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Sterol Accumulation and Composition in Developing *Zea mays L.* Kernels[1]

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ABSTRACT

Kernels were collected from three maize (*Zea mays* L.) inbreds from 10 days after pollination until kernel maturity. Sitosterol, campesterol, and stigmasterol were the major sterols at all stages of kernel development. Cholesterol was less than 1% of the dry weight. The three major sterols accumulated during kernel development, but at a rate slower than dry weight. The ratio of the sterols did not vary greatly among the inbreds. At maturity, the three inbreds, Wf9, Oh43, and Ky226, had sterol levels of 325, 228, and 173 micrograms per kernel, respectively. Sitosterol accounted for 75 to 85% of the sterol. The relative amount of stigmasterol decreased during the linear phase of development, while sitosterol increased in the free fraction and campesterol increased in the steryl ester fraction.

Free sterols and steryl esters were the major sterol fractions and steryl glycosides and acylated steryl glycosides were only minor components during kernel development. Free sterol content decreased rapidly in two maize inbreds between 10 and 26 days after pollination, but partially recovered in one of the inbreds during final stages of development. In the same two inbreds the steryl ester content reached a maximum during the late stages of linear kernel growth.

Sterols have been monitored during germination of maize (*Zea mays* L.) seedlings and sitosterol was the major sterol in the shoot, scutellum, and endosperm (9, 10). Stigmasterol was the major sterol in the unesterified sterol fraction extracted from the roots after 9 days (9). The free sterols increased in developing shoot and root; however, the endosperm and scutellum maintained a rather constant level. The level of sterol esters did not change in shoot and root during germination, but increased 3-fold in the scutellum. During germination of *Nicotiana tabacum* seeds, total sterols increased and stigmasterol and campesterol accounted for the major portion of the increase (1). The free and esterified sterols increased, while steryl glycosides decreased and sitosterol was the predominate sterol.

Sterol composition has not been determined during the development of the seeds with the exception of one study designed to determine changes in lipid classes and fatty acid composition of developing maize kernels (17). Sterols were found to be minor components of the lipid fraction and decreased relative to other lipids during kernel development.

Hou *et al.* (7) synthesized steryl glucosides by using a particulate enzyme preparation from immature soybean seeds. They suggested that since maturing soybean seeds had the enzymes for biosynthesis of steryl glucosides and possibly esterified steryl glucosides, these compounds may represent storage forms for sterol in mature seeds.

The objective of our research was to examine sterol accumulation and composition during maize kernel development.

MATERIALS AND METHODS

Plant Material. Three inbred corn (*Zea mays* L.) lines, Oh43, Wf9, and Ky226, were grown during two seasons at the University of Kentucky Agricultural Experiment Station farm, Lexington. These inbreds were selected because of their differences in carotenoid levels. Ky226 is a white endosperm inbred line low in total pigments, whereas Oh43 is a yellow endosperm inbred with relatively high total pigment and Wf9 is an intermediate (14). Fifty plants of each inbred were self-pollinated on the same day. Ear samples were taken at 10, 14, 18, 22, 26, 30, 34, 38, 42, 50, and 60 DAP[2] and after the kernels had fully matured and dried on the cob. During the second season samples of Oh43 and Ky226 were collected at 15, 26, 30, 45, 50, 60, and 90 DAP. At each sampling date three ears were collected for each inbred, and kernels from each sample ear were removed with a curved wood gouge and stored at −65 C. Moisture was removed from the kernels by lyophilization. Dry weight accumulation patterns were determined on a per kernel basis by weighing 20 kernels from each sampling date.

Sterol Analysis. For total sterol analysis the freeze-dried kernels were ground in a Wiley Mill to pass a 20-mesh screen and 5-g samples were used for the gravimetric method determinations with digitonin developed by Stedman and Rusaniwskyj (16). This method allows for hydrolysis of esters and glycosides, thus yielding total sterol values.

