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- Short communication

CHANGES IN INTERNAL STRUCTURE AND NUTRITIONAL QUALITY WITH DIFFERENT TYPES OF MAIZE KERNELS DURING GRAIN FILLING PERIOD

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Abstract

To understand the changes in grain internal structure and nutrients during maize filling, Jingkenuo 2000 (waxy corn), Chaosweet 2000 (sweet corn), and Zhengdan 958 (common corn) were used as test materials. This study observed the internal structure of the corn grain and measured the change in nutritional quality during the filling period. Throughout the determination stage, the starch granules did not fill the whole endosperm cell in Chaosweet 2000, and the cell wall was clearly visible. By contrast, the starch granules filled the whole endosperm cell in Jingkenuo 2000 and Zhengdan 958. The protein content in Chaosweet 2000 was significantly (P<0.05) higher than those in Jingkenuo 2000 and Zhengdan 958. With the extension of post-pollination time, the content of soluble sugar decreased significantly (P<0.05) in Chaosweet 2000, Jingkenuo 2000, and Zhengdan 958.

Corn is the second most produced crop in China (Shi *et al*. 2009, Bai *et al*. 2023, Shen *et al*. 2023). In recent years, with the improvement of people's living standards and the change in dietary consumption structure, sweet and waxy corn have increasingly been favored by consumers (Li *et al*. 2022). Nutritional quality is an important index to evaluate fresh corn. However, the evaluation of the nutritional quality of fresh corn is mainly based on the appearance for visual inspection and taste (Sun *et al.* 2019), and there are few studies on the internal microstructure. Therefore, exploring the relationship between grain nutrient change and internal ultrastructure is beneficial to understanding grain morphogenesis and nutrient accumulation in fresh corn. Lu *et al*. (2011) analyzed the effects of starch morphology and structure on grain hardness in waxy maize. Xia *et al*. (2018) studied the changes in starch granule distribution and gelatinization characteristics in the endosperm of common maize under different potassium fertilizer levels. Hu *et al*. (2021) and Qu *et al*. (2021) observed the morphology and distribution characteristics of starch grain with different types of corn. Zhong *et al*. (2021) studied the distribution characteristic, particle size, and physicochemical characteristics of starch granules in corn grains. Few studies have involved the changes in matrix proteins in the ultrastructure, except starch grains, and the relationship between the changes in ultrastructure and the accumulation of main nutritional quality in corn grains (Lu *et al*. 2012, Li *et al*. 2021). Corn grain morphogenesis and quality formation occur in the filling period, which is also a period exhibiting intense changes in the internal ultrastructure of grains (Beckles and Thitisaksakul, 2014; Yang *et al*. 2014).

Considerable research has been conducted on the grain filling characteristics and nutrient accumulation in a single type of maize (Xu *et al*. 1991; Liu. 2007; Zhang *et al*. 2014), but there are few studies on different types of maize (Wu 2008, Liu *et al*. 2023). In this study, three types of corn, namely, Jingkenuo 2000 (waxy corn), Chaosweet 2000 (sweet corn), and Zhengdan 958 (common corn) were selected. Jingkenuo 2000, Chaosweet 2000 and Zhengdan 958 are the

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largest waxy maize variety, sweet corn variety and common corn variety in China. The changes in the internal ultrastructure and accumulation process of main nutrients in different types of corn during the filling stage were investigated, which can provide a certain basis for grain quality improvement in diverse types of corn.

Jingkenuo 2000 (waxy corn), Chaosweet 2000 (sweet corn), and Zhengdan 958 (common corn) were selected as test materials. Jingkenuo 2000 was bred by the Maize Research Center of Beijing Academy of Agriculture and Forestry Sciences, which was approved by the state in 2006. This variety has excellent quality and high yield. Chaosweet 2000 was bred by Wanquan Huasui Special Corn Seed Company, which was approved by Hebei Province in 2002. This variety has good commoditization, pleasant flavor, and high sweetness and crispness. Zhengdan 958 was bred by the Henan Academy of Agricultural Sciences, which was approved by the state in 2000. This variety has high and stable yield and high resistance. Zhengdan 958 was designated as a key promotion variety by the Ministry of Agriculture and Rural Affairs, PRC. Jingkenuo 2000 and Chaosweet 2000 were provided by Heyuan Seed Industry Co., Ltd., and Zhengdan 958 was provided by Henan Qiule Seed Industry Co., Ltd.

