Delayed pollination and low availability of assimilates are major factors causing maize kernel abortion

Si Shen\(^1\), Li Zhang\(^1\), Xiao-Gui Liang\(^1\), Xue Zhao\(^1\), Shan Lin\(^1\), Ling-Hua Qu\(^1\), Yun-Peng Liu\(^1\), Zhen Gao\(^1\), Yong-Ling Ruan\(^2\), and Shun-Li Zhou\(^1,3,\)

\(^1\) College of Agronomy and Biotechnology, China Agricultural University, Beijing, 100193, China
\(^2\) School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia
\(^3\) Scientific Observation and Experimental Station of Crop High Efficient Use of Water in Wuqiao, Ministry of Agriculture, Wuqiao 061802, China

* Current address: School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW, 2308, Australia

Correspondence: zhoushl@cau.edu.cn or yong-ling.ruan@newcastle.edu.au

Received 05 July 2017; Editorial decision 08 January 2018; Accepted 09 January 2018

Abstract

Selective seed abortion is a survival strategy adopted by many species that sacrifices some seeds to allow the remaining ones to set. While in evolutionary terms this is a successful approach, it causes huge losses to crop yields. A pollination time gap (PTG) has been suggested to be associated with position-related grain abortion. To test this hypothesis, we developed a novel approach to alter the natural pattern of maize (\textit{Zea mays} L.) pollination and to examine the impact of PTGs on kernel growth and the underlying physiological basis. When apical and basal kernels were synchronously pollinated, the basal kernels set and matured but the apical kernels were aborted at an early stage. Delaying pollination to the basal ovaries suppressed their development and reduced invertase activity and sugar levels, which allowed the apical kernels to set and grow normally. \textit{In situ} localization revealed normal cell wall invertase activity in apical and basal kernels under synchronous pollination but reduced activity in the delayed-pollinated kernels independent of their position. Starch, which was abundant in basal kernel areas, was absent in the apical kernel regions under synchronous pollination but apparent with delayed pollination. Our analyses identified PTG-related sink strength and a low level of local assimilates as the main causes of grain abortion.

Keywords: Abortion, ethylene, fructose, glucose, invertase, kernel set, maize, pollination time, sucrose.

Introduction

The angiosperms are known to produce more flowers and ovules than mature fruits and seeds. Competition for survival among developing progenies results in selective fruit and seed abortion to allow the remaining ones to survive (Stephenson, 1981; Ganeshaiah and Shaanker, 1988; Ruan, 2014; Tardieu \textit{et al.}, 2014). Ovule abortion occasionally occurs during the pre-fertilization stage owing to (i) silk senescence (Bassetti and Westgate, 1993; Bassetti and Westgate, 1994) or (ii) insufficient or feeble pollen grains (Campbell and Halama, 1993; Ehrlien and Eriksson, 1995; Goldingay, 2000). However, pollen limitation is not a common occurrence in many angiosperms (Bawa and Webb, 1984; Burd, 1994; Larson and Barrett, 2000; Sakai and Harada, 2001). Instead, post-fertilization seed abortion can frequently be induced due to limitations in the available...
resources (Snow and Spira, 1991; Arathi et al., 1999; Yang et al., 2005; Brookes et al., 2008; Arathi, 2011), and it can become more severe under unfavorable circumstances, including drought (Otegui et al., 1995; Andersen et al., 2002; McLaughlin and Boyer, 2004a, b), heat (Cheikh and Jones, 1994; Hays et al., 2007; Liu et al. 2016), and other environmental stresses (Andrade et al., 2002; Hiyane et al., 2010). Given the increased frequency and severity of climate-change-related stresses, seed abortion has become a central issue for food security in the 21st century (Grassini et al., 2013; Ray et al., 2013).

Maize (Zea mays L.) is one of the most important crops in the world, ranking third in production acreage and first in grain production worldwide. In practical production, the maize ear shows a base-to-apex gradient of abortion frequency, in that kernels in the apical area have a higher probability of abortion (Cárcova and Otegui, 2001; Uribelarrea et al., 2008; Feng et al., 2011). The phenomenon of non-random kernel abortion is similar to that in those species with a linear arrangement of seeds, such as legumes (Rocha and Stephenson, 1990, 1991), wheat (Yang et al., 2006; Hays et al., 2007), and rice (Wang et al., 2011; Chen et al., 2013). In these species, the probability of seed abortion is highly related to the seed position in the ear or panicle. However, seeds from the disadvantageous position can develop normally on culture medium containing sufficient nutrients (Nakamura, 1986), or if some of the seeds from the more advantageous position are removed (Rocha and Stephenson, 1991; Feng et al., 2011). These observations indicate that the linear probability of abortion is not due to poor seed vigor. Instead, position-related abortion may relate to poor access to maternal resources (Watson and Casper, 1984) and the time of fertilization (Cárcova and Otegui, 2001). However, there is still a lack of experimental evidence to support these theories, despite the existence of a large number of studies investigating the possible basis of seed abortion at the physiological and molecular levels, including sugar deprivation (Hiyane et al., 2010; Kakumanu et al., 2012), ethylene regulation (Hays et al., 2007; Feng et al., 2011), and deficiency in invertase activity (Ruan et al., 2012). In this context, the pollination time gap (PTG) was recently suggested to be an initial and primary cause of maize kernel abortion, which occurred earlier than abortion due to sugar depletion in response to drought (Oury et al., 2016a, b). Whether these putative factors are the causes or the consequences of seed abortion is still disputed, and little is known about their relationships with PTGs in linear-arranged seed or grain abortion (Boyer and McLaughlin, 2007; Ruan et al., 2012; Oury et al., 2016a). We aimed to address these issues by using the maize kernel as an experimental model.

