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Enzyme Activities of Starch and Sucrose Pathways and Growth of Apical and Basal Maize Kernels[1]

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ABSTRACT

Apical kernels of maize (*Zea mays* L.) ears have smaller size and lower growth rates than basal kernels. To improve our understanding of this difference, the developmental patterns of starch-synthesis-pathway enzyme activities and accumulation of sugars and starch was determined in apical- and basal-kernel endosperm of greenhouse-grown maize (cultivar Cornell 175) plants. Plants were synchronously pollinated, kernels were sampled from apical and basal ear positions throughout kernel development, and enzyme activities were measured in crude preparations. Several factors were correlated with the higher dry matter accumulation rate and larger mature kernel size of basal-kernel endosperm. During the period of cell expansion (7 to 19 days after pollination), the activity of insoluble (acid) invertase and sucose concentration in endosperm of basal kernels exceeded that in apical kernels. Soluble (alkaline) invertase was also high during this stage but was the same in endosperm of basal and apical kernels, while glucose concentration was higher in apical-kernel endosperm. During the period of maximal starch synthesis, the activities of sucrose synthase, ADP-Glc-pyrophosphorylase, and insoluble (granule-bound) ADP-Glc-starch synthase were higher in endosperm of basal than apical kernels. Soluble ADP-Glc-starch synthase, which was maximal during the early stage before starch accumulated, was the same in endosperm from apical and basal kernels. It appeared that differences in metabolic potential between apical and basal kernels were established at an early stage in kernel development.

The mechanisms which control development and maintenance of kernel sink capacity in cereal grain species are not well understood. Sink capacity is determined in part by the rate of photosynthate utilization. Starch is the major constituent in mature maize kernels and thus the starch synthesis pathway could be an important site of regulation. It has been determined that starch synthesis in cereal grains involves the synthesis of ADP-Glc by ADPG-PPase[2] and the incorporation of ADP-Glc into starch by ADP-Glc starch synthase (13). The assimilation by endosperm of sucrose imported via phloem could involve sucrose synthase operating in the direction of ADP-Glc or UDP-Glc and fructose formation (3) or involve invertase to form free hexose (20). The importance of sucrose synthase was suggested by studies in which the *shrunken*-1 maize mutant, having 10% as much sucrose synthase as normal endosperm, formed 40% less starch than normal genotypes (3). Possible importance of invertase was suggested by radiotracer studies which indicated that sucrose was hydrolyzed in the cell wall free space prior to hexose uptake by endosperm cells (19, 20).

Earlier-forming kernels on a plant usually have higher survival probability (22), longer growth duration (24), and higher growth rates (21) than later forming kernels. In maize ears, the apical florets develop and pollinate several days after basal florets (24, 25). The timing of pollination in maize can be controlled to synchronize all kernels on an ear, but even with this treatment apical kernels were found to have lower growth rates than did basal kernels (6). The objective of this study was to compare the activities of starch pathway enzymes in apical and basal kernels during development on synchronously pollinated ears.

MATERIALS AND METHODS

Plant Material. Maize (*Zea mays* L., cv Cornell 175) kernels were planted on February 1, 1984 into 11 L plastic pots (two plants/pot) containing a 1:1 silt loam soil:vermiculite medium. Plants were grown in a greenhouse at 27/23°C day/night temperature with natural sunlight supplemented 12 h/d with 1000 W metal halide lamps (Duraglow, General Electric). Plants were watered as needed and 2.5 to 5 g/pot of fertilizer (Peters 20-20-20 [W. R. Grace, Fogelsville, PA]) was applied weekly. Ear shoots were covered at silk emergency. Four to 6 d after silk emergence, they were cut flush with the husk leaves and were synchronously pollinated 2 d later. 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. Ears were not used for sampling if apical and basal regions had less than 90% pollination. Plants were randomly assigned to sampling dates. Ears were sampled between 8 and 11 AM (Eastern Standard Time), frozen in liquid N_2 and kernels from two ears (7–16 DAP) or one ear (19–53 DAP) were counted and pooled to form each replicate.

