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STARCH ACCUMULATION, SIZE DISTRIBUTION AND RELATED ENZYME ACTIVITY IN SUPERIOR AND INFERIOR KERNELS OF MAIZE UNDER DIFFERENT NITROGEN RATES

WENYANG LI[1], ZHI TAN[1], RUI LI[1], JIANXIN YUAN[1], SUHUI YAN[1*] AND CONGFENG LI[2*]

[1]*College of Agronomy, Anhui Science and Technology University, Fengyang 233100, China;*
[2]*Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China*
[*]*Corresponding author's email: yansh@ahstu.edu.cn; licongfeng@caas.cn*

Abstract

Compared to inferior kernels, superior kernels had significantly higher amount of starch accumulation in maize at maturity. The logistic simulation showed that the starting potential and mean accumulation rate were affected by kernel position in the regulation of starch accumulation. The contents of sucrose in inferior kernels were significantly higher than those in superior kernels during kernel growth. The activities of related enzymes, sucrose synthase (SS) and ADPG pyrophosphorylase (AGPase), in superior kernels were much higher compared to inferior kernels. The results suggested that high starch accumulation was caused by high activities of SS and AGPase but not by high starch synthase substrate levels in superior kernels compared to inferior kernels. Nitrogen could increase the degradation and utilization of sucrose and the level of starch synthesis, as well as accelerate the formation of small granules in maize. The volume percentages of granules <18 and >18 μm in superior kernels were significantly higher and lower than those in inferior kernels, respectively. However, kernel position had little influence on the number percentages of granules <3, 3-18 and >18 μm. These results suggest that it is more conducive to increase of individual volume of small and midsized starch granules in superior kernels within a maize ear. There was small difference on the mean AGPase activities during filling between superior and inferior kernels under normal nitrogen compared with low nitrogen. Thus, normal application decreased the variation on the starch accumulation and volume percentage of large starch granules in both kernels.

Key words: *Zea mays* L., Particle size distribution, Kernel position, Kernel development.

Introduction

There are significant differences in the kernel weight within a maize (*Zea mays* L.) ear (Xu *et al.*, 2013). The middle kernels of an ear have greater kernel weight and are regarded as the superior kernels, whereas the upper kernels of an ear have lower kernel weight and are considered the inferior kernels in cereal crops (Jellum, 1967; Yan *et al.*, 2014; Dong *et al.*, 2014). Superior kernels usually exhibit a faster filling rate than do inferior kernels in rice (Liang *et al.*, 2001) and wheat (Jiang *et al.*, 2003). The slow kernel filling rate has been attributed to the limited carbohydrate supply in inferior kernels of wheat (Jiang *et al.*, 2003). However, it is unclear whether an alike mechanism exists in different kernels within a maize ear.

Starch is the major carbohydrate in maize kernels and serves as multifunctional ingredients for the food and non-food uses (Zobel, 1988; Zhang *et al.*, 2017; Ali *et al.*, 2017). Starch is deposited in detached granules in amyloplasts during kernel development. The size distribution of starch granule is one of the main factors determining kernel quality in maize (Cui *et al.*, 2014). Starch granule distribution is controlled by genotype and cultured environment (Wilson *et al.*, 2010; Paterson *et al.*, 2001).

The process of sucrose transforming into starch is controlled by a series of related enzymes after photosynthate is transported to kernel (Braun, 2012). It has been documented that starch synthesis related enzymes, such as sucrose synthase (SS) and adenosine diphosphate glucose pyrophosphorylase (AGPase), play a key role in starch biosynthesis in maize kernels (Hannah, 2005). Various studies have reported that activities of SS and ADPase are associated with starch accumulation of inferior kernels in rice (Yang *et al.*, 2003) and wheat (Jiang *et al.*, 2003; Li *et al.*, 2013), but little information is available on starch biosynthesis in kernels at distinct positions within a maize ear.

The aims of this experiment were to (1) investigate starch accumulation, size distribution and the activities of related enzymes in kernels at different positions within a maize ear and (2) determine whether the variations on starch accumulation amount in kernels within an ear were attributed to assimilate supply and sink activities in inferior kernels of maize. This information should help elucidate of the physiological and biochemical mechanism on regulating spatial difference of individual kernel development within a maize ear.

Materials and Methods

The field experiment was carried out in growing seasons from June 2016 to October 2016 and from June 2017 to October 2017 at the planting park (32°51'N, 117°33'E) of Anhui Science and Technology University, Fengyang, China. Two maize cultivars, Longping206 (during the maize growing seasons of 2016 and 2017) and Zhongdan909 (during the maize growing season of 2017), were chosen in this study.

