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REGULATION OF STARCH BIOSYNTHESIS IN NORMAL AND OPAQUE-2 MAIZE DURING ENDOSPERM DEVELOPMENT*

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Key Word Index—*Zea mays*: Gramineae; maize endosperm; Opaque-2; sucrose-UDP glucosyltransferase; glucose phosphate isomerase; starch synthetase.

Abstract—The absolute activities of sucrose-UDP glucosyltransferase, glucose-6-phosphate ketoisomerase and soluble and bound ADPG-starch glucosyltransferase have been studied in normal and Opaque-2 maize endosperms during development. In general, the activities of these enzymes except sucrose-UDP glucosyltransferase were higher up to 20 days post-pollination and lower at the 30 day stage in Opaque-2 than in normal maize endosperms. However, sucrose-UDP glucosyltransferase activity was higher in normal maize endosperm up to the 20 day stage while it was lower at subsequent stages than in Opaque-2. It is suggested that the lower level of these enzymes, except sucrose-UDP glucosyltransferase, might be responsible for the reduced accumulation of starch in Opaque-2 endosperm during later stages of endosperm development.

INTRODUCTION

Opaque-2 maize varieties, although nutritionally superior, have lower grain yield than normal maize. In addition, Opaque-2 maize has a dull appearance due to the loose packing of starch granules [1]. Starch and protein are the two major components of grain yield. Opaque-2 endosperms have been shown to contain less protein [2], as well as starch [3], than normal maize endosperms. The biochemical basis of improved protein quality and reduced protein accumulation in Opaque-2 is well established [4–8]. The starch biosynthesis in normal maize kernels [9] and endosperms [10] during development has been studied by examining the changes in key enzymes of the pathway of starch biosynthesis. However, a detailed study indicating the regulation of starch biosynthesis in high-lysine Opaque-2 maize endosperm has not been carried out. In our earlier study [11], changes in hexokinase, starch phosphorylase, inorganic pyrophosphatase, ADPG(UDPG) pyrophosphorylase and soluble ADPG starch synthetase in normal and Opaque-2 maize endosperms have been reported during development. In the present study, further investigations of the changes in sucrose UDP-glucosyltransferase, glucose-6-phosphate ketoisomerase, and soluble and bound ADPG-starch glucosyltransferase in the developing endosperms of normal and Opaque-2 have been undertaken to understand the regulation of starch biosynthesis.

RESULTS

Sucrose UDP-glucosyltransferase

Sucrose UDP-glucosyltransferase activity per endosperm as shown in Fig. 1 increased initially up to 20 days post-pollination and then declined up to the 30 day stage in normal maize, while in Opaque-2 it showed a slight increase up to 30 days post pollination. At the 15 and 20 day stages, the activity was higher in normal compared to Opaque-2 maize, while at later stages the trend reversed.

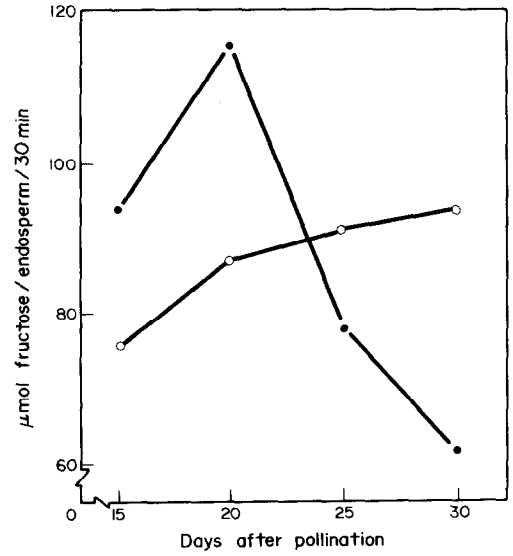

Fig. 1. Activity of sucrose-UDP glucosyltransferase in the developing endosperm of normal (●—●) and Opaque-2 (○—○) maize.

