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LIPID SYNTHESIS BY GERMINATING SOYA BEAN

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Abstract—Lipid content and changes during the first 2 days of germination have been examined in soya bean *Glycine max* (L.) Merr variety Fiskeby V. Triacylglycerol, the principle storage lipid, is reduced on germination and this is accompanied by a rise in phospholipid content. The relative amounts of phospholipids rise equally, apart from the disappearance of *N*-acylphosphatidylethanolamine and significant increases in the proportions of phosphatidic acid and phosphatidylglycerol.

Incorporation of acetate-[^{14}C] into lipids occurs after a brief lag phase. Labelling is almost entirely (94–100%) confined to the acyl portion of the major complex lipids. Triacylglycerols had low specific radioactivities and, of the phospholipids, phosphatidylglycerol, cardiolipin, and phosphatidylinositol had the highest specific radioactivities. Incorporation was somewhat reduced by protein synthesis inhibitors and was mainly into palmitic, stearic and oleic acids. There were minor differences in their distribution within lipid types.

INTRODUCTION

Although many workers have noted the rapid changes in lipid composition which accompany germination [1] there have been surprisingly few studies of complex lipid synthesis in these tissues. Katayama and Funashi [2] measured orthophosphate-[^{32}P] labelling of phospholipids in *Phaseolus radiatus* seedlings. They noted high labelling of phosphatidylcholine and phosphatidylethanolamine in the cotyledons and phosphatidylethanolamine in the hypocotyls and radicles. *Pisum sativum* showed rapid incorporation of acetate-[^{14}C] into phosphatidylinositol. At longer germination times phosphatidylcholine accounted for more total radioactivity [3]. This seed was also remarkable for showing extremely high turnover of the acyl groups of phosphatidylglycerol. Acylation of phospholipids has also been studied in hazel seeds with ^{14}C-fatty acids [4]. Apart from these studies little is known about the relationship of lipid biosynthesis to germination.

The soya bean [*Glycine max* (L.)] provides a high lipid seed of great economic importance. This seed was chosen for use in part of a study on the function of lipids during germination. The complex lipid content [5], fatty acid composition [6] and distribution [7] have been determined for the developing or mature seeds of North American varieties. Studies on the biosynthesis and composition of lipids during germination, using a recently developed North European variety, Fiskeby V, are now reported.

RESULTS AND DISCUSSION

As with most mature seeds [8] the quantitatively major class of lipid in soya bean is triacylglycerol [1,5,9,10]. The soya bean variety, Fiskeby V, was found to contain 195 mg/g dry wt representing 88% of the total lipid extract (Table 1). Phospholipids (9%) were, by far, the most important of the remaining classes. The levels of lipid were also measured during the initial stages of germination (Table 1). Total lipid content per seed remained fairly constant but with an increase in total phospholipids at the expense of triacylglycerol. Catabolism of stored triacylglycerol is a common feature of germination [11] and is due to an increase in lipase activity [12,13]. In pea (*Pisum sativum*) which contains relatively little triacylglycerol, it was found that over an 11-day

Table 1. Composition of total seed lipids during germination

	% Total lipid		
	2 hr germination	24 hr germination	48 hr germination
Triacylglycerol	87·8 ± 0·6	73·6 ± 3·9	66·6 ± 2·6
Free fatty acid	2·7 ± 0·3	6·9 ± 1·1	8·6 ± 1·6
Phospholipid	9·2 ± 0·9	19·6 ± 3·8	24·8 ± 4·9

Figures are mean ± S.D. (three experiments). Lipid content (mg/g dry wt. seed) = 195, 201, 213 at 2, 24 and 48 hr germination respectively.

period the seeds lost half of their phospholipid [14] in contrast to hazel (*Corylus avellana* L.) where phospholipid content increased over a 15 day period [15].

The individual phospholipids were characterized (see Experimental) and their levels in soya bean seeds during germination estimated (Table 2). The major phospholipids were phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol with smaller quantities of phytoglycolipid, cardiolipin and phosphatidic acid. Phosphatidylserine and *N*-acylphosphatidylethanolamine were present in small amounts. During the first 24 hr of germination there was little change in the percentage distribution of these phospholipids (Table 2). The overall content and composition of the phospholipids in the dry seed is similar to results obtained for Anoka variety [5] and to the data of Wagner and Wolff [16].

