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Full Length Article

Kernel Filling Characteristics at Different Grain Positions on an Ear and their Relationship with Stalk Sugar Content in Maize

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Abstract

Kernel filling is related directly to kernel weight and yield. To analyze filling characteristics at different kernel positions on an ear and their relationship with stalk sugar in maize, we investigated kernel filling rate, kernel water content and stalk sugar content at different kernel position at 10, 20, 30, 40 and 50 days after pollination using two hybrids and their parents in 2015 and 2016, respectively. The results showed that filling rate at apical kernels of ear was significantly lower than that of middle and basal kernels, and the difference was maximum when filling rate reached its peak value. The relative differences between the middle and basal kernel filling rate is small, however, before the peak value, the basal kernel filling rate was higher than that of middle, and after the peak value, the filling rate of basal kernel was a little bit lower than that of middle. The kernel filling rates basically presented the tendency of low-high-low, but the genotype differences were also existed. 50 days after pollination, filling rates of upper part kernels still showed an increasing trend for some varieties. The stalk sugar had a close relationship with kernel filling rate, when the consumption of stalk sugar was in maximum value, filling rates of kernels was basically at its peak value, and the performance of hybrid was especially obvious. The utilization of hybrids with high stalk sugar and appropriate delay of harvest may be helpful for further increasing kernel weight in maize.

Keywords: Maize; Kernels positions on an ear; Grain filling rate; Assimilate distribution pattern

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops in the world, and is also the grain-forage crop with the biggest cultivated area and the highest total yield in China (Li *et al*., 2017). Maize yield is mainly determined by the kernel number per unit land area and the kernel weight (Chapman and Edmeades, 1999). Weight of individual kernels of an ear varies for different floret positions on the rachis because of the internal competition for assimilates and, therefore, results differ in the final kernel weight. Generally, the basal and middle kernel on the rachis have higher filling rate and longer duration of kernel filling which results in a higher final kernel weight, thus called as superior kernel. The apical kernels on maize ear are called as inferior kernel because of lower filling rate and shorter duration of kernel filling which results in a lower final kernel weight (Chen *et al*., 2013; Xu *et al*., 2013) and the apical kernels of maize ear (inferior kernel) have a higher possibility of abortion prior to the onset of kernel-filling (Wang *et al*., 2014).

Many studies in filling of inferior spikelets of rice had been carried out, however, there are many explanations to the poor grain-filling of inferior spikelets, including the source limitation (Wang, 1981; Murty and Murty, 1982), sink capacity limitation (Kato, 2004; Yang *et al*., 2006), low sink activities (Nakamura *et al*., 1989; Ishimaru *et al*., 2005) and “flow" impediment (Xiao *et al*., 1993; Huang *et al*., 2005). However, the physiological mechanisms of poor grain-filling of inferior spikelets in rice are still unknown. Recently, studies of positional effect of maize kernel filling have focused primarily on the development of kernels (Tollenaar and Daynard, 1978; Chen *et al*., 2013), nitrogen fertilizer strategies (Shen *et al*., 2005; Yang *et al*., 2014), grain physical characters (Zhang *et al*., 2015), hormone contents (Xu *et al*., 2013), plant density (Wang *et al*., 2011) and genotype (Zhang *et al*., 2007), etc.

A systematic study on physiological mechanism of filling differences of different kernel positions on maize is very important. The sucrose is the important photosynthate, main substance transported inside the plants, and major component of the stalk sugar content (Bian *et al*., 2006).

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The photosynthate needs to make the translocation constantly for maize kernels through the stalks to accomplish processes like kernel filling and dry matter accumulation. At present, information about the relationship between the maize stalk sugar content and kernel filling is scarce. Therefore, this study investigates the difference in filling rate along the rachis of the ear and determines the impact of the maize stalk sugar content on filling characteristics of the different kernel positions on maize ear.

Materials and Methods

Plant Materials

Two maize hybrids, Zhengdan 958 and Xianyu 335, and their parents were used in this study, and both hybrids have the largest planting acreage in China since 2000. Sowing, tasseling and silking dates for each tested materials in 2015 and 2016 are shown in Table 1.

Experimental Design

The field experiments were conducted in 2015 and 2016 at agricultural college of Yangzhou University (32°24' N, 119°26' E,), Yangzhou, China. During maize growth in 2015 (July–September), the accumulated temperature and rainfall were 2369.7°C and 439.3 mm, respectively; in 2016 (July–September), the accumulated temperature and rainfall were 2517.3°C and 789.9 mm, respectively (Table 2). The soil of the experimental plot was sandy loam.