Technically, it is extremely difficult to appreciate the effects of PTGs on maize kernel growth. PTGs depend on the time differences between the pollination of early- and late-appearing silks. The pattern is difficult to change because maize silks emerge and elongate under the protection of husks. Some previous studies attempted to change the PTGs by altering growth conditions via heat or water deprivation treatments during the silking period (Cárcova and Otegui, 2001; Oury et al., 2016a, b). However, altering growth conditions influences not only the PTGs, but also kernel growth and metabolism. In this study, we tested the effect of PTGs on maize kernel development by using an innovative approach in which we cut the basal silks to prevent natural pollination and then provided them with fresh pollen grains at the specified time points to conduct delayed pollination (DP) of the basal kernels. In this way, the inherent pollination pattern was changed without altering the environmental conditions. The effects of PTGs on kernel growth, invertase activity, and sugar and ethylene levels were investigated.

Materials and methods

Plant materials and management

Maize plants were grown at the Wuqiao experimental station of China Agricultural University (37°29 ’–37°47’N; 116°19 ’–116°42’E) in Hebei Province, China. Two maize hybrids, ZhengDan 958 (ZD958) and DengHai 605 (DH605), with normal and large ear sizes, respectively, were chosen for use in the experiments. Seeds were sown at a density of 90000 plants/ha with basal compound fertilizer (750 kg/ha; N 15%, P2O5 15%, K2O 15%) and top-dressed with urea (245 kg/ha; N 46%) applications at the 13-leaf stage. The ears of chosen plants were bagged before the silking stage and then were hand-pollinated. Pesticides were applied as necessary to protect the plants from insects and diseases. There was no evidence of either water or nutrient stress during the growth period.

Identification of basal silks, basal kernel delayed pollination, and validation of the approach

To verify the location of basal silks among the silk cluster, we carefully pulled off husks to observe the spatial structure of silks. As the silks were arranged in an orderly fashion, the silks from the basal area were found to locate at the outer rings of the silk cluster (Supplementary Fig. S1 at JXB online). To further verify that the silks were derived from the basal position of the cobs, we cut the silk cluster to a length of 2 cm from the husks at 5 days after the emergence of all silks, so that the silks could be uniformly and neatly arranged. We used a red dye to mark the silks located in the outer rings of the cluster, and then carefully pulled off the husks without breaking off the silks. The positions from which the marked silks initiated on the ear were observed and identified (Supplementary Fig. S1).

After identifying the basal silk position, we applied the DP treatment to the basal kernels (Fig. 1; Supplementary Fig. S2). At the junction of the middle and basal kernels, early pollinated and delayed pollinated kernels in the same ear could be easily distinguished by their color and size at an early stage (Fig. 2) or by their size and shape at maturity (Supplementary Fig. S3). Synchronously pollinated kernels (with normal basal kernels) were used as a positive control, and incompletely pollinated kernels as a negative control (unfertilized ovaries were covered by the glumes; see Fig. 2 and Supplementary Fig. S3).

To assess the level of silk receptiveness to DP, we conducted further experiments to expose emerged silks to fresh pollen grains from the day of silk emergence to 14 days thereafter at 1-day intervals (Supplementary Fig. S4). These experiments revealed that the number of fertilized basal kernels remained unaffected until 9 days after silk emergence, indicating the receptiveness of these silks to DP (Supplementary Fig. S4). Even with pollination at 11 days after silk emergence, we still observed at least 50% of grain set at the basal region, demonstrating the receptiveness of the basal silks at this stage, which is equivalent to pollination delayed by 6 days (see Figs 1 and 3).

Pollination treatments and sampling

First, an experiment (experiment I) was designed to demonstrate the relationship between pollination and kernel growth in different ear regions during the early growth period (Fig. 3A). Five different pollination treatments were used: synchronous pollination (SP), in
Delayed pollination represses maize kernel development

which all silks were hand-pollinated synchronously; incomplete pollination (ICP), in which the basal silks were prevented from being pollinated; and three DP treatments, in which the pollination of basal kernels was delayed for 2 (DP-2D), 4 (DP-4D), and 6 days (DP-6D), respectively (Fig. 3A).

Sampling was performed at 4, 8, 12, and 16 days after the first pollination. Apical kernels and all middle kernels were sampled from five rings of kernels on the apical and middle ear regions; the delayed-pollinated basal kernels were identified and sampled. Specifically, the SP apical kernels were chosen from those apical kernels whose growth ceased and were aborted from 4 to 16 days after pollination (DAP).

In the DP-6D treatment, owing to the extensive failure of fertilization, only the delayed-pollinated and fertilized kernels were sampled (Fig. 2; Supplementary Fig. S3). The delayed-pollinated basal kernels were identified based on their size and color or shape (as described above). Four biological replicates were used in each case.

A second experiment (experiment II) was conducted to investigate the effect of PTGs on cell wall invertase (CWIN) activity. In this experiment, the DH605 hybrid was exposed to the SP treatment and basal and apical kernel DP treatments, in which pollination was delayed for 6 days for the basal and apical kernels separately. Fresh kernels were sampled 2 days after the apical or basal kernels were pollinated (Fig. 3B) and were sliced to localize CWIN activity as described by McLaughlin and Boyer (2004a) (described below); at this time point, there was no obvious difference in kernel weight or size between apical or basal DP kernels and SP kernels from the same region of the ear.

**Determination of fresh and dry weights**

The fresh weight of kernels was measured when they were detached from the ear on the sampling day. The dry weight of the kernel sample was determined after drying the samples at 80°C for 48 h to a constant weight.

