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RESEARCH ARTICLE

Effects of changing assimilate supply on starch synthesis in maize kernels under high temperature stress

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Highlights

- The middle and apical kernels in a maize ear were all in the same high temperature treatment, but with different starch accumulation and enzymes activities.
- The apical pollination treatment could restore the activity of cell-wall invertase (CWIN) and the synthesis of starch in the apical kernels, while the shading treatment aggravated the inhibition on starch synthesis.
- High temperature showed little effect on the activity of CWIN and the synthesis of starch in the middle kernels.

Abstract

High temperature stress (HT) significantly reduces maize yield by impairing starch accumulation in kernels. However, the mechanism by which HT affects starch synthesis remains controversial — whether through reduced assimilate supply or direct inhibition on kernel metabolism. To clarify these mechanisms, a heat-sensitive maize hybrid, Xianyu 335 (XY), was exposed to 30°C/20°C (maximum/minimum temperature, control) and 40°C/30°C for seven consecutive days during the seed setting stage. Synchronous pollination (SP), apical pollination (AP), and shading treatments were applied to manipulate the inherent source–sink ratio in maize plants. Results showed that apical kernel weight decreased by 11.9% under 40°C in the SP treatment. The ^{13}C content, starch accumulation, and cell-wall invertase (CWIN) activity also declined by 15.9, 36.7, and 16.4%, respectively, under HT. In the shading treatment, 40°C/30°C caused even greater reductions in ^{13}C content, starch accumulation, and CWIN activity due to diminished assimilate supply. Conversely, in the AP treatment, starch content and CWIN activity increased by 22.0 and 18.5%, respectively, under 40°C/30°C, resulting in kernel weight and ^{13}C content similar to those in SP and shading treatments regardless of temperature. Consistent with apical kernels under AP, HT did not negatively affect middle kernels in either SP or shading treatments, as kernel weight and starch content remained unchanged under HT. Although all kernels were exposed to the same HT or control environment, their responses varied a lot. The impaired starch synthesis in apical kernels under HT was rescued by increasing carbon supply *via* AP treatment. The contrasting performance among middle kernels, apical kernels under AP, and apical kernels under SP or shading indicates that reduced carbon supply is a critical factor underlying inhibited starch accumulation. Our findings provide a theoretical basis for further understanding kernel abortion under HT.

Keywords: high temperature stress, maize, seed setting stage, cell wall invertase, starch synthesis

1. Introduction

Maize plays an increasingly important role in meeting the growing global demand for food, fuel, and feed (Edgerton 2009). However, under the context of climate change, unpredictable climatic conditions pose significant challenges to maize production (Knox *et al.* 2012). Numerous studies

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have predicted that more frequent and extreme heat stress (HT) will reduce maize yields at both global and national scales in the future (Meehl and Tebaldi 2004; Hawkins *et al.* 2013; Lobell *et al.* 2013; Lesk *et al.* 2016). This yield reduction poses a serious threat to global food security. Therefore, understanding the underlying mechanisms of HT-induced yield loss in maize is essential for developing improved breeding strategies and targeted management practices.

Starch is the primary determinant of maize yield. In mature maize kernels, starch constitutes over 70% (w/w) of total yield (Thitisaksakul *et al.* 2012; Dai *et al.* 2021). Starch accumulation occurs in two main phases: kernel enlargement and grain filling. During early kernel development, carbohydrates allocated to kernels are primarily used for amyloplast formation, which is crucial for subsequent starch synthesis and storage (Emes *et al.* 2003; Larkins 2017). Extensive research has been conducted to elucidate the mechanisms influencing starch accumulation during the seed setting stage (Hanft and Jones 1986a; Duke and Doehlert 1996; Oury *et al.* 2016a). For many years, disruption of carbon metabolism in kernels has been considered a key mechanism contributing to HT-induced seed abortion (Hanft and Jones 1986a). Supporting this view, several *in vitro* culture experiments demonstrated that kernels failed to develop normally under HT (T<40°C), even when sufficient substrates were provided in the culture medium (Shimamoto and Nelson 1981; Jones *et al.* 1985; Hanft and Jones 1986a, b; Liu *et al.* 2022). Further studies identified altered enzyme activities — such as those of cell wall invertase (CWIN), soluble invertase (Cheikh and Jones 1995; Jain *et al.* 2010), and starch synthesis-related enzymes (Yang *et al.* 2018) — as contributing factors. Recently, genetic evidence indicated that HT-induced kernel number loss during the seed setting stage results from insufficient assimilates (Niu *et al.* 2021). Additional studies under drought stress similarly concluded that competition for assimilates is the primary cause of apical kernel abortion (Oury *et al.* 2016b; Turc and Tardieu 2018; Shen *et al.* 2020). These studies suggest that changes in carbon metabolism during flowering may be a consequence rather than a cause of kernel developmental failure (Oury *et al.* 2016a). Thus, there remains controversy regarding whether HT-induced alterations in kernel carbon metabolism are causative or secondary (Appendix A). The mechanisms underlying HT-induced kernel abortion during the seed setting stage therefore require further investigation.

