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CHANGES IN LIPID CLASSES AND FATTY ACID COMPOSITION IN DEVELOPING *PSOPHOCARPUS TETRAGONOLOBUS* SEEDS

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Key Word Index—*Psophocarpus tetragonolobus*; winged bean; Leguminosae; lipid classes; fatty acid composition; oilseed.

Abstract—The accumulation of lipids, the changes in lipid classes and the changes in fatty acid composition of total lipids and major lipid classes in developing winged bean (*Psophocarpus tetragonolobus*) seeds from three to seven weeks after flowering (WAF) have been studied. Seed lipids accumulated progressively from three to six WAF and then stabilized. Neutral lipids, mainly triacylglycerols (TG), accumulated progressively in the developing seeds whereas polar lipids, glyco- and phospholipids decreased rapidly as the seeds developed and matured. Analysis of the fatty acid composition of seed total lipids and major lipid classes indicated substantial changes in the proportion of certain fatty acids during seed development. The amounts of palmitic (16:0), stearic (18:0) and linolenic acids (18:3) decreased whilst those of linoleic (18:2) and behenic acids (22:0) increased progressively during seed development. Most of the 22:0 was found in the TG fraction whereas the polar lipids were found to contain more unsaturated fatty acids.

INTRODUCTION

Winged bean is a potential source of protein and oil for the tropics [1–2]. The oil of mature winged bean seeds from various sources has been analysed [3–7]. Winged bean seed oil contains *ca* 30–40% saturated and 60–70% unsaturated fatty acids. *ca* 10–20% of the saturated fatty acids are behenic acid (22:0) and *ca* 50% of the unsaturated fatty acids are oleic (18:1) and linoleic (18:2) acids. The high level of 22:0 in winged bean seed oil has caused some concern in developing the seeds as a source of edible oil because this acid present at a concentration of 1–3% in peanut oil has been suspected to contribute to the higher atherogenicity of this oil in animal experiments [8–11]. Therefore, it is of nutritional interest to study the formation and distribution of this fatty acid in different classes of winged bean seed lipids. In this paper we have examined the development and accumulation of oil as well as the changes of lipid classes and fatty acid composition in developing winged bean seeds.

RESULTS AND DISCUSSION

The oil contents and fatty acid composition of the seed total lipids at different stages of seed development are given in Table 1. At three weeks after flowering (WAF), the lipid content accounts for 4.9% of the seed fresh weight and it increased progressively until six WAF; thereafter it stabilized at *ca* 9.5%. Changes in the fatty acid composition in the developing winged bean seeds are evident (Table 1). In the younger seeds (3 WAF), the linolenic acid (18:3) content was relatively high (23.1%) but its concentration subsequently dropped. Concomittantly, the amount of 18:1 increased sharply from 12.2% at three WAF to 36.4% at four WAF; after this time its content decreased slightly. The concentration of 18:2 was relatively high (31.4%) at three WAF and it increased slightly to reach a plateau after six WAF. On the other hand, the amounts of saturated fatty acids, palmitic acid (16:0) and stearic acid (18:0) were 18.1 and 8.3%, respectively, at three WAF but both decreased progressively as the seeds developed and matured. The concentration of 22:0 was low (3.9%) at three WAF but it increased rapidly *ca* to 9% by six WAF remaining constant thereafter.

Fractionation of the seed total lipids on an acid-treated Florisil column indicated that neutral lipids predominated at three WAF increasing progressively until six WAF (Table 2). The amounts of polar lipids, glycolipids and phospholipids, decreased progressively as the seeds developed toward maturity.

Separation of the neutral lipid, glycolipid and phospholipid fractions from the Florisil column either by one- or two-dimensional TLC revealed changes in the proportion of the major lipid classes at different stages of seed development (Table 2). Triacylglycerols (TG) predominated in the neutral lipid fraction at three WAF and increased progressively until six WAF. The increase in TG content was followed by a simultaneous decrease in diacylglycerols (DG) and unesterified fatty acids (FA). There was no consistent changes in the pattern of sterols and sterol esters during seed development.

