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LIPID CHANGES IN MATURING AND GERMINATING COTTONSEEDS

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Key Word Index—*Gossypium hirsutum*; *G. barbadense*; Malvaceae cottonseed; lipid changes; cyclopropene fatty acids.

Abstract—Developing cottonseeds (*Gossypium hirsutum*) show a rapid rise in oil synthesis after the fifth week including cyclopropene fatty acids (CPFA) and free fatty acids. During subsequent slow oil synthesis, the proportion of saturated fatty acids remains stationary, that of oleic acid decreases marginally, that of linoleic acid rises and that of CPFA declines CPFA occurs only as triacylglycerol and may arise by modification of preformed triglycerides. During cottonseed germination, even as the oil content declines, the major fatty acid composition remains fairly constant Disappearance of CPFA has no relationship with that of major fatty acids or with oil mobilization, suggesting metabolism by a different mechanism.

INTRODUCTION

Lipid accumulation in developing cottonseed occurs with a rapid increase after *ca* four to six weeks These lipids are characterized by an elevated initial free acidity, absence of cyclopropene fatty acids (CPFA) at immaturity, uniform fatty acid composition throughout, and desaturation in the final maturing stages [1, 2]. Later studies showed a decrease in CPFA after 10 days of flowering [3] and a rise in gossypol and tocopherol contents with increasing maturity [4]. During the germination of cottonseeds (as indeed of all seeds), reserve carbohydrates are rapidly consumed [5] and unsaturated fatty acids disappear rapidly [6]. CPFA are of course very minor constituents of cottonseed and they disappear much faster during germination than do normal fatty acids [7]. CPFA occurs at a much higher concentration in *Sterculia foetida* seed oil, from which abnormal disappearance during germination has been reported [8].

Lipid changes in maturing and germinating cottonseeds, with special reference to CPFA, are reported in this paper.

RESULTS AND DISCUSSION

Maturation studies

For both cultivars studied, seed and kernel weights reach maximal values in the seventh week of growth (Table 1). Lint content decreases with maturity. Free gossypol content increases with the period of rapid growth and then levels off, but total gossypol continues to increase steadily. The protein content, expressed on a defatted kernel basis, also rises rapidly at first, but slowly thereafter All these trends are shown by both cultivars, though to different degrees.

The changes in oil content are striking. For the cultivar G Cot 10, there is an abrupt increase within the seven days between the fifth and sixth week from 0.2 to 22.1%, and only a slight increase thereafter to 23.8% in the twelfth week. In the other cultivar G. Cot 11, this increase is from 0.05 to 12.5% and occurs over 14 days, thereafter rising to 22.8% in the twelfth week.

The major fatty acids (Table 2) show no spectacular changes during the rapid growth period; changes with maturity are steady and consistent. Saturated and oleic acid percentages in the oil present decrease marginally and the linoleic acid content rises. These changes stand out much more sharply when the quantities of individual fatty acids present in 100 of dry whole seeds at each stage are considered (Fig. 1). In the slower-maturing cultivar G Cot 11, all developments occur more slowly Linoleic acid appears to be generated by desaturation of oleic or saturated acids during seed maturation

It is seen from Table 2 that the free fatty acid content declines gradually all through, showing no reflection of the sudden oil spurt phase. Such a decrease of free acids during fat synthesis is to be expected.

The CPFA contents at any time are extremely low but do fall fourfold during maturation, from 0.47 to 0.13% in one cultivar and from 0.38 to 0.10% in the other. Such a decrease must reflect some biological disturbance. Analysis of total lipids, extracted from G Cot 11 seeds at 35, 42 and 63 days, followed by TLC fractionation of lipid classes and CPFA estimation, showed no CPFA in diacylglycerol, monoacylglycerol, free fatty acids, glycolipid or phospholipid. Only in the triacylglycerol component was CPFA present to the extent of 0.43, 0.41 and 0.11%, respectively, at the three growth stages In cottonseed, acylglycerols carrying CPFA appear to arise by modification of existing compounds.

Germination studies

During germination of cultivars Hybrid 4 and Suvin, roots emerged on the second day, leaves on the fourth and rootlets on the sixth. In slower-germinating Laxmi and Varalaxmi, leaves appeared two days later. These differences in germination rate are reflected in slower rates of oil disappearance in the last two cultivars, the actual order being Hybrid 4, Suvin, Laxmi and Varalaxmi (Table 3). An apparent parallel protein increase which

Table 1 Changes in seed characteristics of two varieties of cottonseed during maturation