Enzyme Extraction. The embryo and pericarp were removed and 5 gfw of endosperm were homogenized (model 45, VirTis) with 10 ml buffer in an ice bath for 2 min. The extraction buffer contained 50 mM Hepes-NaOH (pH 7.5), 20 mM KCl, 1 mM DTT, 10% (v/v) glycerol, and 0.1% (w/v) BSA (10). This extract was centrifuged at 3300*g* and 0° to 4°C for 20 min. Supernatant and resuspended precipitant were dialyzed against extraction buffer overnight at 4°C for all enzyme preparations except ADPG-PPase which was asssayed in undialyzed extracts to reduce loss in activity (11).

Enzyme Assays. In all assays, the formation of reaction product was linear with time and with the amount of added enzyme preparation. Invertase (β-D-fructofuranoside fructohydrolase; E.C. 3.2.1.26) activity was assayed in a mixture containing 40 mM Na-citrate, 10 mM sucrose, and 0.05 ml of enzyme preparation in either 75 mM Hepes-NaOH (pH 7.5) for soluble-alkaline invertase or 75 mM Mes-NaOH (pH 5.0) for insoluble (acid) invertase. The total reaction volume was 0.15 ml and was incubated at 25°C for 15 to 30 min. Reactions were begun by adding

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

enzyme preparation and stopped by heating with boiling water for 10 min. Control assays with denatured enzyme preparation were used to correct for glucose not produced by invertase. Glucose released was measured by a glucose oxidase-peroxidase coupled reaction method (18).

Sucrose synthase (UDP-Glc: D-fructose 2-α-glucosyltransferase; E.C. 2.4.1.13) activity was determined as the UDP-Glc-dependent sucrose formation in the presence of fructose substrate. Supernatant enzyme preparations were incubated with 7.5 mM UDP-Glc, 7.5 mM fructose, 15 mM $MgCl_2$ in 50 mM Hepes-NaOH buffer (pH 7.5) at 25°C for 15 to 30 min (17). Reactions were terminated by heating with boiling water for 10 min, then samples were incubated 1 h with 10 IU of invertase (25°C, pH 4.5) and glucose was measured as described for the invertase method.

ADPG-PPase (ATP: α-D-glucose-1-phosphate adenylyltransferase; E.C 2.7.7.27) activity was determined by a coupled spectrophotometric assay (14, 17). Contents of the reaction mixture were 50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 5 mM ADP-Glc, 2 mM PPi, 0.3 mM $NADP^+$, 1.5 IU phophoglucomutase, 1 IU 6-phosphogluconate dehydrogenase, 5 IU glucose-6-P dehydrogenase, and 25 μl of soluble enzyme preparation. The reaction was initiated by the addition of enzyme preparation and the production of NADPH was monitored by the change in A at 340 nm.

ADP-Glc starch synthase (ADP-glucose: α-1,4-glucose α-4-glucosyltransferase; E.C. 2.4.1.21) was measured as adenosine diphospho-D-[U-^{14}C]glucose (25 cpm/nmol) incorporation into starch primer (10). The reaction mixture contained 50 mM Hepes-NaOH (pH 7.5), 1 mM Na-citrate, 2 μmol glutathione, 0.1% (w/v) BSA, 1 mg corn starch, 400 nmol ADP-Glc, and 50 μl of either soluble enzyme extract or starch-granule-bound enzyme fraction. After incubating at 25°C for 15 min, the reaction was terminated by adding 2 ml of 90% (v/v) methanol (containing 1% KCl) followed by centrifugation at 3300g for 20 min. The pellet collected by centrifugation was washed 5 times with 90% (v/v) methanol. After resuspension in 1 ml of distilled H_2O, 10 ml of scintillation cocktail with a toluene/Triton X-100 base (18) and 5 to 10 mg of thixotropic gelatenizing powder (Packard) were added. The incorporated [^{14}C]ADP-Glc was determined by liquid scintillation spectrometry (model 100, Beckman) and corrected for counting efficiency which was 80 to 90% as estimated by the external standard method.