The sp. act. of the enzyme was higher in normal than in Opaque-2 up to the 25 day stage, whereas at the 30 day stage it was higher in Opaque-2 maize endosperms (Table 1). During endosperm development, sp. act. increased in Opaque-2 whereas in normal maize endosperms it

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Table 1. Specific activity of sucrose-UDP glucosyltransferase (μmol fructose released/mg protein/30 min) in developing normal and Opaque-2 maize endosperms

Days after pollination	Specific activity	
	Normal	Opaque-2
15	36.1	28.2
20	72.2	34.2
25	55.2	45.5
30	57.5	73.3

increased at the 20 day stage and decreased thereafter at the 25 day stage.

Glucose-6-phosphate ketoisomerase

In both normal and Opaque-2 maize, the enzyme activity per endosperm did not show much variation during development, except that in Opaque-2 at the 30 day stage the activity declined appreciably (Fig. 2). The activity in Opaque-2 was slightly higher compared to normal maize at the 15 and 25 day stages whereas it was only half that of normal maize endosperm at 30 day stage. Sp. act. followed an erratic pattern during endosperm development (Table 2). Sp. act. in Opaque-2 was higher at 15 day stage, while it was lower at the 20 and 30 day stages than normal maize endosperms.

Soluble ADPG-starch glucosyltransferase (starch synthetase)

(a) *Primed activity.* The primed activity of soluble starch synthetase per endosperm was *ca* 25% higher in Opaque-2 compared to normal maize at the 15 day stage and more or less comparable in both at the 20 and 25 day stages (Fig. 3). At the 30 day stage the activity in Opaque-2 endosperm was only 50% that of normal maize. During development

Table 2. Specific activity of glucose-6-phosphate ketoisomerase (μmol fructose formed/mg protein/5 min) in developing normal and Opaque-2 maize endosperms

Days after pollination	Specific activity	
	Normal	Opaque-2
15	17.8	23.8
20	25.5	17.0
25	21.1	20.5
30	23.5	14.6

the enzyme activity increased up to the 20 day stage in Opaque-2, and thereafter decreased, whereas in normal maize the activity showed a general increase during this period. The sp. act. of the enzyme (Table 3) from normal maize and Opaque-2 followed a pattern similar to that obtained on a per endosperm basis.

(b) *Unprimed activity.* Unprimed starch synthetase activity per endosperm in the case of normal maize increased consistently from the 15 to the 30 day stage, whereas in the case of Opaque-2 endosperms it increased up to the 25 day stage and then declined at the 30 day stage (Fig. 4). At all stages of endosperm development, activity was lower in Opaque-2 endosperms compared to normal maize endosperms. However, the difference was maximal at the 30 day stage where the activity was 6.5-fold more in normal than in Opaque-2 endosperms. Sp. act. in both normal and Opaque-2 maize endosperms increased up to the 25 day stage and then decreased at the 30 day stage (Table 3). The activity in normal maize was higher than in Opaque-2 endosperms.

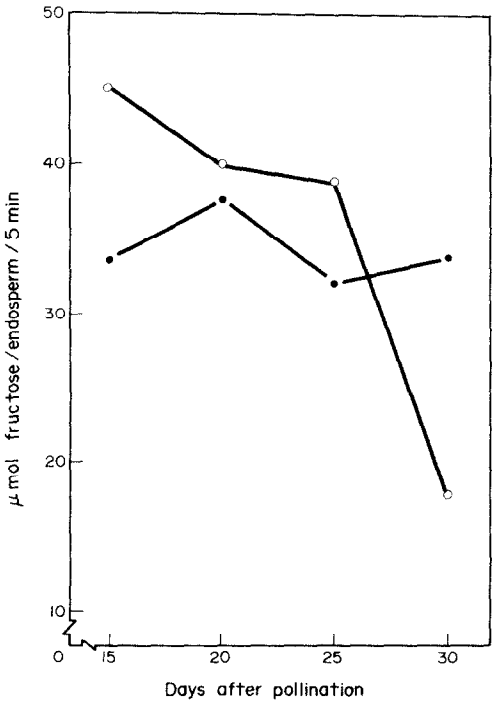

Fig. 2. Activity of glucose-6-phosphate ketoisomerase in the developing endosperm of normal (●—●) and Opaque-2 (○—○) maize.

