Plant Physiol. (1985) 79, 852–855
0032-0889/85/79/0852/04/$01.00/0

Effect of Increased Temperature in Apical Regions of Maize Ears on Starch-Synthesis Enzymes and Accumulation of Sugars and Starch[1]

Received for publication May 3, 1985 and in revised form July 29, 1985

TSAI-MEI OU-LEE AND TIM LLOYD SETTER*
Department of Agronomy, Cornell University, Ithaca, New York 14853

ABSTRACT

Apical florets of maize (*Zea mays* L.) ears differentiate later than basal florets and form kernels which have lower dry matter accumulation rates. The purpose of this study was to determine whether increasing the temperature of apical kernels during the dry matter accumulation period would alter the difference in growth rate between apical and basal kernels. Apical regions of field-grown maize (cultivar Cornell 175) ears were heated to 25 ± 3°C from 7 days after pollination to maturity (tip-heated ears) and compared with unheated ears (control). In controls, apical-kernel endosperm had 24% smaller dry weight at maturity, lower concentration of sucrose, and lower activity of ADP-Glc starch synthase than basal-kernel endosperm, whereas ADP-Glc-pyrophosphorylase (ADPG-PPase) activities were similar. In tip-heated ears apical-kernel endosperm had the same growth rate and final weight as basal-kernel endosperm and apical kernels had higher sucrose concentrations, higher ADP-Glc starch synthase activity, and similar ADPG-PPase activity. Total grain weight per ear was not increased by tip-heating because the increase in size of apical kernels was partially offset by a slight decrease in size of the basal- and middle-position kernels. Tip-heating hastened some of the developmental events in apical kernels. ADPG-PPase and ADP-Glc starch synthase activities reached peak levels and starch concentration began rising earlier in apical kernels. However, tip-heating did not shorten the period of starch accumulation in apical kernels. The results indicate that the lower growth rate and smaller size of apical kernels are not solely determined by differences in prepollination floret development.

Apical kernels on maize ears have lower growth rates and smaller final size than basal kernels (9, 15, 16). Apical florets differentiate later and maternal tissues are smaller at pollination than basal florets in maize (4) and other cereal grains (5, 6). Thus, it is possible that basal florets have a larger supply of nutrients in nucellus and associated tissues for use in early development of endosperm (11, 12) or that the vascular transport pathway is better developed in basal than apical kernels (7, 18).

Increased fruit or inflorescence temperature can increase the rate of metabolism and sink strength (14), thus selective temperature treatment might be an effective method to alter partitioning between competing sink organs. If the rate of metabolism and photosynthate transport into developing kernels determines the apical/basal difference in mature kernel size, enhancing apical kernel temperature might alter this difference. However, if prepollination floret development solely determines the apical/basal kernel difference, temperature treatments applied after pollination might have little or no effect. This study was performed to determine whether increasing the temperature of apical kernels during the period from 7 DAP[2] to maturity would alter the difference in growth rate and final size between apical and basal kernels.

MATERIALS AND METHODS

Plant Material and Heat Treatment. Maize (*Zea mays* L., cv Cornell 175) seed was planted into Darien gravelly silt loam (fine loamy mixed mesic aeric ochraqualfs) at the Varna research site near Ithaca, NY on June 6, 1984. Ammonium nitrate fertilizer (200 kg N ha^{-1}) was applied immediately before plowing and 280 kg fertilizer ha^{-1} was applied at planting (analysis: 10% N, 9% P, 17% K [w/w]). Plants were in 76 cm rows with 7.3 plants m^{-2}. Ear shoots were covered at silk emergence. Four to 6 d after silk emergence, they were cut flush with husk leaves and were synchronously pollinated 2 d later. The apical regions of randomly selected primary ear shoots were thermostatically heated to 25 ± 3°C throughout the period from 7 to 65 DAP with heat tape. The heat tape consisted of plastic-insulated nichrome wire 28 cm in length providing 5.5 W/ear, which was wrapped around the apical region of each ear. A 0.7 cm layer of polyester blanket was placed around the heat tape for thermal insulation followed by an inverted 390 ml polystyrene cup to exclude rainwater. Control ears were also protected with polystyrene cups.