**Assay of sucrose, glucose, and fructose levels**

Carbohydrate extraction was performed according to Hanft and Jones (1986) with slight modifications. Sucrose, glucose, and fructose were then separated using high-performance liquid chromatography (HPLC). Before injection, the carbohydrate extraction solution was filtered through a 0.22 μm Millipore filter. The HPLC system consisted of a Waters 2414 Refractive Index Detector, a Waters 600 Pump, a Waters 600 Controller, and Waters XBridge Amide Columns. The mobile phase was 80% acetonitrile and 20% ultrapure water (containing 0.1% ammonium hydroxide); the pump was set to a flow rate of 1.0 ml min⁻¹. The sugar concentrations were quantified using external standards of sucrose, glucose, and fructose (Sigma-Aldrich).

**Starch determination and localization**

Starch was extracted according to Hanft and Jones (1986). Starch localization was performed for freshly detached kernels at 8 DAP. The kernels were sliced by hand and then stained with I₂-KI solution.
Shen et al. [0.2% I$_2$(w/w) and 0.53% KI (w/w)] for 1 min, briefly washed with water, and viewed under a dissecting microscope.

Measurement of invertase activity

Cell wall and soluble acid invertase extracts were prepared according to Zinselmeier et al. (1999). Invertase activity was determined at pH 4.8 with a reaction in 100 mM sucrose at 30 °C for 30 min. The reaction was terminated by boiling the samples in water for 5 min. Invertase activity was quantified by using a spectrophotometer (Persee Corporation) at a wavelength of 540 nm after reaction with dinitrosalicylic acid solution at 100 °C for 2 min.

In situ localization of cell wall invertase activity

CWIN activity was localized in situ according to the method described in McLaughlin and Boyer (2004a). Briefly, kernel slices were washed for 5 h with constant stirring, using flowing water to ensure that the soluble sugars in the slices were removed. The washed slices were exposed to the reaction medium for 30 min at 25 °C. The sections were then washed briefly with water, fixed in a 4% solution of formaldehyde, viewed under a dissecting microscope and imaged with a camera.

Ethylene measurement

Ethylene was collected and measured according to Cheng and Lur (1996) and Feng et al. (2011). A gas chromatograph (Shimadzu GC-17A) with a flame ionization detector and an alumina column was used to measure the ethylene emissions. The column, injector, and detector temperatures were 50, 120, and 120 °C, respectively. The carrier gas was N$_2$ with a flow rate of 50 ml min$^{-1}$, the burning gas was H$_2$ with a flow rate of 70 ml min$^{-1}$, and the assist gas was air with a flow rate of 500 ml min$^{-1}$.

Statistical analyses and illustration drawing

All measurements described comprised at least four biological replicates. Statistical analyses were conducted by using Student’s $t$-test. For data presented in bar charts, one-way analysis of variance was conducted by using Duncan’s new multiple range test, with statistical significance accepted at $P<0.05$. Statistical analyses were performed using SPSS Statistics and Excel software. Illustrations were drawn in Origin, Adobe Photoshop, and PowerPoint software.

Results

Delayed pollination suppressed kernel development

ZhengDan 958 (ZD958) and DengHai 605 (DH605) maize exhibited similar patterns of early kernel development (Fig. 2). The asynchronous hand-pollination treatments broke the temporal patterns of natural pollination (Fig. 2). Basal
Delayed pollination represses maize kernel development

kernels derived from DP were smaller in size and lighter in color compared with the preferentially pollinated middle kernels and with synchronously pollinated basal kernels in the SP treatment; this difference in appearance was more profound in the treatments with increased PTGs (Fig. 2K–T). The development of apical kernels was obviously improved by delaying or preventing the pollination of basal filaments (Fig. 2A–D, F–I). Specifically, in the DP-6D treatment, some of the basal kernels failed to develop from the fertilization stage (Fig. 2D, I). This failure in kernel development may be due to decreased silk receptivity when pollination is delayed (Bassetti and Westgate, 1993). However, there were still some delayed-pollinated kernels, which were successfully fertilized, developed, and evidently larger than the unfertilized basal ovaries in the ICP treatment (Fig. 2N, O, S, T). At maturity, the fertilized aborted basal kernels were smaller and had abnormal shapes compared with the basal kernels in the SP treatment (Supplementary Fig. S3).

Some of the apical kernels in the SP treatment were aborted in the early growth stage (Fig. 4). Delaying or stopping pollination of the basal kernels in the DP and ICP treatments restored the growth of the apical kernels to different degrees. At maturity, the ICP treatment yielded the highest fresh weight of apical kernels, followed by the DP-6D, DP-4D, DP-2D, and SP treatments in order (Fig. 5). For kernels in the middle of the ear, there were no obvious differences in fresh weight across the treatments, in which all showed rapid growth (Fig. 4B, E) and developed into normal grains (Fig. 5; Supplementary Fig. S3). The fresh weight of the basal kernels in the DP treatments increased slowly compared with those in the SP treatment, and the degree of this growth retardation correlated with the length of delay of pollination (Fig. 4C, F). The basal kernels derived from the DP-2D and DP-4D treatments developed into normal seeds with slightly reduced weight at maturity (Fig. 5; Supplementary Fig. S3), whereas basal kernels from the DP-6D treatment displayed the same growth-inhibition phenotype as the aborted SP apical kernels, whose growth ceased early in development (Fig. 4C, F; Supplementary Fig. S3).

Sugar concentrations were reduced in response to delayed pollination of basal kernels

Among apical kernels, the sucrose concentration was lowest in the SP treatment at the early growth stage (Fig. 6A, D), but was significantly increased by delayed or stopped pollination of the basal kernels in the DP and ICP treatments (Fig. 6A, D). The sucrose concentration in the middle kernels was maintained at a higher level than in the apical kernels (Fig. 6). In basal kernels, the sucrose concentration was similar in the SP, DP-2D, and DP-4D treatments for both ZD958 and DH605 (Fig. 6C, F), whereas in both hybrids the DP-6D treatment was associated with the lowest sucrose concentration at 12 DAP (Fig. 6F). Thus, delaying the pollination time
of the basal kernels reversed the pattern in the SP treatment, in which sucrose was preferentially allocated to the basal kernels. Interestingly, the SP apical kernels and the DP-6D basal kernels were aborted with different sucrose concentrations (Fig. 6), implying that different mechanisms underlie kernel abortion in these two kernel types.