There were two nitrogen rates of 135 kg $N \cdot ha^{-1}$ (LN) and 270 kg $N \cdot ha^{-1}$ (NN). Seventy percent of the nitrogen fertilizer was applied as the basal manure, and another 30% was topdressed at the stem elongation stage of maize. In addition, basal manure was used at the rates of 120 $kg \cdot ha^{-1}$ P_2O_5 and 120 $kg \cdot ha^{-1}$ K_2O. Winter wheat (*Triticum aestivum* L.) was the previous crop. Seeds were sown on 12 June 2016 and 11 June 2017 with a density of 67500 $plants \cdot ha^{-1}$. The size of experimental plot was 6 × 6.7 m with 10 rows (60 cm between rows). Other cultural practices followed in maize production field were used in this study.

Sampling: At anthesis, ears silking on the same date were labeled. From 7 days after silking (DAS), five labelled ears of each experimental plot were sampled every seven days until maturity.

The middle kernels on maize ear were divided as superior kernels, whereas the upper kernels on the same ear were divided as inferior kernels. These kernels were dried at 70°C for 48 h for sucrose and starch determination. Additional kernels were frozen in liquid nitrogen for 20 min and then stored at -40°C for enzyme activity assay. Maize kernels were dried at 70°C for 48 h for the size analysis of starch granules in the maturity.

Starch determination: The starch contents were determined using a DA7200 Continuous Spectrum Near-infrared Analyzer (Perten Instruments, Sweden).

The starch accumulation process in individual kernel were fitted by the logistic equation as described by Birch (1999) and Yan *et al.,* (2000):

$$W = K/(1+e^{A+Bt})$$

The parameters of starch accumulation were calculated according to the following equations: the maximum starch accumulation = K, the starting potential (C_0) = $K/(1+e^A)$, the active duration (approximately 95% of total starch accumulation) (D) = [ln0.053-A]/B, the mean starch accumulation rate (R_{mean}) = K/D, and the maximum starch accumulation rate (R_{max}) = -KB/4.

Starch separation, purification and size distribution analysis: Starch was extracted and purified from maize kernels following the methods of Peng *et al.*, (1999). The granule size distribution of starch was measured by using a LS13320 Laser Diffraction Particle Size Analyzer (Beckman Coulter Inc, USA).

Sucrose determination: Sucrose content was determined following the methods described by Zhang & Qu (2003).

Enzyme extraction and assays: The samples and reagents were prepared following the methods described by Nakamura & Yuki (1992). The sucrose synthase (SS) and adenosine diphosphate glucose pyrophosphorylase (AGPase) activity was analysed following the methods of Nakamura & Yuki (1992).

Statistical analyses

The data were analysed with SPSS. The means were compared by the least significant difference test, when the F-test was significant at the 0.05 level.

Results

Starch accumulation: The starch accumulation in kernels at different position within a maize ear showed S-shaped curves (Fig. 1). The starch accumulation weights among treatments were significantly higher in superior kernels than in inferior kernels at maturity. The processes of starch accumulation were well fitted by the logistic equation (R^2>0.999) (Table 1). The coefficients K, C_0 and R_{mean} were much higher in superior kernels, while R_{max} and D were lower in superior kernels than in inferior kernels. The logistic simulation showed that it was the starting potential and the mean accumulation rate, but not the maximum rate and the duration of starch accumulation affected by kernel positions to regulate starch accumulation in maize kernels.

Normal nitrogen could increase the amount of starch accumulation in superior and inferior kernels than low nitrogen, which indicates that nitrogen application could accelerate starch accumulation. Greater mean starch accumulation rates were observed under appropriate nitrogen application than under low nitrogen application, suggesting that appropriate nitrogen application was responsible for the higher kernel filling rate. The variations on starch accumulation amount in superior and inferior kernels were 33.5 and 31.7 mg·kernel^{-1} (average of two cultivars in two grow seasons) under low and normal nitrogen. And the variations on the coefficients C_0 in superior and inferior kernels were 2.05 and 1.81 mg·kernel^{-1} (average of two cultivars in two grow seasons) under low and normal nitrogen treatment.

Table 1. Starch accumulation characteristic parameters in the superior and inferior kernel of maize.

Season	Cultivar	Nitrogen rate	Kernel position	K /(mg·kernel^{-1})	C_0 /(mg·kernel^{-1})	R_{mean} /(mg·kernel^{-1}·d^{-1})	R_{max} /(mg·kernel^{-1}·d^{-1})	D /d
2016	Longping206	LN	S	262.06	2.84	4.82	8.09	54.3
			I	225.78	0.87	4.10	7.94	55.1
		NN	S	274.49	3.97	4.91	7.88	55.9
			I	239.93	1.05	4.29	8.17	55.9
2017	Longping206	LN	S	254.84	2.60	4.70	7.96	54.2
			I	215.95	0.55	4.05	8.27	53.3
		NN	S	275.87	3.78	4.86	7.86	56.8
			I	232.94	0.80	4.24	8.35	54.9
	Zhongdan909	LN	S	250.06	2.06	4.78	8.35	52.3
			I	210.68	0.41	4.10	8.65	51.4
		NN	S	258.48	2.37	4.92	8.47	52.5
			I	219.68	0.49	4.35	9.02	50.5

K, the maximum starch accumulation; C_0, the starting potential; R_{mean}, the mean starch accumulation rate; R_{max}, the maximum starch accumulation rate; D, the active duration

Table 2. Volume distribution of starch granules in the superior and inferior kernel of maize. /%.