Carbohydrate Assay. Sugars were extracted from 30 mg of freeze dried ground samples with 3 ml of 80% (v/v) ethanol at 50 to 55°C for 48 h. The extract was filtered through Whatman No. 42 paper, and the residue was washed three times with 1 ml of 80% ethanol at 25°C and the extracts were combined. Glucose in each extract was determined with a glucose oxidase method as described above. Sucrose was determined by the amount of glucose released following hydrolysis with 10 IU of invertase in 50 mM Mes buffer (pH 5.0) at 25°C for 1 h. Starch was determined following enzymic hydrolysis of the insoluble residue. Samples were suspended in 1 ml of water, heated for 1 h at 90°C to gelatinize starch, and cooled. One ml of citrate buffer (100 mM, pH 4.5) containing 10 IU of amyloglucosidase (Boehringer) and 20 mM NaF were added, and samples were incubated for 48 h at 40°C (18). Glucose in the hydrolysate was measured as described above. Parallel sets of standards containing 0 to 125 μg glucose or sucrose were used for calibration.

Radiolabeled ADP-Glc was obtained from Amersham. Other chemicals except as noted were obtained from Sigma Chemical Company. Results shown are means of two replicates; bars above or below symbols represent SE and where bars are absent, SE limits are within symbol dismensions.

RESULTS

Water, Dry Weight, Glucose, Sucrose, and Starch in Endosperms during Development. Water content per kernel, which provides a measure of kernel expansion growth, increased rapidly in the period from 10 to 15 DAP (Fig. 1A). Basal kernel water content was similar to that of apical kernels at 7 DAP, but their contents diverged during this period of rapid expansion. Water content decreased more rapidly in apical kernels than in basal kernels, thus at 34 DAP it was less than 30% (physiological maturity) in apical kernels (Fig. 1B). Apical kernels had lower dry weight accumulation rates and apparently shorter accumulation duration compared to basal kernels (Fig. 1C), so that at maturity apical kernels had only 44% of the dry weight of basal kernels.

Endosperm glucose, sucrose, and starch concentrations had distinct developmental patterns (Fig. 2, left). In both apical and basal kernels, glucose had its highest concentration at the earliest sampling date (7 DAP), and declined rapidly (Fig. 2A). Prior to 15 DAP, glucose was slightly higher in apical kernels. Sucrose concentration in apical and basal kernel peaked at 13 DAP (Fig. 2B), which was later than the glucose maximum. From 7 to 16 DAP, sucrose in basal kernels was significantly higher than in apical kernels. Starch concentrations reached a maximum at about 20 DAP in both apical and basal kernels, and were about 15 to 20% lower in apical kernels (Fig. 2C). Carbohydrate levels were examined on a kernel basis (Fig. 2, right) to identify developmental patterns which might escape detection on a dry weight basis, such as might occur in late stages when a large share of the dry weight is in nonmetabolic starch granule material. Glucose content in apical kernels was maintained at moderate levels from 7 to 40 DAP, while in basal kernels it peaked at 13 DAP and declined rapidly between 13 and 30 DAP (Fig. 2A). As found on a dry weight basis, sucrose on a kernel basis was substantially higher in basal than in apical kernels from 10 to 30 DAP. Net accumulation of sucrose per kernel occurred until 13 DAP in basal kernels and 19 DAP in apical kernels (Fig. 2B), thus this process overlapped the period of starch accumulation which began about 13 DAP and continued until 40 to 45 DAP (Fig. 2C).

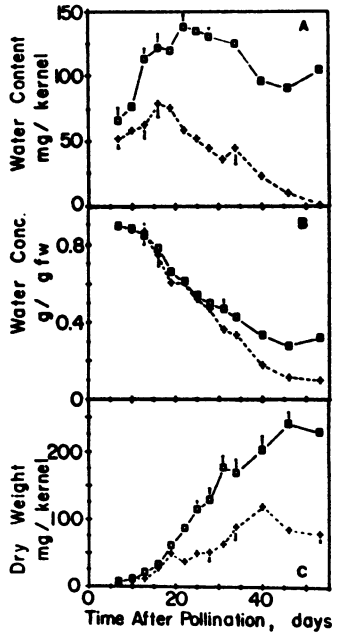

FIG. 1. Endosperm water content on a kernel basis (A) and on a fresh weight basis (B), and dry weight accumulation(C) during development of apical (+) and basal (□) kernels.