Maturation time (days)	Seed index		Lint, %		Kernel, %		Oil content, %		Gossypol content, %						Protein*, %	
									Free		Total					
	G Cot 10	G Cot 11	G Cot 10	G Cot 11	G Cot 10	G Cot 11	G Cot 10	G Cot 11	G Cot 10	G Cot 11	G Cot 10	G Cot 11	G Cot 10	G Cot 11	G Cot 10	G Cot 11
21	1 7	—	14 7	—	20 9	-	0 03	-	0 1	-	0 8	-	28 1	- -		
28	3 2	1 9	10 2	11 0	43 2	13 5	0 14	0 02	0 5	0 6	1 2	1 1	34 4	29 0		
35	4 3	2 2	9 6	9 6	53 8	17 6	0 21	0 05	0 7	0 7	1 3	1 3	35 2	31 9		
42	4 6	3 7	8 7	6 2	55 8	40 0	22 12	12 5	0 8	0 7	1 6	1 3	37 4	33 8		
49	4 9	5 1	8 3	5 0	56 0	46 1	22 90	19 7	0 9	0 7	1 8	1 5	39 4	36 1		
56	4 9	5 1	7 5	3 9	56 8	47 3	22 90	22 4	0 9	0 8	1 9	2 0	40 8	39 7		
63	4 9	5 1	5 2	3 7	56 6	47 6	23 50	22 8	0 9	0 9	1 9	2 0	41 2	40 4		
72	4 9	5 1	5 1	3 7	56 5	47 6	23 80	22 8	0 9	0 9	2 0	2 1	40 9	40 2		

*Percentage in decoiled dry meal of kernel

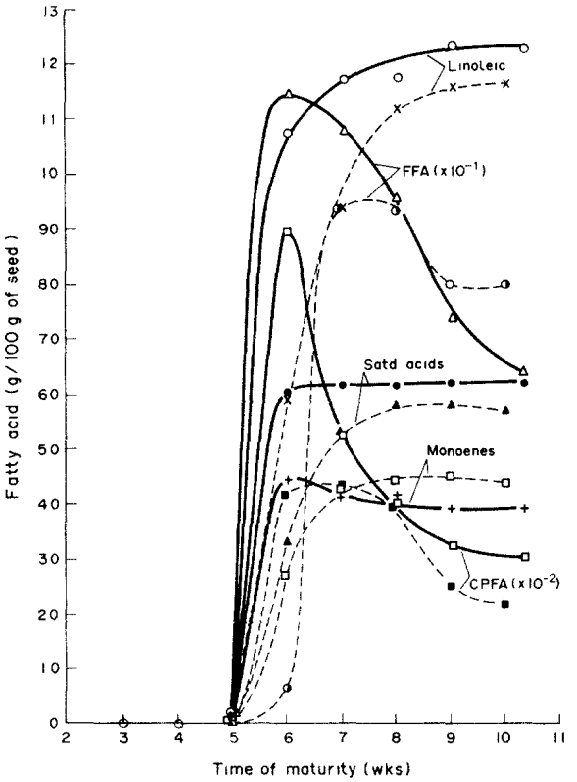

Fig 1 Changes in amount of fatty acids in seed during maturation of cottonseed (Bold lines for G Cot 10 and dotted lines for G Cot 11)

varies from 30 to 11, on the sixth day, is obviously a consequence of the well-known mobilization of carbohydrates during germination [5] Both free and total gossypol levels decrease progressively as germination advances

Apparent free fatty acid levels in oils rise as germination advances (Table 4). However, when calculated on a whole seed basis, the two cultivars are seen to have attained a constant amount of free fatty acids and the two others are moving towards one

An important factor is that even as the oil content decreases, the major fatty acid composition remains essentially constant showing that saturated, oleic and linoleic acids are mobilized equally for germination (Table 5) The actual proportions mobilized vary from one cultivar to another, being related to the rates of individual germination

It is evident that disappearance of CPFA is independent of cultivar and unrelated to the mobilization of the major fatty acids The extreme example is Varalaxmi when 92% of CPFA has gone on the sixth day, but only 6–7% of normal fatty acids Normal fatty acids are known to be metabolized during germination after hydrolysis of their acylglycerols

EXPERIMENTAL

For the maturation study, seeds from two cultivars of *G hirsutum* namely G Cot 10 and G Cot 11, derived from bolls detached at weekly intervals after setting, were harvested and analysed

Table 2. Characteristics of oils during maturation of G Cot 10 and G. Cot. 11 varieties of cottonseed

Maturation time,	FFA, % as oleic		CPFA, % as malvalic		Fatty acid composition, %					
					Total satd		Monoenes		Linoleic	
days	G Cot. 10	G Cot. 11	G. Cot. 10	G. Cot. 11	G Cot. 10	G Cot. 11	G Cot. 10	G Cot. 11	G Cot. 10	G. Cot. 11
21	—	—	—	—	33 7	—	23.6	—	42 7	—
28	8 9	8 4	0.47	0 38	30.5	30 8	22.8	24.4	46.7	44.8
35	7.7	8 2	0 46	0 36	30 3	29.8	21.9	22 4	47 8	47.8
42	5 1	5.9	0 40	0 34	28 4	27.9	20.8	22.4	50.8	49.7
49	4 7	4 8	0.23	0.22	28 0	27.8	18 5	22.1	53.5	50 1
56	4.2	4.2	0.17	0.17	27 8	26 8	18 9	20 8	53.3	52.4
63	3 1	3 5	0 14	0.11	27 8	26.2	17.4	20.7	54 8	53 1
72	2 7	3 5	0 13	0.10	27 7	25.8	17 4	20 4	54 9	53 8