Altering the source–sink ratio is an effective approach to explore the relationship between assimilate availability and carbon metabolism in kernels. Various techniques have been employed to modify the source–sink balance, including stem sucrose feeding (McLaughlin and Boyer 2004), leaf shading, or defoliation (Egharevba *et al.* 1976; Setter and Flannigan 1986; Reed *et al.* 1988). More recently, a non-destructive method was introduced: cutting basal silks to prevent natural pollination and selectively pollinating only apical kernels (Shen *et al.* 2018). Through apical pollination (AP), the natural pollination pattern is altered, allowing apical kernels — which typically receive limited assimilates under normal conditions — to access a greater share of photosynthates. Manipulating sink capacity and assimilate supply thus provides valuable insights into source–sink dynamics under stress conditions.

Based on the above, we hypothesize that when adequate carbon supply is provided to apical kernels *via* AP treatment: (i) kernel weight and starch content will be maintained; and (ii) metabolic enzyme activities in kernels will be preserved under HT. To test this hypothesis, a series of pot experiments were conducted in a temperature-controlled greenhouse, applying shading and AP treatments under different temperature regimes during the seed setting stage. The effects of HT and source–sink manipulations on starch content, carbon allocation, and invertase activity were examined.

2. Materials and methods

2.1. Plant materials and growth conditions

Pot experiments were carried out in a temperature-controlled greenhouse at the Wuqiao Experimental Station of China Agricultural University, Hebei Province, China, in 2022. The maize hybrid Xianyu 335 (XY), previously shown to exhibit poor growth and yield under HT (Li *et al.* 2020), was used. Three seeds were sown at a depth of 4 cm in barrel pots (35 cm diameter×40 cm height). A total of 60 pots were prepared for experimental treatments. Each pot was filled with a mixture of clay soil, standard growing media (Zhonghe Agricultural Technology Co., Jiangsu Province, China), and vermiculite in a volume ratio of 1:2:1. Prior to sowing, 6 g of compound fertilizer ($N/P_2O_5/K_2O$=23%/15%/18%) was added per pot, with an additional 5 g of urea applied at the 12-leaf stage. At the V3 (the third fully expanded leaf) stage, seedlings were thinned to one plant per pot. Plants were well-watered throughout the growing period to avoid water stress, and weeds were controlled manually. Upon husk emergence, ears were covered with paper bags to prevent natural pollination.

2.2. Experimental treatments

Synchronous pollination, apical pollination and shading treatments To alter assimilate distribution within the ear, synchronous pollination (SP) and AP were implemented following Shen *et al.* (2018), with minor modifications. When silks had fully emerged, paper bags were removed. For SP and shading treatments, silks were carefully trimmed to ensure uniform exposure, and all silks were hand-pollinated simultaneously using abundant fresh pollen (Fig. 1-A). For AP treatment, silks from middle and basal ovaries (located in the outer and middle rings of the silk cluster, according to Shen *et al.* (2020)) were excised, and only silks in the central cluster were pollinated (Fig. 1-C). This ensured fertilization only of apical kernels, which consequently received a higher proportion of assimilates compared to SP. All artificial pollinations were performed between 10:00 and 11:00 a.m. on the 4th day after silk emergence. To reduce assimilate supply from the “source,” a partial shading treatment was applied. In this treatment, selected SP plants had all leaves except the ear leaf wrapped in aluminum foil, completely enclosing each leaf from petiole to tip. Shading materials were applied daily at 6:00 a.m. and removed at 8:00 p.m. to minimize physiological damage from prolonged darkness

(Fig. 1-B). This reduced total assimilate delivery to kernels. Through SP, AP, and shading treatments, distinct assimilate supply levels were established: normal supply to both middle and apical kernels in SP; reduced supply in shading; and enhanced supply to apical kernels in AP. Each treatment included 20 pots.

Temperature treatments After pollination, plants were maintained under outdoor conditions for 2 days to ensure completion of fertilization. On the 6th day after silk emergence (the 2nd day after artificial pollination), 30 maize plants with similar growth characteristics (10 for SP, 10 for AP, and 10 for the shading treatment) were selected for subsequent temperature treatments. Specifically, five pots from each treatment (SP, AP, and shading) were transferred to a greenhouse maintained at 30°C/20°C (maximum/minimum temperature; control), and an equivalent number of pots were placed in a greenhouse set at 40°C/30°C. A smart temperature control system was used to regulate temperature fluctuations in the greenhouses, simulating atmospheric temperature trends. Air temperature was recorded every 10 min using a GSP-6 temperature data logger (Elitech Technology Co., Hangzhou, China) (Wang *et al*. 2019). Temperature treatments lasted for 7 days, during which maize plants were exposed to sinusoidal temperature variations during daylight hours and stable temperatures at night. Details of the experimental treatments are provided in Table 1. To ensure equal air humidity across temperature treatments, water basins were placed in the 30°C/20°C greenhouse, and the floor was sprayed with water to increase relative humidity.

