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Starch granule size distribution and morphogenesis in maize (*Zea mays L.*) grains with different endosperm types

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Abstract

In this study we investigated starch granule size distribution and morphogenesis in the grains of maize with different endosperm types. Four maize varieties including super-sweet (Huawei NO. 6), pop (Tebao NO. 2), waxy (Xixinghuangnuo NO. 6), and dent corns (Zhengdan 958) were examined for starch granule size distribution and arrangement within the grains using a laser diffraction grain size analyzer, transmission electron, and scanning electron microscopies. The distribution of starch granule volume formed a triple humped curve in which granules with a diameter of less than 2 μm were abundant, while greater than 15 μm were most prevalent. Super-sweet corn bucked the trend slightly, with granules from 2-15 μm predominating. Average diameter showed the following order: waxy>pop>dent>super-sweet. Correlation analysis indicated that grain weight and starch content were significantly correlated (0.649*, 0.764*) with volume percentage in granules from 2-15 μm predominating, whereas other grain quality were not correlated with granule volume for size ranges. Transmission electron microscopy revealed a marked difference in the development of starch granules, protein and lipid bodies. In first layer endosperm cells, there were many protein and lipid bodies in waxy, super-sweet and dent corns, but none in the equivalent region of popcorn, which appeared to have a higher number of plastid starch granules. Vacuoles and most protein bodies were large in super-sweet corn. In second layer endosperm cells, pop, waxy, and dent corns contained many starch granules. In third layer endosperm cells, almost all starch granules were patterned in popcorn. In deep layer endosperm cells, patterned starch granules were plentiful in dent and super-sweet corn, but scarce in waxy and pop varieties. Lipid bodies and cell inclusions were more abundant in pop, dent, and waxy types than in super-sweet corn.

Keywords: Maize (*Zea mays* L); Starch granule; Cell size; Morphogenesis; Endosperm.

Abbreviations: Dent_dent corn；Pop_popcorn；S-S_super-sweet corn; Waxy_waxy corn; First: first layer (outermost) endosperm cells; Second_ second layer endosperm cells；Third_ third layer endosperm cells；Deep_deep layer endosperm cells; DPA_days post-anthesis; S_ starch granule; PB_ protein body; SDS_ Sodium dodecyl sulfate.

Introduction

Maize (*Zea mays* L.) kernel dry weight contains about 70% starch. Many studies have focused on the effects of sowing date, variety and nitrogen top-dressing on starch granule distribution in single or double varieties (Hunter et al., 2002; Zhang et al., 2011; Sheng et al., 2004). However, starch granule size distribution in the grains of different maize endosperm types has not been extensively investigated to date. Understanding the anatomical and physiological factors affecting grain texture is important for developing corn germplasm with high grain quality. The size of the starch granules is one of the most important qualities of corn starch. The structure and distribution of starch grains varies in different corn varieties (Ji et al., 2003a; Ji et al., 2003b; Hou et al., 2009). In general, starch grain diameter varies from 7–25 μm, with an average diameter of 10 μm (Ji et al., 2003b; Paterson et al., 2001; Li et al., 1999), and grain shape is also variable (Wang et al., 1993). Starch granule volume in maize grains is typically 14.5–18.2 μm3 (Brown et al., 1971; Wilson et al., 2000). The size of starch granules is significantly correlated with the initial, peak, and ultimate gelatinization temperatures of corn starch (Knutson et al., 1982). Larger granules contain more amylase (Ji et al., 2003b), and granule size distribution influences the physical and chemical properties of starch (Wu et al., 2006). The objectives of the current study were to evaluate the starch granule content, size distribution, and morphogenesis in maize, and identify differences in starch development between four different endosperm types.