Sampling Procedure. Apical kernels were obtained from the region of fertilized kernels on each ear measuring 80 to 90% of the distance from base to tip of the ear. The basal kernels were from the region 10 to 20% of this distance.

Before sampling, ears were randomly assigned to harvest dates. Ears were sampled at 7 to 8 AM (Eastern Standard Time), chilled on ice, and kernels from two ears (11–17 DAP) or one ear (20–65 DAP) were counted and pooled to form each replicate. Results shown are means of three replicates; bars above or below symbols represent SE and where bars are absent SE limits are within symbol dimensions.

Enzyme Assay. Kernels were subsampled from each pooled replicate for enzyme extraction. The embryo and pericarp were removed and 5 gfw of endosperm were homogenized and extracted as previously described (9). Remaining kernels were frozen at −18°C, lyophilized, and ground (Wiley mill, A. H. Thomas Scientific, Philadelphia, PA) for use in carbohydrate assays as described below. ADPG-PPase (ATP: α-D-glucose-1-phosphate adenyltransferase; E.C.2.7.7.27) activity in the undi-

[1] Supported in part by United States Department of Agriculture/Competitive Research Grants Organization grant 59-2361-1-1-727-0.

[2] Abbreviations: ADPG-PPase, ADP-Glc-pyrophosphorylase; DAP, days after pollination; gdw, grams dry weight; gfw, grams fresh weight.

alyzed supernatant of crude extracts was assayed as previously described (9). ADP-Glc starch synthase (ADP-glucose; α-1,4-glucose α-4-glucosyltransferase; E.C. 2.4.1.21) in the starch granule-bound (insoluble) fraction was assayed as previously described (9) except that after terminating the reaction the insoluble fraction was washed by adding 2 ml of 90% (v/v) methanol (containing 1% KCl), filtered through Whatman No. 42 paper and rinsed 8 times with 2 ml of 90% methanol (13).

Carbohydrate Assay. Sugars were extracted from lyophilized samples, and glucose, sucrose, and starch were analyzed as previously described (9).

RESULTS

Temperature Treatment. The record of daily high and low air temperature for the field site (Fig. 1) indicates the magnitude of the tip-heated treatment. Although the daily highs frequently exceeded the thermostatic set-point for tip region heating (indicated by the horizontal line at 25°C), the daily low temperatures were always less than the set-point. Thus, the treatment was greatest with respect to the ambient temperatures during the night period and during the late season when temperatures were low.

Water, Dry Weight, Glucose, Sucrose, and Starch. Water concentration per gfw declined similarly for both apical and basal kernels of control ears, while in tip-heated ears it declined earlier for apical than basal kernels (Fig. 2A). Data showing water content on a kernel basis (Fig. 2B) indicate the time course of kernel volume increase during the expansion-growth stage. The period of water accumulation and thus volume expansion occurred from 10 to 23 DAP.

Apical kernels of control ears had a lower dry matter accumulation rate than basal kernels resulting in a smaller weight per kernel at maturity (Fig. 2C). In tip-heated ears, however, apical kernels had essentially the same accumulation rate and weight per kernel at maturity as the basal kernels. Despite the larger size of apical kernels in tip-heated ears, the total grain dry weight per ear was only slightly larger and not statistically significant in tip-heated compared to control ears (Fig. 3). This can be explained in part by the small fraction which apical kernels constitute of the total kernel weight per ear. In addition, the basal and middle kernels in tip-heated ears were slightly smaller than in control ears (Fig. 4; 221 and 237 mg/kernel, respectively; SE = 2.3 mg). Tip-heated ears did not have a smaller number of kernels per ear than controls, thus the treatment did not increase the extent of apical kernel abortion.

In both apical and basal kernels, glucose concentration was highest at the earliest sampling date (11 DAP) and declined rapidly in both control and tip-heated ears (Fig. 5A). Glucose content per kernel was higher between 14 and 23 DAP in apical than basal kernels of control ears; however, it was the same in apical and basal kernels of tip-heated ears (Fig. 5B).