Maize hybrids and its parents were planted in the experimental field in July 2015 and July 2016, respectively. A randomized block design was used with two replications of six lines in each trial. In both years, a one-row length of 2.5 m per plot and an inter-row space of 0.6 m were applied. The seeds were sown at 0.03 m depth and 0.25 m intervals. In each replication, each line was planted in six rows, and each row comprised 10 plants. The seeds were over-sown and then thinned to 66,670 plants hm^{-2} in 16–20 d after sowing. Nitrogen (N) fertilizer in the form of urea (with 46% N) was basally applied at a rate of 241.5 kg N hm^{-2}, the application amount of P (P_2O_5) and K (K_2O) were both 67.5 kg hm^{-2}.

Sampling Method

About 40 single plants (except the plants at the beginning and the ending of the row) whose growth status was relatively regular for each experimental material (six sets) were selected for determining relevant growth states (tasseling, silking, etc). Before silking, the ear was covered with the sulfuric paper bags uniformly to get segregated; all the materials had adopted the artificial self-pollination; each material was given saturated pollination to make it sure that the grain numbers of each ear were basically consistent; made a record for the pollination dates of different materials to determine the sampling time. The plants determining filling rate were also used to measure stalk sugar content.

Determination of Filling Rate and Dehydration Rate

Samples were taken respectively from each test materials after 10, 20, 30, 40 and 50 days of pollination, and 8 ears of same growing status were selected each time. Samples were taken every day between 7 a.m. and 8 a.m. and rapidly threshing.

According to the grain positions, kernels of upper 3 to 10 rings as apical part sample kernels (counting from the highest part of the ear) were taken, kernels of middle 13 to 22 rings (counting from the lowest part of the ear) as middle part sample kernels and kernels of lower 8 to 12 rings (counting from the lowest part of the ear) as basal part sample kernels were collected. Each part was randomly selected 50 (inbred lines) or 100 (hybrids) kernels to measure their fresh weight after the separate threshing and mixing of each part. Fresh weight of materials were calculated and then kept in an oven of 105°C for 30 min and then dry them to constant weight at a temperature of 80°C for dry weight.

Following formula was used to calculate

Kernel filling rate = (fresh weight of 100 kernels in the latter sample-taking time - fresh weight of 100 kernels in the former sample-taking time)/ Sampling interval.

Kernel water content (g/per 100 kernels) refers to the weight of water in 100 maize kernels.

Determination of Stalk Sugar Content

The leaves, sheathes and tassel of each sample were separated from the plant and juice was extracted with the help of juice extractor (Henglian TYZ-8.0) and put it in the culture dishes. After intensive mixing, 100 μL was taken to measure its sugar content with sugar meter (PAL-1, Japan).

Statistical Analysis

Use Microsoft Excel 2007 for data processing and mapping, use SPSS 16.0 software for data statistics and analysis. The trend of date in 2015 and 2016 is similar, so the data analysis is mainly based on 2015, and part of the data is the mean of 2015 and 2016 (Shi *et al*., 2013).

Results

Change of Stalk Sugar Content (Brix)

The stalk sugar content of hybrids and their parents showed decreasing trends from 10 to 50 days after pollination (Fig. 1). The stalk sugar content of inbred lines was generally higher than their hybrids. From 10-50 days after pollination, stalk sugar content of two hybrids, Xianyu335 (Fig. 1A)

Table 1: Sowing, tasseling, flowering and silking dates of each tested varieties

Material	Sowing (month/day)	Tasseling (month/day)	Flowering (month/day)	Silking (month/day)
2015				
Zhengdan958	7/13	9/3	9/5	9/4
Zheng58	7/13	9/6	9/10	9/9
Chang7-2	7/13	9/8	9/11	9/12
Xianyu335	7/13	9/5	9/5	9/4
PH6WC	7/13	9/7	9/11	9/12
CPH4CV	7/13	9/8	9/10	9/10
2016				
Zhengdan958	7/11	8/31	9/2	9/2
Zheng58	7/11	9/4	9/7	9/8
Chang7-2	7/11	9/5	9/10	9/12
Xianyu335	7/11	9/2	9/9	9/8
PH6WC	7/11	9/4	9/8	9/8
CPH4CV	7/11	9/7	9/10	9/11