The experimental materials were planted in the Northwest A&F University farm (Latitude: 34.2636, Longitude:108.0634, Altitude: 400 m, Yangling, Shannxi, China), which was sown on May 15, 2022. The planting density was 52500 plants/hm^2. Pure nitrogen (225 kg/hm^2), P_2O_5 (90 kg/hm^2), and K_2O (90 kg/hm^2) were applied once as base fertilizers at sowing time. A single factor randomized block design was used in the experiment. Each treatment was repeated 3 times. There were nine plots, each covering 30 m^2 (5 m × 6 m). Fifty individual plants with the same flare opening stage (July 10) were bagged in each treatment cell before spinning. Artificial self-cross pollination was carried out on the same day as the peak of loose powder, and the pollination date was marked. Samples were collected 15, 20, 25, 30, and 35 days after pollination. Three rows existed in the middle of each cell, and four complete bracts were collected randomly every time.

On the day after pollination, kernels were taken in the middle of the ear, weighed fresh, dried at 105 °C for 20 min, and then dried again in the oven at 80°C to constant weight. The dry weight of the kernels was measured, and the water content was calculated. The contents of starch and protein in grains were determined by DA7200 near-infrared spectroscopy. Soluble sugar was measured by anthrone colorimetric method (Li *et al.* 2011).

The middle seeds in the ear were taken and mixed well for use. The seeds were front fixed using 4% glutaraldehyde and then rinsed with phosphoric acid buffer (pH 6.8). After rinsing, they were frozen in liquid nitrogen for 50 s. The endosperm near the middle of the grain was cut with a double-sided blade, dehydrated with 30, 50, 70, 80 and 90% ethanol by volume. Again dehydrated with 100% ethanol for 3 times, dried using a freeze dryer, and plated on an ion sputtering apparatus. A JSM-6360LV scanning electron microscope was used to photograph.

All statistical analyses were performed with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). ANOVA was conducted, and significant differences for all statistical tests were calculated at the least significant difference of 0.05.

From 15 days to 35 days after pollination, the water content in grains showed a decreasing trend in all the studied maize varieties (Table 1). The descending gradient was 0.63% per day for Chaosweet 2000, 1.21% per day for Jingkenuo 2000, and 1.61% per day for Zhengdan 958. The decrease in daily average water content in grains from 15 days to 20 days after pollination was greater than that in other periods in the different types of maize. Previous studies indicated that the rate of seed dehydration is proportional to dry matter accumulation (Chen *et al*. 2022; Xiao *et al*. 2022).

Table 1. Changes in water content in maize grains

	Chaosweet 2000		Jingkenuo 2000		Zhengdan 958	
Days after pollination	Water content (%)	Average daily decline (%)	Water content (%)	Average daily decline (%)	Water content (%)	Average daily decline (%)
15	$(78.2 \pm 8.3)^{a}$	0	$(68.2 \pm 8.2)^{a}$	0	$(68.9 \pm 7.1)^{a}$	0
20	$(72.6 \pm 6.4)^{b}$	1.12	$(58.1 \pm 6.1)^{b}$	2.02	$(57.9 \pm 4.2)^{b}$	2.20
25	$(71.1 \pm 6.8)^{c}$	0.30	$(51.6 \pm 5.2)^{c}$	1.30	$(48.3 \pm 5.2)^{c}$	1.92
30	$(66.3 \pm 5.2)^{d}$	0.96	$(45.3 \pm 5.0)^{d}$	1.26	$(40.6 \pm 3.8)^{d}$	1.54
35	$(65.7 \pm 7.1)^{e}$	0.12	$(44.1 \pm 4.3)^{e}$	0.24	$(36.8 \pm 4.6)^{e}$	0.76
Average		0.63		1.21		1.61

In a column means having similar letter are statistically similar and those having different letter differ significantly at P 0.05 according to LSD test.