In apical kernels, hexose concentrations rapidly decreased after pollination in the SP treatment, which indicates sugar depletion in the aborted apical kernels. In contrast, hexose concentrations in the normally set apical kernels in the DP and ICP treatments increased in ZD958 and were maintained in DH605 (Fig. 6). In DP-6D basal kernels, the reduction in hexose concentrations was much larger than the reduction in sucrose concentrations (Fig. 6); this finding indicates that the degradation of sucrose into hexoses in basal kernels was suppressed by the DP treatments, especially by DP-6D.

**Starch quantity and localization differed in kernels exposed to different pollination treatments**

Localization analysis revealed abundant starch in the basal to middle kernels in all pollination treatments but, surprisingly, no starch in the apical kernels in the SP treatment, where the ovaries in the apical region were pollinated at the same time as those in the cob base (Fig. 7). Significantly, delayed or no pollination of the basal ovaries allowed starch to be synthesized in the apical kernels (Fig. 7). Quantification of the starch content confirmed an increase in the starch level in apical kernels and a decrease
in basal kernels associated with DP of the basal kernels (Supplementary Fig. S5). These data indicate a deficient supply of assimilates to the apical kernels and a sufficient supply to the basal kernels (Fig. 7).

**Delayed pollination inhibited invertase activity**

Soluble invertase and CWIN play major roles in controlling sink strength and plant fertility (Ruan, 2014; Wan et al.,...
Enzyme assay revealed that the activities of these enzymes were altered by the different pollination treatments (Fig. 8). Invertase activities were lowest in the apical kernels from the SP treatment, and were higher to various degrees in the ICP and DP treatments (Fig. 8). In the basal kernels, the activities of the two invertases were reduced by different degrees in response to DP, with the lowest activities being detected in the DP-6D treatment (Fig. 8). This observation corresponded with a slight reduction in sucrose concentrations and a more pronounced reduction in hexose concentrations (Fig. 6). Considering that the duration of growth of DP-6D basal kernels sampled at 12 and 16 DAP were practically 6 and 12 days, respectively (i.e. if comparisons were performed according to the duration of growth instead of the sampling date), the activities of the invertases were dramatically reduced in the DP-6D treatment, whereas invertase activities in the DP-2D and DP-4D treatments were somewhat lower than those recorded in the SP treatment.

To further verify this relationship between the PTGs and CWIN in the apical and basal kernels, experiment II was conducted using synchronous pollination and a 6-day delay in apical and basal pollination (Fig. 3B). In the SP treatment, strong CWIN activity was detected in both apical and basal kernels (Fig. 9A, D). However, DP of basal ovaries dramatically reduced CWIN activity in the basal kernels compared with the activity in kernels in the same position from the SP treatment (Fig. 9D, E). Application of the DP treatment to apical ovaries also reduced CWIN activity in the kernels that developed in this region (Fig. 9C) without affecting the activity in the basal region (Fig. 9F). Overall, CWIN activity was suppressed by DP independent of kernel position (Fig. 9) and was evident prior to the emergence of differences in sugar contents (Fig. 9).

**Endogenous ethylene release in aborted kernels**

As one of the plant hormones, ethylene has been implicated in grain abortion in cereals (Young and Gallie, 1999; Hays et al., 2007). Here, we found that, in aborted SP apical and DP-6D basal kernels, ethylene levels remained at a high level early in development; in contrast, in normally set grains, ethylene emission decreased rapidly from 4 DAP and then remained at a low level (Fig. 10).

---

Fig. 7. Staining of starch in sections of fresh kernels of ZhengDan 958 (ZD958) and DengHai 605 (DH605) hybrid maize at 8 days after pollination. Starch was located around the nucellus and vascular tissues in the pedicel. Abundant starch was present in the middle and basal kernels in all pollination treatments; in the apical kernels, starch was absent in the synchronous pollination (SP) treatment but recovered in the delayed pollination (DP) and incomplete pollination (ICP) treatments. DP-2D, pollination time for basal kernels delayed by 2 days; DP-4D, pollination time for basal kernels delayed by 4 days; DP-6D, pollination time for basal kernels delayed by 6 days. Scale bar=5 mm.
Delayed pollination represses maize kernel development

Discussion

Weak competition for assimilates among kernels on different ear regions is the primary driver of post-fertilized kernel abortion

From an evolutionary perspective, the advantage of overproduction of ovaries relative to the number of seeds set could be explained by (i) a ‘bet-hedging’ strategy in which plants could quickly adjust the number of seeds in response to unpredictable environments (Lloyd, 1980; Kozlowski and Stearns, 1989; Burd, 1998; Arathi, 2011) and (ii) a mechanism for eliminating ovaries of low quality and survival probability, in order to efficiently invest resources in more competitive ovaries (de Jong and Klinkhamer, 2005; Mena-Ali and Rocha, 2005; Arathi, 2011), especially when resources are deficient, for example, under abiotic stresses (Ruan, 2014; Tardieu et al., 2014). This hypothesis predicts that kernel set or abortion closely relates to the amount of resources available. Consistent with this hypothesis, many previous studies have demonstrated that limitation of assimilates drives fruit and seed abortion (Reynolds et al., 2009; Egli, 2010; Ruan et al., 2012). However, several recent studies have suggested that sugar deprivation is a consequence rather than a cause of kernel abortion, because genes affecting expansive growth were influenced earlier than the genes affecting sugar metabolism in aborted kernels (Oury et al., 2016a, b). Nevertheless, these conclusions were reached under conditions of water deficit, which could independently influence expression of genes related to kernel growth (Kakumanu et al., 2012).