Table 2: Total monthly precipitation (mm), monthly average temperature (ºC) and total monthly sunlight (hour) during the growth period of maize in 2015 and 2016

Year	Month								
	7			8			9		
	Total precipitation (mm)	Average temperature (℃)	Total sunlight (hour)	Total precipitation (mm)	Average temperature (℃)	Total sunlight (hour)	Total precipitation (mm)	Average temperature (℃)	Total sunlight (hour)
2015	122.3	26.6	153.7	261.6	27.4	197.7	55.4	23.3	201.4
2016	385.1	29.0	180.4	138.5	29.0	241.4	266.3	24.1	166.9

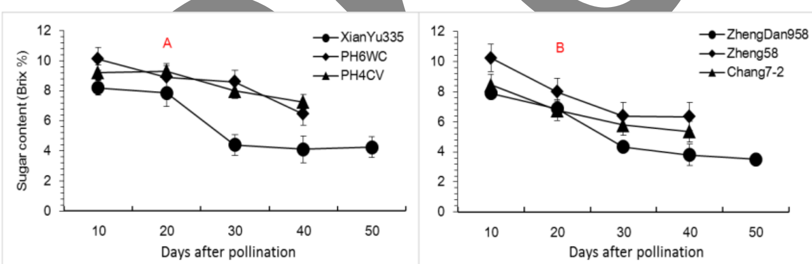

Fig. 1: Maize stalk sugar content in different measurement stage

and Zhengdan985 (Fig. 1B), had similar decreasing trends; However, change of stalk sugar content for their parents (four inbred lines) were different, compared with parents of Zhengdan958 (Zheng58 and Chang7-2), the stalk sugar content of two parents of Xianyu335, PH6WC and CPH4CV decreased slowly (Fig. 1). Variance analysis showed that there was a significant difference in stalk sugar content among 6 tested materials (F=79.55, P < 0.001), and the difference of stalk sugar content at different measurement stages were also significant (F=9.25, P < 0.001).

Filling Dynamic of Kernels at Different Kernel Positions

Filling rates of kernels at different kernel positions (apical, middle, basal) had few differences at the early filling stage. With the extension of filling period, the differences enlarged while the difference between filling rates of kernels at middle and basal positions remained small. When the filling rate was peak value, the difference between filling rates of kernels at apical part and the middle and basal part became the biggest (Fig. 2). The analysis of variance showed that filling rates of different kernel positions was very significant different (F=5.99, P < 0.01). As can be seen from Fig. 2, the filling rate of hybrids reached the peak at 30 days after the pollination, and the filling rate peak of parental inbred lines except PH4CV (20 days after the pollination) was earlier than that of hybrids. Before and after the peak, the filling rates of kernels at different kernel positions showed differences. Before and at the peak, the filling rates of hybrids and parental inbred lines were shown basically as kernels at basal part > kernels at middle part > kernels at apical part. After the peak, the filling rate of middle part kernels was higher than that of basal part kernels, and that of apical part kernels remained the lowest. For filling rates of apical part kernels, one hybrids and two parental inbred line including Xianyu335 (Fig. 2A), PH4CV (Fig. 2C) and Zheng58 (Fig. 2E) still showed an increasing trend in the

