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TRIGLYCERIDE METABOLISM IN GERMINATING *ANDROPOGON GAYANUS* SEEDS*

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Key Word Index—*Andropogon gayanus*; Gramineae; savannah grass; triglycerides; metabolism; germination.

Abstract—Seed triglycerides of *Andropogon gayanus* contained 17 fatty acid moieties, principally palmitic, oleic and linoleic acids. These were distributed in an essentially random manner amongst the triglycerides to form the following main types: POL, PLL, OOL, LLO and LLL. Triglycerides decreased during both light and dark germination but there was no evidence for selective hydrolysis. Free fatty acids appear to be derived from triglyceride hydrolysis but the free and triglyceride fatty acid composition differed. Less palmitic, oleic and linoleic acids and more stearic, linolenic and C_{20}-acids were found in the free state than combined in the triglycerides. Free fatty acids did not accumulate during germination.

INTRODUCTION

THE TRIGLYCERIDE composition of natural fats has been studied by fractional crystallization,[1] counter current distribution,[2–4] various oxidation techniques,[5–8] lipase hydrolysis[9–13] and TLC–GLC.[14,15] The results obtained from these methods have given rise to the 'even',[16] 'random',[17,18] 'restricted random'[19] and 'positional'[11,12] theories of triglyceride structure.

Triglyceride metabolism during germination has been described for several seeds; e.g. water melon,[20] soy beans[21] and wheat grains.[22] In these studies, individual triglyceride

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types were not determined, and only the total weight and fatty acid compositions were reported.

This investigation presents the results of TLC–GLC studies of the changes in the triglyceride amounts and composition of germinating seeds of *Andropogon gayanus* var. *bisquamulatus* (Hochst.) Hack., a large, perennial African, savannah grass.[23,24] The triglyceride composition of *A. gayanus* seeds determined experimentally is compared to that calculated from the fatty acid data according to the 'even' and 'random' theories of fatty acid distribution.

RESULTS AND DISCUSSION

Lipid isolates, obtained by homogenization of the tissue with $CHCl_3$–MeOH,[25,26] were separated into classes by TLC[27] and the triglyceride and free fatty acid fractions collected. Triglycerides were then separated according to degree of unsaturation by argentation TLC,[28] and each triglyceride band analysed by GLC. The sum of these quantities was taken to be the total weight of triglyceride present (Table 1).

TABLE 1. THE TRIGLYCERIDE AND FREE FATTY ACID CONTENT OF GERMINATING *Andropogon gayanus* SEEDS

Germination period (days)	Triglyceride (mg/g dry wt)		Free fatty acids (μg/g dry wt)	
	Light	Dark	Light	Dark
0	58·8	58·8	83·5	83·5
0·5	57·5	54·1	90·4	95·0
1	48·3	49·1	95·9	106·6
2	36·8	31·2	95·0	99·5
3	20·4	16·7	91·8	103·6
5	14·6	11·2	88·8	103·4
7	9·8	8·9	91·8	111·4
9	7·2	7·1	102·9	115·8

The triglyceride fraction decreased in a similar fashion in both light and dark grown seedlings, the most rapid loss being in the initial 3 days of germination. In soy beans[29] and water melon[20,30] seeds, the triglycerides appear to decrease somewhat faster in light than in dark.

Triglycerides comprised approximately 68% of the seed lipid extract and 31 and 13% of the 9-day dark and light grown seedling lipid extract respectively. This latter result is probably due to the synthesis of galactolipids during illuminated growth. Such lipids are characteristically high in α-linolenic acid[31–33] and their synthesis would account for the increase of this acid in the total lipid extract of illuminated seedlings.[34]

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The methyl esters of the fatty acids of the total triglyceride fraction and of each unsaturated class of triglyceride were analysed by GLC. *Andropogon gayanus* seed triglycerides contained 17 fatty acids ranging from lauric (12:0) to behenic acid (22:0), with palmitic (16:0), oleic (18:1) and linoleic (18:2) acids being the principal components (Table 2). During germination the fatty acid composition remained essentially unaltered except for a small increase in α-linolenate (18:3), this increase being more pronounced in the light (Table 2).

The GLC data of the triglyceride classes and of their constituent fatty acids enabled individual triglyceride types to be calculated. Palmito-oleo-linolein (POL), palmito-dilinolein (PLL), linoleo-diolein (OOL), oleo-dilinolein (LLO), and trilinolein (LLL) were the major components (Table 3). For both light and dark grown seedlings, no evidence was obtained of selective hydrolysis of the triglycerides (Table 3) as found in germinating castor beans.[35]

Free fatty acids formed 0·1% of the total seed lipid. Their amounts increased only slightly in the initial 24 hr of germination (Table 1), thereafter remaining constant and forming 0·5 and 0·2% of the 9-day dark and light germinated seedling lipid respectively. Free fatty acid accumulation has been reported in germinating sunflower[36,37] seeds and Crombie and Comber[30] noted a rise in free acidity of the oil during the germination of water melon seeds. However, these latter workers showed that only a fraction of this acidity was due to extractable free fatty acids, the remainder being due to phosphatides.