Sucrose concentration in control ears was maximal in basal kernels from 11 to 23 DAP, but it was lower and declined earlier in apical kernels (Fig. 6A). In contrast, the sucrose concentration in apical kernels of tip-heated ears was higher than in basal kernels throughout this period. Sucrose accumulated in both apical and basal kernels of both the control and tip-heated treatments from 11 to 23 DAP (Fig. 6B). Both sucrose and glucose contents per kernel were highest during the period of rapid expansion growth from 11 to 23 DAP (compare Figs. 6B

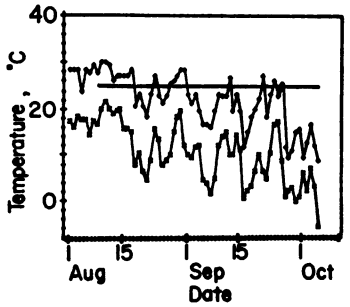

FIG. 1. Daily high (+) and low (×) air temperature. Tip-heated thermostatic temperature (25°C) is shown as a horizontal line.

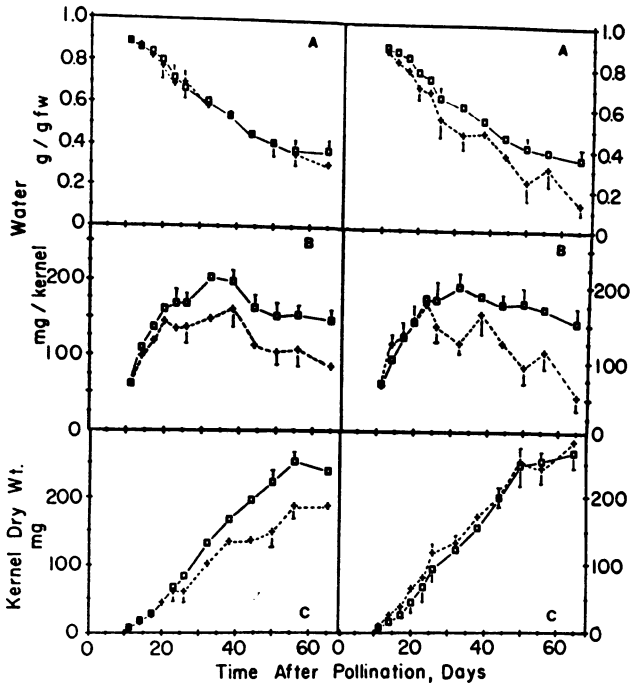

FIG. 2. Endosperm water content on a fresh weight basis (A) and on a kernel basis (B) and dry weight accumulation per kernel (C) during development of apical (+) and basal (□) kernels of control (left) and tip-heated (right) ears.

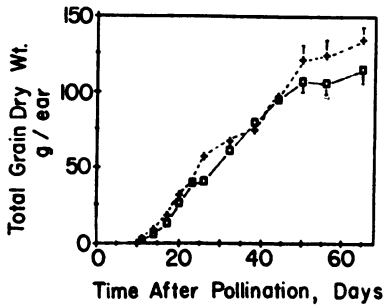

FIG. 3. Grain dry weight accumulation per ear during development of control (□) and tip-heated (+) ears.

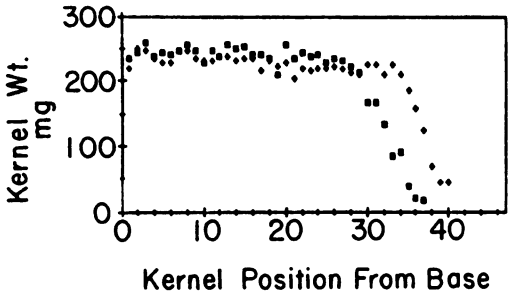

FIG. 4. Dry weight of kernels in control (□) and tip-heated (+) ears as a function of position number, counted acropetally from the ear base.