The sterol-digitonide precipitate was broken using a 12-hr treatment with 2 ml of pyridine containing cholestane as the internal standard. Digitonin was precipitated with diethyl ether and the sterols were analyzed by GLC (4). Calculations by computer were carried out using the internal standard for quantitation and external standard to correct for flame ionization detector response.

To separate the sterols into their free, ester, glycoside, and acylated glycoside classes 5 g were Soxhlet-extracted in acetone and fractionated by serial elution column chromatography (3, 13). Five grams of silica gel were added to the corn acetone

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[2] Abbreviation: DAP: days after pollination.

extract prior to drying under partial vacuum. The dried material was transferred to a 25-g silica gel (70–325 mesh) column. Serial elution was as follows: 150 ml of *n*-hexane, 200 ml of 10% benzene in *n*-hexane, and 700 ml of 40% benzene in *n*-hexane to elute steryl esters; 150 ml of benzene and 800 ml of chloroform to elute free sterols; 700 ml of 2% methanol in chloroform to elute acylated steryl glycosides; and 600 ml of 5% methanol in chloroform to elute steryl glycosides. Esters were hydrolyzed by refluxing for 30 min with 15 ml of 10% KOH in 95% ethanol. The hydrolysate was brought to pH 7.0 with dilute H_2SO_4 and extracted four times with *n*-hexane. The acylated steryl glycosides and steryl glycosides were hydrolyzed by refluxing for 15 hr with 25 ml of 95% ethanol which contained 0.13 ml H_2SO_4. Each fraction was taken to dryness under reduced pressure. Sterol analysis of each fraction was by gas chromatography (4, 5).

RESULTS

The patterns of sterol accumulation for the maize inbred lines were the same for the 2 years. Each of the three inbreds rapidly accumulated dry weight from 10 to 40 DAP (Fig. 1). The rate of increase appeared to be slightly faster for Wf9. After 40 DAP, the kernels slowed in dry weight accumulation and finally showed a decrease for inbreds Wf9 and Oh43. Ky226, a late maturing inbred, was still increasing in weight at the final sampling date.

On a mg/g dry weight basis, sterol levels in the kernels of the three inbreds decreased as the kernels developed (Table I). This decrease occurred mainly over the first 30 DAP and was at a relatively constant level for the remainder of the period. Oh43 and Ky226 had similar levels at 10 DAP; however, the white endosperm inbred, Ky226, decreased in sterol level at a faster rate and at 60 DAP had a much lower sterol content. Wf9 had the highest sterol level at 10 DAP, but at 60 DAP had nearly the same sterol level as Oh43. Sterols in Wf9 and Oh43 continued to accumulate on a per kernel basis until maximum dry weight was reached. The late maturing Ky226 continued to accumulate sterol and dry weight until 60 DAP; however, sterol per kernel was less than for the other two inbreds.

During the second year of these experiments, samples of the yellow endosperm inbred (Oh43) and white endosperm inbred (Ky226) were collected for free, esterified, and glycosidated sterol analysis (Table II). The free and steryl esters were the major fractions in developing maize kernels. Steryl glycosides and acylated steryl glycosides accounted for small amounts of the total sterol. Free sterols decreased rapidly between 15 and 26 DAP in both inbreds, while the steryl esters increased in Oh43 during the linear phase of kernel growth. Steryl esters decreased during the final part of the growth period.

Sitosterol, campesterol, and stigmasterol were the major sterols in developing maize kernels (Table III), accounting for over 99% of the total sterols. Cholesterol amounted to less than 1% in the kernels. Sitosterol, the major sterol in the free and steryl ester fractions, accounted for a slightly higher percentage of the steryl ester than the free fraction (Table III).