$^{13}CO_2$ feeding A $^{13}CO_2$ feeding experiment was conducted between 9:00 and 11:00 a.m. to determine carbon allocation to apical or middle kernels under SP, AP, and shading treatments across two temperature regimes. Briefly, after exposure to HT for 6 days, three plants with consistent growth were selected from the five plants in each of the SP, AP, and shading treatments. The ear leaf was enclosed in a transparent plastic bag with similar leaf area. Then, 50 mL of $^{13}CO_2$ was injected into the bag, and the bag was removed from the leaf 1 h later. All labeled plants were harvested 24 h after the end of $^{13}CO_2$ assimilation. Further details can be found in Wang *et al*. (2019).

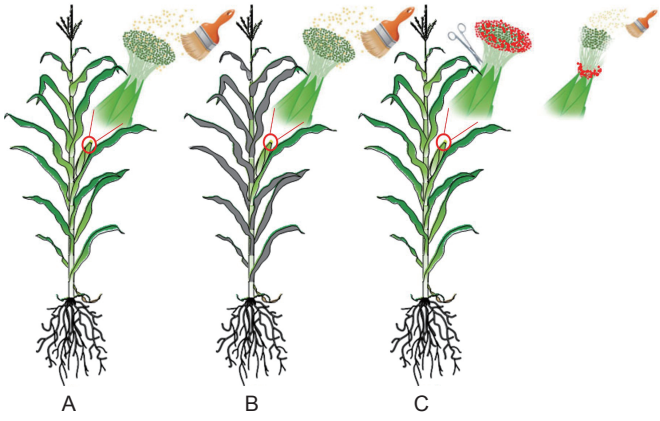


Fig. 1 Schematic diagram of synchronous pollination (SP) (A), shading treatments (B), and apical pollination (AP) (C). A, all emerged silks were directly pollinated with fresh pollen. B, the pollination procedure was identical to that in A, but leaves other than the ear leaf were shaded. C, the silks marked in red represent the outer and middle rings of the silk cluster, which were excised, while the remaining silks were pollinated with fresh pollen. Additional details on the AP treatment are available in Shen *et al*. (2018).

2.3. Sampling and measurements

Samples collection After 7 days of HT treatment, all plants were harvested and sampled. Plants were immediately separated into ear leaf, other leaves, stem, and ear. All vegetative organs were maintained at 105°C for 30 min and then oven-dried at 80°C to constant weight. After weighing and pulverizing, the samples were stored for subsequent measurements. The ears were transported and stored at 4°C for further sampling procedures. Two representative and intact kernels (with pedicel) were selected from the apical and middle positions and stored at –80°C in liquid nitrogen for enzyme activity assays. All remaining apical kernels were carefully separated from the ear cob and the pedicel at the base of each kernel was completely removed. The carbon in the pedicel was not included in kernel carbon measurements in this study. The remaining apical kernels were maintained at 105°C for 30 min, oven-dried at 80°C, and weighed. After pulverization, the samples were stored under dry conditions for additional analyses. In this study, all fertilized kernels within the top 5 cm of the ear were defined as apical kernels, and those located in the middle 10 cm of the ear were defined as middle kernels. The sampling and processing methods for middle kernels were identical to those for apical kernels.

Soluble invertase and cell-wall invertase The determination of soluble invertase and cell-wall invertase (CWIN) activities in apical and middle kernels followed the protocol of Zinselmeier *et al.* (1999). The two stored kernels were weighed and ground using a grinder in 1 mL of soluble extraction buffer. The crude enzyme extract was centrifuged at 12,000 r min^{-1} for 10 min at 4°C. The residual insoluble matter was washed three times with the soluble extraction buffer, and the combined washings were transferred to a volumetric flask to measure soluble invertase activity. To measure CWIN, the remaining insoluble matter was extracted with 1 mL of soluble extraction buffer and incubated overnight. After centrifugation, the supernatant was collected to assess CWIN activity. Soluble invertase and CWIN activities were quantified using assay kits from Suzhou Grace Biotechnology Company Ltd. (Suzhou, China).

Starch and sugar Oven-dried and pulverized kernels were used to measure starch and sugar content. Starch and

Table 1 Temperature and source–sink treatment lasted for 7 days at seed-setting stage

Temperature treatment	Source–sink treatment[1)]
30°C	SP
	AP
	Shading
40°C	SP
	AP
	Shading

[1)] SP, synchronous pollination; AP, apical pollination.

sugar extractions were performed according to Dubois *et al.* (1956). Sugar was extracted three times with boiling water. After centrifugation, the combined supernatants constituted the sugar extract for analysis. For starch measurement, 3 mol L^{-1} HCl was added to the insoluble residue and hydrolyzed in boiling water for 60 min. The supernatant collected after centrifugation was used to determine starch content. The anthrone reagent method was employed, with spectrophotometric detection at 620 nm.