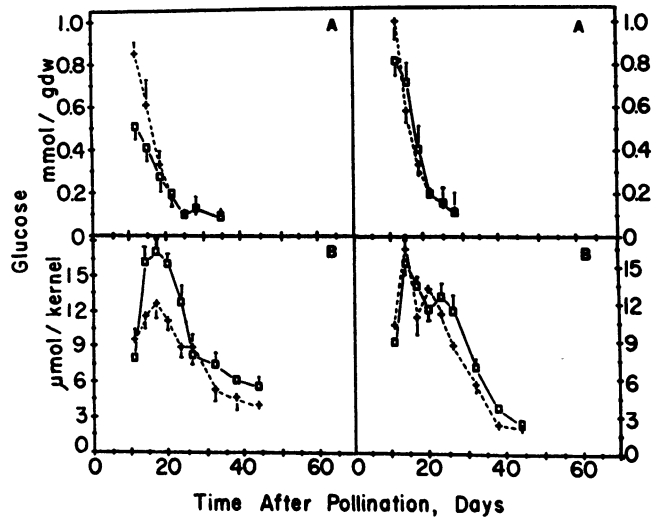

FIG. 5. Endosperm glucose content on a dry weight basis (A) and on a kernel basis (B) in apical (+) and basal (□) kernels of control (left) and tip-heated (right) ears.

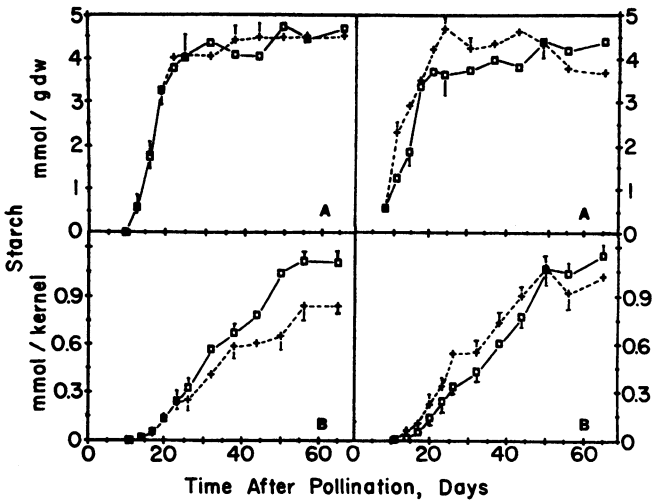

FIG. 7. Endosperm glucose equivalents of starch content on a dry weight basis (A) and on a kernel basis (B) in apical (+) and basal (□) kernels of control (left) and tip-heated (right) ears.

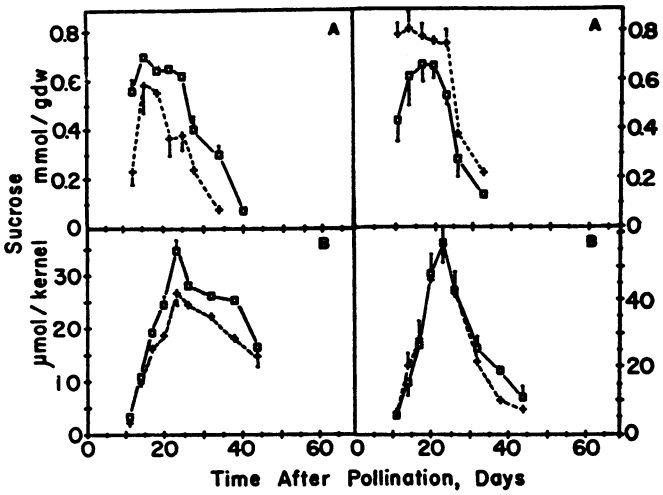

FIG. 6. Endosperm sucrose content on a dry weight basis (A) and on a kernel basis (B) in apical (+) and basal (□) kernels of control (left) and tip-heated (right) ears.