Season	Cultivar	Nitrogen rate	Kernel position	Particle diameter of starch granule/μm		
				<3	3-18	>18
2016	Longping206	LN	S	9.12 ± 0.13b	58.93 ± 0.04ab	31.96 ± 0.09c
			I	8.6 ± 0.08c	56.46 ± 0.02c	34.95 ± 0.1a
		NN	S	10.42 ± 0.14a	59.35 ± 0.3a	30.24 ± 0.16d
			I	9.12 ± 0.14b	58.36 ± 0.01b	32.52 ± 0.16b
2017	Longping206	LN	S	9.2 ± 0.03c	59.54 ± 0.32ab	31.27 ± 0.35e
			I	8.68 ± 0.09d	56.49 ± 0.19d	34.84 ± 0.1b
		NN	S	10.27 ± 0.18b	59.83 ± 0.35a	29.9 ± 0.17f
			I	9.04 ± 0.18cd	58.68 ± 0.57b	32.29 ± 0.39d
	Zhongdan909	LN	S	9.95 ± 0.04b	57.52 ± 0.08cd	32.53 ± 0.13d
			I	9 ± 0.16cd	54.33 ± 0.01e	36.68 ± 0.16a
		NN	S	10.92 ± 0.06a	58.53 ± 0.25bc	30.56 ± 0.19ed
			I	9.8 ± 0.11b	56.73 ± 0.01d	33.47 ± 0.13c

Means within columns at the same season followed by different letters are significantly different at $P = 0.05$

Starch granule size distribution: Compared with inferior kernels, superior kernels had a higher volume percentage of small granules (<3 μm) and midsized granules (3-18 μm) and a lower volume percentage of large granules (>18 μm) (Table 2). Nitrogen application increased volume percentage of granules <3 μm in kernels at the same position within the rates from 135 to 270 $kg{\cdot}ha^{-1}$ nitrogen, indicating that nitrogen fertilizer could quicken the formation of small starch granules. The variations on volume percentage of large starch granules in superior and inferior kernels were 3.57 and 2.45% (average of two cultivars in two grow seasons) under low and normal nitrogen.

The proportions of small granules (<3 μm) ranged from 98.24% to 99% of the total number of granules (Table 3), which suggested that the starch granules in maize kernels were composed of small granules. This indicated that small granules possessed most of the number although fewer volume compared with midsized and large starch granules in maize kernels. Kernel position had little influence on the numbers of small, midsized and large starch granules in maize.

Sucrose content: The change in the sucrose content in kernels from different treatments and different kernel positions showed a declining trend (Fig. 2). Inferior kernels had a higher sucrose content than did inferior kernels during kernel filling among the treatments, which showed that the inferior kernels had a good substrate supply. Nitrogen application could increase the sucrose content in kernels at the same position when the rates varied from 135 to 270 $kg{\cdot}ha^{-1}$, which indicated that nitrogen fertilizer could increase the substrate supply during starch accumulation.

SS activity: Compared with inferior kernels, superior kernels showed higher SS activities (Fig. 3), indicating that superior kernels had an advantage in sucrose degradation and utilization. At 14 days after silking (DAS), the SS activities in the same kernel position under both nitrogen rates were not obviously different. However, the SS activities in the same kernel position at normal nitrogen (270 $kg{\cdot}ha^{-1}$) were significantly higher than those at low nitrogen (135 $kg{\cdot}ha^{-1}$) after 28 DAS. The results showed that nitrogen application could increase the degradation and utilization of sucrose in the middle- and late-filled kernels in maize.

AGPase activity: The AGPase activities in superior kernels were signicantly higher than those in inferior kernels during kernel filling at the same nitrogen rate (Fig. 4), which suggested that a higher level of starch synthesis in superior kernels than did inferior kernels in maize. Nitrogen application slightly increased the AGPase activities at the same kernel position. This finding indicated that nitrogen application could increase the level of starch synthesis. The variations on the mean AGPase activities during filling in superior and inferior kernels were 1.33 and 0.95 μmol $G1P{\cdot}g^{-1}{\cdot}min^{-1}$ (average of two cultivars in two grow seasons) under low and normal nitrogen.

Discussion

Many researchers have reported that there are differences in carbohydrate supply, starch synthesis and grain filling among grains within wheat (Calderini & Reynolds, 2000; Wang *et al.*, 2003; Li *et al.*, 2013; Liang *et al.*, 2017) and rice (Mohapatra *et al.*, 1993; Yang *et al.*, 2006; Wang *et al.*, 2012) spikes. The results from the existing studies on whether the sucrose content (the substrate of starch synthesis) is one of the limiting factors for starch synthesis in inferior kernels have been inconsistent in cereal crops. Zhang *et al.*, (2000) reported that the source supply was satisfied by filling of kernels at different position within a wheat spike. However, Jiang *et al.*, (2003) reported that superior kernels had greater contents of sucrose and thus a higher level of starch synthesis than did inferior grains in wheat. In this study, the contents of sucrose in inferior kernels were significantly higher than those in superior kernels during kernel development, indicating that there was not a causal link between sucrose content (assimilate supply) and starch accumulation within a maize ear. Compared with low nitrogen, normal nitrogen decreased the variation on the amount and the starting potential of starch accumulation in both kernels.