The changes in starch, protein, and soluble sugar content in corn kernels are indicated in Table 2. The content of starch increased rapidly in the early stage of grouting (15-20 days) in the three types of maize. From 15 days after pollination, waxy and common corn grains have higher starch content than sweet corn. The content of starch increased slowly or decreased slightly in the middle and late stages of grouting (25-35 days). The starch content of sweet corn was always lower than those of waxy and ordinary corn, and that of ordinary corn was lower than that of waxy corn (Gu *et al.* 2010; Shamixinuer *et al.* 2023). Thirty-five days after pollination, the starch contents of Chaosweet 2000, Jingkenuo 2000, and Zhengdan 958 were 50.7, 67.9 and 65.7%, respectively.

The protein content of Chaosweet 2000 was always significantly higher than those of Jingkenuo 2000 and Zhengdan 958, and that of Jingkenuo 2000 was slightly higher than that of Zhengdan 958. The protein content of common corn decreased significantly ($P < 0.05$). Thirty-five days after pollination, the protein contents of Chaosweet 2000, Jingkenuo 2000, and Zhengdan 958 were 11.2, 7.8 and 7.4%, respectively.

The soluble sugar contents of Jingkenuo 2000, Chaosweet 2000, and Zhengdan 958 showed a significant ($P<0.05$) decreasing trend with the extension of post-pollination time (Table 2). The soluble sugar contents of Jingkenuo 2000 and Zhengdan 958 decreased rapidly at 15-20 days after pollination and slowly after 25 days. The soluble sugar content of Chaosweet 2000 decreased rapidly at 20-25 days and 30-35 days after pollination, specifically to 6.3% after 35 days. Meanwhile, the soluble sugar contents of Jingkenuo 2000 and Zhengdan 958 were 6.2% and 6.0%, respectively.

The Internal ultrastructure of endosperm with Jingkenuo 2000 was observed. Fifteen days after pollination (Fig. 1), the starch granules in the endosperm cells were larger in volume and more in number. The starch grains were irregular polyhedral and elliptic, and there were some concaves on the surface. Flocculent matrix proteins appeared faintly between starch granules . The Internal ultrastructure of endosperm with Chaosweet 2000 was observed. Fifteen days after pollination (Fig. 1), substantial starch granules existed in the endosperm cells, and the volume was small. The starch granules were always spherical and were closely arranged. Twenty days after pollination (Fig. 2), there were obvious filamentous sugars in the endosperm, while soluble sugars were abundant. Thirty-five days after pollination (Fig. 5), some concaves appeared on the surface of the starch grains, which might be related to starch digestion (Yu *et al*. 2015).

With the extension of pollination time, the inclusion contents of the endosperm cells increased gradually. Proteins and sugars formed a honeycomb where the starch grains were embedded. The Internal ultrastructure of endosperm with Zhengdan 958 was observed. Fifteen days after pollination (Fig. 1), the starch grains became compact and uneven in size, and a few starch grains had a concave surface. Twenty-five days after pollination (Fig. 3), flocculent matrix proteins appeared in the endosperm. The volume of starch granules continued to increase from 15 days to 30 days after pollination. With the extension of crop growth period, the starch grains gradually changed from being nearly round to having an irregular shape.

The volume of starch granules in Jingkenuo 2000 and Zhengdan 958 were similar and larger than that of Chaosweet 2000 when 15-25 days after pollination (Fig. 1, 2, 3). The starch grains became small and substantial in Chaosweet 2000 when 30-35 days after pollination (Figs 4, 5). The volume of starch grains in Zhengdan 958 was larger than that in Jingkenuo 2000 and Chaosweet 2000. In the middle and late stages of the filling period, the density of starch grains was Chaosweet 2000 > Zhengdan 958 > Jingkenuo 2000. During the measurement period, the starch granules in Chaosweet 2000 did not fill the whole cell, and the cell wall was clearly visible. On the contrary, the endosperm cells of waxy and common corn were filled with starch grains.

