighed into 10 ml (Eppendorf) tubes and suspended with 5 ml of double-distilled water. The tubes were vortexed and kept at 4°C for 1 h, during which time the tubes were vortexed every 15 min. Then 1 ml of the starch suspension was transferred into the dispersion tank of a laser diffraction particle size analyser containing double-distilled water for size measurements. The assessments of equivalent volume, equivalent surface area and number proportions of starch granule were made automatically by the laser diffraction particle size analyser and represented the diameter of a sphere with the same volume as the starch grain and the diameter of a circle with the same projected area as the real starch grain. Amylose and amylopectin contents in wheat grain were determined with a coupled spectrophotometer assay. 100 mg of milled grain was stirred with 10 ml of 0.5 M KOH for 15 min at 100°C and then diluted to a volume of 50 ml with distilled water. Of this solution, 2.5 ml were diluted with 30 ml distilled water and adjusted to pH 3.5 with 0.1 mol HCl L⁻¹, and then 0.5 ml of I₂-KI reagent was added to the solution, which was diluted with distilled water to a final volume of 50 ml. After blending for 20 min, the mixture was monitored with a UV-2450 Shimadzu spectrophotometer at 471, 553, 632 and 740.3 nm. Standard amylase and amyllopectin were purified from wheat. The absorption peaks of purified amylase which reacted with the I₂-KI reagent were 632 and 471 nm, whereas those of amyllopectin were 740.3 and 553 nm.

1.5. Data analysis

The statistical model included sources of variation due to 2 yr, Duncan's new multiple range test was employed after a preliminary F-test to assess differences between the treatment means at the 0.05 probability level. All determinations were replicated three times, the experimental and data were performed using SPSS statistical procedures.

Results

2.1. Differences in Experimental Factors

Analysis of variance for the kernel number per spikelet (KN), weight per spikelet (WS), grain weight per spikelet (GW), starch content (ST), amylase content (AM), volume distribution of starch granules <9.9 (V1), volume distribution of starch granules 9.9–22.8 (V2), volume distribution of starch granules 22.8–42.8 (V3), number distribution of starch granules <0.6 (N1), number distribution of starch granules 0.6–2.8 (N2), number distribution of starch granules 2.8–9.9 (N3) made it possible to identify the sources of variation (Table 1). Year (Yr) main effects and C x Yr, P x Yr interaction failed to influence those traits suggesting Year (Yr) was not a significant factor in the total reaction (Table 1). Cultivar (C)
and Position (P) main effects were significantly affected those traits suggesting they were important to determine the quality and yield of grain starch. It also suggested the interaction was a complicated network (Table 1).

### 2.2. Spatial distribution of grain number, spikelet weight and grain weight

The grain number (Fig. 2A, B), spikelet weight (Fig. 2C, D) and grain weight (Fig. 2E, F) with the spikelet positions from the bottom to the top increased first and then decreased, showed parabolic change. And this was according with medial dominant in grain development. The spikelet weight and grain weight in different positions were showed the trend: upper spikelet < basal spikelet < middle spikelet.

### 2.3. Starch content and accumulation

There were significant differences in starch and amylase content in grains differing in spikelet (Fig. 3). Upper spikelets had the highest starch and amylase content followed by basal spikelets, whereas middle spikelets had the lowest. Starch and amylase accumulation showed the reverse form with their contents (Fig. 4).

### 2.4. Starch granule distribution

#### 2.4.1. Volume distribution

The volume distribution of starch granules is given in Table 2. For the two cultivars, upper spikelets and middle spikelets had the highest and the lowest percentage of starch granules 22.8–42.8 μm, respectively. However, for Jimai20, the volume percentage of starch granules of 9.9–22.8 μm showed upper spikelets > middle spikelets > basal spikelets; but there was no significant difference between the three in Shannong1391. For the two cultivars, middle spikelets had

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**Table 1. Mean square significance among treatments and interactions for test factors.**

| variation | KN | WS | GW | ST | AM | V1 | V2 | V3 | N1 | N2 | N3 |
|-----------|----|----|----|----|----|----|----|----|----|----|----|
| Cultivar (C) | ns | ns | ns | * | * | ns | ns | * | * | * | ns |
| Position (P) | ** | ** | ** | ** | ** | ns | ns | * | * | * | ns |
| Year (Yr) | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| C × P | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| C × Yr | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| P × Yr | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

*Significantly different at the 0.05 probability level.
**Significantly different at the 0.01 probability level.