Results

Starch granule volume distribution

The distribution of starch granule volume in the grains of the four maize varieties formed a triple humped curve (Fig. 1). The peak values of each hump were 2.107 μm, 5.355 μm, and 18.000 μm (pop), 16.40 (18.00) μm, 45.76 μm, and 96.49 μm (waxy), 1.919 μm, 5.355 μm, and 14.940 μm (super-sweet), and 1.919 μm, 5.355 μm, and 18.000 μm (dent). The proportion of pop, waxy, super-sweet and dent corn starch granules with a diameter below 2 μm was only 5.0%, 5.5%, 7.6% and 6.2%, respectively, while the proportion of granules with a diameter greater than 15 μm were 68.9%, 61.3% and 60.3% for pop, waxy, and dent corns, respectively. Super-sweet corn had the highest proportion of granules with a diameter between 2–15 μm (63.9%).

Table 1. Starch granule volume distribution in different maize endosperm types (%).

Variety	<2 μm	2–15 μm	>15 μm	Mean
Pop	4.95 ± 0.203 d	26.13 ± 0.697 c	68.9 ± 2.036 a	17.18 ± 0.263 b
Waxy	5.55 ± 0.167 c	31.48 ± 1.236 b	63.06 ± 2.117 ab	33.86 ± 0.524 a
Super-sweet	7.65 ± 0.315 a	63.78 ± 1.679 a	28.49 ± 0.379 c	11.90 ± 0.236 c
Dent	6.22 ± 0.219 b	33.49 ± 0.964 b	60.3 ± 2.069 b	16.02 ± 0.224 b

Data in the table are means of nine replications. Values followed by different letters are significantly ($P \leq 0.05$) different among different varieties.

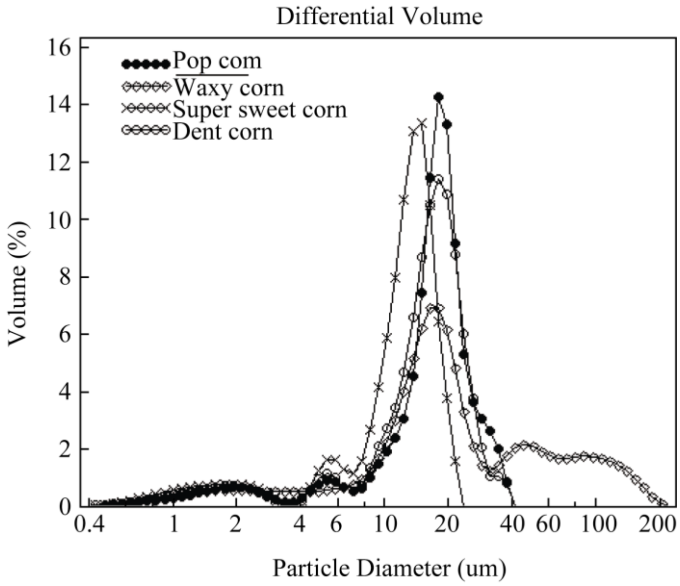


Fig 1. Distribution of starch granule volume in the endosperm of grains from different maize types.

The overall average diameter showed the following trend: waxy>pop>dent>super-sweet (Table 1). Grain weight and protein content were ordered dent>waxy>super-sweet>pop (Table 2), while starch content and test weight were ordered >pop>dent>waxy>super-sweet, and amino acid content was ordered waxy>super-sweet>dent>pop. Finally, crude fat content showed following order: dent>pop>waxy>super-sweet.

Correlation of starch granule volume and grain quality

Both grain weight and starch content were significantly correlated with the volume percentage of starch granules from 2-15 μm predominating. In contrast, protein, amino acids, and crude fat content, were not correlated with granule volume, and neither was test weight. This was the case across all size ranges.