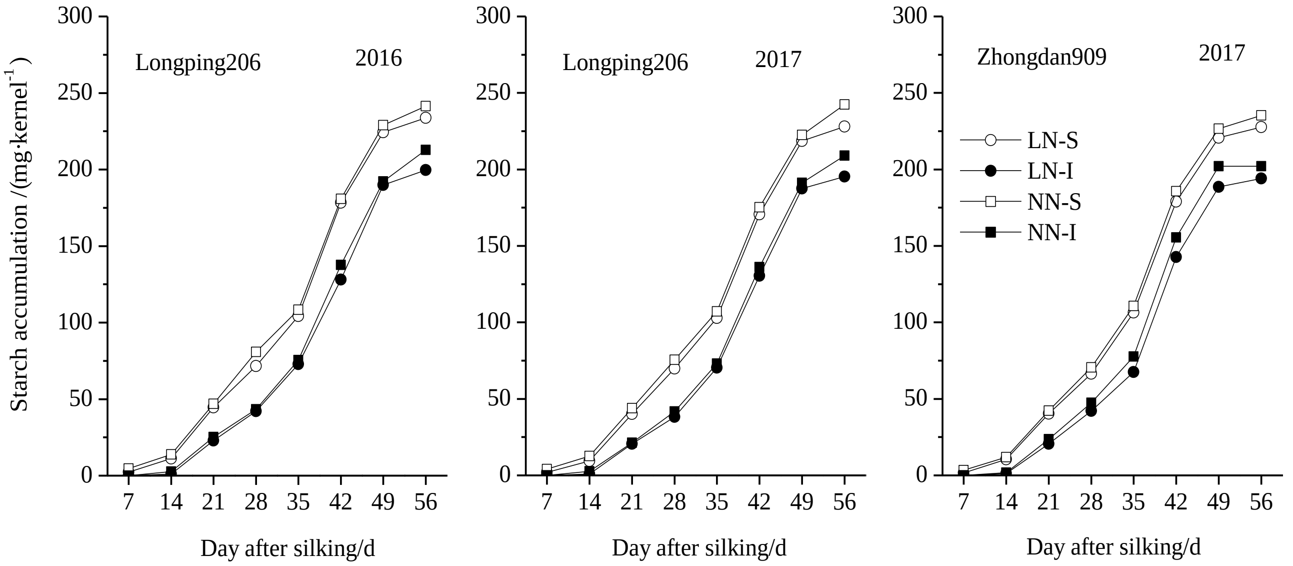

Fig. 1. Starch accumulation in the superior and inferior kernel of maize. LN, Low nitrogen; NN, Normal nitrogen; S, Superior kernels; I, Inferior kernels.