The free fatty acid profiles of light and dark grown seedlings differed mainly in their relative linoleic and linolenic acid contents. In dark grown seedlings, linoleic acid increased during germination to become the major component whereas in light grown seedlings, linolenic acid increased to become the major component (Table 4). In seed lipid, 14% of the C_{20} acid was found in the free state and this proportion increased during dark germination to 90% by the 9th day. In light grown seedlings the percentage of 20:- reached 99% after 3 days germination, after which it decreased to 36% by the 9th day. Current theories of lipid metabolism involve hydrolysis of the triglyceride by lipases and subsequent β-oxidation of the liberated fatty acids. On this basis, assuming β-oxidation proceeds at rates proportionate to the amounts of free fatty acids present, one would expect the profiles of triglyceride and free fatty acids to be similar. However, the results show (see Tables 2 and 4) that less palmitic, oleic and linoleic acids, and more stearic, linolenic and 20:- acids are present in the free state than combined in the triglycerides. However, there were indications that the two were related since the linolenate content of illuminated seedlings increased in both the triglyceride and the free state during germination. Furthermore, the 20:- acid was shown to be present entirely in the triglyceride molecules or as the free acid.[34] The relationship was emphasized by simultaneous reversed fluctuations in its levels between these two states. Since no evidence was obtained for selective hydrolysis of the triglycerides (Table 3), the different fatty acid composition of the triglycerides and free state may suggest an imbalanced equilibrium of the β-oxidation system, preferential channelling of fatty acids into metabolic pathways or interconversions.

No shorter chain fatty acids were detected in the free state than those present in the

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TABLE 2. TRIGLYCERIDE FATTY ACID COMPOSITION

Fatty acid	Amount of acid (% total)* *Dark germination* Ungerm. seed	12 hr	1 day	2 day	3 day	5 day	7 day	9 day
12:0	T	T	T	T	T	T	0·1	0·1
12:-	T	T	T	T	T	T	T	T
14:0	0·2	0·1	0·1	0·2	0·2	0·2	0·4	0·4
14:-	T	T	T	T	T	T	T	T
15:0	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1
15:-	T	T	T	T	T	T	T	T
16:0	16·0	15·5	14·7	13·2	12·6	13·8	14·0	13·9
16:1	0·4	0·5	0·4	0·2	0·4	0·5	0·6	0·7
17:0	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·2
17:-	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·2
18:0	1·6	1·8	2·0	2·0	2·1	2·2	2·0	2·3
18:1	31·0	31·1	31·1	32·2	32·0	30·4	31·1	30·6
18:2	47·9	48·3	49·1	49·3	49·5	49·3	48·5	48·6
18:3	1·6	1·6	1·7	1·8	1·8	1·8	2·4	2·4
20:0	0·6	0·5	0·2	0·5	0·5	0·6	0·4	0·6
20:-	0·3	0·2	0·2	0·2	0·5	0·4	0·1	—
22:0	0·1	0·1	0·2	0·1	0·1	0·1	0·1	0·1

* T—Trace.

TABLE 3. MAIN TRIGLYCERIDE TYPES OF *Andropogon gayanus* SEEDLINGS DURING GERMINATION

Triglyceride*	Amount of triglyceride (mole %) Ungerm. seed	12 hr	1 day	2 day	3 day	5 day	7 day	9 day
				Dark germination				
PPO	3·1	3·2	2·5	1·7	2·3	1·8	1·9	2·6
PPL	3·6	4·1	3·7	2·5	3·3	4·1	3·7	2·9
POO	6·3	5·6	5·4	4·3	5·0	6·4	4·9	5·0
POL	15·3	16·9	15·5	13·5	15·5	15·2	13·8	12·4
OOO	7·2	5·4	5·5	5·1	5·2	4·5	6·0	4·4
PLL	10·5	11·7	11·8	11·7	11·9	12·9	11·9	12·4
OOL	18·4	17·4	18·1	17·4	17·8	17·9	19·7	19·1
LLO	21·3	22·4	25·0	27·6	26·8	23·6	24·1	25·5
LLL	14·3	13·3	12·5	16·2	12·2	13·6	14·0	15·7
				Light germination				
PPO		2·1	1·6	1·6	2·6	1·8	1·7	1·9
PPL		3·6	3·3	2·3	3·8	4·3	2·7	2·2
POO		5·6	4·7	4·9	5·6	6·7	4·7	5·3
POL		13·9	13·0	13·0	16·2	16·6	12·9	11·8
OOO		5·1	5·1	5·7	5·8	5·7	4·0	3·5
PLL		13·5	12·2	12·3	11·3	13·3	12·3	9·8
OOL		18·3	17·5	18·1	17·6	16·7	15·4	18·4
LLO		24·3	26·3	27·6	26·1	22·1	28·6	29·2
LLL		13·6	16·3	14·5	11·0	12·8	17·7	17·9