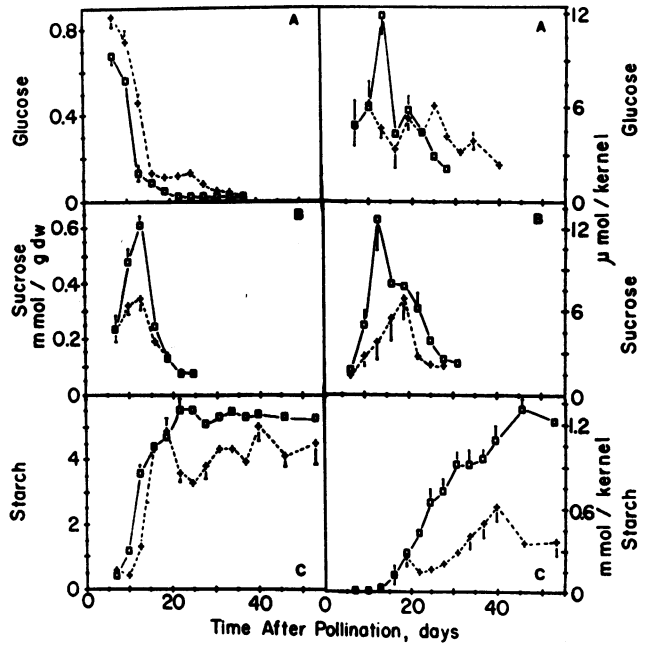

FIG. 2. Endosperm glucose (A), sucrose (B), and glucose equivalents of starch (C) on a dry weight basis (left) and a kernel basis (right) during development of apical (+) and basal (□) kernels.

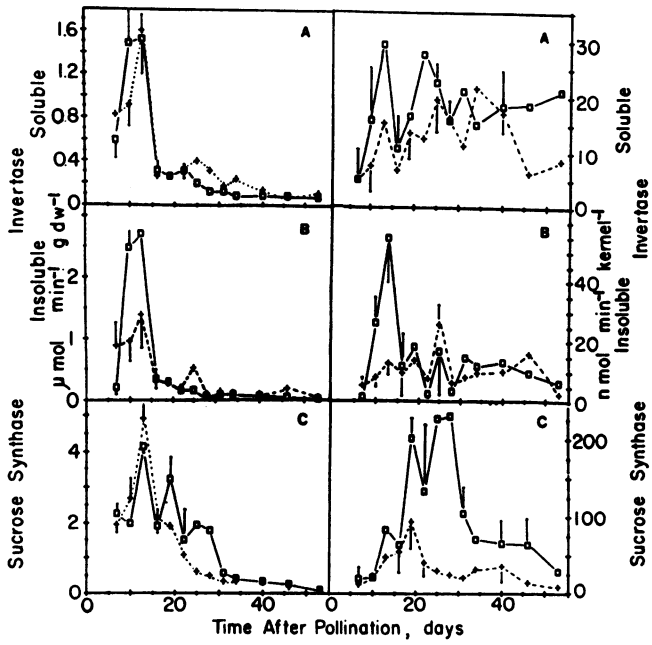

FIG. 3. Endosperm soluble (alkaline) invertase (A), insoluble (acid) invertase (B), and sucrose synthase (C) activities on a dry weight basis (left) and a kernel basis (right) during development of apical (+) and basal (□) kernels. The soluble and insoluble preparations from enzyme extracts were assayed for invertase activity at pH 7.5 and 5.0, respectively, by determining the glucose released from sucrose substrate. Sucrose synthase activity was determined as the UDP-Glc-dependent sucrose formation in the presence of fructose substrate. See text for details.