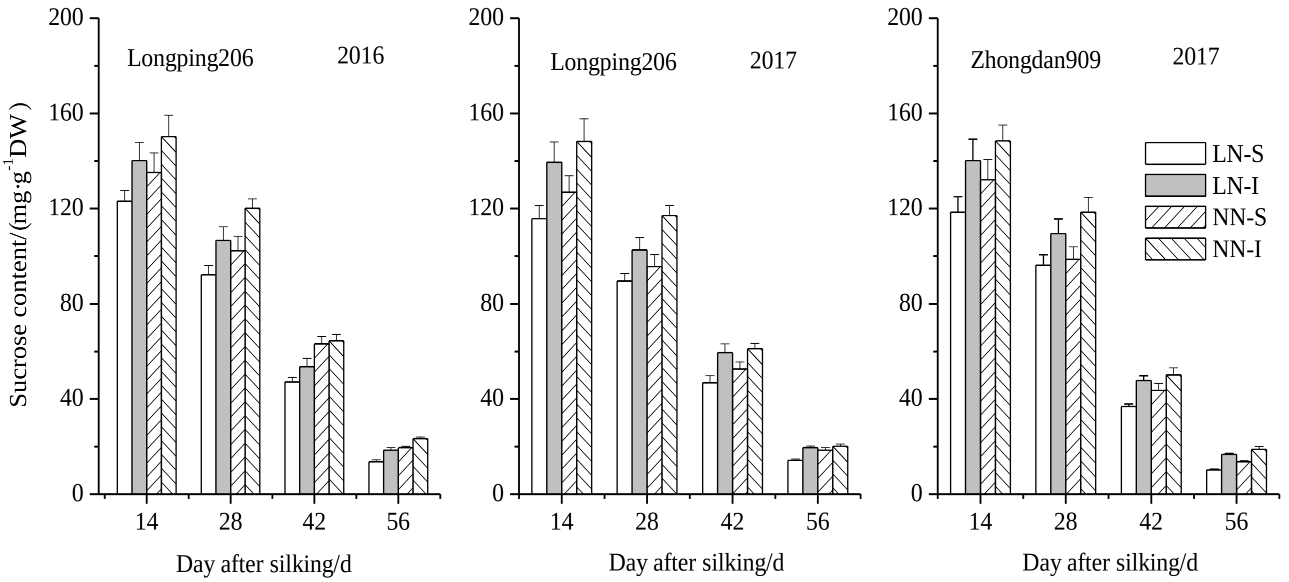

Fig. 2. Sucrose content in the superior and inferior kernel of maize. LN, Low nitrogen; NN, Normal nitrogen; S, Superior kernels; I, Inferior kernels.

Table 3. Number distribution of starch granules in the superior and inferior kernel of maize. /%

Season	Cultivar	Nitrogen rate	Kernel position	Particle diameter of starch granule/μm		
				<3	3-18	>18
2016	Longping206	LN	S	98.86 ± 0.08a	1.1 ± 0.08bc	0.04 ± 0.01a
			I	99 ± 0.08a	0.95 ± 0.07c	0.04 ± 0.01a
		NN	S	98.4 ± 0.11b	1.56 ± 0.1a	0.04 ± 0.01a
			I	98.65 ± 0.09ab	1.31 ± 0.08ab	0.04 ± 0.01a
2017	Longping206	LN	S	98.81 ± 0.08ab	1.15 ± 0.07de	0.04 ± 0.01a
			I	98.98 ± 0.04a	0.98 ± 0.04e	0.04 ± 0.01a
		NN	S	98.24 ± 0.06d	1.72 ± 0.06a	0.04 ± 0.01a
			I	98.45 ± 0.09cd	1.51 ± 0.08bc	0.04 ± 0.01a
	Zhongdan909	LN	S	98.62 ± 0.04bc	1.34 ± 0.03cd	0.04 ± 0.01a
			I	98.82 ± 0.02ab	1.14 ± 0.01de	0.04 ± 0.01a
		NN	S	98.28 ± 0.06d	1.68 ± 0.06ab	0.04 ± 0.01a
			I	98.39 ± 0.02d	1.57 ± 0.01ab	0.04 ± 0.01a

Means within columns at the same season followed by different letters are significantly different at $P = 0.05$

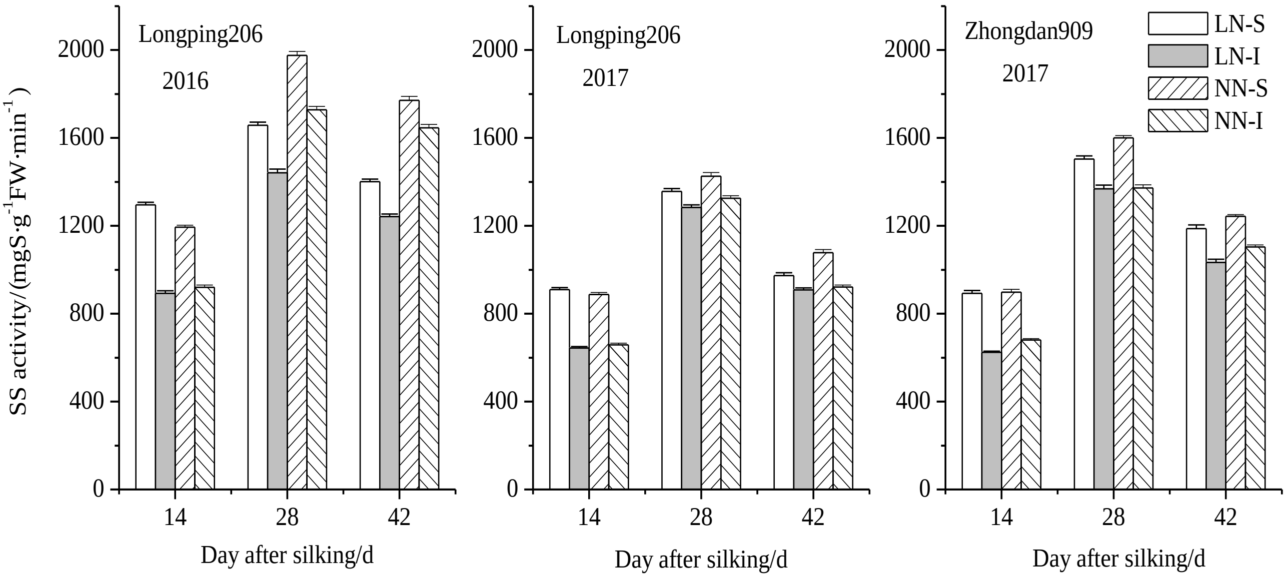

Fig. 3. SS activity in the superior and inferior kernel of maize. LN, Low nitrogen; NN, Normal nitrogen; S, Superior kernels; I, Inferior kernels.