1Parameters were: kernel number per spikelet (KN), weight per spikelet (WS), grain weight per spikelet (GW), starch content (ST), amylase content (AM), volume distribution of starch granules <9.9 (V1), volume distribution of starch granules 9.9–22.8 (V2), volume distribution of starch granules 22.8–42.8 (V3), number distribution of starch granules <0.6 (N1), number distribution of starch granules 0.6–2.8 (N2), number distribution of starch granules 2.8–9.9 (N3).

1Not significant at P=0.05 (ns).

Position–Year interaction (P × Yr), Cultivar–Year interaction (C × Yr), Cultivar-Position interaction (C × P).
the highest the volume percentage of starch granules of $<2.8 \, \mu m$ and $2.8–9.9 \, \mu m$, followed by basal spikelets, whereas upper spikelets had the lowest. The results showed that middle spikelets had the highest percentage of B-type starch granules.

### 2.4.2. Number distribution

Proportions of starch granules $<2.8 \, \mu m$ and $<9.9 \, \mu m$ were in the ranges of 96.4%–96.6% and 99.9% of the total number, respectively (Table 3), which showed that number of granules in wheat grain analyzed was made up of B-type starch granules. These results implied that small starch granules are the main
Fig. 3. Effect of Spikelet position on starch content (A and B) and amylose content (C and D) in wheat grains in winter wheat in growing seasons 2010/2011 (left) and 2011/2012 (right). US, upper spikelets; MS, middle spikelets; BS, basal spikelets.

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Fig. 4. Effect of spikelet position on starch content (A) and amylase (B) accumulation in wheat grain in the growing season 2010/2011. US: Upper spikelets; MS: Middle spikelets; BS: Basal spikelets.

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component although they contribute less to the total volume. Spikelets exerted little effect on the alteration of percentage of starch granules.

### Table 2. Effect of Spikelet position on the proportion by volume distribution of starch granules in wheat grain (d.f. = 17).

| Seasons | Cultivars | Spikelet positions | Particle equivalent diameter of starch granule (μm) |
|---------|-----------|---------------------|-----------------------------------------------------|
|         |           | <2.8                | 2.8–9.9     | <9.9     | 9.9–22.8 | 22.8–42.8 |
| 2010/11 | Jimai20   | US                  | 0.107bc     | 0.308c   | 0.415c   | 0.287b    | 0.308b    |
|         |           | MS                  | 0.128a      | 0.342a   | 0.472a   | 0.289b    | 0.239a    |
|         |           | BS                  | 0.112b      | 0.324b   | 0.436b   | 0.320a    | 0.244c    |
|         | Shannong1391 | US          | 0.093bc     | 0.252c   | 0.345c   | 0.308a    | 0.347a    |
|         |           | MS                  | 0.112a      | 0.280a   | 0.392a   | 0.304a    | 0.304c    |
|         |           | BS                  | 0.101b      | 0.273ab  | 0.374b   | 0.305a    | 0.321b    |
| 2011/12 | Jimai20   | US                  | 0.110b      | 0.308c   | 0.418c   | 0.285c    | 0.297a    |
|         |           | MS                  | 0.124a      | 0.339a   | 0.463a   | 0.306b    | 0.231c    |
|         |           | BS                  | 0.113b      | 0.321b   | 0.434b   | 0.317a    | 0.249b    |
|         | Shannong1391 | US          | 0.108a      | 0.234c   | 0.342c   | 0.307a    | 0.351a    |
|         |           | MS                  | 0.088b      | 0.302a   | 0.390a   | 0.306a    | 0.304c    |
|         |           | BS                  | 0.102ab     | 0.275b   | 0.377b   | 0.308a    | 0.315b    |

