Phytochemistry, 1975, Vol. 14, pp. 1201–1203. Pergamon Press. Printed in England.

STEROLS IN DEVELOPING SEED FROM LOW AND HIGH OIL *ZEA MAYS* STRAINS

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(*Revised Received* 13 *August* 1974)

Key Word Index—*Zea mays* L.; Gramineae; maize; phytosterols; steryl esters; sterol glycosides; fatty acids; seed development and biosynthesis.

Abstract—Two strains of maize, one developed for high oil seed and one for low oil seed, where examined for sterol and fatty acid composition during seed development. During development of high oil seed the free sterol concentration decreased approx 24% while the steryl esters increased over 200%. In the low oil seed, the concentration of the free sterols decreased almost 300% while steryl esters decreased over 200%. On a per seed basis, the high oil seed accumulated higher levels of free sterol and steryl esters. Acylated steryl glycosides were highest in the high oil seed during the first phase of development. Linoleic and oleic acids were the major fatty acids in the oil of both strains.

INTRODUCTION

Free sterols and steryl esters are the major sterol fractions of developing maize kernels [1]. In two maize inbreds the free sterol concentration decreased rapidly between 10 and 26 days after pollination and the steryl esters appeared to reach a maximum concentration during the late stages of linear kernel growth. In an earlier report steryl esters were found to decrease in relation to other lipids during kernel development [2]. The presence of enzymes for the biosynthesis of steryl glucosides, and possibly esterified steryl glucosides, in maturing soybean seeds has been reported [3] and it is suggested that these compounds may be storage forms of sterols in mature seeds.

The fatty acid composition of the oil changes during maize kernel development [4–6]. Palmitic and linolenic acid percentages decrease while oleic and stearic acid percentages increase with maturity. During kernel development the amount of linoleic acid varies among genotypes and the rate and time of accumulation vary among the inbreds [6]. These variations may influence the pattern of steryl ester accumulation in seeds. The objective of our research was to examine the relationship among the sterol fractions, particularly the free and esterified fractions in genotypes that varied in oil content. We hypothesized that the amount of esterified sterols should be related to fatty acid levels in the seed.

RESULTS AND DISCUSSION

The free sterols and steryl esters were the major sterol fractions in low (ILO) and high (IHO) oil maize seeds (Table 1). Sitosterol, stigmasterol, and campesterol were the major sterols in all sterol fractions. The component sterol percentages were similar to those reported previously for commercial inbreds with normal oil content (4–6% oil) [1]. In both strains sitosterol varied from 75 to 82% of the total while campesterol accounted for 10–15% and stigmasterol from 4 to 10%. The high oil strain had from 15 to 20% more campesterol than the low oil strain. The relative amount of stigmasterol decreased as the seeds developed.

The two strains differed greatly in the pattern of free sterol and steryl ester accumulation. The concentration of free sterol in the low oil seed decreased from 15 to 60 days after pollination (DAP). Steryl esters decreased in the low oil seed from 15 to 45 DAP, but increased slightly from 45 to 60

Table 1. Free sterol, steryl esters, steryl glycosides and acylated steryl glycosides in developing seeds of low and high oil *Zea mays* strains

Strain	DAP*	Free sterol		Steryl esters		Steryl glycosides		Acylated steryl glycosides	
		μg/g dry wt	μg/seed	μg/g dry wt	μg/seed	μg/g dry wt	μg/seed	μg/g dry wt	μg/seed
ILO	15	1152	41·3	444	15·9	49	1·8	5	Trace
	30	678	133·9	299	59·1	5	Trace	5	Trace
	45	459	168·5	158	58·0	5	Trace	15	5·5
	60	398	137·3	194	66·9	25	8·6	5	Trace
IHO	15	1386	35·6	395	10·1	30	0·1	160	4·1
	30	1140	122·2	650	69·7	5	Trace	105	11·3
	45	1209	226·9	778	146·0	5	Trace	20	3·8
	60	1060	213·6	926	186·6	25	5·0	5	1·0

* Days after pollination.

DAP (Table 1). In the high oil seed the free sterol concentration decreased during kernel development but remained much higher than that of the low oil seed. Steryl esters in the high oil seed increased in concentration as the kernels developed, whereas, a decrease was found for the low oil seed. Steryl glycosides were found in small but similar quantities in both strains. The high oil strain had much higher quantities of acylated steryl glycosides than did the lower oil strain but they decreased as the kernels matured. On a per kernel basis, the high oil strain accumulated higher amounts of free sterols and steryl esters (Table 1).