^{13}C analysis The ^{13}C abundance and total carbon content in the samples were determined using a stable isotope analyser (Vario EI, Germany). The amount of labeled C in the plant samples was calculated using the following eq. according to Sawada *et al.* (1995):

$$\text{Total } ^{13}C = {}^{13}C\% \times TC$$

$$TC = C\% \times W$$

where ^{13}C% represents ^{13}C abundance in the sample; TC represents the total amount of C in the sample; C% represents the abundance of C in the sample; W represents the sample weight.

2.4. Statistical analyses

Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Significance was assessed using the least significant difference test. Figures in the article were prepared using OriginPro2019 (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Kernel weight in different ear positions

The SP, AP, shading treatments, and temperature regimes significantly affected maize kernel weight. Compared with SP, the shading treatment significantly reduced kernel weight in both apical and middle ear positions. In contrast, the AP treatment increased apical kernel weight regardless of temperature regime (Fig. 2). Regarding temperature effects, under SP, apical kernel weight decreased by 11.9% under HT; however, middle kernel weight increased under HT, being 21.4% higher at 40°C than at 30°C. A similar trend was observed under shading. In contrast, under AP, no reduction was observed, and apical kernel weight was slightly enhanced by HT (Fig. 2). Middle florets in the AP treatment remained unpollinated; therefore, no middle kernels developed under AP.

3.2. ^{13}C partitioning in different kernel positions

The total amount of ^{13}C was quantified and is presented in Fig. 3. In both the SP and shading treatments, the middle kernels accumulated significantly more 13C than the apical kernels. Under SP conditions, the total ^{13}C content in apical kernels decreased by 15.9% at 40°C compared to 30°C. In contrast, middle kernels exhibited an increasing trend in ^{13}C content at 40°C. A similar pattern was observed under shading, where apical kernels showed a greater reduction (21.1%) at 40°C, while middle kernels displayed only slight increases. In the AP treatment, neither apical nor middle kernels showed significant temperature effects on ^{13}C content. Notably, the total ^{13}C content in apical kernels was significantly higher under AP than under SP or shading treatments (Fig. 3).

In contrast to total ^{13}C content, the ^{13}C atom% excess was similar between apical and middle kernels across all treatments. Furthermore, the response of ^{13}C atom% excess to high temperature differed from that of total ^{13}C content under SP, shading, and AP. Under SP, exposure to 40°C had minimal impact on ^{13}C atom% excess in either kernel position. However, under shading, significantly higher ^{13}C atom% excess was observed at 40°C in both apical and middle kernels. In the AP treatment, apical kernels showed an increasing trend in ^{13}C atom% excess at 40°C (Appendix B). The combination of higher ^{13}C atom% excess but lower total ^{13}C content in apical kernels at 40°C can be attributed to reduced kernel weight at this temperature (Fig. 3).

3.3. Content of starch and soluble sugar

Consistent with kernel weight and ^{13}C content patterns, starch content was substantially higher in middle kernels than in apical kernels. HT effects on starch accumulation were observed only in apical kernels. In SP, HT reduced starch content by 36.7% compared to 30°C, and a similar decreasing

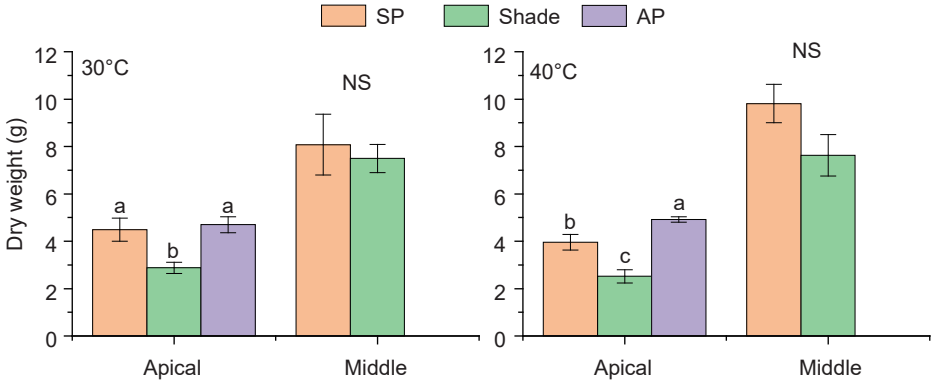


Fig. 2 High temperature effects on the kernel dry weight in different ear positions under synchronous pollination (SP) and shading and apical pollination (AP). Bars mean SD (n=3). Different letters above columns indicate statistical significance at P<0.05 within SP, shading and AP treatments, and NS means not significant in each treatment.