Comparison of all grain positions:

|                  | P<0.05 | P<0.01 | P<0.01 | P<0.05 | P<0.05 |
|------------------|--------|--------|--------|--------|--------|
| S.E.D.           | 0.0022 | 0.0024 | 0.0035 | 0.0026 | 0.0024 |

Means within cultivar followed by a different letter are significantly different at P<0.05. US: Upper spikelets; MS: Middle spikelets; BS: Basal spikelets.

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### Table 3. Effect of Spikelet position on the proportion by number distribution of starch granules in wheat grain (d.f. = 17).

| Seasons | Cultivars | Spikelet positions | Particle equivalent diameter of starch granule (μm) |
|---------|-----------|---------------------|-----------------------------------------------------|
|         |           | <0.6                | 0.6–2.8     | <2.8     | 2.8–9.9     |
| 2010/11 | Jimai20   | US                  | 0.416b      | 0.549b   | 0.965a     | 0.034a     | 0.99a     |
|         |           | MS                  | 0.412c      | 0.553a   | 0.965a     | 0.034a     | 0.99a     |
|         | Shannong1391 | BS          | 0.421a      | 0.545c   | 0.966a     | 0.033a     | 0.99a     |
|         |           | US                  | 0.405b      | 0.560ab  | 0.965a     | 0.034a     | 0.99a     |
|         |           | MS                  | 0.401c      | 0.563a   | 0.964a     | 0.035a     | 0.99a     |
|         |           | BS                  | 0.415a      | 0.550b   | 0.965a     | 0.034a     | 0.99a     |
| 2011/12 | Jimai20   | US                  | 0.418b      | 0.547b   | 0.965a     | 0.034a     | 0.99a     |
|         |           | MS                  | 0.411c      | 0.554a   | 0.965a     | 0.034a     | 0.99a     |
|         |           | BS                  | 0.425a      | 0.541c   | 0.966a     | 0.033a     | 0.99a     |
|         | Shannong1391 | US          | 0.407b      | 0.558b   | 0.965a     | 0.034a     | 0.99a     |
|         |           | MS                  | 0.401c      | 0.563a   | 0.964a     | 0.035a     | 0.99a     |
|         |           | BS                  | 0.419a      | 0.546c   | 0.965a     | 0.034a     | 0.99a     |

Comparison of all grain positions:

|                  | P<0.01 | P<0.01 | NS     | NS     | NS     |
|------------------|--------|--------|--------|--------|--------|
| S.E.D.           | 0.018  | 0.016  | 0.002  | 0.01   | 0.001  |

Means within cultivar followed by different letter are significantly different at P<0.05. US: Upper spikelets; MS: Middle spikelets; BS: Basal spikelets.

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starch granules 0.6–2.8 μm, middle spikelets were the highest, followed by upper spikelets, and the basal spikelets were lowest, which showed that it is more conducive to the development of small starch granule in the middle spikelets.

2.5. Starch content

Starch content and starch component content at maturity were significant different among the grains in different spikelet positions (Table 4). For both cultivars, the 3 parameters as starch, amylase and amylopectin in the different grains had a trend of upper spikelet > basal spikelet > middle spikelet. However, amyllose/amylopectin (AM/AP) in grains had different trends between the two cultivars. In Jimai20, AM/AP in upper spikelet was the highest, followed by that in basal spikelet, and the middle spikelet was the lowest. In addition, for Shannong1391, the middle spikelet was the lowest, but there were no significant differences between upper spikelet and basal spikelet.

The proportion of granules by volume (volume proportion) of starch granules <2.8 μm and 2.8–9.9 μm were negatively correlated with the ratio of amyllose to amylopectin (r = −0.67, P<0.05; r = −0.89, P<0.01; respectively; Fig. 5A and B). A significantly positive correlation (r=0.92, P<0.01; Fig. 5D) was found between volume proportions of starch granules 22.8–2.8 μm and the ratio of amyllose to amylopectin. Volume proportions of starch granules 9.9–22.8 μm were not significantly correlated with the ratio of amyllose to amylopectin (r=−0.15; P>0.05; Fig. 5C).