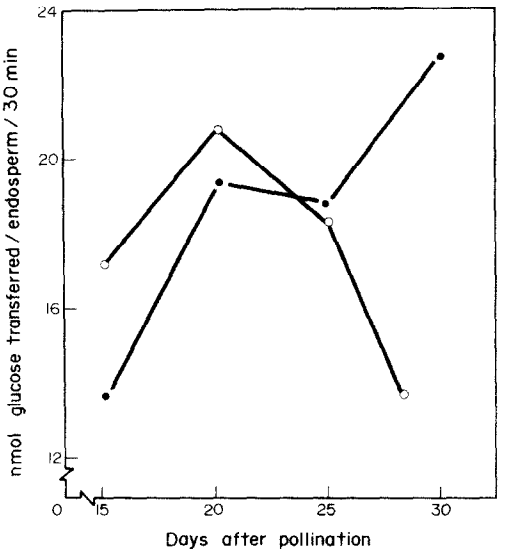

Fig. 3. Primed activity of soluble ADPG-starch glucosyltransferase in the developing endosperm of normal (●—●) and Opaque-2 (○—○) maize.

Table 3. Specific activity of soluble ADPG-starch glucosyltransferase (nmol glucose transferred/mg protein/30 min) in normal and Opaque-2 maize developing endosperms

Days after pollination	Primed activity		Unprimed activity	
	Normal	Opaque-2	Normal	Opaque-2
15	4.0	5.2	0.22	0.07
20	7.3	8.2	1.45	1.24
25	7.8	7.6	4.04	1.39
30	13.3	5.2	2.66	1.02

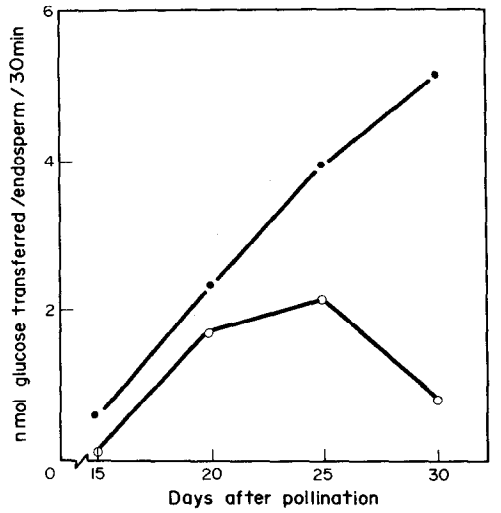

Fig. 4. Unprimed activity of soluble ADPG-starch glucosyltransferase in the developing endosperm of normal (●—●) and Opaque-2 (○—○) maize.

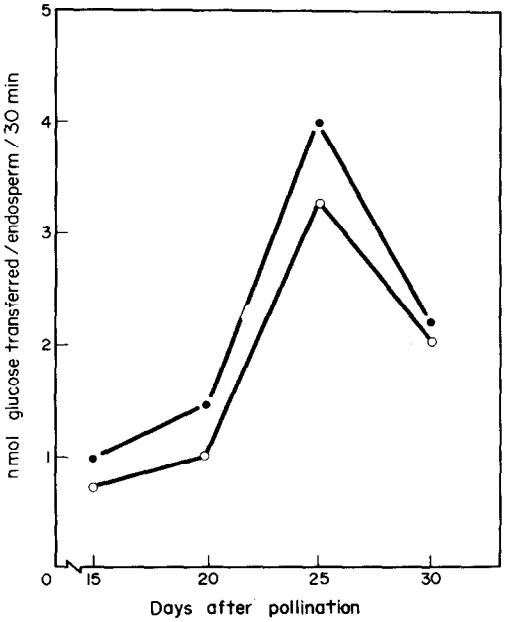

Fig. 5. Primed activity of bound ADPG-starch glucosyltransferase in the developing endosperm of normal (●—●) and Opaque-2 (○—○) maize.