Compared with those of common corn, the starch grain morphology and distribution space of sweet and waxy corn were different; thus, the thermal and gelatinization characteristics of starch also showed (Li *et al*. 2014, Xu *et al*. 2019). The results of this study indicated that during the whole grouting process (Figs 1, 2, 3, 4, 5), the volume of starch grains in waxy and common corn was larger than that in sweet corn. The uniformity of starch grains in waxy and common corn was better than that in sweet corn; this was similar to the results of Yi and Zhang (2015).

Table 2. Contents of starch, protein, and soluble sugar in corn kernel.

	Starch content (%)		
Days after pollination	Chaosweet 2000	Jingkenuo 2000	Zhengdan 958
15	$(37.1\pm3.9)^{c}$	$(55.2\pm6.1)^{e}$	$(38.2\pm4.5)^{e}$
20	$(50.8\pm4.5)^{b}$	$(63.7\pm5.6)^{d}$	$(62.5\pm5.3)^{d}$
25	$(51.9\pm4.6)^{a}$	$(66.6\pm6.4)^{c}$	$(65.5\pm7.2)^{b}$
30	$(51.8\pm5.2)^{a}$	$(67.6\pm6.6)^{b}$	$(64.4\pm6.9)^{c}$
35	$(50.7\pm5.0)^{b}$	$(67.9\pm7.3)^{a}$	$(65.7\pm6.4)^{a}$
	Protein content (%)		
15	$(10.7\pm1.2)^{b}$	$(8.8\pm0.9)^{b}$	$(9.1\pm1.2)^{a}$
20	$(10.1\pm0.8)^{c}$	$(9.2\pm1.3)^{a}$	$(8.3\pm0.8)^{b}$
25	$(9.7\pm1.3)^{d}$	$(8.1\pm0.7)^{d}$	$(7.8\pm0.7)^{c}$
30	$(10.8\pm1.1)^{b}$	$(8.4\pm1.3)^{c}$	$(7.6\pm0.8)^{d}$
35	$(11.2\pm1.4)^{a}$	$(7.8\pm0.9)^{e}$	$(7.4\pm0.7)^{e}$
	Soluble sugar content (%)		
15	$(13.4\pm2.2)^{a}$	$(13.2\pm2.6)^{a}$	$(13.5\pm1.4)^{a}$
20	$(13.1\pm2.0)^{b}$	$(7.5\pm1.0)^{b}$	$(6.9\pm0.7)^{b}$
25	$(8.6\pm0.8)^{c}$	$(6.9\pm0.8)^{c}$	$(6.5\pm0.8)^{c}$
30	$(8.1\pm0.7)^{d}$	$(6.4\pm0.7)^{d}$	$(6.3\pm0.6)^{d}$
35	$(6.3\pm0.6)^{e}$	$(6.2\pm0.6)^{e}$	$(6.0\pm0.7)^{e}$

In a column means having similar letter are statistically similar and those having dissimilar letter differ significantly at P 0.05 according to LSD test.