We showed in this study that artificially preventing basal kernel set by delaying pollination activated the apical kernels, which would otherwise have been aborted, to grow normally. We also observed that even under severe water stress, apical kernels could develop when basal kernels were repressed by blocking pollination (data not shown). These findings demonstrate the existence of competition between kernels in the basal and apical regions for the limited assimilates. Notably, the apical kernels still aborted in the SP treatment when no environmental stress was imposed (Fig. 2A, F; Supplementary Fig. S3), indicating that kernel abortion could be triggered even if pollination takes place. Collectively,
these findings strongly suggest that limited availability of assimilates or resources is the driver of kernel abortion.

Pollination time and ear region are early determinants of assimilate partitioning and selective kernel abortion

Sucrose is the major photoassimilate translocated from source to sink organs such as developing kernels. Sucrose deficiency has been proposed to be the major cause of growth suppression of maize kernels (Hanft and Jones, 1986; Boyle et al., 1991; McLaughlin and Boyer, 2004a; Hiyane et al., 2010).

Consistent with this proposal, sucrose was significantly lower in aborted SP apical kernels at the early growth stage and higher in developed apical kernels following ICP to the basal ovaries. Phloem-unloaded sucrose could be used in maize kernels for synthesizing starch as a carbon reserve in the maternal tissue pedicel if it is not immediately catabolized (Zinselmeier et al., 1999). Thus, starch abundance in the pedicel is indicative of the supply of assimilates to maize kernels. In this study, starch was abundant in the basal to middle kernels in all pollination treatments, whereas no starch was present in the apical kernels in the SP treatment (Fig. 7). The findings indicate that apical kernel abortion may be caused in part by sucrose depletion. Interestingly, there seems to be a certain level of sucrose available to aborted basal kernels following DP-6D treatment (Figs 6 and 7), suggesting that in addition
to deficient sucrose supply, other factors may contribute to basal kernel abortion. Indeed, previous studies have demonstrated that mild water deficit can cause kernel abortion even when the sucrose supply is not depleted (Schussler and Westgate, 1995; Andersen et al., 2002; Muller et al., 2011). The differences between aborted and set basal kernels in glucose and fructose concentrations were more pronounced than in sucrose concentration, indicating that breakdown of sucrose into glucose and fructose was suppressed in the aborted basal kernels. Therefore, our data suggest that kernel abortion is induced by low availability of assimilates and poor ability of the kernels to cleave sucrose into hexoses.

Invertases, including CWIN and soluble invertase, hydrolyze sucrose into glucose and fructose. The invertases in sinks are particularly relevant to sucrose phloem unloading, generation of the hexose-to-sucrose ratio, sugar signaling in the fruit, and the seed set process (Koch, 2004; Ruan et al., 2012; Ruan, 2014). Significantly, in this study the invertase activities were detected at low levels in aborted kernels, including the SP apical kernels and the DP-6D basal kernels (Fig. 8). The extremely low hexose and normal sucrose levels in DP-6D aborted basal kernels further point to low invertase activity as a major factor responsible for basal kernel abortion.

Invertases might control kernel growth in several ways. First, high CWIN could facilitate apoplasmic phloem unloading of sucrose to the developing grains (Ruan et al. 2012). Second, strong CWIN activity produces adequate glucose as a signal to activate cell cycle genes, contribute to the maintenance of reactive oxygen species homeostasis, and inhibit expression of programmed cell death genes to allow seed set to proceed (Rolland et al., 2006; Ruan et al., 2010; Liu et al., 2016). Third, CWIN may control seed growth through other signaling pathways independent of sugars (Cheng and Chourey, 1999; Muller et al., 2011). Therefore, higher CWIN facilitates higher utilization of sucrose, which promotes preferential flow of the sucrose supply to, and its utilization within, developing kernels.

In species with linearly arranged ovules and kernels, the probability of abortion is often non-random with respect to position (Hossaert and Valéro, 1988; Rocha and Stephenson, 1991; Guittain, 1994; Munier-Jolain et al., 1998; Jing et al., 2000). The effect of seed position may involve competition for maternal resources (Watson and Casper, 1984; Rocha and Stephenson, 1991) or the timing of fertilization (Rocha and Stephenson, 1991; Cárcova and Otegui, 2001; Obeso, 2002; Uribelarrea et al., 2008; Arathi, 2011; Oury et al., 2016b). Interestingly, in maize kernel abortion preferentially occurs on the apical region of ears, which are the furthest from maternal resources, whereas abortion tends to occur on the basal end of fruits, which are the nearest to maternal resources, in cucumber (Varga and Bruinsma, 1990; Cheng et al., 2015) and legumes (Hossaeart and Valéro, 1988; Rocha and Stephenson, 1990, 1991; Guittain, 1994). The common feature among these species is that abortion preferentially occurs in the kernels or ovules that are pollinated later. This phenomenon may be due to a ‘head start’ effect of fertilization leading to the formation of a strong resource sink, independent of kernel position (Arathi, 2011). In this study, delaying pollination changed the partitioning of assimilates and the pattern of abortion in maize, providing strong experimental evidence for the vital role of the PTG in non-random kernel abortion and in rebalancing the trade-off between apical and basal kernel growth.