Starch granule morphogenesis

Transmission electron microscopy revealed a marked difference in the development of starch granules, protein bodies and lipid bodies (Fig. 2). In the first (outermost) layer of endosperm cells, development was most advanced with dent corn, and ordered dent>waxy>super-sweet>pop, while the number of protein bodies was highest in waxy corn, and ordered waxy>super-sweet>dent>pop. Protein bodies were largest in super-sweet corn, and ordered super-sweet>dent>waxy>pop, while starch granules were most abundant in popcorn and ordered pop>dent>waxy>super-sweet. Lipid bodies and cell inclusions were both most numerous in popcorn and ordered pop>waxy>dent>super-sweet. Both protein and lipid bodies were abundant in the first layer endosperm cells of waxy, super-sweet, and dent corn, but completely absent in this region of popcorn, which instead contained many starch granules, and vacuoles and most of protein bodies were was large as those in super-sweet corn. The second layer endosperm cells were largest in waxy corn, and ordered waxy>super-sweet>dent>pop, while the number of starch granules was highest in popcorn and ordered pop>waxy>dent>super-sweet. Starch granules were largest in waxy corn and ordered waxy>pop>dent>super-sweet. Lipid bodies and cell inclusions were most abundant in dent corn and ordered dent>pop>super-sweet>waxy. Unlike the other corns, starch granules were absent in super-sweet corn. Lipid bodies and cell inclusions were plentiful in pop and dent corns, but much scarcer in waxy and super-sweet corns, which contained many areas in which these features were completely absent.

The third layer endosperm cells were largest in popcorn and ordered pop>waxy>super-sweet>dent. Starch granules were most abundant in popcorn and ordered pop>waxy>dent> super-sweet, and the size of starch granules also followed this trend. Lipid bodies and cell inclusions were most abundant in popcorn and ordered pop>super-sweet>dent>waxy. Popcorn had the highest percentage of patterned starch granules, followed by dent, super-sweet, and waxy, in that order. There were almost no unpatterned starch granules in popcorn, while dent and super-sweet corns showed a mixture of patterned and unpatterned, and all were unpatterned in waxy corn. In deep layer endosperm cells, the highest number of starch granules were observed in super-sweet corn, followed by dent, waxy, and pop, in that order. Starch granule size was ordered pop>waxy>dent>super-sweet. Patterned starch granules were most prevalent in dent corn, followed by super-sweet, pop, and waxy. Lipid bodies and cell inclusions were most abundant in popcorn, followed by dent, waxy, and super-sweet. Although plentiful in dent and super-sweet corns, patterned starch granules were much scarcer in waxy and pop varieties. Super-sweet corn was particularly lacking in lipid bodies and cell inclusions compared to the other three corns. Scanning electron microscopy showed that the starch granule arrangement was dense in popcorn, with little empty space

Table 2. Grain quality traits of the tested maize cultivars.

Cultivar	Grain weight (mg)	Starch content (%)	Test weight (g L^{-1})	Protein content (%)	Amino acid content (g kg^{-1})	Crude fat content (%)
Pop	109.3 ± 3.22d	71.37 ± 2.69a	0.722 ± 0.013a	2.371 ± 0.132d	5.680 ± 0.136c	5.882 ± 0.346b
Waxy	249.4 ± 13.64b	57.63 ± 1.49b	0.683 ± 0.009b	4.292 ± 0.162b	9.192 ± 0.267a	5.193 ± 0.267c
Super-sweet	134.8 ± 14.22c	49.90 ± 1.57c	0.647 ± 0.008c	3.673 ± 0.129c	6.629 ± 0.155b	5.074 ± 0.436c
Dent	348.9 ± 21.37a	67.60 ± 3.11a	0.712 ± 0.011a	5.224 ± 0.137a	5.817 ± 0.209bc	6.290 ± 0.419a

Data in the table are means of nine replications. Values followed by different letters are significantly ($P \leq 0.05$) different among different varieties.