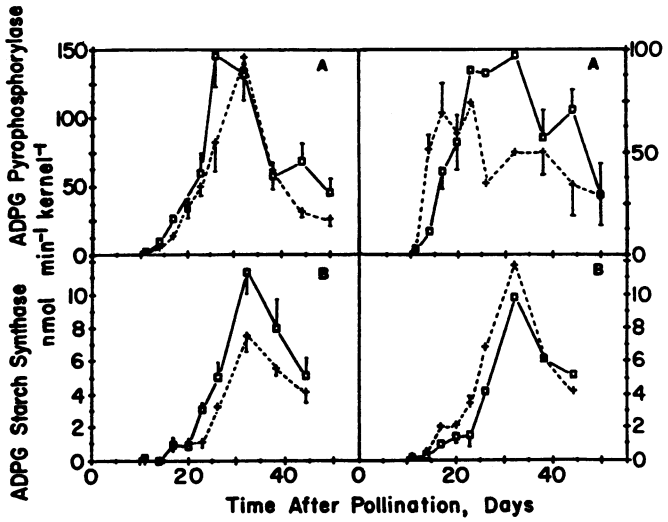

FIG. 8. Endosperm ADP-Glc-pyrophosphorylase (A) and insoluble starch synthase (B) activities in apical (+) and basal (□) kernels of control (left) and tip-heated (right) ears. ADP-Glc-pyrophosphorylase was determined by measuring glucose-1-P formation from ADP-Glc and PPi using a coupled spectrophotometric assay. The insoluble preparation from enzyme extracts was assayed for starch synthase activity by measuring [^{14}C]ADP-Glc fixation to starch primer. See text for details.

and 5B with 2B). At this time, however, sucrose level was substantially greater than glucose and it continued to accumulate from 17 to 23 DAP when glucose levels were declining.

Starch accumulation began at about 11 DAP and starch concentration reached a maximum at about 25 DAP for both apical and basal kernels of both treatments (Fig. 7A). As expected, starch accumulation on a kernel basis (Fig. 7B) had a similar pattern to that of dry weight accumulation, because their composition was 70 to 80% starch. Thus, the effect of tip-heating on dry matter accumulation patterns (Fig. 2C) could largely be attributed to differences in starch accumulation rate.

Enzyme Activities. ADPG-PPase activity was similar in apical and basal kernels of control ears throughout development (Fig. 8A). In tip-heated ears, ADPG-PPase activity was initially (14 DAP) higher in apical than basal kernels; however, it was less in apical than basal kernels between 23 and 32 DAP when the bulk of starch synthesis occurred.

Starch synthase activity was highest in both treatments at 32 DAP (Fig. 8B) coinciding with the period of most rapid starch accumulation. Basal kernels in control ears had higher activity than did apical kernels; however, in tip-heated ears the apical kernels had higher activity than did basal kernels.

DISCUSSION

The tip-heated treatment, while increasing the size of apical kernels, appeared to slightly decrease the size of the more numerous, basal- and middle-position kernels. This suggests that grain yield per plant was restricted by the supply of photosynthate or other nutrients and is consistent with studies which have shown that enhancing light flux density in maize canopies has increased the grain yields and number of kernels per plant (10, 17).

The tip-heated ear treatment hastened some of the developmental events in apical kernels. The dates when ADPG-PPase activity peaked and starch synthase activity was rising occurred

earlier in tip-heated apical kernels and corresponded with an earlier rise of starch concentration in apical kernels of tip-heated compared to control ears (Fig. 7). Higher ambient temperature has been found to hasten maize development and shorten time to maturity when treatments have been applied to whole plants (2, 15) and to *in vitro* cultured kernels (8). Consistent with this, the developmental time course in the current study was much less rapid than in our earlier study of cultivar where it was grown at warmer greenhouse temperatures (27/23°C, day/night) (9). However, tip-heating did not appear to shorten the period of starch accumulation in apical kernels. Light and moisture conditions, photosynthetic status, and genotype also influence the response of kernel development to temperature (15). Perhaps these interacting influences were responsible for the observed response.

The tip-heated treatment substantially increased apical-kernel glucose and sucrose contents per kernel during the period of volume expansion. Sugars might have a role during cell expansion in providing a more negative osmotic potential and in turn a steeper water potential gradient between xylem and expanding endosperm (3). Although tip-heating did not appear to increase the rate of water accumulation (Fig. 2B), it is possible that basipetally progressing maturation and desiccation (1) were more rapid in apical kernels of tip-heated ears (Fig. 2A) and that this obscured an enhancement of kernel expansion at higher temperatures.