In the glycolipid fraction (Table 2), five glycolipids were identified. Cerebrosides (CE) predominated in young seeds (3 WAF) but not in mature seeds (7 WAF). The amounts of CE, esterified steryl glycosides (ESG), steryl glycosides (SG) and diglycosyl diglycerides (DGDG) decreased progressively as the seeds developed and matured. Monoglycosyl diglycerides (MGDG), on the other hand, increased from 0.4% at three WAF to 0.9% at four WAF. The concentration decreased gradually thereafter.

Table 1. Lipid contents and fatty acid composition of developing winged bean seeds

		Weeks after flowering (WAF)				
		3	4	5	6	7
Fresh seed wt (g)		52.8	55.8	40.1	48.6	35.3
Total lipids (wt %)		4.9	6.3	8.5	11.1	12.2
Fatty acids (%)	12:0	0.1	0.1	t	t	t
	14:0	0.2	0.1	t	t	t
	16:0	18.1	9.9	6.6	6.5	7.5
	16:1	t	0.1	t	t	0.1
	18:0	8.3	4.2	3.0	2.6	3.3
	18:1	12.2	36.4	33.2	31.6	31.4
	18:2	31.4	33.7	38.3	39.5	38.2
	18:3	23.1	4.4	2.7	3.9	3.4
	20:0	1.0	1.9	1.8	1.6	1.8
	20:1	0.9	1.9	2.8	2.0	2.3
	22:0	3.9	6.3	9.0	9.3	9.5
	22:1	t	t	1.6	1.4	1.1
	24:0	0.8	1.0	1.5	1.6	1.4
Total saturates		32.4	23.4	21.9	21.6	23.5
Total unsaturates		67.6	76.5	78.6	78.4	76.5

t trace <0.1%

In the phospholipid fraction, six components were identified. Phosphatidylcholine (PC) was the largest phospholipid component at three and seven WAF. The amounts of PC, phosphatidylethanolamine (PE) and phosphatidylinositol. (PI) increased progressively and reached a plateau at *ca* six WAF. The concentrations of phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) decreased as the seeds developed.

Analysis of the fatty acid profiles of major neutral lipids, TG, DG and FA showed that most of the 22:0 was found in the TG fraction (Table 3). In this fraction, the major fatty acids were 18:1 and 18:2 which accounted for more than 50% of the total fatty acids. The 18:1 content was high, 39.4%, at three WAF and increased further to 42% at four WAF; thereafter its level decreased slightly as the seeds matured. The 18:2 content fluctuated but altered little during development. The amount of 18:3 was low, 1.8%, at three WAF and decreased at seven WAF. The concentrations of 16:0 and 18:0 decreased progressively as the seeds developed and matured. The amount of 22:0, in contrast to other saturated fatty acids, increased steadily from 10.2% at three WAF to 15.4% at seven WAF. The patterns of changes of major unsaturated fatty acids, 18:1 and 18:2, in DG were the opposite of that seen in TG. However, the trend of changes in 16:0, 18:0 and 22:0 in DG was similar to that observed in TG. The pattern of changes in fatty acids in FA was less consistent compared to that of TG and DG. It is interesting to note that the content of 22:0 was very low at three WAF but rose rapidly to 21.9% at seven WAF.

The fatty acid profiles of the major glycolipids were also analysed. The concentrations of 16:0 and 18:0 in all glycolipids fluctuated during seed development. The 18:1, except in ESG, and 18:2 contents, except in CE, also fluctuated during seed development. The concentration of 18:3 was in general higher than that observed in the neutral lipids. Its content was highest in DGDG but dropped consistently in all glycolipids as the seeds developed and matured.

The fatty acid profiles of the major phospholipids in developing winged bean seeds were also analysed. The concentrations of 16:0 in all major phospholipids fluctuated whilst those of 18:0 in PC, PE and PI decreased progressively during seed development. The content of 22:0 remained very low in all phospholipids. The amounts of 18:1 in PC and PE increased progressively to reach a plateau at *ca* six WAF whereas those of the same acid in PI, PG and DPG fluctuated. The concentration of 18:2 in PE and PI increased progressively to peak at six WAF whereas that of the same acid in PC, PG and DPG fluctuated. The 18:3 content decreased at seven WAF.