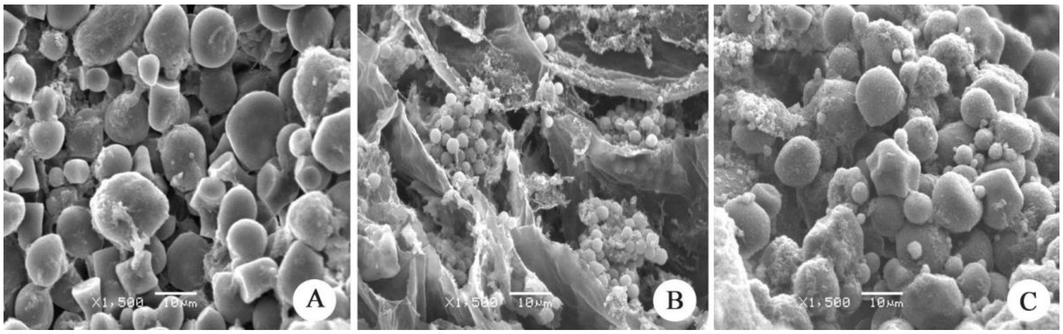

Fig. 1. Changes in starch granules and matrix proteins with different types of maize. A-C. 15 days after pollination. A. Jingkenuo 2000 (waxy corn); B. Chaosweet 2000 (sweet corn); C. Zhengdan 958 (common corn).

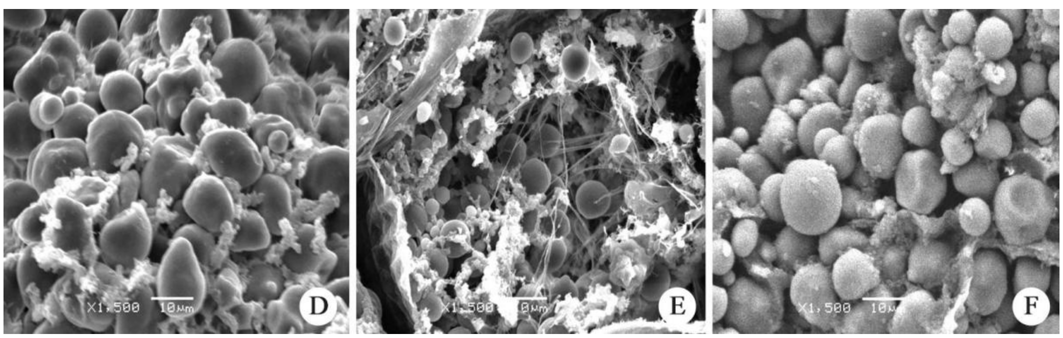

Fig. 2. Changes in starch granules and matrix proteins with different types of maize. D-F. 20 days after pollination. D. Jingkenuo 2000 (waxy corn); E. Chaosweet 2000 (sweet corn); F. Zhengdan 958 (common corn).

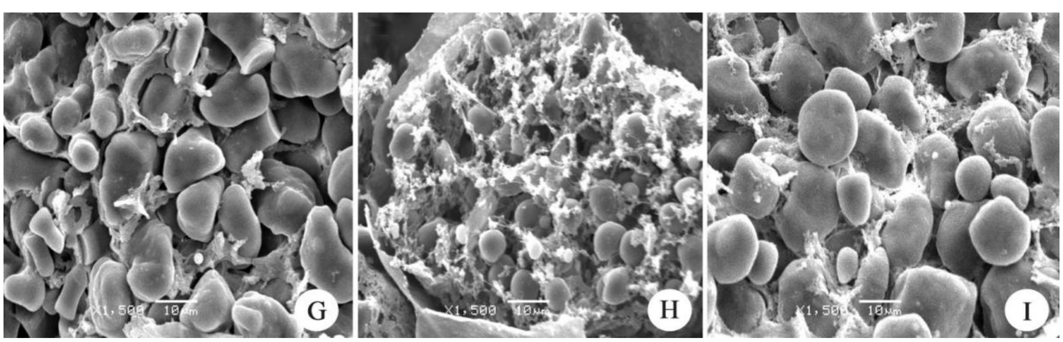

Fig. 3. Changes in starch granules and matrix proteins with different types of maize. G-I. 25 days after pollination. G. Jingkenuo 2000 (waxy corn); H. Chaosweet 2000 (sweet corn); I. Zhengdan 958 (common corn).