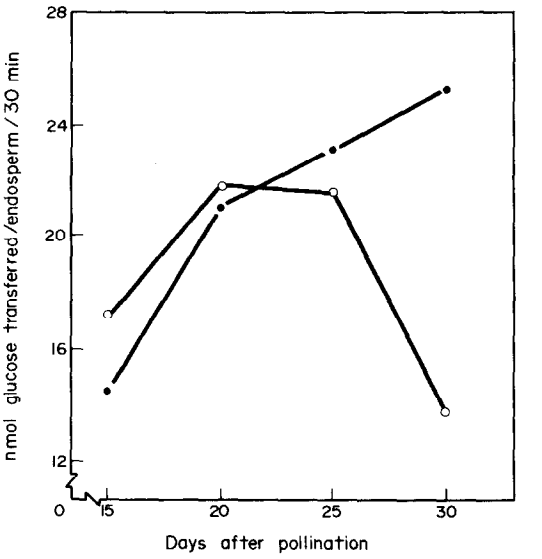

Fig. 6. Total activity of ADPG-starch glucosyltransferase (soluble + bound) in the developing endosperm of normal (●--●) and Opaque-2 (○—○) maize.

Bound ADPG-starch glucosyltransferase

The activity of bound starch synthetase per endosperm in both normal and Opaque-2 maize showed a similar trend during development (Fig. 5). The activity increased up to the 25 day stage and then decreased at the 30 day stage. At all stages the activity in normal maize was slightly higher than in Opaque-2. Bound starch synthetase activity was considerably lower than soluble starch synthetase activity during development in both normal and Opaque-2 maize endosperms (Figs. 3 and 5).

Total starch synthetase activity (soluble + bound) per endosperm increased in both normal and Opaque-2 endosperms up to the 20 day stage and decreased in Opaque-2 from 25 day to the 30 day stage, while in normal maize it increased slightly during this period (Fig. 6). However, the total activity in both Opaque-2 and normal maize was more or less comparable up to the 25 day stage. At the 30 day stage, the total starch synthetase activity in Opaque-2 was nearly half that of normal maize.

DISCUSSION

Improved nutritional quality in Opaque-2 maize appears to be due to the premature termination of the grain development processes which results in a decreased accumulation of protein [2, 8, 12] and starch [3, 11]. This mainly results in a decrease in grain wt. On a per endosperm basis, Opaque-2 contains *ca* 15% less starch compared with normal maize. The reduced level of starch accumulation in Opaque-2 could be due to a lower activity of the enzymes involved in starch biosynthesis.

The turning point with respect to starch accumulation in Opaque-2 appears to be *ca* 25 days post-pollination because starch accumulation is higher in Opaque-2 than normal maize during early endosperm development, whereas it is lower than normal maize towards the later stages [3]. A similar trend has been observed in the present study for the activities of glucose-6-phosphate ketoisomerase and starch synthetase during endosperm development.

Translocated sucrose is the main raw material for the synthesis of starch in the grain [13]. Sucrose is converted to UDP (ADP) glucose by the action of sucrose-UDP (ADP) glucosyltransferase and the fructose liberated may be converted to glucose-1-phosphate via hexokinase, glucose-6-phosphate ketoisomerase (GPI) and phosphoglucomutase [14, 15]. Since during later stages of endosperm development the absolute level of sucrose-UDP glucosyltransferase increased slightly in Opaque-2 endosperm while it decreased in normal maize endosperm, this enzyme is not limiting the starch biosynthesis in Opaque-2. Lower levels of GPI observed in the present study and that of hexokinase observed earlier [11] during later stages of Opaque-2 endosperm development might be responsible for the less efficient conversion of fructose liberated from sucrose to glucose-1-phosphate. Not only this, but also subsequent conversion of glucose-1-phosphate to ADP (UDP) glucose or direct polymerization to amylose by starch phosphorylase appears to be less efficient because of lower levels of ADP (UDP) glucose pyrophosphorylase and starch phosphorylase in Opaque-2 during later stages of endosperm development [11].

Biosynthesis of α-1,4-glucosidic linkages of starch in developing maize kernels is catalysed by ADPG-starch glucosyltransferase [9]. In the present study the activity of bound starch synthetase was only 5–15% that of soluble starch synthetase. A similar observation has also been made by Ozbun *et al.* [9] while studying starch synthetase in waxy (wx) and amylose extender (ae) maize during kernel development. The lower levels of starch synthetase observed in Opaque-2 during later stages of endosperm development appear to be further responsible for the reduced synthesis of amylose.