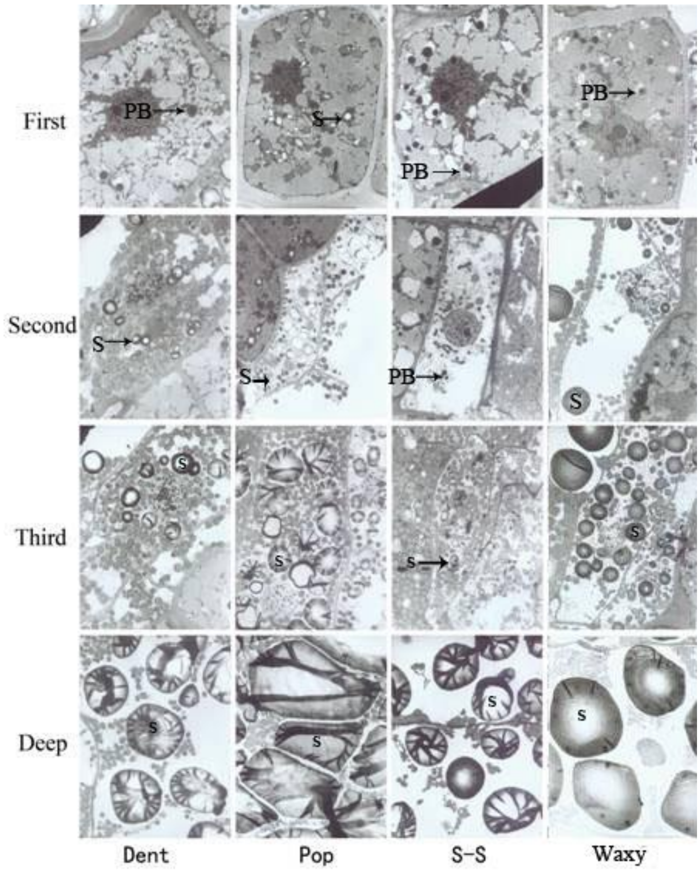


Fig. 2. Transmission electron microscopy of starch granule arrangement in the endosperm of grains from different maize types (×2,500). Dent, Pop, S-S (super-sweet) and Waxy refer to maize type. First, Second, Third, and Deep refer to endosperm cell layer. S, starch granule. PB, protein body.

(Fig. 3). In contrast, starch granules were much more diffuse in super-sweet corn, with many empty spaces between granules. Intermediate density was shown by dent and waxy varieties.