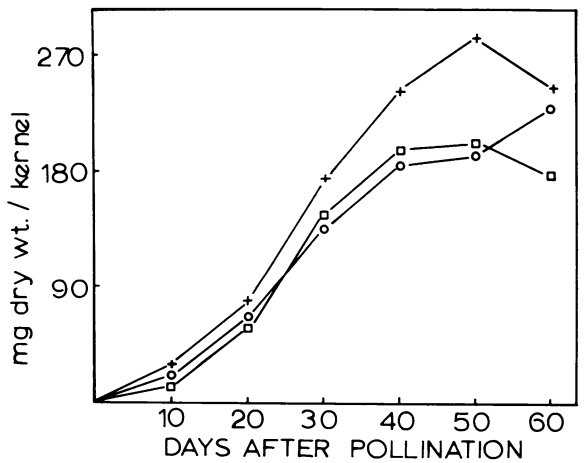

FIG. 1. Dry weight accumulation per kernel of three maize inbreds. Oh43 (□), Wf9 (+), and Ky226 (○).

Table I. *Sterol in Kernels of Three Inbreds from 10 to 60 Days after Pollination*

After Pollination	Ky226		Wf9		Oh43	
days	*mg g dry wt*	*μg/kernel*	*mg/g dry wt*	*μg/kernel*	*mg/g dry wt*	*μg/kernel*
10	2.36	30.4	2.97	29.1	2.40	26.9
14	1.82	47.7	2.19	53.2	2.09	56.4
18	1.31	66.3	1.73	91.2	1.52	83.8
22	1.03	85.9	1.44	126.0	1.45	122.5
26	1.02	120.5	1.59	220.5	1.52	172.9
30	0.90	129.9	1.36	232.4	1.32	190.9
34	0.78	126.0	1.40	279.9	1.28	207.1
38	0.82	150.1	1.36	325.6	1.32	259.5
42	0.79	143.5	Not Collected		1.31	270.5
50	0.83	159.4	1.33	378.1	1.29	261.7
60	0.76	173.4	1.32	324.5	1.31	228.3

Table II. *Free Sterol, Steryl Esters, Steryl Glycosides, and Acylated Steryl Glycosides in Developing Maize Kernels*

After Pollination	Free Sterol		Stery Esters		Steryl Glycosides		Acylated Steryl Glycosides	
	Oh43	Ky226	Oh43	Ky226	Oh43	Ky226	Oh43	Ky226
days	*mg/g dry wt*							
15	1.27	1.62	0.52	0.42	0.03	0.09	0.16	0.04
26	0.40	0.42	0.90	0.29	0.02	0.03	0.04	0.05
30	0.47	0.33	1.07	0.43	0.02	0.03	0.02	0.03
45	0.56	0.37	0.69	0.19	<0.01	0.04	<0.01	0.02
50	0.25	0.35	0.82	0.55	0.06	0.03	<0.01	0.04
60	0.65	0.23	0.54	0.19	0.01	<0.01	0.02	0.03
90	0.62	0.39	0.53	0.16	0.02	<0.01	<0.01	0.03

Table III. *Sterol Composition of Free and Steryl Ester Fractions from Developing Maize Kernels*

Fraction	Days after Pollination					
	15	26	30	45	50	60
	%					
Free sterol						
Campesterol	12.3	12.8	11.8	10.0	8.7	11.8
Stigmasterol	11.3	7.3	6.3	8.6	5.6	7.8
Sitosterol	76.4	79.9	81.9	81.4	85.7	80.4
Steryl esters						
Campesterol	9.1	12.3	12.8	9.9	12.2	9.4
Stigmasterol	5.9	5.4	3.7	4.5	3.7	6.0
Sitosterol	85.0	82.3	83.5	85.6	84.1	84.6

Sitosterol, campesterol and stigmasterol were also found in the steryl glycoside and acylated steryl glycoside fractions. The relative amount of stigmasterol in the free and steryl ester fractions decreased between 15 and 50 DAP. In both fractions the relative percentage of stigmasterol increased during final maturation of the kernel. The free sterol fraction decreased from 1.27 to 0.40 mg/g dry weight between 15 and 26 DAP, while the relative amounts of sitosterol, stigmasterol, and campesterol remained within 2 to 3 actual percentage points.