The accumulation of lipids and the changes in fatty acid composition in developing winged bean essentially confirmed previous findings [12]. The trend in oil accumulation is similar to that observed in other developing oilseeds [13–15]. The changes in fatty acid composition, with the exception of 22:0, are broadly similar to those observed in developing safflower seeds [13] and in certain cultivars of soybean [14, 16–18]. The rapid synthesis of 22:0 in developing winged bean seeds suggests the presence of very active elongation systems in the seed cotyledons. Microsomes prepared from developing winged bean seed cotyledons actively incorporated ^{14}C-malonyl CoA into 22:0 acid *in vitro*. (Khor, H.-T., unpublished data) Extraction of winged bean seed oil on a pilot scale has been successfully attempted [7]. Because of its high 22:0 content this oil offers an excellent opportunity to test the hypothesis that behenic acid contributes to the high atherogenicity [8–11].

The changes in neutral, glyco- and phospho-lipids in developing winged bean seeds are similar to those observed in soybean [13]. The accumulation of lipids in the

Table 2. Changes in the proportion of various lipid classes of developing winged bean seeds*

Lipid class	Weeks after flowering (WAF) 3	4	5	6	7
Neutral lipids	80.0	83.5	90.0	92.4	90.1
TG	54.2	62.0	77.5	77.6	76.7
FA	16.7	8.7	4.5	5.5	5.2
DG	8.5	5.4	4.4	3.5	3.6
S	2.9	2.2	0.9	2.1	1.4
SE	2.8	3.3	2.3	3.8	3.3
Glycolipids	7.8	5.6	2.1	1.6	1.7
ESG	1.9	1.5	0.6	0.4	0.5
MGDG	0.4	0.9	0.4	0.2	0.2
SG	1.3	1.1	0.5	0.5	0.6
CE	3.2	1.5	0.4	0.3	0.3
DGDG	0.8	0.5	0.2	0.1	0.1
Phospholipids	7.5	7.2	7.0	6.5	6.0
PC	1.9	2.0	2.2	2.3	2.2
PE	1.2	1.3	1.6	1.6	1.6
PG	0.7	0.6	0.5	0.4	0.5
DPG	1.6	1.1	0.9	0.4	0.4
PI	0.6	0.8	1.0	1.1	1.1
PS	1.2	0.4	0.3	0.4	0.1
X	0.2	0.5	0.4	0.3	0.1

*Expressed as wt % of the total lipids. TG = triacylglycerols; FA = fatty acids; DG = diacyglycerols; S = sterols; SE = sterol esters; ESG = esterified steryl glycosides; SG = steryl glycosides; MGDG = monoglycosyl diglycerides; CE = cerebrosides; DGDG = diglycosyl diglycerides; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosophatidylglycerols; PI = phosphatidylinositols; DPG = diphosphatidylglycerols; PS = phosphatidylserine; X = unknown.

former is due primarily to increased deposition of neutral lipids, mainly TG, in the seed cotyledons. Most of the 22:0 present in winged bean seed oil is found in TG. Recent analysis in our laboratory indicated that almost all of the 22:0 is esterified to the sn-3 position of the TG (Khor, H.-T., unpublished data)

The changes in polar lipids, glycolipids and phospholipids, in developing winged bean seeds are also broadly similar to those observed in soybean [13]. Analysis of the fatty acid profiles of the major polar lipids has confirmed previous observations that polar lipids in winged bean seeds contain high concentrations of 18:1 and 18:2 but low ones of 18:3 and 22:0 [1].

EXPERIMENTAL

Winged bean (*Psophocarpus tetragonolobus*) was grown in the field; pods were harvested and processed as described in ref. [12].