Table 3 Changes occurring in composition of cottonseed during germination

Cultivar	Hybrid-4				Suvin				Laxmi				Varalaxmi			
No of days of germination	0	2	4	6	0	2	4	6	0	2	4	6	0	2	4	6
Oil content, %	20 9	17 4	15 3	12.3	24.9	22 4	21.7	18.6	20.6	20.2	19 0	17.7	26.0	25 8	25 5	24 6
Free gossypol, %	1 44	1 28	1.11	0.98	1 72	1 63	1.35	1.29	1.11	0.84	0 72	0.56	1 68	1 44	1.35	1.28
Total gossypol, %	1 67	1 49	1 30	1.16	2.02	1 93	1 63	1 57	2.02	1.57	1.36	1.10	2.36	2.06	1.96	1.85
Protein, %	37 1	40 6	45.3	48.3	35 5	39 5	42.9	45.1	37.0	39.5	40.2	43.9	37.9	40 3	41.1	42 1

For the germination study, mature seeds of three cultivars of *G. hirsutum*, namely 'Hybrid 4' 'Laxmi' and 'Varalaxmi', and one of *G. barbadense* termed 'Suvin' were obtained. The latter seeds were used as obtained. The three *G hirsutum* seeds were soaked in conc. H_2SO_4 for a few min, washed with H_2O and sound, healthy seeds which did not float were isolated and dried on filter paper. For germination, seeds of known wt were soaked in H_2O for 10 min and kept between wet filter papers. For each cultivar, one set of seeds served as the O-day control; three other sets were germinated for 48, 96 and 144 hr followed by analysis

Analytical procedures. As is customary in cottonseed analysis [9], results were expressed as in Table 4

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Table 4

Characteristics	Basis of expression	Details of determination
Seed index (g)	Weight of 100 seeds	Manual
Lint content (%)	Whole seed, as is	AOCS [9]
Kernel content (%)	Whole seed, as is	Manual
Moisture content (%)	Whole seed, as is	AOCS [9]
Protein content (%)	Kernel, on moisture free and oil free (m f.b and o.f.b)	ECO Norma Automatic N Analyser (% N × 6 25)
Oil content (%)	Whole seed, on m.f.b.	AOCS [9]
Total gossypol (%)	Kernel, on m f.b	AOCS [9]
Free gossypol (%)	Kernel, on m f b.	AOCS [9]
Free fatty acids as % oleic	Extracted oil	AOCS [9]
CPFA as % malvalic	Extracted oil	AOAC [10]
Fatty acids composition (%)	Mixed fatty acids of oil	GC, FID Detector, 2 m FFAP column

Table 5 Changes* in characteristics of oil during germination of cottonseed

Cultivar	Hybrid-4				Suvin				Laxmi				Varalaxmi			
No of days	0	2	4	6	0	2	4	6	0	2	4	6	0	2	4	6
F F A,	2 7	7 4	8 1	9 9	2 8	6 4	8 9	10 2	2 7	3 4	5 6	10 3	2 5	5 9	8 0	10 0
%	(0 6)	(1 3)	(1 2)	(1 2)	(0 7)	(1 4)	(1 9)	(1 9)	(0 6)	(0 7)	(1 1)	(1 8)	(0 7)	(1 5)	(2 0)	(2 5)
CPFA	0 36	0 35	0 25	0 02	0 43	0 36	0 12	0 05	0 39	0 33	0 20	0 05	0 46	0 40	0 29	0 03
%	(0 08)	(0 06)	(0 04)	(0 003)	(0 11)	(0 08)	(0 03)	(0 01)	(0 08)	(0 07)	(0 04)	(0 01)	(0 12)	(0 10)	(0 07)	(0 01)
Total satd,	27 8	27 0	27 0	26 4	25 5	26 5	24 7	24 7	27 7	27 8	27 6	27 1	26 7	26 5	26 5	26 2
%	(5 6)	(4 5)	(3 8)	(3 1)	(6 1)	(5 2)	(5 1)	(4 4)	(5 5)	(5 3)	(5 0)	(4 7)	(6 6)	(6 6)	(6 5)	(6 2)
Monoenes,	18 0	18 3	18 4	21 4	20 8	21 7	22 3	22 7	18 8	19 1	19 2	19 6	18 3	18 5	18 6	19 4
%	(3 8)	(3 0)	(2 7)	(2 5)	(4 9)	(4 7)	(4 6)	(4 1)	(3 7)	(3 7)	(3 6)	(3 5)	(4 6)	(4 6)	(4 5)	(4 3)
Dienes,	54 2	54 7	54 6	52 2	53 7	53 8	53 0	52 6	53 5	53 1	53 2	53 3	55 0	55 0	54 9	54 4
%	(10 6)	(9 1)	(8 0)	(6 1)	(12 8)	(11 5)	(11 0)	(9 3)	(10 6)	(10 3)	(9 6)	(8 7)	(13 7)	(13 6)	(13 4)	(12 8)

*Figures in parentheses are the calculated amounts of the respective components as g per 100 g of seeds (moisture free basis)

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