The activities of ADPG-PPase and insoluble (granule-bound) starch synthase were determined in crude preparations of tissue homogenate to provide a preliminary indication of the relative enzyme activities in apical and basal kernels. Tip-heating appeared to increase insoluble starch synthase activity in apical kernels, corresponding with the effect of the treatment on starch accumulation rate. However, tip-heating did not appear to increase ADPG-PPase activity during the period of rapid starch accumulation in apical kernels. It is possible that the activities reported in this study underestimate the maximum catalytic potential of these enzymes due to incomplete extraction and activation or other factors, as previously discussed (9). The observed rate of starch accumulation by basal kernels of both treatments was about 20 nmol of glucose equivalents per min (Fig. 7). Thus, although ADPG-PPase activities were sufficient to account for the calculated flux through the pathway, the starch synthase activities were insufficient. Soluble starch synthase, which was not measured in the current study, also constitutes a substantial portion of the total starch synthase catalytic potential (9, 13). Further studies will be needed to establish whether the *in vitro* activities of these enzymes are partially responsible for the differences between apical and basal kernels in starch accumulation rate.

The nutrient reserves in maternal tissues surrounding the embryo sac are accumulated before pollination and are depleted during early endosperm growth (11). The tip-heating treatment was begun after these processes were essentially complete. Thus, the observed enhancement of apical kernel size with heating suggests that such factors do not solely determine the kernel size at maturity.

LITERATURE CITED

1. AFUAKWA JJ, RK CROOKSTON 1984 Using the kernel milk line to visually monitor grain maturity in maize. Crop Sci 24: 687–691
2. BADU-APRAKU B, RB HUNTER, M TOLLENAAR 1983 Effect of temperature during grain filling on whole plant and grain yield in maize (*Zea mays* L.). Can J Plant Sci 63: 357–363
3. BARLOW EWR, JW LEE, R MUNNS, MG SMART 1980 Water relations of the developing wheat grain. Aust J Plant Physiol 7: 519–525
4. CHENG PC, RI GREYSON, DB WALDEN 1983 Organ initiation and the development of unisexual flowers in the tassel and ear of *Zea mays*. Am J Bot 70: 450–462
5. COTTRELL J, JE DALE 1984 Variation in size and development of spikelets within the ear of barley. New Phytol 97: 565–573
6. Fisher JE 1973 Developmental morphology of the inflorescence in hexaploid wheat cultivars with and without the cultivar Norin 10 in their ancestry. Can J Plant Sci 53: 7–15
7. HANIF M, RHM LANGER 1972 The vascular system of the spikelet in wheat (*Triticum aestivum*). Ann Bot 36: 721–727
8. JONES RJ, S OUATTAR, RK CROOKSTON 1984 Thermal environment during endosperm cell division and grain filling in maize: effects on kernel growth and development *in vitro*. Crop Sci 24: 133–137
9. OU-LEE T-M, TL SETTER 1985 Enzyme activities of starch and sucrose pathways and growth of apical and basal maize kernels. Plant Physiol 79: 848–851
10. PENDLETON JW, DB EGLI, DB PETTERS 1967 Response of *Zea mays* L. to a "light rich" field environment. Agron J 59: 395–397
11. RANDOLPH LF 1936 Developmental morphology of the caryopsis in maize. J Agr Res 53: 881–916
12. REDDY VM, TB DAYNARD 1983 Endosperm characteristics associated with rate of grain filling and kernel size in corn. Maydica 28: 339–355
13. RIJVEN AHGC 1984 Use of polyethylene glycol in isolation and assay of stable, enzymically active starch granules from developing wheat endosperms. Plant Physiol 75: 323–328
14. THORNE JH 1982 Temperature and oxygen effects on ^{14}C-photosynthate unloading and accumulation in developing soybean seeds. Plant Physiol 69: 48–53
15. TOLLENAAR M 1977 Sink-source relationships during reproductive development in maize. A review. Maydica 22: 49–75
16. TOLLENAAR M, TB DAYNARD 1978 Kernel growth and development at two positions on the ear of maize (*Zea mays*). Can J Plant Sci 58: 189–197
17. TOLLENAAR M, TB DAYNARD 1978 Relationship between assimilate source and reproductive sink in maize grown in a short-season environment. Agron J 70: 219–223
18. WHEELER AW 1976 some treatments affecting growth substances in developing wheat ears. Ann Appl Biol 83: 455–462