Grain weight at different spikelet position was significantly different (Table 1). And, a positively correlated was founded with Grain weight at different spikelet position and the volume proportion of starch granules <9.9 μm. A negatively correlation was found between grain weight and the volume proportion of starch granules 22.8–42.8 μm, while no significant relationships were observed with the volume proportion of starch granules 9.9–22.8 μm. However, there was no significant relationship between grain number and the number proportion of starch granules (Table 5).

Discussion

It has been well reported in the literature that the degree and rate of grain filling in wheat spikelets differ largely with their positions on a panicle [30]. Markedly differences were also reported in the floret development, carbohydrate supply and endogenous hormones and starch synthesis among grains within a rice and wheat spike [16, 41]. Nevertheless, results of previous studies mainly took the sucrose content as a limiting factor for starch synthesis in inferior grains, further reason for this observation was unclear and needed to be investigated [42, 43]. For instance, Jiang et al. [25] suggested that superior grains had higher sucrose contents (substrate of starch synthesis) at early and middle grain filling compared to inferior ones. However, Zhang et al. [44] found that the source supply was
abundant during grain filling stage, while the sink size and ability of assimilation conversion were the main limiting factors for starch accumulation. Based on the present study and previous work, we proposed that the starch accumulation was

### Table 4. Effect of Spikelet position on the proportion of starch and its components in wheat grain.

| Seasons | Cultivars | Spikelet positions | ST (/100) | AM (/100) | AP (/100) | AM/AP |
|---------|-----------|--------------------|----------|-----------|----------|-------|
| 2010/11 | Jimai20   | US                 | 62.41a   | 16.42a    | 45.99a   | 0.357a |
|         |           | MS                 | 59.01c   | 14.71c    | 44.30b   | 0.332c |
|         |           | BS                 | 61.34b   | 15.82b    | 45.52a   | 0.347b |
|         | Shannong1391 | US             | 64.55a   | 17.84a    | 46.71a   | 0.382a |
|         |           | MS                 | 62.39c   | 16.91c    | 45.48b   | 0.372b |
|         |           | BS                 | 63.18b   | 17.45ab   | 45.73b   | 0.381a |
| 2011/12 | Jimai20   | US                 | 62.57a   | 16.55a    | 46.02a   | 0.359a |
|         |           | MS                 | 59.11c   | 14.71c    | 44.40b   | 0.331c |
|         |           | BS                 | 61.41b   | 15.91b    | 45.50a   | 0.349b |
|         | Shannong1391 | US             | 65.15a   | 17.95a    | 47.71a   | 0.380a |
|         |           | MS                 | 62.15c   | 16.61c    | 45.54b   | 0.364b |
|         |           | BS                 | 63.12b   | 17.54ab   | 45.58b   | 0.383a |

Comparison of all grain positions

| Means within cultivar followed by different letter are significantly different at *P*<0.05. US: Upper spikelets; MS: Middle spikelets; BS: Basal spikelets; ST, starch; AM, amylase; AP, amylopectin; AM/AP, amylose/amylopectin.

Means within cultivar followed by different letter are significantly different at *P*<0.05. US: Upper spikelets; MS: Middle spikelets; BS: Basal spikelets; ST, starch; AM, amylase; AP, amylopectin; AM/AP, amylose/amylopectin.

**Fig. 5. The relationship between distribution by volume of starch and the ratio of amylose to amylopectin granules in grain.**

[Graphs showing the relationship between starch granule distribution and Amylose/Amylopectin ratio]
much higher in middle spikelets (the 6th to 16th spikelet) which behaved preferential growth and stronger positions compared with basal and upper spikelets. It was also confirmed the results of Tan et al. [45].