Specifically, strong CWIN activity level was apparent in SP apical kernels (Fig. 9), which means that the abortion of SP apical kernels was not triggered by weak CWIN. However, DP of the apical and basal kernels reduced both CWIN activity and kernel set (Fig. 9), directly demonstrating the negative
In this study, we observed two different responses to DP: DP-2D and DP-4D basal kernels developed to seeds with a slight reduction in fresh weight, whereas DP-6D basal kernels stopped development and aborted (Fig. 4C, F; Supplementary Fig. S3). Weight reduction or abortion represent two phenotypes that may be under different metabolic regulatory control (Fig. 11B). Here, the switch to kernel abortion likely depends on the extent of the competition for assimilates at the most sensitive stage; when invertase activity was suppressed by DP, those kernels were inferior to normally pollinated kernels in terms of sugar metabolism. Notably, when this inferiority was intensified by increasing the PTG to 6 days, a much-reduced hexose concentration was observed in the basal kernels and the abortion process was triggered. Therefore, the mechanism by which inferior kernels switch from weight reduction to abortion may involve sugar signaling (Kakumanu et al., 2012). To this end, ethylene has been suggested to be involved in ovary abortion in maize and wheat (Young et al., 1997; Young and Gallie, 1999; Hays et al., 2007; Cicchino et al., 2013). Glucose could play a vital role in suppressing ethylene biosynthesis (Hong et al., 2004; Wang et al., 2014) and its signaling pathway (Yanagisawa et al., 2003; Iqbal et al., 2013). In this study, endogenous ethylene emission was significantly higher both in the SP apical and DP-6D basal aborted kernels (Fig. 10), which represents an opposite pattern to that observed for glucose and sucrose concentrations (Fig. 6). This observation is consistent with the view that sufficient glucose and sucrose could suppress endogenous ethylene emission (Hong et al., 2004; Feng et al., 2011). Thus, we suggest that a process by which sugars antagonize ethylene emission and signaling is involved in the process of kernel abortion (Fig. 12).

**Apical kernel abortion in practical maize production is determined by poor supply of assimilates and the PTG**

As discussed above, we propose that a shortage of assimilates determines kernel abortion, whereas the PTG-induced decline in invertase activity further determines which kernels should be aborted, and to what extent. In practical crop production, maize kernels are pollinated naturally and asynchronously. Although the silks of the apical kernels must elongate by the smallest distance to extent out of the bracts, they are the latest-emerging silks according to the base-to-apex initiation pattern. Thus, the apical ovaries are pollinated last among all the ovaries in the cob, rendering them the weakest sinks for assimilates. Moreover, the supply of assimilates to the apical kernels is inferior to the supply to the middle and basal kernels. Owing to the concurrence of the two limiting factors,
Delayed pollination represses maize kernel development

Kernel abortion occurs frequently in the apical ear region in practical maize production. Future efforts should be made to alleviate these limiting factors to synchronize maize kernel development and improve grain yield.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. Images showing the process of identifying the basal silks in a silk cluster.

Fig. S2. Images showing the processes used to conduct the basal kernel delayed pollination treatment.

Fig. S3. Ears and kernels of maize exposed to the different pollination treatments at the maturity stage.

Fig. S4. Images showing the results of an experiment to assess the level of silk receptiveness of DengHai 605 (DH605) and ZhengDan 958 (ZD958) to delayed pollination.

Fig. S5. Starch content in apical, middle, and basal kernels of maize hybrids ZhengDan 958 (ZD958) and DengHai 605 (DH605) at 8 and 12 DAP.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant no. 31371558), the China Agriculture Research System (grant no. CARS-02-13), the Australian Research Council (grant no. DP180103834); the National Key Research and Development Program of China (grant no. 2016YFD0300301), and the Chinese Universities Scientific Fund (grant no. 2015QC095). We thank Prof. Jun-Ping Gao and his group for providing assistance with the ethylene emission measurements; Prof. Zhi-Min Wang and Ying-Hua Zhang for their advice on the experimental design; and Jian-He Yan and Han-Yu Feng for support with the techniques. We also thank the editor and two anonymous reviewers for their valuable suggestions that improved the manuscript.

References

Andersen MN, Asch F, Wu Y, Jensen CR, Naested H, Mogensen VO, Koch KE. 2002. Soluble invertase expression is an early target of drought stress during the critical, abortion-sensitive phase of young ovary development in maize. Plant Physiology 130, 591–604.

Andrade FH, Echarte L, Rizzalli R, Della Maggiora A, Casanovas M. 2002. Kernel number prediction in maize under nitrogen or water stress. Crop Science 42, 1173–1179.

Arathi HS. 2011. Selective embryo abortion in a perennial tree-legume: a case for maternal advantage of reduced seed number per fruit. Journal of Plant Research 124, 675–681.

Arathi HS, Ganeshaiah KN, Shaanker RU, Hegde SG. 1999. Seed abortion in Pongamia pinnata (Fabaceae). American Journal of Botany 86, 659–662.

Bassetti P, Westgate ME. 1993. Emergence, elongation, and senescence of maize silks. Crop Science 33, 271–275.

Bassetti P, Westgate ME. 1994. Floral asynchrony and kernel set in maize quantified by image analysis. Agronomy Journal 86, 699–703.

Bawa KS, Webb C. 1984. Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. American Journal of Botany 71, 736–751.

Boyer JS, McLaughlin JE. 2007. Functional reversion to identify controlling genes in multigenic responses: analysis of floral abortion. Journal of Experimental Botany 58, 267–277.

Boyle MG, Boyer JS, Morgan PW. 1991. Stem infusion of liquid culture medium prevents reproductive failure of maize at low water potential. Crop Science 31, 1246–1252.

Brookes RH, Jesson LK, Burd M. 2008. A test of simultaneous resource and pollen limitation in Stylidium armeria. New Phytologist 179, 557–565.

Burd M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. The Botanical Review 60, 83–139.
Burd M. 1998. “Excess” flower production and selective fruit abortion: a model of potential benefits. Ecology 79, 2123–2132.

Campbell DR, Halama KJ. 1993. Resource and pollen limitations to lifetime seed production in a natural plant population. Ecology 74, 1043–1051.