Sucrose Assimilation Enzyme Activities. Soluble invertase activities per gdw in both apical and basal kernels were high in the early stage from 7 to 13 DAP, corresponding with the period of maximum sucrose concentration (Fig. 3A). On a kernel basis, activities at both positions were variable and lacked a seasonal trend. Activities of insoluble invertase per gdw in both regions were also high at the early stage from 7 to 13 DAP and the activity in basal kernels was significantly higher than in apical kernels (Fig. 3B). Insoluble invertase activity was amost twice the activity of the soluble form. Sucrose synthase activity per gdw (Fig. 3C) appeared slightly higher in basal compared to apical kernels from 19 to 31 DAP, and on a kernel basis it was significantly higher in basal kernels from 19 DAP to maturity. Sucrose synthase activity per kernel peaked at 19 to 28 DAP for basal and at 19 DAP for apical kernels, which was after the period of net sucrose accumulation (Fig. 2B) and coincident with the period of most rapid starch accumulation (Fig. 2C).

Starch Synthesis Pathway Enzyme Activities. Activity of ADPG-PPase was maximal during the starch accumulation stage and was higher in basal than in apical kernels from 28 to 40 DAP both on a dry weight and a kernel basis (Fig. 4A). Soluble ADPG starch synthase per gdw was greatest at the early stage before substantial starch accumulation was discerned (Fig. 4B). Between 9 and 18 DAP, its activity declined rapidly in both apical and basal kernels. On a kernel basis, activity was quite variable and did not have a distinct seasonal maximum. The activity per gdw of the insoluble (starch-granule-bound) form of ADPG starch synthase was appreciably higher in basal kernels than in apical kernels from 15 to 40 DAP (Fig. 4C). At about 28 DAP, its activity in apical kernels declined to near 20% of the maximum, similar to the pattern which was found for ADPG-PPase (Fig. 4A). On a kernel basis, ADPG starch synthase in basal kernels was significantly higher than in apical kernels throughout the entire period of development (Fig. 4C).

DISCUSSION

This study showed that slower growing apical kernels differed from basal kernels in several respects. During the early stage

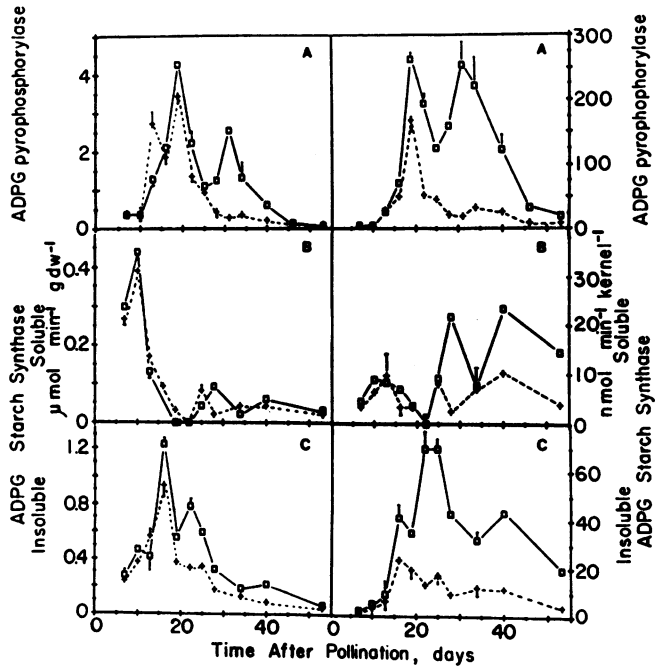

FIG. 4. Endosperm ADP-Glc pyrophosphorylase (A), soluble starch synthase (B), and insoluble starch synthase (C) activities on a dry weight basis (left) and a kernel basis (right) during development of apical (+) and basal (□) kernels. ADP-Glc pyrophosphorylase was determined by measuring glucose-1-P formation from ADP-Glc and PPi using a coupled spectrophotometric assay (14, 17). The soluble and insoluble preparations from enzyme extracts were assayed for starch synthase activity by measuring [^{14}C]ADP-Glc fixation to starch primer (10). See text for details.