Steryl esters may be involved in sterol transport [7] although this proposal is not supported by the work of Bush and Grunwald [8] who found that two sterols had higher specific radioactivities in the ester form than in the free form when MVA-[2-^{14}C] was administered to *Nicotiana tobacum*. Goad [9] suggested that steryl esters may be associated with sterol synthesis. Rather than involvement solely in sterol transport or sterol synthesis, we postulate that the steryl esters are involved in some phase of lipid biosynthesis in developing maize seed since the higher oil strain had a correspondingly higher level of sterol. Their role is possibly correlated with fatty acid transport, stability, or biosynthesis. Nonesterified fatty acids are enzyme inhibitors [10] and they are present in small quantities in cells. Further, it has been suggested that fatty acids are carried on phosphotidyl choline during fatty acid desaturation [10]. Sterols could also serve as fatty acid carriers in a lipid complex. Rapid turnover rates of certain steryl esters occur in young seedlings [8, 11] which could result from an active role in lipid synthesis. In the high oil-high steryl ester seed the amount of free sterol was also high and this could serve as a pool for the esterified fraction.

Palmitic, stearic, oleic, linoleic, and linolenic acids were found in the oil of the two strains (Table 2) in comparable amounts to those reported pre-

Table 2. Fatty acid composition in developing seeds of high and low oil strains at four dates after pollination

Strain	DAP*	Fatty acid composition (%)				
		Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
ILO	15	16·3	1·2	35·1	44·7	2·7
	30	14·7	1·4	19·3	61·1	3·5
	45	14·4	2·1	13·4	66·1	4·0
	60	15·7	2·5	14·8	62·4	4·6
IHO	15	16·8	1·3	32·3	47·2	2·4
	30	12·9	1·0	37·8	47·4	0·9
	45	12·8	1·3	34·8	50·2	0·9
	60	12·5	1·3	31·3	54·9	Trace

* Days after pollination.

viously for these strains [4, 5] and for other inbreds [6, 12]. Palmitic acid decreased from 15 to 60% DAP for both strains whereas the linoleic acid percentages increased in agreement with the results of Curtis *et al.* [5] who examined immature and mature kernels of these two oil strains. In our samples oleic acid decreased from 15 to 45% DAP for the ILO seeds. The fatty acid composition of the steryl esters was not determined for the developing seeds in this study. However, Kemp and Mercer [7] found that the composition of the fatty acid fraction derived from the steryl esters of maize shoots varied from C_{12} to greater than C_{22} and palmitic, palmitoleic, lauric, myristic and linolenic acids were the most abundant.

EXPERIMENTAL

Plant material. Two maize strains that differed greatly in oil content were grown at the University of Kentucky Agricultural Experiment Station farm, Lexington, Kentucky in 1970. Illinois High Oil (IHO), 15–17% oil, and Illinois Low Oil (ILO), 1–2% oil [5], developed by the University of Illinois, were sampled at 15, 30, 45 and 60 days after pollination (DAP). A composite sample of kernels from 3–5 ears was removed by a wood gouge and stored at −65° prior to lyophilization. Kernel dry wt was determined on 20 kernels from each sampling date. Similar samples of several strain/date combinations were collected the following year in order to confirm the prior results.

Sterol analysis. Samples (5 g) of ground, dried seeds were extracted with Me_2CO (150 ml) for 24 hr in a Soxhlet apparatus and the lipid fractionated by column chromatography [13, 14]. Si gel (5 g) was added to the Me_2CO extract, dried under partial vacuum, and transferred to a 25 g Si gel (70–325 mesh) column. Serial elution with *n*-hexane (150 ml), 10% C_6H_6 in *n*-hexane (200 ml), and 40% C_6H_6 in *n*-hexane (700 ml) gave steryl esters; C_6H_6 (150 ml) and $CHCl_3$ (800 ml) eluted free sterols; 2% MeOH in $CHCl_3$ (700 ml) eluted acylated steryl glycosides; and 5% MeOH in $CHCl_3$ (600 ml) eluted steryl glycosides. Esters were hydrolyzed for 30 min with 10% KOH in 95% EtOH (15 ml). The hydrolysate was adjusted to pH 7·0 with dilute H_2SO_4 and extracted with *n*-hexane (×4). The acylated steryl glycosides and steryl glycosides were hydrolyzed by refluxing for 15 hr with 95% ethanol (25 ml) and H_2SO_4 (0·13 ml) [8, 11]. Sterol analysis was by GLC [15].

Fatty acid analysis. The oil was homogenized (30 sec) with petrol (20 ml), filtered, and evaporated to dryness under reduced pressure. The fatty acids were subjected to a base-catalyzed interesterification prior to GLC on 12% diethylene glycol succinate Anakrom ABS (70/80 mesh) [6, 12].

Acknowledgement– The investigation reported in this paper (No. 73-3-126) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

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