Cárcoles J, Otegui ME. 2001. Ear temperature and pollination timing effects on maize kernel set. Crop Science 41, 1809–1815.

Cheikh N, Jones RJ. 1994. Disruption of maize kernel growth and development by heat stress (role of cytokinin/abscisic acid balance). Plant Physiology 106, 45–51.

Chen T, Xu Y, Wang J, Wang Z, Yang J, Zhang J. 2013. Polyamines and ethylene interact in rice grains in response to soil drying during grain filling. Journal of Experimental Botany 64, 2523–2538.

Cheng CY, Lur HS. 1996. Ethylene may be involved in abortion of the maize caryopsis. Physiologia Plantarum 98, 245–252.

Cheng J, Wang Z, Yao F, Gao L, Ma S, Sui X, Zhang Z. 2015. Down-regulating CaHT1, a cucumber pollen-specific hexose transporter, inhibits pollen germination, tube growth, and seed development. Plant Physiology 168, 635–653.

Cheng WH, Chourey PS. 1999. Genetic evidence that invertase-mediated release of hexoses is critical for appropriate carbon partitioning and normal seed development in maize. Theoretical and Applied Genetics 98, 485–495.

Cicchino MA, Rattalino Edreira JI, Otegui ME. 2017. Maize physiological responses to heat stress and hormonal plant growth regulators related to ethylene metabolism. Crop Science 53, 2135–2146.

de Jong T, Klinkhamer P. 2005. Evolutionary ecology of plant reproductive strategies. Cambridge: Cambridge University Press.

Egli DB. 2010. SOYPOD: a model of fruit set in soybean. Agronomy Journal 102, 39–47.

Ehrlein J, Eriksson O. 1995. Pollen limitation and population growth in a herbaceous perennial legume. Ecology 76, 652–656.

Feng HY, Wang ZM, Kong FN, Zhang MJ, Zhou SL. 2011. Roles of carbohydrate supply and ethylene, polyamines in maize kernel set. Journal of Integrative Plant Biology 53, 388–398.

Ganeshaiah KN, Shaanker RU. 1988. Seed abortion in wind-dispersed pods of Dalbergia sissoco: maternal regulation or sibling rivalry? Oecologia 77, 135–139.

Goetz M, Guivarth A, Hirsche J, et al. 2017. Metabolic control of tobacco pollination by sugars and invertases. Plant Physiology 173, 994–997.

Goldingay RL. 2000. Further assessment of pollen limitation in the waratah (Telopea speciosissima). Australian Journal of Botany 48, 209–214.

Grassini P, Eskridge KM, Cassman KG. 2013. Distinguishing between yield advances and yield plateaus in historical crop production trends. Nature Communications 4, 2918.

Guittian J. 1994. Selective fruit abortion in Prunus mahaleb (Rosaceae). American Journal of Botany 81, 1555–1558.

Hanft JM, Jones RJ. 1986. Kernel abortion in maize: I. carbohydrate concentration patterns and acid invertase activity of maize kernels induced to abort in vitro. Plant Physiology 81, 503–510.

Hays DB, Do JH, Mason RE, Morgan G, Finlayson SA. 2007. Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar. Plant Science 172, 1113–1123.

Hiyane R, Hiyane S, Tang AC, Boyer JS. 2010. Sucrose feeding reverses shade-induced kernel losses in maize. Annals of Botany 106, 395–403.

Hong JH, Cowan AK, Lee SK. 2004. Glucose inhibits ACC oxidase activity and ethylene biosynthesis in ripening tomato fruit. Plant Growth Regulation 43, 81–87.

HossaNt M, Valéro M. 1988. Effect of ovule position in the pod on patterns of seed formation in two species of Lathyrus (Leguminosae: Papilionoideae). American Journal of Botany 75, 1714–1731.

Iqbal N, Trivellini A, Masood A, Ferrante A, Khan NA. 2013. Current understanding on ethylene signaling in plants: the influence of nutrient availability. Plant Physiology and Biochemistry 73, 128–138.

Jing HC, Bergervoet JH, Jalink H, Klooster M, Du SL, Bino RJ, Hilhorst HW, Groot SP. 2000. Cucumber (Cucumis sativus L.) seed performance as influenced by ovule and ovary position. Seed Science Research 10, 435–445.

Kakumanu A, Ambavaram MM, Klumas C, Krishnan A, Batlang U, Myers E, Grene R, Pereira A. 2012. Effects of drought on gene expression in maize reproductive and leaf meristem tissue revealed by RNA-Seq. Plant Physiology 160, 846–867.

Koch K. 2004. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current Opinion in Plant Biology 7, 235–246.

Kozlowski J, Stearns SC. 1989. Hypotheses for the production of excess zygotes: models of bet-hedging and selective abortion. Evolution 43, 1369–1377.

Larson BM, Barrett SC. 2000. A comparative analysis of pollen limitation in flowering plants. Biological Journal of the Linnean Society 69, 503–520.

Liu YH, Offler CE, Ruan YL. 2016. Cell wall invertase promotes fruit set under heat stress by suppressing ROS-independent cell death. Plant Physiology 172, 163–180.

Lloyd DG. 1980. The distributions of gender in four angiosperm species illustrating two evolutionary pathways to dioecy. Evolution 34, 123–134.

McLaughlin JE, Boyer JS. 2004a. Glucose localization in maize ova ries when kernel number decreases at low water potential and sucrose is fed to the stems. Annals of Botany 94, 75–86.

McLaughlin JE, Boyer JS. 2004b. Sugar-responsive gene expression, invertase activity, and senescence in aborting maize ova ries at low water potentials. Annals of Botany 94, 675–689.

Mena-Ali JI, Rocha OJ. 2005. Selective seed abortion affects the performance of the offspring in Bauhinia ungulata. Annals of Botany 95, 1017–1023.