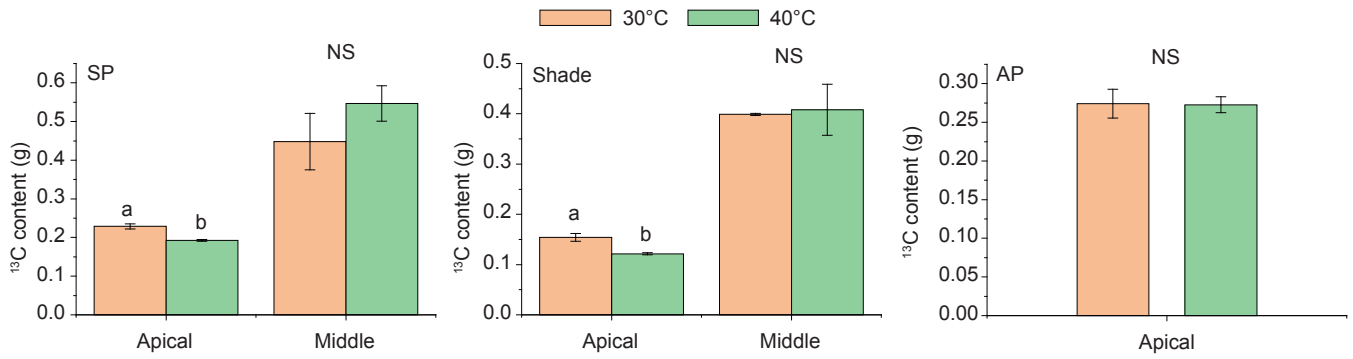


Fig. 3 Changes in the ^{13}C contents in different temperature regimes under synchronous pollination (SP), shading, and apical pollination (AP). The middle florets in the AP were not pollinated, so no data were shown. Bars mean SD (n=3). Different letters above columns indicate statistical significance at P<0.05, and NS means not significant in each treatment.

trend was observed under shading. However, in the AP treatment, starch content did not decrease; instead, it was 22.0% higher at 40°C than at 30°C (Fig. 4-A). This increase contributes to the greater kernel weight observed under HT in AP (Fig. 2). The maintenance of starch accumulation in both middle and apical kernels under AP suggests that starch synthesis is not impaired by 40°C HT when sufficient carbon supply is available.

For soluble sugar content, both temperature regimes and source–sink treatments had less pronounced effects compared to starch. Nevertheless, HT increased soluble sugar content in all treatments except for middle kernels under shading. Notably, in apical kernels, soluble sugar content increased by 13.0% in SP and by 11.3% under shading (Fig. 4-B).

3.4. Invertase activity

Enzyme assays revealed that the activities of CWIN and soluble invertase were altered by source–sink manipulations and temperature regimes. First, CWIN activity was substantially higher in middle kernels than in apical kernels under both SP and shading treatments. HT positively influenced CWIN activity in middle kernels. This observation aligns with the increased ^{13}C content and slight starch accumulation in middle kernels under HT (Figs. 3 and 4-A). In apical kernels, CWIN activity decreased by 16.4% under SP and was significantly inhibited when carbon supply was reduced during shading (Fig. 5-A). When carbon supply to apical kernels was restored under AP, CWIN activity recovered — even exceeding levels observed under SP. Similarly, HT enhanced CWIN activity under AP, reaching levels comparable to those in middle kernels (Fig. 5-A).

For soluble invertase activity, temperature regimes exerted more pronounced effects compared to CWIN. Soluble invertase activity was reduced under 40°C HT across all treatments, particularly under AP, where it was 64.8% lower than at 30°C (Fig. 5-B). Decreasing trends were also evident in middle kernels, contrasting with the response of CWIN activity.

4. Discussion

In the present study, HT was applied at the seed-setting stage — after pollination and fertilization — in combination with SP, shading, and AP treatments. We primarily investigated the physiological mechanisms by which carbon supply influences starch synthesis and source–sink relationships under HT. After a 7-day exposure to 40°C, the weight of apical kernels was significantly reduced (Fig. 2). This reduction resulted from decreased carbon allocation and impaired starch synthesis, associated with reduced CWIN activity. In contrast, in the middle kernels and apical kernels under SP treatment, ^{13}C allocation, starch content, and CWIN activity were unaffected by HT (Figs. 3–5). These findings enhance our understanding of the mechanisms underlying HT-induced kernel abortion and provide new insights into regulatory networks during the seed-setting stage.

4.1. Effects of HT on carbon allocation

Crop yield depends not only on photosynthetic assimilate production at the “source” but also on the proportion of assimilates allocated to “sink” organs (Suwa *et al*. 2010). Both processes are sensitive to stress conditions such as HT. While the effects of HT on crop photosynthesis have been extensively studied (Mathur *et al*. 2014; Rotundo *et al*. 2019; Moore *et al*. 2021), carbon allocation under HT remains less clear.