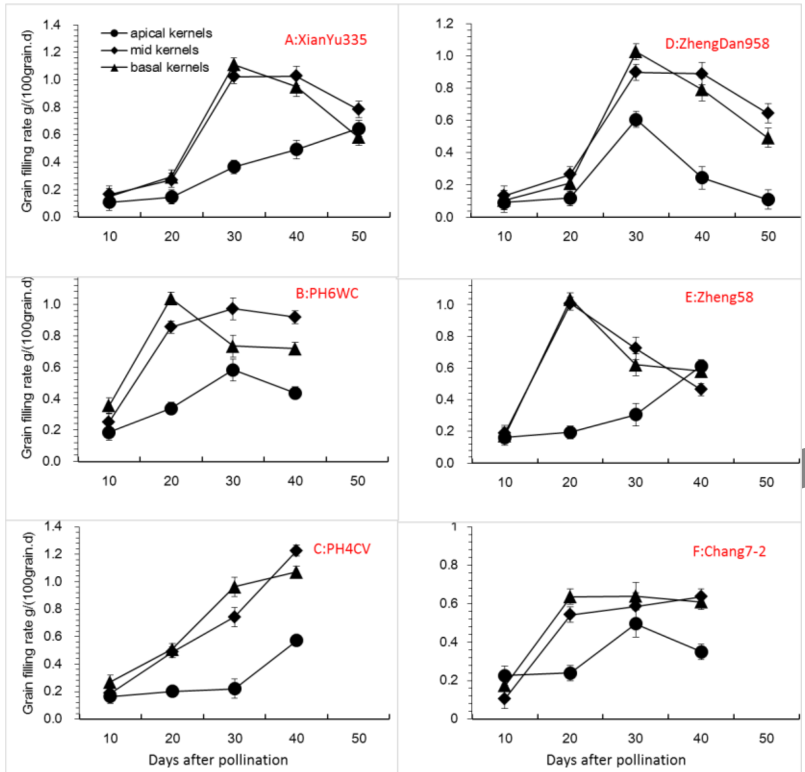

Fig. 2: Kernel filling rate at different grain positions of maize ear

later period. It is possible to further increase weight of apical position kernels at the later stage of growth if the growth status of maize plant is good.

There was genotype difference of kernel position effect for filling rate. The filling rates of kernels at different kernel positions for inbred line PH4CV (Fig. 2C) showed an increasing trend from 10 to 40 days after pollination, but kernel filling rates of other tested materials showed a low-high-low trend basically. The kernel filling characteristics of PH4CV probably had some effects on the continuous increase of filling rate of the kernels at the ear's apical part of its hybrid Xianyu335 (Fig. 2A).

Water Dynamic of Kernels at Different Grain Positions

Among different varieties, there was significant difference for kernel water content ($P < 0.001$); the significant level was the same as different varieties for different measurement stage. However, among different kernel position, there was no significant difference for kernel water content (F=0.12, P=0.8832). Form 10 to 50 day after pollination, the performance of kernel water content was low-high-low trend; water content of kernel was peak value at 20 days after the pollination (Fig. 3). 15 to 20 days after pollination was formation stage of maize kernels, and kernel size increased rapidly, while dry matter accumulated little and water content increased rapidly. Therefore, the results were consistent with the formation and enrichment characteristics of maize kernels.

The Relationship between Stalk Sugar Content and Kernel Filling Rate

Sucrose is an important photosynthetic product, which is the main substance of transportation in plant, and also the main component of sugar (Nakamura *et al.,* 1989). Maize stalk is the main organ of transportation of photosynthate to kernel, and the relationship between sugar content and kernel filling rate shall be concerned. At the tenth day after pollination, the kernel filling rate was a very low level, while stalk sugar content was a very high level; with the increase of kernel filling rate, the sugar content of stalk decreased (Fig. 1 and 2). In performance of the relationship between sugar consumption and kernel filling rate, hybrids Xianyu335 and Zhengdan958 showed no differences (Fig. 4A and D and Table 3); at the thirtieth day after pollination, the kernel filling rate was the highest, and the sugar consumption was the highest too. The other 3 parental inbred lines except PH4CV had also the same performance in the relationship between sugar consumption and kernel filling rate, and when stalk sugar consumption was the maximum, their filling rates basically reached the highest value (Fig. 4B, E and F and Table 3).