DISCUSSION

Sterol accumulation continued as maize kernels developed, although at a rate slower than the accumulation of dry weight. It has been reported that cell division in the endosperm of Wf9 is essentially completed within 28 days after pollination; however, dry weight accumulation continued for 45 days (8).

The highest level of free sterol in the maize kernels occurred during the most rapid rate of endosperm mitosis and decreased rapidly about the time of the last wave of mitosis which is completed between 17 and 25 DAP (2). During this same period the epidermal layer of scutellum has become differentiated, but some of the cells remain meristematic until 1 week before maturity (11). Our results indicate that it is not necessary for the kernels to maintain the initial concentration of free sterol during the early stages of linear growth. In a previous study the embryo accumulated dry weight and chemical constituents at a linear rate during the entire 45-day period (8). The endosperm increased faster in dry weight and accounted for most of the weight at maturity. The endosperm development was divided into two phases: the first was characterized by a rapid accumulation of soluble constituents, and the second phase by a utilization of the soluble constituents to cause an increase in protein. Our data indicate that sterols do not behave as the second phase soluble constituents of the endosperm, but are accumulated on a per kernel basis throughout the period of development. Kiesselbach and Walker (11) reported that the endosperm, embryo, and pericarp account for 84.3, 9.7, and 6.0% of the kernel dry weight, respectively.

Examining the figures of Kemp and Mercer (10) it appears that the endosperm contained about twice the amount of sterol as did the scutellum after 4 days of germination. Root and shoot tissue contained even less at that stage. Based on these observations and the fact that the major part of the kernel is endosperm, a major part of the sterol accumulation found in our study would be in the endosperm. It is apparent that sterol synthesis continues until maximum dry weight of the kernel is reached. Recently, Knights (12) suggested that the concentration of specific sterols in seeds might be locally determined by selective biosynthesis or selective migration. In the case of the corn kernel it appears likely that the sterols required by the embryo would be synthesized locally and those for the endosperm synthesized in that tissue.

Reasons for relatively large amounts of sitosterol and trace quantities of cholesterol in the developing corn kernels cannot be given presently. However, Heftman (6) has suggested that sitosterol may function as a reserve supply for other phytosterols. Cholesterol has been described as a key intermediate of other plant steroids (6) and Kemp and Mercer (10) found the esterified cholesterol to be the major sterol of the nuclear and chloroplastic fraction in maize shoots.

Kemp and Mercer (10) found, in endosperm 4 days after germination of maize seed, the amount of free sterol to be more than three times greater than the esterified sterol. We did not find this magnitude of difference in the whole, mature kernels. The genotypes we studied ranged from the extreme case of Ky226 which had over two times more free sterol than steryl ester at 90 DAP, to Oh43 which had only 1.2 times as much free sterol as steryl ester at the last sampling date. If the free sterols are associated with membranes, as has been suggested (5, 6, 10), one might expect the level to remain higher during the period of mitosis and cell enlargement; the latter continues well after mid-maturity (2). However, the ratio of membrane to other cellular material in developing maize kernels has not been determined. Grunwald (5) suggested that the free sterols play a role in controlling membrane permeability. In our studies free sterol did continue to accumulate during kernel development.

The suggestion by Hou *et al.* (7) that steryl glucosides and probably acylated steryl glucosides are storage forms of sterols in seeds is not valid for maize kernels since these were present in such small quantities. It is doubtful that they serve as carriers for sterol transport during maize kernel development as has been suggested in *Mycoplasma* (15).

Furthermore, our data would indicate that the relative amounts of the sterol components are in equilibrium in the free and esterified fractions with the possible exception of stigmasterol. This gives support that the free sterols form a pool for the steryl ester formation with a random process of esterification available to convert sterols to steryl esters. This equilibrium is in contrast to the sterol changes reported during germination of maize (9) and tobacco seedlings (1).

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