from 7 to 19 DAP, when cell division and expansion occurs (5), apical kernels had substantially lower sucrose concentration and insoluble (acid) invertase activity. During the period of maximal starch synthesis, the activities of sucrose synthase, ADPG-PPase, and insoluble ADP-Glc-starch synthase were lower in apical kernels. The enzyme activities reported in this study were measured using crude preparations of tissue homogenate. It is possible that other factors might change to varying extents in endosperms of apical and basal kernels during development and influence the activities as assayed. For example, a proteinaceous inactivator of invertase was found to increase during development of maize endosperm (8) and an inhibitor of ADPG-PPase was found in crude homogenate of endosperm (7). Thus, the activities in the current study provide a preliminary indication of the relative enzyme potentials in apical and basal kernels. Further studies will be needed to establish their *in vivo* relevance.

Radiotracer kinetic studies have indicated that in maize endosperm sucrose is rapidly resynthesized from hexose (19). In growing tissues, sugars accumulate into vacuoles (9) and contribute to osmotic water potential, thus facilitating water flow into expanding cells (1). We observed that apical kernels had substantially lower sucrose concentrations and slightly higher glucose concentrations than basal kernels during the cell expansion stage (7–19 DAP). Similar differences in the sucrose concentration of endosperm from apical and middle-position kernels have been reported recently for field-grown maize (23). This suggests that the capacity for sucrose synthesis or accumulation into storage vacuoles was less developed in apical kernels. However, when apical kernels were excised and cultured *in vitro*, their peak sucrose concentrations increased (23). This suggests that the accumulation of sugar by apical-kernel endosperm is also restricted by competitive interactions between kernels or availability of photosynthate and other nutrients. Further studies will be needed to test these alternative possibilities.

Sucrose synthase activity per kernel was highest during the period after 10 DAP when the starch synthesis rate was maximal, consistent with a previous report (26). During this period, there was a net decline in sucrose per kernel. This pattern suggests that the enzyme was involved in the assimilation of sucrose for the synthesis of starch, as indicated by other studies in maize (3, 13). ADPG-PPase and insoluble starch synthase were also present at highest activity after 10 DAP, as previously reported for maize endosperm (11, 26).

The average rate of starch synthesis, calculated from the time course of starch accumulation per kernel (Fig. 2C), was 25 and 38 nmol of glucose equivalents per min in apical and basal endosperms for the period of most rapid accumulation from 28 to 40 DAP and 16 to 31 DAP, respectively. The measured activities of sucrose assimilation enzymes (Fig. 3) were adequate to account for these rates of starch accumulation. The measured activities of ADPG-PPase and starch synthase were adequate to account for the starch accumulation rate in basal kernels; however, they were slightly less than adequate in apical kernels (Fig. 4). Ozbun *et al.* (11) reported that the ADPG-PPase and starch synthase activities in normal genotypes of maize endosperm were 4 to 8 and 2 to 8 times greater, respectively, than the rate of starch synthesis during the period of rapid starch accumulation. Although ADPG-PPase from maize endosperm is relatively insensitive to regulation by D-glycerate-3-P and Pi (4, 26), it is possible that the current assays underestimated its potential activity due to incomplete extraction, presence of inhibitors (7), incomplete activation, or other factors. Soluble starch synthase activity may have been underestimated due to the presence of amylase in the extracts (10, 12, 16) or may not have been fully stimulated compared to rates with PEG in extraction and assay buffers (16). Further study will be needed to determine whether differences in maximum catalytic activities of these starch pathway enzymes were partially responsible for the differences between apical and basal kernels in starch accumulation rate.

Cell division, amyloplast multiplication and starch granule formation are some of the early events in endosperm development (5,. 15). Correlations have been found between the mature kernel size and number of endosperm cells (2, 15), and starch granules (15) per kernel. Such differences might also give rise to differences at later stages, such as the differences in enzyme activities of the starch synthesis pathway and starch accumulation rates observed in the current study. Studies which we will describe in another paper were conducted to determine whether the potential for larger size of basal-kernel endosperm is established solely during the early endosperm development period.

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