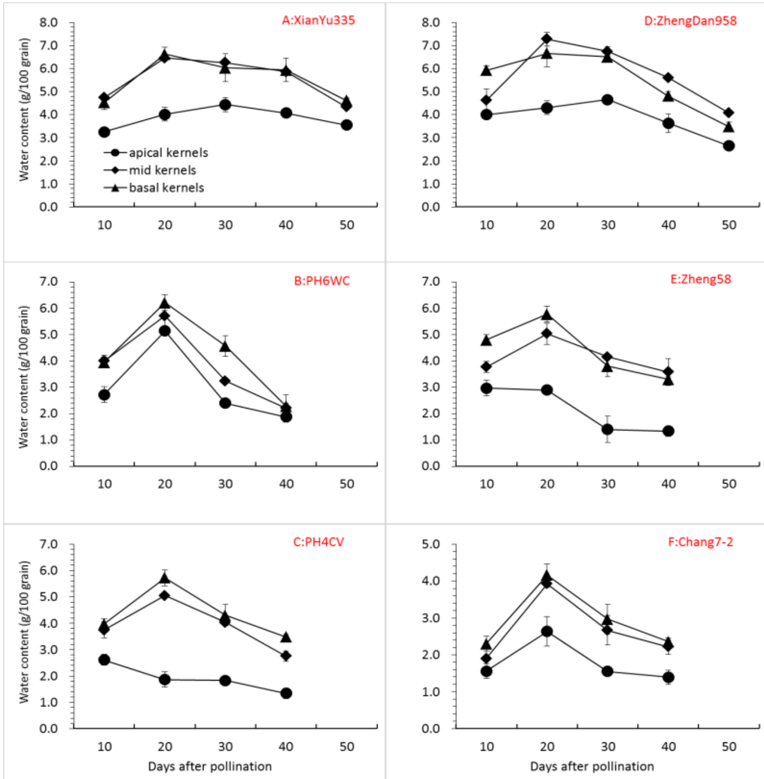

Fig. 3: Kernel water content at different grain positions of maize ear

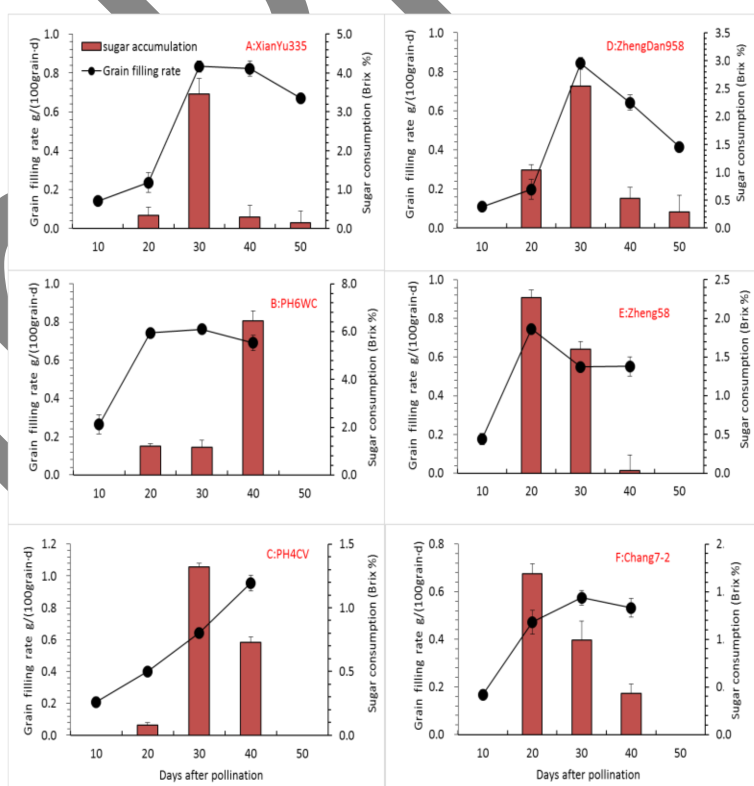

Fig. 4: The relations between grain filling rate and stalk sugar consumption

Table 3: Stalk sugar consumption at different grain filling stage

Materials	Characters	Number of days after pollination (d)				
		10	20	30	40	50
Xianyu335	Filling rate g/100grain.d	0.14	0.24	0.83	0.82	0.67
	Sugar consumption Brix %		0.34	3.46	0.29	0.14
PH6WC	Filling rate g/100grain.d	0.26	0.74	0.76	0.69	
	Sugar consumption Brix %		1.21	1.16	6.46	
PH4CV	Filling rate g/100grain.d	0.21	0.40	0.64	0.96	
	Sugar consumption Brix %		0.08	1.32	0.73	
Zhengdan958	Filling rate g/100grain.d	0.11	0.20	0.84	0.64	0.42
	Sugar consumption Brix %		1.04	2.54	0.53	0.29
Zheng58	Filling rate g/100grain.d	0.18	0.75	0.55	0.55	
	Sugar consumption Brix %		2.27	1.60	0.03	
Chang7-2	Filling rate g/100grain.d	0.17	0.47	0.57	0.53	
	Sugar consumption Brix %		1.69	0.99	0.43	

Discussion

Maize is the largest food forage crop in China, so the high yield of maize is an eternal topic. The yield of maize was composed of three factors: ear number per unit area, grain number per ear and grain weight. In agricultural production, the number of ears and the grain number per ear are the most easily adjusted factors of three yield components (Cao *et al.*, 1993), and the way of increasing density has been widely used to obtain high maize yield. In the Huang Huai Hai region, the filling time of summer maize is short, and there are plenty of rainy and cloudy days during the filling period. Besides, with the habit of early harvest of farmers, grain weight instability becomes the main reason for the large fluctuation of summer maize yield in Huang Huai Hai region among years (Wang *et al.*, 2012). Therefore, it is necessary to set grain weight improvement as the main direction of maize super-high yield.