The results of the present study along with those of a previous study [11] clearly indicate decreased levels of most of the enzymes relating to starch biosynthesis in Opaque-2 compared to normal maize during later stages of endosperm development. It has been shown earlier [2] that after the 25 day stage the rate of protein accumulation in Opaque-2 endosperm is very slow compared to that in normal maize endosperm. The increase in protein accumulation in the endosperm was only 7% in the former compared to 36% in the latter after this stage. Therefore, the decreased enzyme levels in Opaque-2 compared to normal maize endosperm towards later stages of development may be a direct result of decreased rate of protein synthesis in it. The major constraints limiting grain yield in Opaque-2 maize thus appear to be a slow rate of protein synthesis, including that of the enzyme related to starch biosynthesis during later stages of endosperm development.

EXPERIMENTAL

One of the high combining, well-adapted normal maize inbred line Fla 3H 94 and its Opaque-2 version, were used. Self-pollinated ears were harvested at 15, 20, 25 and 30 days post-pollination, immediately chilled, endosperms were collected and stored in liquid N_2. The kernels mature at 38 days post-pollination.

Enzyme extracts were prepared by grinding the endosperm samples in liquid N_2 using a pestle and mortar with appropriate buffers (1:5 w/v). The supernatant obtained after centrifugation of the homogenate at 20 000 *g* for 20 min was collected. Residue was re-extracted and pooled supernatant used for enzyme assay. All operations, unless otherwise stated, were carried out at 4°.

Sucrose UDP-glucosyltransferase. Enzyme extract was prepared in 50 mM Tris–Cl (pH 7.3) containing 0.1 mM EDTA and 0.1 mM DTT. It was assayed according to ref. [16]. The reaction mixture in final vol. of 0.9 ml contained: sucrose, 250 μmol; UDP (pH 7), 2.5 μmol; NaF, 5 μmol; enzyme extract, 10 μl. Assay was carried out at 37° for 30 min. The fructose released was measured by Nelson's reagent [17] against a control in which UDP was omitted.

Glucose-6-phosphate ketoisomerase activity was assayed by incubating the enzyme prepn with glucose-6-P and measuring the formation of fructose-6-P according to ref. [18]. The enzyme extract was prepared in 50 mM Tris–Cl buffer, pH 7.5. The reaction mixture in a final vol. of 1.2 ml contained: HEPES buffer (pH 8), 100 μmol; glucose-6-P, 4 μmol; enzyme prepn, 50 μl. It was incubated at 30° for 5 min. In the control, enzyme was omitted.

ADPG-starch glucosyltransferase (starch synthetase). (i) *Soluble* ADPG-starch glucosyltransferase was extracted in 50 mM Tris–Cl buffer (pH 7.5) containing 0.01 M EDTA and 1 mM DTT. The pellet was again extracted with extraction buffer and soluble enzyme pooled. Primed activity of the soluble enzyme was assayed by the incorporation of [^{14}C]-glucose into starch from ADP glucose by slight modification of method of ref. [19]. The reaction mixture in a final vol. of 150 μl contained: ADP [U-^{14}C]-glucose (50 μCi/5 ml; 295 mCi/mmol), 1 μl; ADPglucose, 3 μmol; BICINE (pH 8.5), 20 μmol; EDTA (pH 8), 1 μmol; KOAc, 5 μmol; reduced glutathione, 2 μmol; amylopectin, 1 mg and enzyme extract, 50 μl. The enzyme was assayed at 30° for 30 min, after which the reaction was stopped by the addition of 2 ml of chilled MeOH (75%) containing 1% KCl and further processed as in ref. [19]. The values were corrected for blank counts obtained where MeOH–KCl was added before adding enzyme extract in assay. Unprimed enzyme activity was assayed according to ref. [20]. The reaction mixture for assay and blank was similar to that used for primed activity, except that amylopectin and KOAc were replaced by BSA (100 mg) and Na citrate (100 μmol). (ii) *Bound* starch synthetase was prepared by suspending the pellet, obtained after extracting soluble enzyme, in the extraction buffer. Only the primed activity of the enzyme was assayed as in case of soluble enzyme.

For all enzymes two independent extractions were done for each sample and then analysed in duplicate. The values reported in this study are an average of values for two independent extractions which agreed closely.

Protein was estimated as given in ref. [21] using BSA as standard.

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