In this study, HT significantly reduced kernel weight and total ^{13}C content in apical kernels (Figs. 2 and 3). This observation aligns with previous studies in maize (Suwa *et al*. 2010) and rice (Ito *et al*. 2009), which demonstrated that HT reduces carbon allocation to sink organs. Suwa *et al*. (2010) showed that the reduction in ear weight under HT was primarily due to a lower ^{13}C export rate from the ear leaf. This reduced export may stem from disrupted transport tissues (Zhang *et al*. 2018) or altered carbon metabolism in sink organs (Paul and Foyer 2001). For instance, CWIN activity in apical kernels was inhibited under HT in the present study (Fig. 5-A). CWIN plays a critical role in sucrose unloading prior to transport into kernels. Due to suppressed sink activity under HT, the synthesis of cellulose and hemicellulose declined (Suwa *et al*. 2010), which likely impeded starch synthesis. Studies under drought and HT conditions suggest that inhibited CWIN activity is regulated by signaling molecules (Liu *et al*. 2020, 2022). When assimilates

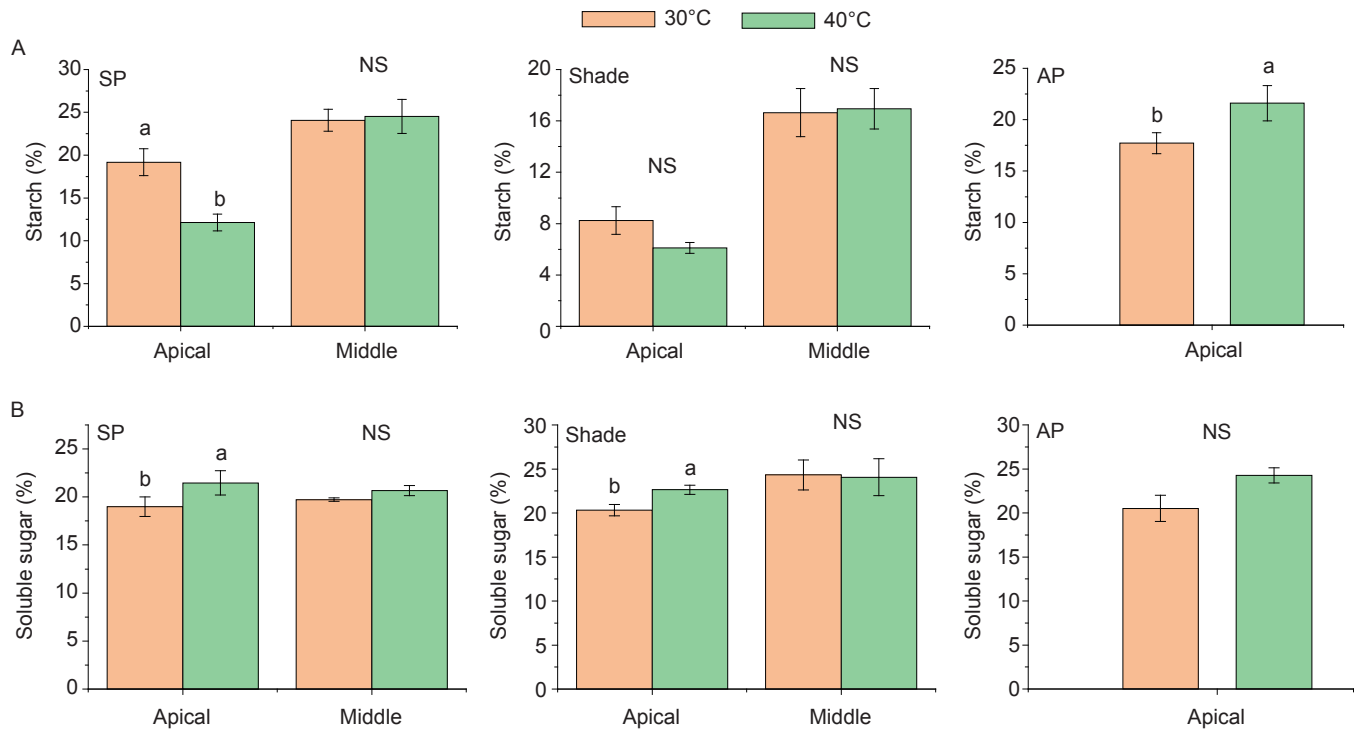


Fig. 4 Changes in the concentrations of starch (A), and soluble sugar (B) in different temperature regimes under synchronous pollination (SP), shading, and apical pollination (AP). The middle florets in the AP were not pollinated, so no data were shown. Bars mean SD (n=3). Different letters above columns indicate statistical significance at P<0.05; NS indicates no significant difference within each treatment.