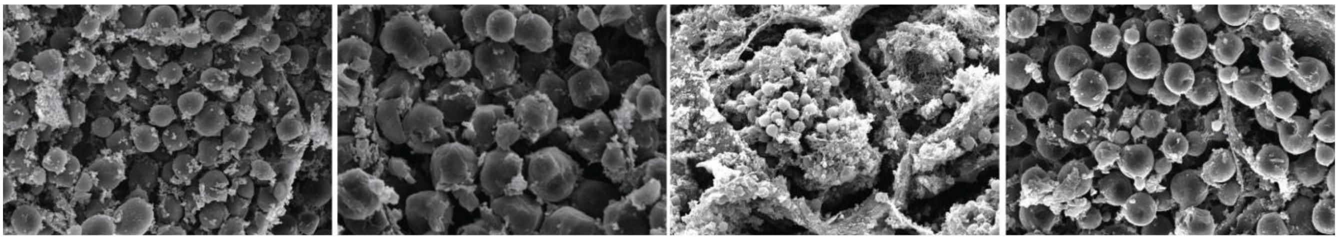
Discussion

Maize (*Zea mays* L.) kernel endosperm dry weight contains about 70% starch. Many factors can affect the grain physical structure, including the physical arrangement of the cellular components, thickness of the cell wall, size of the cells in the endosperm storage parenchyma, thickness of the proteinaceous matrix in contact with starch granules, and the strength of adhesion between the proteinaceous matrix and starch granules (Simmonds et al., 1973; Abdelrahman and Hoseney, 1984). The variety, cultivation practices, and the environment may all affect the size and distribution of the starch granules (Lu et al., 2011; Kaur et al., 2007). In wheat, starch granules have been divided into A type (>16 μm), B type (5–16 μm) and C type (<5 μm) (Bechtel et al., 1990; Bechtel et al., 1993; Wilson et al., 2006). In corn, these have been divided into small (<2 μm), medium (2-15 μm) and large (>15 μm) (Zhang et al., 2011). The distribution of starch granules in maize grains fell into three discrete size ranges, as shown by the triple humped curve (Fig. 1). Starch granules with a diameter below 2 μm were the least abundant, while those with a diameter greater than 15 μm were the most prevalent. Super-sweet corn was the exception, in which granules from 2-15 μm predominated. The average diameter showed following order: waxy>pop>dent> super-sweet. The variation in the range of diameters of starch grains in a single variety has previously been shown to be less than 5% (Sandhu et al., 2004). Starch granules of corn contain amylase and amylopectin, but little protein or lipids (Ellis et al., 1998). Granule proteins are important for determining the distribution of endosperm compounds (Glenn et al., 1991). In a previous study, popcorn endosperm was almost vitreous in texture (Li, 1999) and starch granules were arranged densely. This may be one of the reasons that the expansion coefficient of popcorn was high. Super-sweet corn contained a large amount of soluble sugar (Liu et al., 2005), as the name would suggest, and starch granules were much less densely packed, which gives rise to the low starch content of this variety. The starch content of dent and waxy corns was intermediate between that of pop and super-sweet varieties, and the starch granule arrangement was highly similar, although granule shape differed

Table 3. Correlation coefficients of starch granule volume and grain quality traits.

Quality index	Starch granule diameter			Average diameter (μm)
	<2 μm	2–15 μm	>15 μm	
Grain weight (mg)	0.362	0.649*	–0.672*	0.467
Starch content (%)	0.444	0.764*	0.569*	0.794*
Test weight (g L^{-1})	0.202	0. 526	0.336	0.511
Protein content (%)	0.310	0.084	0.091	0.261
Amino acid content (%)	0.094	0.049	0.010	0.123
Crude fat content (%)	0.198	0.558	0.082	0.517

Data in the table are means of nine replications. Values followed by different letters are significantly (P<0.05) different among different varieties. *, ** represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.



Dent corn Pop corn Super sweet corn Waxy corn

Fig 3. Scanning electron microscopy of starch granule arrangement in the endosperm of grains from different maize types (×1,500).

significantly. The second layer endosperm cells contained starch granules, suggesting that cells in this layer developed earlier than those of the first endosperm layer. Drape starch granules were present in third layer endosperm cells, suggesting an even earlier developmental process. These granules were also detected in deep layer endosperm cells of corns grown under different conditions, suggesting these cells were the earliest to develop. Starch granules therefore accumulated from the inside to the outside of the maize endosperm. This is consistent with the reports of Wang et al. (2004), and around 20 DAP is the most appropriate for manipulating starch content.

Materials and Methods

Materials and experimental design

Field experiments were conducted from 2009 to 2011 at the Corn Research Center of Shandong Agricultural University, China (35°50´N, 117°30´E; 100 m above sea level). Laboratory tests were carried out at the State Key Laboratory of Crop Biology, Shandong Agricultural University, China.

Plant materials

Four maize varieties, Huawei NO. 6 (super-sweet corn), Tebao NO. 2 (popcorn), Xixinghuangnuo NO. 6 (waxy corn) and Zhengdan 958 (dent corn), which were used as initial materials, are widely cultivated as the major varieties in the local area. Huawei NO. 6, Tebao NO. 2, Xixinghuangnuo NO. 6 and Zhengdan 958 ears were harvested 79, 95, 99 and 96 days after sowing, respectively.