Muller B, Pantin F, Généar M, Turc O, Freixes S, Piques M, Gibon Y. 2011. Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. Journal of Experimental Botany 62, 1715–1729.

Munier-Jolain NG, Munier-Jolain NM, Roche R, Neya B, Duthion G. 1998. Seed growth rate in grain legumes I. Effect of photosynthate availability on seed growth rate. Journal of Experimental Botany 49, 1963–1969.

Nakamura RR. 1986. Maternal investment and fruit abortion in Phaseolus vulgaris. American Journal of Botany 73, 1049–1057.

Obeso JR. 2002. The costs of reproduction in plants. New Phytologist 155, 321–348.

Otegui M, Andrade F, Suero E. 1995. Growth, water use, and kernel abortion of maize subjected to drought at silking. Field Crops Research 40, 87–94.

Oury V, Caldeira CF, Prodhomme D, Pichon JP, Gibon Y, Tardieu F, Turc O. 2016a. Is change in ovary carbon status a cause or a consequence of maize ovary abortion in water deficit during flowering? Plant Physiology 171, 997–1008.

Oury V, Tardieu F, Turc O. 2016b. Ovary apical abortion under water deficit is caused by changes in sequential development of ovaries and in silk growth rate in maize. Plant Physiology 171, 986–996.

Ray DK, Mueller ND, West PC, Foley JA. 2013. Yield trends are insufficient to double global crop production by 2050. PLoS One 8, e66428.

Reynolds M, Foulkes MJ, Slauer GA, Perry P, Parry MA, Snape JW, Angus WJ. 2009. Raising yield potential in wheat. Journal of Experimental Botany 60, 1899–1918.

Rocha OJ, Stephenson AG. 1990. Effect of ovule position on seed production, seed weight, and progeny performance in Phaseolus coccineus L. (Leguminosae). American Journal of Botany 77, 1320–1329.

Rocha OJ, Stephenson AG. 1991. Effects of nonrandom seed abortion on progeny performance in Phaseolus coccineus L. Evolution 45, 1196–1208.

Roitsch T, Balibrea ME, Hofmann M, Proels R, Sinha AK. 2003. Extracellular invertase: key metabolic enzyme and PR protein. Journal of Experimental Botany 54, 513–524.
Delayed pollination represses maize kernel development

Rolland F, Baena-Gonzalez E, Sheen J. 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. Annual Review of Plant Biology 57, 675–709.

Ruan YL. 2014. Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annual Review of Plant Biology 65, 33–67.

Ruan YL, Jin Y, Yang YJ, Li GJ, Boyer JS. 2010. Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. Molecular Plant 3, 942–955.

Ruan YL, Patrick JW, Bouzayen M, Osorio S, Fernie AR. 2012. Molecular regulation of seed and fruit set. Trends in Plant Science 17, 656–665.

Sakai S, Harada Y. 2001. Why do large mothers produce large offspring? Theory and a test. The American Naturalist 157, 348–359.

Schussler J, Westgate M. 1995. Assimilate flux determines kernel set at low water potential in maize. Crop Science 35, 1074–1080.

Snow AA, Spira TP. 1991. Pollen vigour and the potential for sexual selection in plants. Nature 352, 796–797.

Stephenson A. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12, 253–279.

Tardieu F, Parent B, Caldeira CF, Welcker C. 2014. Genetic and physiological controls of growth under water deficit. Plant Physiology 164, 1628–1635.

Uribelarrea M, Cárcova J, Borrás L, Otegui ME. 2008. Enhanced kernel set promoted by synchronous pollination determines a tradeoff between kernel number and kernel weight in temperate maize hybrids. Field Crops Research 105, 172–181.

Varga A, Bruinsma J. 1990. Dependence of ovary growth on ovule development in Cucumis sativus. Physiologia Plantarum 80, 43–50.

Wan H, Wu L, Yang Y, Zhou G, Ruan YL. 2017. Evolution of sucrose metabolism: the dichotomy of invertases and beyond. Trends in Plant Science. doi: 10.1016/j.tplants.2017.11.001.

Wang Y, Zhang C, Wang X, Wang W, Dong L. 2014. Involvement of glucose in the regulation of ethylene biosynthesis and sensitivity in cut Paeonia suffruticosa flowers. Scientia Horticulturae 169, 44–50.

Wang Z, Xu Y, Wang J, Yang J, Zhang J. 2011. Polyamine and ethylene interactions in grain filling of superior and inferior spikelets of rice. Plant Growth Regulation 66, 215–228.

Watson MA, Casper BB. 1984. Morphogenetic constraints on patterns of carbon distribution in plants. Annual Review of Ecology and Systematics 15, 233–258.

Yanagisawa S, Yoo SD, Sheen J. 2003. Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. Nature 425, 521–525.

Yang CF, Sun SG, Guo YH. 2005. Resource limitation and pollen source (self and outcross) affecting seed production in two louseworts, Pedicularis siphonantha and P. longiflora (Orobanchaceae). Botanical Journal of the Linnean Society 147, 83–89.

Yang J, Zhang J, Liu K, Wang Z, Liu L. 2006. Abscisic acid and ethylene interact in wheat grains in response to soil drying during grain filling. New Phytologist 171, 293–303.

Young TE, Gallie DR. 1999. Analysis of programmed cell death in wheat endosperm reveals differences in endosperm development between cereals. Plant Molecular Biology 39, 915–926.

Young TE, Gallie DR, DeMason DA. 1997. Ethylene-mediated programmed cell death during maize endosperm development of wild-type and shrunken2 genotypes. Plant Physiology 115, 737–751.

Zinselmeyer C, Jeong BR, Boyer JS. 1999. Starch and the control of kernel number in maize at low water potentials. Plant Physiology 121, 25–36.