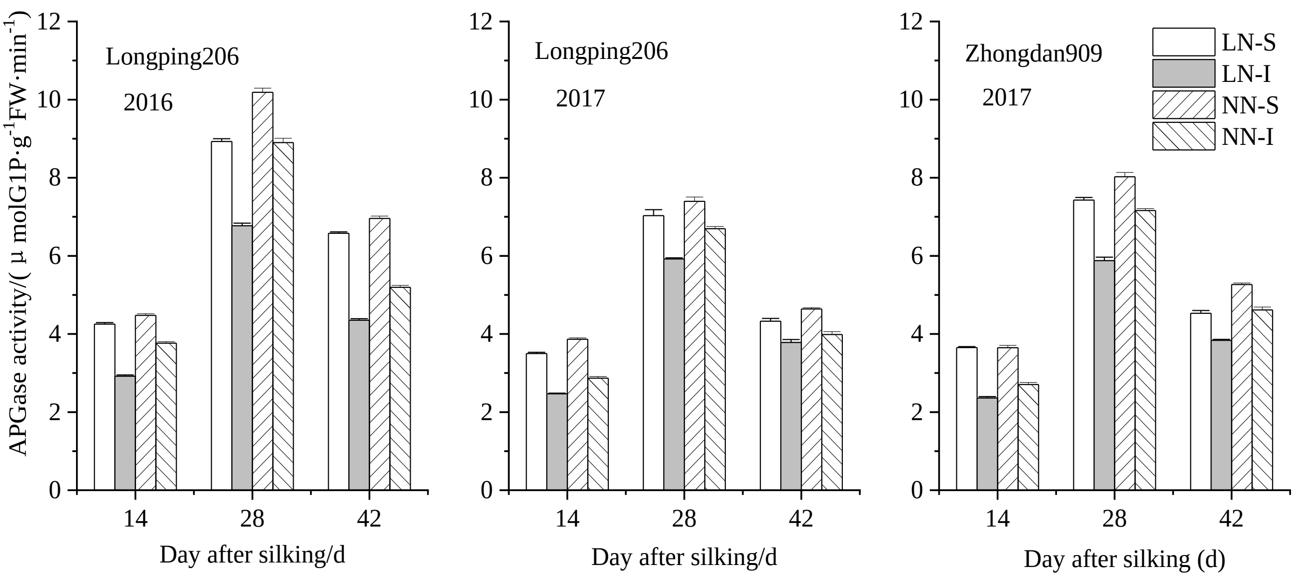

Fig. 4. AGPase activity in the superior and inferior kernel of maize. LN, Low nitrogen; NN, Normal nitrogen; S, Superior kernels; I, Inferior kernels.

It is generally recognized that the kernel filling rate in cereal crops is determined by sink size and sink activity (Hannah & James, 2008; Mohapatra *et al.*, 2009). Sink activity is mainly controlled by the activities of enzymes involved in utilization of photosynthate, such as SS and AGPase in maize kernels (Green & Hannah, 2010; Li *et al.*, 2013). SS is a key enzyme that catalyses the cleavage of sucrose (Smyth & Prescott, 1989). Adenosine diphosphate glucose (ADPG) is the substrate for the biosynthesis of the starch components (Preiss & Sivak, 1998). AGPase catalyses the synthesis of ADPG from ATP and glucose-1-phosphate (G1P) (Stark, 1992). In this study, the amounts of starch accumulation in superior kernels were significantly higher than that in inferior kernels within a maize ear. By integrating the change in the activities of SS and AGPase, we could regard that there was a difference in the transformation capability of sucrose to starch components resulting in differences of starch accumulation amount between both kernels within a maize ear; the higher starch accumulation in the superior kernels within a maize ear was due to the better sink activities during kernel filling. Meanwhile, these results suggested that the transformation of sucrose to starch rather than carbohydrate supply might be a limiting step in starch biosynthesis in inferior kernels in maize. Nitrogen application could reduce the variation on the mean AGPase activities during filling of both kernels.

In wheat, mature grains include small B-type starch granules and large A-type starch granules (Peng *et al.*, 1999). A-type granules begin form 4-5 days after anthesis, and B-type starch granules begin form approximately 12-14 days after anthesis in wheat (Bechtel & Wilson, 2003; Li *et al.*, 2016). Starch biosynthetic enzymes involve in the formation of starch granules in kernel in cereal crops (Mu-forster *et al.*, 1996; Preiss & Sivak, 1998). The formation and development of starch granules comprise the increase in individual volume and their number in cereal crops. This study also showed that the volume percentages of granules <18 μm and >18 μm were remarkably higher and lower in superior kernels than in inferior ones, respectively. However, there were no marked difference in the number percentages of granules <3 μm (small), 3-18μm (midsize) and >18 μm (large) in both kernels at different kernel positions. These results suggest that it is easier to increase the individual volumes of small starch granules in superior kernels within a maize ear. The reason for this effect may be the high capacity of substrate degradation and high activity of starch biosynthetic enzymes that are also participated in the formation of small and midsize granules in superior kernels compared with inferior kernels. Nitrogen application could reduce the variation on volume percentage of large starch granules in both kernels. The reason may be the smaller differences on the mean AGPase activities during filling between superior and inferior kernels under normal nitrogen, compared with low nitrogen.