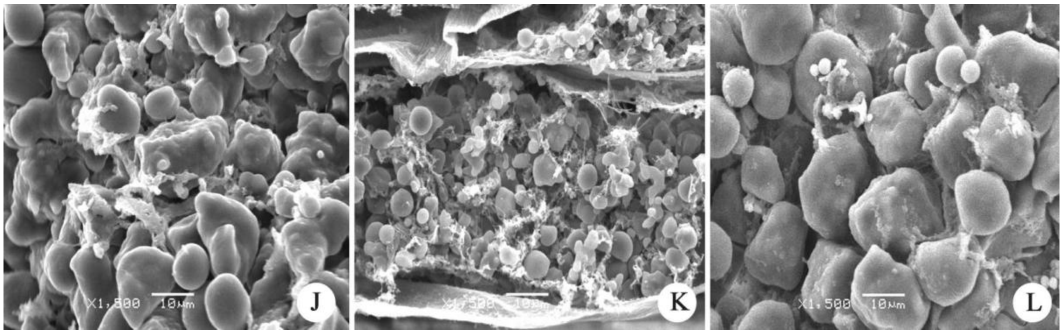

Fig. 4. Changes in starch granules and matrix proteins with different types of maize. J-L. 30 days after pollination. J. Jingkenuo 2000 (waxy corn); K. Chaosweet 2000 (sweet corn); L. Zhengdan 958 (common corn).

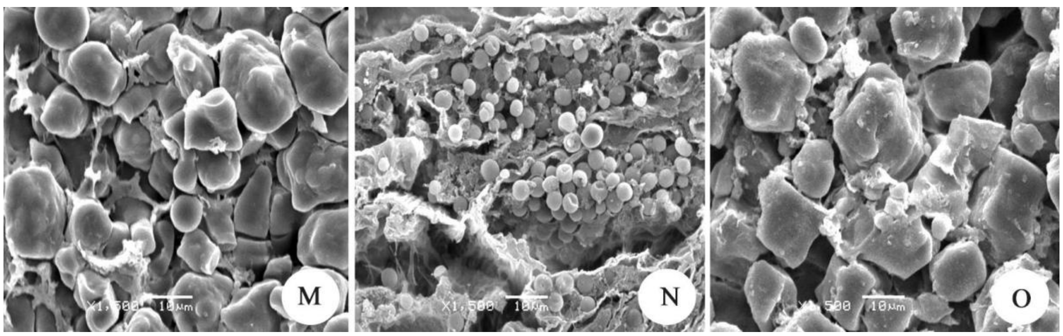

Fig. 5 Changes in starch granules and matrix proteins with different types of maize. M-O. 35 days after pollination. M. Jingkenuo 2000 (waxy corn); N. Chaosweet 2000 (sweet corn); O. Zhengdan 958 (common corn).

At the early grouting stage, starch granules were observed in endosperm cells in the three types of maize. From 20 days to 35 days after pollination (Figs 2, 3, 4, 5), a large number of matrix proteins filled into the starch space in sweet and waxy maize. Filamentous sugar appeared in sweet corn, while the matrix protein was not obvious in common corn. Hence, there were significant differences in internal ultrastructure in grain among the three types of maize, which might be the reason for the difference in grain quality among the different types of maize.

Sweet corn had flatter kernels, and its 100-grain weight was low. The starch grains were mostly spherical and closely arranged. Although there was no obvious correlation between starch grain shape and grain shape, starch grain shape and grain quality had a correlation. In the rice endosperm, because the starch grains and protein bodies are not closely arranged, the white opaque parts between them form chalk. Chalky rice has poor transparency, low milling rate, and poor commodity quality (Wang *et al.* 2022; Chen *et al.* 2023). In this study, 20-25 days after pollination (Figs 2, 3), the distribution of starch grains in sweet corn was closer than that in waxy and ordinary corn. The inclusions increased, the matrix proteins and sugars formed a honeycomb, protein and soluble sugar were rich, and the keratinization degree was high. These contributed to the sweet and crisp character of sweet corn. The volume of starch grains in common corn was larger, the arrangement was closer than that in waxy corn, and the content of protein and soluble sugar was lower. The starch grains in the endosperm cells were arranged loosely in waxy corn, and the starch content was always higher than those in ordinary and sweet corn during the whole testing stage, leading to the soft taste and stickiness of waxy corn (Zheng *et al.* 2021).

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