Grain weight of maize is different because of difference in the position of ears. Normally, the kernels grown in the basal and the middle part of the ear are filled better and have higher grain weight, thus identified as superior kernels; the kernels grown in the apical part of the ears are filled worse and have lower grain weight, which are identified as inferior kernels (Xu *et al.*, 2013). The effect of kernel position existed in filling rates in different parts of the maize ears (Shen *et al.*, 2005; Yan *et al.*, 2007; Xu *et al.*, 2013; Qiao *et al.*, 2016), this study showed that the grain position effect of filling rate was different before and after the peak value of filling rate. Before and at the peak, the filling rates were basically showing as kernels in the basal part＞kernels in the middle part ＞ kernels in the apical part, after the peak, the filling rate of kernels in middle part was higher than that of kernels in basal part, and the filling rate of kernels in the apical part remained the lowest.

The top flowers of ear developed late, and the silking extracted late. In the case of natural pollination, pollination and fertilization were late for top flowers of ear and the early fertilized ovaries in the middle and basal parts had location inhibition on that of top flowers (Otegui *et al.*, 1995; Jorgelina *et al.*, 2000). In this study, the effects of pollination and fertilization on the development of top flowers were excluded by the method of artificial pollination, but the filling rate of kernels in the apical part was still in an inferior state. The sugar content (photosynthetic product) of the kernels in the upper part was not enough, which resulted in low accumulation of final material of kernels in apical part (Qiao *et al.*, 2016). The imbalance in the supply of assimilates may be an important reason for the differences in kernel development between upper and middle and lower parts (Shen *et al.*, 2005). This study found out that the kernel filling rate of different materials had a different downtrend at later stage, the authors believed that a higher filling rate at later stage such as PH4CV (Fig. 4C) was beneficial for improving the weight of kernels in the upper part.

Maize ears were generally in cylindrical or conical shape, and the apical diameter was always smaller than the middle and basal parts. The distribution of geometric space of kernels in maize ears have also affected the kernel storage capacity (kernel size) in the apical part; the previous studies (Tollenaar and Daynard, 1978; Borrás *et al.*, 2004) also confirmed that the small storage capacity was the main cause of slow accumulation of dry matters of kernels in apical part, therefore, selection of cylindrical ears was beneficial for improving the weight of kernels in upper part of ears. Grain weight was an important component of maize yield, and the advantages or disadvantages of kernel filling were directly related to grain weight and yield. Therefore, it was very important to strengthen the study of filling characteristics and mechanism of inferior kernels (kernels in apical part).

Grain weight of maize was closely related to starch accumulation. The content of soluble sugar (sucrose and reducing sugar) is of decisive significance to the accumulation of starch. Shen *et al.* (2005) observed that sucrose and total sugar content in kernels of apical part were significantly lower than those in the middle and basal parts, and the total sugar content was consistent with the decrease of filling rate. It was suggested that the imbalance in the supply of assimilates was an important reason for the difference of grain development between the apical part and the middle and basal part. Hua *et al.* (2016) found that the change trend of sugar content in maize stalk at different growth stages was related to factors such as kernel filling rate, photosynthetic product synthesis and plant consumption, etc. The sucrose is the important photosynthate, main substance transported inside plants (Jang and Sheen, 1997; Zhang and Li, 2002), and major component of stalk sugar content (Bian *et al*., 2006). Sucrose is closely related to the biosynthesis of starch (Li *et al*., 2016). The stalk sugar in maize was an important source of kernel filling, which is consistent with the results of the current study. Of course, the sugar content in maize stalk was the result of the accumulation of photosynthetic products during the whole growth of maize. When the consumption of stalk sugar for inbred line PH4CV was at its peak value, the kernel filling rate was only 0.64 g/(100 grain.d), which was rather low (Fig. 4C), implying that its stalk sugar may be consumed in the growth and development of other organs.

Conclusion

It can be concluded that the filling rate at the apical kernels was significantly lower than that of the middle and basal kernels, and the difference was in maximum when filling rate reached its peak value. Furthermore, the stalk sugar content had a close relationship with kernel filling rate of both maize hybrids. Thus, hybrids with high stalk sugar and appropriate delay of harvest would help in improvement of yield.

Acknowledgments

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