Conclusions

The starch accumulation was significantly higher in superior kernels than in inferior kernels at maturity in this study. And the higher amount of starch accumulation in superior kernels was mainly owing to the higher starting potential and the mean starch accumulation rate. This study showed that the transformation of sucrose to starch rather than carbohydrate supply might be a limiting step in starch biosynthesis in inferior kernels in maize. Superior kernels had a higher volume percentage of small and midsized starch granules and a lower volume percentage of large granules compared with inferior kernels. However, kernel position had little influence on the numbers of small, midsized and large starch granules. Normal nitrogen was beneficial to reduce the variation on the starch accumulation, the starting potential and the mean AGPase activities between both kernels in maize.

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References

Ali, K., M. Arif, F. Shah, A. Shehzad, F. Munsif, I.A. Mian and A.A. Mian. 2017. Improvement in maize (*Zea mays* L) growth and quality through integrated use of biochar. *Pak. J. Bot.*, 49: 85-94.

Bechtel, D.B. and J.D. Wilson. 2003. Amyloplast formation and starch granule development in hard red winter wheat. *Cereal Chem.*, 80: 175-183.

Birch, C.P.D. 1999. A new generalized logistic sigmoid growth equation compared with the richards growth equation. *Ann. Bot.*, 83: 713-723.

Braun, D.M. 2012. Sweet! The pathway is complete. *Science*, 335: 173-174.

Calderini, D.F., M.P. Reynolds and G.A. Slafer. 2006. Source-sink effects on grain weight of bread wheat, durum wheat, and triticale at different locations. *Aust. J. Agric. Res.*, 57: 227-233.

Cui, L., S.T. Dong, J.W. Zhang and P. Liu. 2014. Starch granule size distribution and morphogenesis in maize (*Zea mays* L.) grains with different endosperm types. *Aust. J. Crop Sci.*, 8: 1560-1565.

Dong, M.H., J.R. Gu, L. Zhang, P.F. Chen, T.G. Liu, J.H. Deng, H.Q. Lu, L.Y. Han and B.H. Zhao. 2014. Comparative proteomics analysis of superior and inferior spikelets in hybrid rice during grain filling and response of inferior spikelets to drought stress using isobaric tags for relative and absolute quantification. *J. Proteomics*, 109: 382-399.

Green, T.W. and L.C. Hannah. 2010. Adenosine diphosphate glucose pyrophosphorylase, a rate-limiting step in starch biosynthesis. *Physiol. Plantarum*, 103: 574-580.

Hannah, L.C. 2005. Starch synthesis in the maize endosperm. *Maydica*, 50: 497-506.

Hannah, L.C. and M. James. 2008. The complexities of starch biosynthesis in cereal endosperms. *Curr. Opin. Biotech.*, 19: 160-165.

Jellum, M.D. 1967. Fatty acid composition of corn oil as influenced by kernel position on ear. *Crop Sci.*, 7: 593-595.

Jiang, D., W.X. Cao, T.B. Dai and Q. Jing. 2003. Activities of key enzymes for starch synthesis in relation to growth of superior and inferior grains on winter wheat spike. *Plant Growth Regul.*, 41: 247-257.

Li, J., E. Baroja-Fernandez, A. Bahaji, F.J. Munoz, M. Ovecka, M. Montero, S.M. Teresa, N. Alonso-Casajus, G. Almagro, S.A. Maria, M. Hidalgo, M. Zamarbide and J. Pozueta-Romero. 2013. Enhancing sucrose synthase activity results in increased levels of starch and adp-glucose in maize (*Zea mays* L.) seed endosperms. *Plant & Cell Physiol.*, 54: 282-294.

Li, W., S. Yan and Z. Wang. 2013. Effect of spikelet position on starch proportion, granule distribution and related enzymes activity in wheat grain. *Plant Soil & Environ.*, 59: 568-574.

Li, W., S. Yan and Z. Wang. 2016. Formation and developmental characteristics of and b type starch granule in wheat endosperm and response to nitrogen. *J. Chin. Cereals & Oils Assoc.*, 31: 22-26.

Liang, J.S., J.H. Zhang and X.Z. Cao. 2001. Grain sink strength may be related to the poor grain filling of indica-japonica rice (*Oryza sativa*) hybrids. *Physiol. Plantarum*, 112: 470-477.

Liang, W., Z. Zhang, X. Wen, Y. Liao and Y. Liu. 2017. Effect of non-structural carbohydrate accumulation in the stem pre-anthesis on grain filling of wheat inferior grain. *Field Crop Res.*, 211: 66-76.