* P—palmitic acid; O—oleic acid; L—linoleic acid. The order of fatty acids within the triglyceride molecule is not specified.

OF GERMINATING *Andropogon gayanus* SEEDS

Amount of acid (%total)* *Light germination*						
12 day	1 day	2 day	3 day	5 day	7 day	9 day
T	T	T	T	T	T	T
T	T	T	T	T	T	T
0·1	0·1	0·1	0·2	0·1	0·1	0·2
T	T	T	T	T	T	T
T	T	T	T	0·1	0·1	0·1
T	T	T	T	T	T	T
14·9	14·0	12·5	12·6	12·4	12·3	12·7
0·5	0·6	0·7	0·4	0·5	0·6	0·6
0·2	0·1	0·1	0·1	0·1	0·1	0·6
T	T	T	T	T	T	T
2·0	1·9	1·8	2·5	2·6	2·6	2·7
31·2	31·1	31·8	29·8	28·6	28·5	27·5
48·6	49·9	49·9	50·8	50·2	48·8	48·9
0·5	0·5	1·0	0·7	0·3	0·8	0·9
1·9	1·6	2·0	2·7	5·0	5·8	5·3
—	0·1	—	—	—	0·2	0·4
0·1	0·1	0·1	0·2	0·1	0·1	0·1

triglycerides (see Tables 2 and 4). However, it has been shown in rat liver mitochondria[38] that once attacked, the whole of a palmitic acid molecule disappears very rapidly.

The composition of *A. gayanus* seed triglycerides obtained by TLC–GLC has been compared to that calculated from the fatty acid data (Table 2) according to theories of triglyceride structures. Triglyceride type, according to 'random' distribution of fatty acids were calculated according to Bailey[18] and assuming an 'even' distribution as described by Hilditch and Meara.[39] Results for Kartha's 'restricted random'[19] theory were not calculated, as the result would be essentially the same as that of a pure random distribution. To simplify calculations, only the principal acids, 16:0, 18:1 and 18:2, were considered, the weight percentages being converted to mole percentages.[40] The comparison of triglyceride types found experimentally with those calculated from the fatty acid data is given in Table 5. The results show that the acids are distributed amongst the triglycerides in an essentially random fashion as found in linseed oil,[41] soybean oil,[2] safflower oil[3] and corn oil.[42] Hilditch,[1] however, has shown that for a large number of vegetable fats, the 'even' theory gives a fair approximation. Investigation with pancreatic lipase hydrolysis[13] have shown that unsaturated 18C acids tend to be in position 2 of the triglyceride molecule. Therefore, although the fatty acids appear to be randomly distributed amongst *A. gayanus* seed triglycerides, they may not be randomly distributed within the triglyceride molecules.

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TABLE 4. FREE FATTY ACID COMPOSITIONS OF DARK

Fatty acid	Amount of acid (% of total)							
	Ungerm. seed	12 hr	1 day	2 day	3 day	5 day	7 day	9 day
12:0	0·3	0·3	0·2	0·4	0·3	0·2	0·4	0·4
12:-	0·4	0·3	0·3	0·3	0·2	0·1	0·4	0·4
14:0	0·9	1·2	1·1	3·1	1·3	1·0	2·5	2·0
15:0	0·9	1·1	1·0	2·7	2·6	1·0	1·3	1·3
16:0	11·8	12·2	13·9	10·5	12·2	9·8	9·3	9·8
16:1	1·2	1·2	1·2	1·4	0·8	0·7	0·9	0·7
17:0	1·5	1·5	1·9	3·2	5·5	1·4	2·4	1·9
17:-	0·2	0·2	0·2	0·6	0·1	0·1	0·4	0·3
18:0	6·0	7·3	7·9	5·6	7·9	3·5	3·9	3·6
18:1	16·8	18·1	21·0	9·1	12·6	16·2	10·7	11·6
18:2	22·6	24·8	25·6	22·5	29·3	32·7	35·0	35·6
18:3	4·6	5·0	5·7	7·5	8·5	9·5	13·0	13·4
20:	32·8	26·8	20·0	33·2	18·7	23·7	19·9	19·0