Sampling

The soil in the planting field was brown, had a pH of 6.89, and contained 1.36 mg kg^{-1} organic matter, 90.75 mg kg^{-1} alkaline nitrogen, 0.07% total nitrogen, 74.56 mg kg^{-1} available phosphorus, 81.64 mg kg^{-1} rapid-efficient potash, and 117.41 mg kg^{-1} total potash. Maize varieties were planted on 13 June at a density of 67,500 plants ha^{-1}. The plots consisted of five rows situated 0.60 m apart. Before sowing, 375 kg ha^{-1} of complete fertilizer (N, P, K = 15%, 16%, 17%) was applied to the soil. About 45 days after sowing, the soil was ground-dressed with urea nitrogen (375 kg ha^{-1}). At 20 days post-anthesis (DPA), middle kernels were removed to investigate starch granule morphogenesis by transmission electron microscopy. Starch, protein, amino acid, and crude fat content were recorded, as well as the starch granule size distribution. Kernels were similarly characterized after harvesting, and the grain weight and test weight were also determined. Fresh middle kernels were cut into small pieces that included the starchy endosperm layer, fixed by immersion in 2.5% glutaraldehyde in 0.1 M Na_3PO_4 buffer (pH 7.2 at 0–4ºC for 48 h), and processed for light and transmission electron microscopies. At maturity, kernels were boiled at 105°C for 30 min, dried at 85°C to a constant weight, ground, and their grain weight, test weight, and starch granule size distribution were determined, before passing through a 100-mesh sieve to assess grain quality by determining their starch, protein, amino acid, and crude fat content.

Measurements

Starch granule morphogenesis

Starch granule morphogenesis was investigated according to the method of Haiyan Zhang et al. (2011). For transmission electron microscopy, samples were fixed in ethanol, washed in 0.1 M Na3PO4 buffer (pH 7.2), and post fixed in 2% osmium tetroxide in 0.1M Na3PO4 buffer (pH 7.2) at room temperature for 2 h. Samples were dehydrated and cleared in propylene oxide for 10 min, and embedded in spurr epon-812. Semi-thin sections (1 μm) were prepared using an HM360 slice cutter (ZEISS, Germany) and stained with Schiff's reagent and 1% toluidine blue-O. Ultra-thin sections (500 Å) were prepared with a Nova-V ultramicrotome (LKB, Sweden) and stained with uranyl acetate for 30 min, washed with double distilled water, counter-stained with lead citrate, and rinsed. Development of starch granules and protein bodies were examined using a JEM-1200 EX transmission electron microscope (JEOL, Japan).

Determination of starch, protein, amino acid and crude fat content

Starch, protein, amino acid content and crude fat content were determined using semi-micro Kjeldahl methods (Zhang et al., 2011).

Starch granule size distribution

Starch granules were extracted according to the method of Peng (1999) and Malouf (1992). Ten middle kernels were soaked in deionized water and ground into a homogenate. This was repeated three times, and the homogenate was centrifuged at 3,300 ×*g* for 10 min. The supernatant was added to 25 ml deionized water, mixed, and centrifuged again. These steps were repeated three times each day for four days. The resulting solid was washed with 2% SDS and 0.2% NaOH as described above for seven days, then with deionized water for a further seven days, then once with acetone, air-dried in a fume cupboard to a constant weight, and stored at -20°C. The starch granule size distribution was determined using a LS 13320 laser diffraction particle size analyzer (Beckman Coulter, USA)

Conclusion

In this study four maize varieties were examined for starch granule size distribution and arrangement within the grains using a laser diffraction grain size analyzer, transmission electron, and scanning electron microscopies. The distribution of starch granule volume formed a triple humped curve in which granules with a diameter of less than 2 μm were abundant, while greater than 15 μm were most prevalent. Starch granule arrangement was different in four maize varieties. Starch granule development is from the inside out. Grain weight and starch content were significantly correlated (0.649^{*}, 0.764^{*}) with volume percentage in granules from 2-15 μm predominating.We could infer grain weight and starch content of maize through the arrangement and size of starch granules. After pollination 15-20d is a critical period of corn starch granule formation, we should strengthen the management of fertilizer and water to get high yield.

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