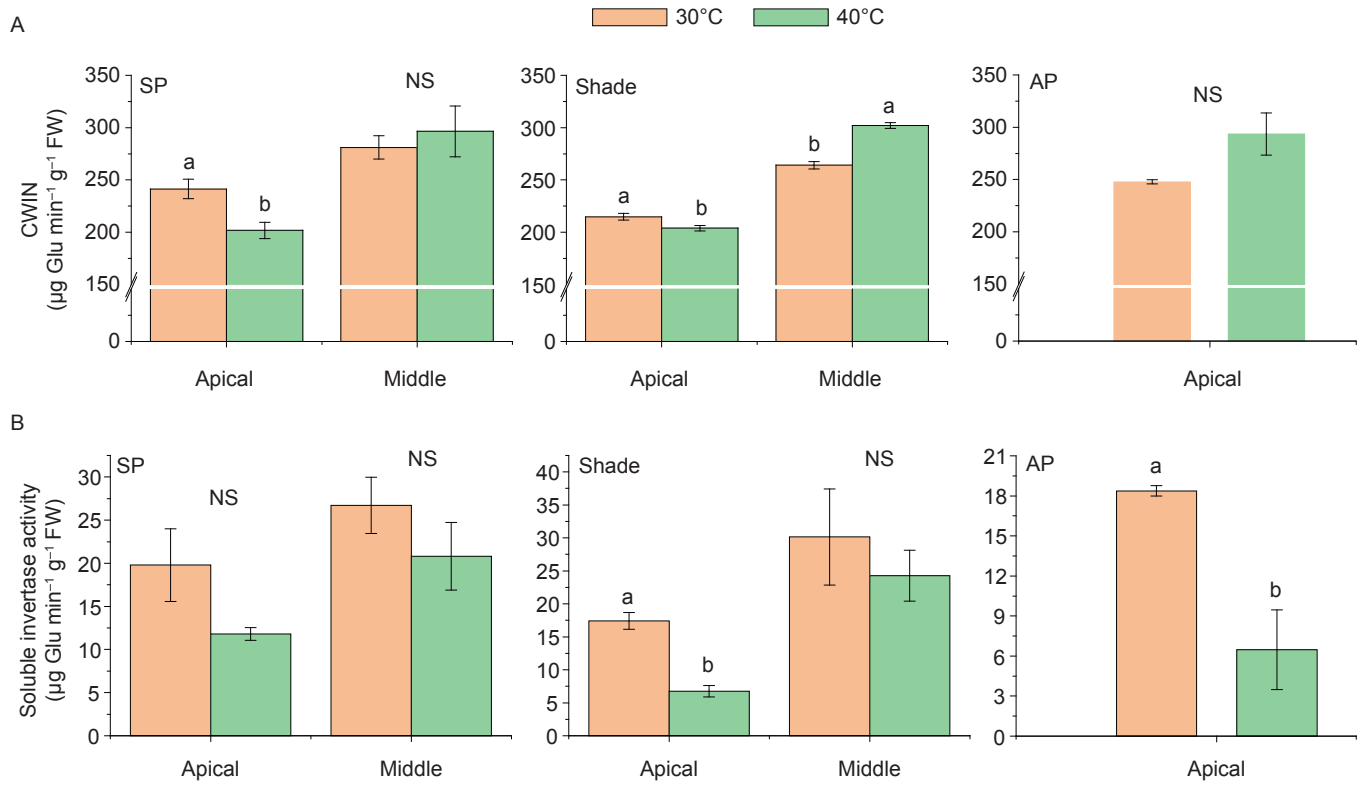


Fig. 5 Changes in the enzyme activities of cell-wall invertase (CWIN) (A) and soluble invertase (B) in the apical and middle kernels under synchronous pollination (SP), shading, and apical pollination (AP) treatments. The middle florets in the AP treatment were not pollinated; therefore, no data are shown. Bars mean SD (n=3). Different letters above the columns indicate statistical significance at P<0.05, and NS indicates no significant difference within each treatment.

are limited under stress, kernels in disadvantaged positions — such as apical kernels in maize — may produce signaling molecules that suppress CWIN activity, allowing remaining kernels to acquire more assimilates (Shen *et al*. 2020; Niu *et al*. 2021). CWIN activity and subsequent starch synthesis were restored when assimilate supply was supplemented via AP treatment (Figs. 4-A and 5-A). A similar phenomenon has been observed under drought stress (Shen *et al*. 2020). Based on these observations, we hypothesize that reduced carbon allocation to apical kernels is the initial event under HT, followed by the release of signaling molecules that inhibit CWIN activity and disrupt downstream starch synthesis.

However, other studies report no effect of HT on carbon allocation to sink organs (Wang *et al*. 2019; Liu *et al*. 2020). This discrepancy may arise from two factors: (i) in the present study, the pedicel at the base of each kernel was carefully removed during sampling. Carbon may accumulate in this region and fail to unload under HT stress, particularly in apical kernels (Suwa *et al*. 2010; Shen *et al*. 2020); (ii) previous studies calculated ^{13}C content in the entire ear. In this study, although apical kernels exhibited lower ^{13}C content, middle kernels showed higher levels, potentially compensating for the loss in apical kernels at the whole-ear level.

4.2. Effects of carbon supply on kernel abortion under stress condition

Starch constitutes the most abundant component in maize kernels. Insufficient starch synthesis during early kernel development directly leads to kernel abortion (Hanft and Jones 1986b; Dai *et al*. 2021). In maize, kernel abortion predominantly occurs in apical positions. Stress conditions typically exacerbate apical kernel abortion but exert relatively minor effects on middle kernels (Shen *et al*. 2020; Liu *et al*. 2022). In this study, HT significantly reduced apical kernel weight, whereas middle kernel weight changed little and was even enhanced under HT in the SP treatment (Fig. 2). The increased weight in middle kernels may result from (i) aborted apical kernels freeing up carbon for allocation to middle kernels (Ruan 2014; Tardieu *et al*. 2014); and (ii) HT-induced increases in grain-filling rate (Xu *et al*. 2013; Zhao *et al*. 2014). Starch synthesis is regulated by hormonal content in kernels, which is sensitive to HT stress (Huang *et al*. 2016). Liu *et al*. (2022) found that mean kernel filling rate correlated positively with zeatin riboside (ZR) content at 10 days after pollination in both basal and apical kernels. Thus, altered kernel weight in the present study may be influenced by disturbed hormone levels.