Lipid extraction and fractionation. Winged bean seed lipids were extd with CH_2Cl_2–MeOH (2:1) as described in ref. [19] and quantified gravimetrically. Seed total lipids were fractionated on an acid-treated Florisil column as described in ref. [20].

TLC. The neutral lipid fraction was further sepd on silica gel G using hexane–Et_2O–HCO_2H (40:10:1). The sepd lipids were visualized by exposure to I_2 vapour and quantified by the acid charring method [21]. Cholesteryl stearate, cholesterol, tripalmitin, palmitic acid and distearin were used to construct calibration curves for the quantitative estimation of neutral lipids.

The glycolipid fraction were sepd on silica gel G using $CHCl_3$–MeOH–HOAc–H_2O (170:25:25:4). All glycolipids were well sepd in this solvent system [20]. Spots were visualized by exposure to I_2 vapour and estimated using the PhOH–H_2SO_4 reagent [22], as modified in ref. [20], and finally expressed as mol%.

The phospholipid fraction was sepd by 2D development using $CHCl_3$–MeOH–28%NH_3 (13:7:1) followed by $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (10:4:2:2:1) [23]. All phospholipid spots were identified by co-chromatography with authentic

Table 3. Fatty acid profiles of major neutral lipids of developing winged bean seeds

Lipid Class	WAF	Fatty acid composition (%) 12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0
TG	3	t	0.8	9.3	0.1	5.6	39.4	26.4	1.8	1.0	2.5	10.2	0.5	2.4
	4	t	0.6	8.2	t	5.2	42.0	24.2	1.2	1.2	2.6	11.6	0.5	2.7
	5	t	t	7.7	t	4.3	37.8	28.3	1.2	1.3	3.5	12.1	1.0	2.8
	6	t	t	7.3	t	3.4	35.8	28.1	1.2	1.6	3.8	14.1	1.2	3.5
	7	t	t	7.8	t	4.6	35.5	26.2	0.9	1.5	3.6	15.4	1.1	3.4
DG	3	t	0.2	13.7	t	6.2	39.1	30.2	0.7	4.3	1.9	2.5	1.0	0.2
	4	t	0.2	12.2	0.2	5.2	35.3	34.8	0.7	2.8	2.0	3.4	1.9	1.3
	5	t	0.1	14.0	t	4.9	36.9	31.5	1.1	1.9	2.4	4.6	1.7	0.9
	6	0.1	0.4	14.9	0.4	4.4	36.7	26.8	1.2	1.5	4.1	6.3	1.5	1.7
	7	t	0.1	9.4	0.3	4.2	37.3	31.2	1.2	1.5	4.0	7.0	1.6	2.2
FA	3	t	0.3	19.1	t	5.9	34.9	31.4	0.5	5.8	0.4	1.1	0.1	0.5
	4	0.1	0.5	24.4	t	7.1	38.1	24.0	0.6	1.8	0.2	2.6	0.4	0.3
	5	0.1	0.6	24.0	t	8.0	38.0	19.1	0.8	4.4	0.2	3.5	0.7	0.6
	6	t	1.3	21.9	t	7.2	31.9	8.9	2.3	4.2	0.2	18.0	2.9	1.3
	7	0.2	2.3	16.9	0.1	5.9	28.9	7.9	2.9	4.9	0.8	21.9	4.6	2.7

t, trace <0.1%.

phospholipid standards and quantified by p estimation according to ref. [24].

Prep. TLC using the solvent systems mentioned above as used to isolate pure individual lipid class for fatty acid analysis. After development in the appropriate solvent system, the lipid bands were visualized by sparying with 2,7-dichlorofluorescein and viewed under UV light. The lipids were then transmethylated directly in the presence of silica gel [25].

GC. Fatty acid Me esters (FAME) were prepared by refluxing with MeOH–HCl generated by mixing AcOCl and dry MeOH as described in ref [20]. Pure FAME were analysed on a FID instrument using a glass column (2.2 m × 4 mm) packed with 12% SP 2300 (Supelco, U.S.A.). All analyses were carried out isothermally at 230°. Peak areas were determined by electronic integration and expressed as % of total fatty acids.

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