Environment conditions affected starch granules distribution obviously. The number of B-type starch granules decreased when exposed to higher temperatures, correspondingly, that of A-type starch granules increased [46, 47]. Similar observation had been reported in heat stress and shade studies [48]. In a comparison of irrigated conditions, rain-fed conditions resulted in the increase of the volume and surface area percents of B-type starch granules, correspondingly, those of A-type starch granules were reduced. Li et al. [49] reported that shading led to a significant reduction in the proportions of B-type starch granules volume distribution, while increased in those of A-type starch granules. It behaved the opposite of P fertilization [50]. We now proposed that the middle spikelets had significant higher percentage of B-type starch granules compared with basal and upper spikelets. Firstly, the B-type granules formed within protrusions from A-type amyloplasts [51]. Secondly, sufficient substrate was propitious to small starch granules polarization and development [52]. Finally, the limited substrate for starch accumulation was mainly partitioned for growing starch granules, not for producing more starch granule numbers in grain of basal and upper spikelets.

Wheat A- and B-type starch granules are reported to have significantly different chemical compositions such as amylose, amylopectin, lipid, and protein concentrations [7, 12, 53]. Thus, starch granule size distribution had a close association with its component content. Various studies reported amylose content was higher in the large starch granules than the small granules. It was suggested that the A-type granules have a 4–10% higher amylose content than the B-type granules [15, 54], while Ames et al. [55] found only minor differences (2–3%) in amylose content. Raeker et al. [56] reported that the starch content was negatively correlated with volume percentage of granule <5 and 9.9 μm. Upper spikelets had the highest amylose content followed by basal spikelets, whereas middle spikelets had the lowest. Integrating with starch granule size distribution, we suggested that amylose content in grain was positively correlated to volume percentage of A-type granule (R=0.96, P<0.01).

This study also showed that number percentage of starch granules 2.8–9.9 μm (midsize) and >9.9 μm (large) in grains had no significant difference among upper, middle and basal spikelets (Table 1). However, the variability in number distribution of the small starch granules (<2.8 μm) among different spikelet position was highly significant. Number percentage of granules 0.6–2.8 μm in

| Cultivars     | Volume diameter of starch granule (μm) | Number diameter of starch granule (μm) |
|---------------|----------------------------------------|----------------------------------------|
|               | <9.9 | 9.9–22.8 | 22.8–42.8 | <0.6 | 0.6–2.8 | <2.8 |
| Jimai20       | 0.90** | 0.56 | −0.98** | −0.20 | 0.25 | 0.14 |
| Shannong1391  | 0.90** | 0.64 | −0.98** | −0.12 | 0.22 | 0.19 |
grains of middle spikelets was remarkably higher than that of basal and upper spikelets, indicating that it was more conducive to the development of small starch granules in middle spikelets. It was resulted in high substrate supply and degradation capacity and high activities of enzymes involving in starch synthesis, especially the development of B-type granules in middle spikelets.

**Conclusion**

In this study, the development of starch granules in grain with spikelet position was investigated. Grain weight was positively correlated with the volume proportion of $<9.9 \text{ }\mu\text{m}$ ($R=0.90^{**}$), but remarkable negative correlations were found between grain weight and the volume proportion of $22.8–42.8 \text{ }\mu\text{m}$ ($R=−0.98^{**}$). There were significantly positive relationship between AM/AP and the volume proportion of $22.8–42.8 \text{ }\mu\text{m}$ and negative correlation between AM/AP and the volume proportion of $<9.9 \text{ }\mu\text{m}$. Thus, the middle spikelets had significant higher percentage of B-type starch granules, yet, the limited substrate for starch accumulation was mainly partitioned for growing starch granules of basal and upper spikelets.

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**Author Contributions**

Conceived and designed the experiments: AY YL YN. Performed the experiments: YL YN. Analyzed the data: AY YN. Contributed reagents/materials/analysis tools: WY DY. Contributed to the writing of the manuscript: AY YL YN. Designed the software used in particles analysis: ZC. Guided the experiments: ZW YY.

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