The differential responses of kernel weight across ear positions were further confirmed by ^{13}C labeling. The amount of ^{13}C in apical kernels decreased, whereas it increased in middle kernels under HT (Fig. 3). This suggests that under HT, when total assimilate supply is limited, apical kernels may cease assimilate uptake to prioritize carbon allocation to middle kernels. A similar pattern has been reported under drought stress (Shen *et al*. 2020). This unequal distribution results in markedly reduced starch accumulation in apical kernels, while middle kernels remain unaffected (Fig. 4). Under HT, normal development and starch accumulation in middle kernels indicate that HT does not impair the starch synthesis process itself. Enzyme activity analysis further supports this: CWIN activity was not inhibited in middle kernels but was significantly reduced in apical kernels (Fig. 5). Therefore, based on the contrasting responses between apical and middle kernels under SP treatment, we propose that restricted carbon supply is a key physiological mechanism underlying HT-induced kernel abortion.

To test this hypothesis, we manipulated assimilate supply to different ear positions using shading and AP treatments. Under shading, which limits assimilate availability, the inhibitory effect of HT on middle kernel weight became evident. Conversely, restoring assimilate supply to apical kernels *via* AP treatment prevented HT-induced reductions in kernel weight and starch content. Recent studies under drought stress show that supplying sufficient assimilates to apical kernels can restore starch synthesis and rescue aborted kernels (Shen *et al*. 2020). Enzyme activity of CWIN and starch content followed similar trends to kernel weight accumulation. This implies that when assimilates are sufficient, starch synthesis proceeds normally despite HT. Furthermore, recent research at enzymatic and transcriptomic levels indicates that post-pollination heat stress in maize primarily reduces carbohydrate availability (Niu *et al*. 2021). Hence, we conclude that assimilate deficiency in aborted kernels is the primary cause of reduced enzyme activity and subsequent kernel abortion under HT.

In contrast to CWIN, soluble invertase activity was reduced under HT, likely due to decreased substrate (sucrose) availability. Because CWIN activity increased (Fig. 5), most assimilates were cleaved into hexoses at the pedicel before entering the kernel. Consequently, reduced soluble invertase activity in apical kernels may reflect diminished assimilate delivery under HT (Fig. 3).

4.3. Opposite results of the current results and previous studies *in vitro*

The impact of HT on kernel abortion has also been examined using *in vitro* kernel culture. In such systems, fertilized kernels are detached from the cob and incubated in culture media. These studies indicate that impaired kernel growth and starch biosynthesis are primarily linked to changes in carbon utilization rather than limitations in carbon supply (Hanft and Jones 1986b; Cheikh and Jones 1995). Subsequent work confirmed that HT inhibits key enzymes in carbon metabolism, such as sucrose synthase, fructokinase, aldolase, and acid invertase (Duke and Doehlert 1996). These findings contrast with the present study and recent reports under HT or drought conditions (Oury *et al*. 2016a; Shen *et al*. 2020; Niu *et al*. 2021). Two factors may explain this discrepancy. First, in *in vitro* systems, kernels are continuously exposed to HT, whereas in this study, peak temperatures occurred only at midday. Prolonged HT exposure causes more severe damage, while intermittent stress allows recovery periods, resulting in milder effects. Second, in this study, kernel internal temperature was likely lower than ambient air temperature due to protection by multiple layers of husks (Bassetti and Westgate 1993). The

actual thermal environment near the kernels warrants further investigation.

In this study, disrupted starch synthesis in apical kernels under HT was rescued by increasing carbon supply through AP treatment. These results support the importance of source-sink dynamics under HT. However, the findings are based on potted experiments. Future research should validate them under field conditions. Although previous studies have documented various impacts on yield during grain filling, final maize yield could not be assessed here due to the experimental manipulation of source–sink relationships. Subsequent studies should refine experimental designs to explore the physiological mechanisms and source–sink interactions affecting yield during the seed-setting stage.

5. Conclusion

In this study, HT treatment significantly reduced apical kernel weight due to decreased starch content and CWIN activity. Reduced apical kernel weight under shading and increased weight under AP treatment demonstrate that altering carbon supply leads to divergent developmental outcomes. Combined with the restoration of starch accumulation in apical kernels under AP treatment and the stable development of middle kernels, we conclude that adequate carbon supply enables HT-compromised kernels to develop normally. Thus, the primary factor limiting starch synthesis under HT is insufficient carbon supply. These results contribute to resolving ongoing debates about the causes of kernel abortion under HT and provide a theoretical foundation for improving understanding of this phenomenon.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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