Mohapatra, P.K., R. Patel and S.K. Sahu. 1993. Time of flowering affects grain quality and spikelet partitioning within the rice panicle. *Aust. J. Plant Physiol.*, 20: 231-242.

Mohapatra, P.K., R.K. Sarkar and S.R. Kuanar. 2009. Starch synthesizing enzymes and sink strength of grains of contrasting rice cultivars. *Plant Sci.*, 176: 256-263.

Mu-Forster, C., R. Huang, J.R. Powers, R.W. Harriman, M. Knight, G.W. Singletary, P.L. Keeling and B.P. Wasserman. 1996. Physical association of starch biosynthetic enzymes with starch granules of maize endosperm. *Plant Physiol.*, 111: 821-829.

Nakamura, Y. and K. Yuki. 1992. Changes in enzyme activities associated with carbohydrate metabolism during the development of rice endosperm. *Plant Sci.*, 82: 15-20.

Paterson, J.L., A. Hardacre, P. Li and M.A. Rao. 2001. Rheology and granule size distribution of corn starch dispersions from two genotypes and grown in four regions. *Food Hyd.*, 15: 453-459.

Peng, M., M. Gao, E.S.M. AbdelAal, P. Huel and R.N. Chibbar. 1999. Separation and characterization of A- and B-type starch granules in wheat endosperm. *Cereal Chem.*, 76: 375-379.

Preiss, J. and M.N. Sivak. 1998. Biochemistry, molecular biology and regulation of starch synthesis. *Genetic Engn.*, 20: 177-223.

Sayed, H.E. 2010. Endosperm development in maize and related to photosynthate supply. *J. Agron. & Crop Sci.*, 168: 278-285.

Stark, D.M., K.P. Timmerman, G.F. Barry, J. Preiss and G.M. Kishore. 1992. Regulation of the amount of starch in plant tissues by ADP glucose pyrophosphorylase. *Science*, 258: 287-292.

Wang, Z., Y. Xu, J. Wang and J. Yang. 2012. Polyamine and ethylene interactions in grain filling of superior and inferior spikelets of rice. *Plant Growth Regul.*, 66: 215-228.

Wang, Z., Y. Yin, M. He, Y. Zhang, S. Lu, Q. Li and S. Shi. 2003. Allocation of photosynthates and grain growth of two wheat cultivars with different potential grain growth in response to pre- and post-anthesis shading. *J. Agron. & Crop Sci.*, 189: 280-285.

Wilson, J.A., D.V. Glover and W.E. Nyquist. 2010. Genetic effects of the soft starch (*h*) and background loci on volume of starch granules in five inbreds of maize. *Plant Breed.*, 119: 173-176.

Xu, Y., D. Gu, H. Qing, H. Zhang, Z. Wang and J. Yang. 2015. Changes in carbohydrate accumulation and activities of enzymes involved in starch synthesis in maize kernels at different positions on an ear during grain filling. *Acta Agron. Sin.*, 41: 297-307.

Yan, S., W. Li, Y. Yin and Z. Wang. 2010. Sink strength in relation to growth of superior and inferior grains within a wheat spike. *J. Agri. Sci.*, 148: 567-578.

Yang, J., J. Zhang, Z. Wang and Q. Zhu. 2003. Hormones in the grains in relation to sink strength and postanthesis development of spikelets in rice. *Plant Growth Regul.*, 41: 185-195.

Yang, J., J. Zhang, Z. Wang, K. Liu and P. Wang. 2006. Post-anthesis development of inferior and superior spikelets in rice in relation to abscisic acid and ethylene. *J. Exp. Bot.*, 57: 149-160.

Zhang, C., D. Jiang, F. Liu, J. Cai, T. Dai and W. Cao. 2010. Starch granules size distribution in superior and inferior grains of wheat is related to enzyme activities and their gene expressions during grain filling. *J. Cereal Sci.*, 51: 226-233.

Zhang, H., X. Zhou, J. He, T. Wang, X. Luo, L. Wang, R. Wang and Z. Chen. 2017. Impact of amylosucrase modification on the structural and physicochemical properties of native and acid-thinned waxy corn starch. *Food Chem.*, 220: 413-419.

Zhang, Z., X. Zheng, J. Yang, J. Messing and Y. Wu. 2016. Maize endosperm-specific transcription factors O2 and PBF network the regulation of protein and starch synthesis. *P. Natl. Acad. Sci. USA*, 113: 10842-10847.

Zhang, Z.L. and W.J. Qu. 2013. Plant Physiology Experiment Instruction. Beijing: Higher Education Press, 127-129.

Zhao, F., L. Jing, D. Wang, F. Bao, W. Lu and G. Wang. 2018. Grain and starch granule morphology in superior and inferior kernels of maize in response to nitrogen. *Sci. Rep.*, 8: 6343.

Zobel, H.F. 1988. Starch crystal transformations and their industrial importance. *Starch-Stärke*, 40: 1-7.

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