TABLE 5. COMPARISON OF AMOUNTS OF *Andropogon gayanus* SEED TRIGLYCERIDES, WITH THOSE EXPECTED ASSUMING A 'RANDOM' AND 'EVEN' DISTRIBUTION OF FATTY ACIDS

Triglyceride types	Experimental	Mole % 'Random'	'Even'
PPO	3·1	3·3	
PPL	3·6	4·9	
POO	6·3	5·7	
POL	15·3	17·5	36·7
OOO	7·2	3·3	
PLL	10·5	15·7	17·9
OOL	18·4	15·4	13·9
LLO	21·3	23·8	31·5
LLL	14·3	12·4	

* The order of fatty acids within the triglyceride molecule is not specified.

EXPERIMENTAL

Seed material. A. gayanus seeds were kindly donated by Mr. R. J. Hagger, Sheika Experimental Station, Samaru, Northern Nigeria.

Methods. 1 g samples (*ca.* 860 seeds) of seed were germinated on moistened filter paper at 30°, either in the dark or under constant illumination (fluorescent, warm white—250 lx). Samples were removed for analysis after 12 hr and 1, 2, 3, 5, 7 and 9 days. Lipids were extracted with $CHCl_3$–MeOH, 2:1,[25] and the solvent removed by rotary evaporation. Extracts were dried over KOH, *in vacuo* and the crude lipid re-extracted 3× 20 ml $CHCl_3$–CH_3OH.[26] After filtration, solvent was removed, as above, and the dried lipid extract weighed. All procedures were carried out under N_2.

TLC. Lipids were separated into classes on 250 μ silica gel G (Merck) plates developed with light petrol.–Et_2O–HOAc (90:10:1).[27] The resulting bands were detected with 0·0012% aq. rhodamine 6G[43] and viewing in UV light. Standards of tripalmitin and palmitic acid were used. Areas corresponding to triglycerides and free fatty acids were removed from the plate and eluted in Et_2O.

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AND LIGHT GROWN SEEDLINGS OF *Andropogon gayanus*

Light germination						
12 hr	1 day	2 day	3 day	5 day	7 day	9 day
0·3	0·2	0·1	0·1	0·2	0·1	0·1
0·3	0·2	0·1	0·2	0·1	0·1	0·1
2·5	2·7	1·3	1·2	1·4	2·6	0·8
0·6	0·5	0·6	0·7	1·0	1·4	0·4
8·8	11·4	10·8	7·4	9·1	9·8	7·3
0·4	0·4	0·3	0·3	0·4	1·0	0·5
1·2	1·1	0·6	1·3	1·7	2·2	0·4
0·6	0·6	0·1	0·5	0·4	0·6	0·3
1·4	1·6	0·6	0·7	2·0	1·7	1·1
13·9	14·8	8·8	10·7	15·3	13·2	8·5
23·0	32·9	31·0	25·9	28·0	23·4	24·1
5·9	5·5	9·5	9·1	11·0	21·9	38·1
41·2	28·0	36·2	41·8	29·5	22·0	18·3

Triglycerides were further separated according to unsaturation on 400 μ silica gel G plates impregnated with 5% $AgNO_3$.[28] These were developed in darkness with CCl_4–$CHCl_3$–HOAc–EtOH (60:40:0·5:1·5).[44] Standard triglycerides (from Applied Science Labs.) were run on either side of the chromatograms. Bands were visualized and eluted as before.

GLC was performed on a Perkin–Elmer F11 gas chromatograph fitted with hydrogen FIDs. Triglycerides were separated on 50 cm × 3 mm stainless steel columns, packed with 2% OV-1 on AW-DMCS treated chromosorb W (100–120 mesh) from 200° to 325° at 2°/min, with N_2 of 100 ml/min. The injection port was maintained at 350°. Peaks were identified, by the relative elution temp. method[45] using trilaurin as an internal standard, and quantified by peak area measurement. Average wt response and molar response factors[46] were assigned to each peak based on its estimated fatty acid composition.[47]

Fatty acids were methylated by the low temperature H_2SO_4 method[48] and chromatographed before and after hydrogenation.[49] Methyl esters were separated by GLC on a 2 m column packed with 20% diethylene glycol succinate on HMDS treated chromosorb W (80–100 mesh), operated at 180°, and N_2 at 15 ml/min; and a 2 m column packed with 10% Apiezon L on AW-chromosorb W (80–100 mesh), at 197° and N_2 at 28 ml/min. Fatty acids were quantified by measurement of corrected peak areas.

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