There were small increases in the levels of phosphatidic acid and phosphatidylglycerol and a loss of *N*-acylphosphatidylethanolamine during the first 48 hr of germination. An increase in phosphatidic acid might be expected since this is a key intermediate in phospholipid synthesis [1,17]. Indeed, incorporation studies with slices from maturing soybean showed a rapid incorporation of acetate-[^{14}C] into phosphatidic acid [18]. A rapid disappearance of *N*-acylphosphatidylethanolamine was also observed on hydration of pea seeds [19]. Some increase in phosphatidylglycerol content might have been expected during germination since this phospholipid is a characteristic component of photosynthetic rather than storage tissue [1]. Increases in the percentage of phospholipid as phosphatidic acid and phosphatidylglycerol were also observed with germinating hazel seeds [15].

Because water imbibition is an integral part of germination, this forms a convenient route for isotopic administration. Harwood and Stumpf [3] found no difference in the pattern of fatty acids formed when either water-[^{3}H] or acetate-[^{14}C] was used as precursor and acetate-[^{14}C] was generally preferred for convenience. The time course of lipid labelling from acetate-[^{14}C] and phosphate-[^{33}P] is shown in Fig. 1. As with germinating pea [3] a lag phase with no appreciable labelling is seen, followed by a steady rise in radioactivity. A similar situation has been found in several seeds and is most likely due to a need for seed hydration to reach a critical stage [3,20]. At 15° soya beans double their dry wt in about 4 hr and this is when lipid labelling begins. At 4° it takes about 20 hr to reach a similar stage and for lipid synthesis from acetate to start. The critical stage for pea seeds is very similar, being when the seeds have reached about 185% of their dry wt [3].

Table 2. Composition of phospholipid fraction during germination

	Germination time (hr)		
Phospholipid class	0	24	48
	% Total phospholipid (w/w)		
Phosphatidic acid	1·5 ± 0·1	3·9 ± 0·1	4·0 ± 0·4
Cardiolipin	3·7 ± 1·4	3·8 ± 0·1	3·5 ± 0·3
Phosphatidylethanolamine	21·4 ± 3·7	19·9 ± 4·7	20·0 ± 2·5
N-acylphosphatidylethanolamine	1·1 ± 0·2	tr.	tr.
Phosphatidylcholine	43·6 ± 3·8	47·3 ± 3·2	45·8 ± 4·9
Phosphatidylglycerol	0·3 ± 0·1	1·4 ± 0·2	1·9 ± 0·1
Phosphatidylserine	0·5 ± 0·2	15·8 ± 2·2	16·8 ± 2·6
Phosphatidylinositol	17·5 ± 1·2		
Phytoglycolipid	6·1 ± 0·5	5·9 ± 0·2	6·0 ± 0·7
Other	4·3 ± 0·4	2·0 ± 0·3	2·0 ± 0·2

Means ± s.d. (three experiments). Phospholipid content (mg/g dry wt. seed) = 18·4, 39·4, 52·8, at 0, 24 and 48 hr germination respectively.

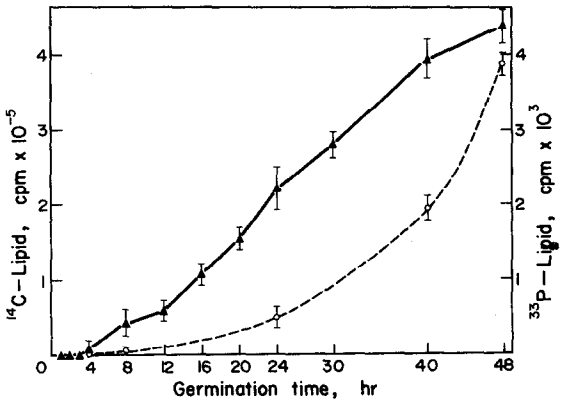

Fig. 1. Time course of acetate-[^{14}C] and phosphate-[^{33}P] incorporation into lipid: ▲——▲ incorporation from acetate-[^{14}C]; O---O incorporation from phosphate-[^{33}P].

Phosphate-[^{33}P] incorporation during the first 48 hr of germination is not linear (Fig. 1) but increases with time. This is probably due to initial dilution of specific radioactivity by phosphate contained within the dry seed.

Acetate-[^{14}C] is known, under certain conditions, to be incorporated into the acyl portions of lipids much better than into the remainder of the molecule [1]. However, the exact distribution in the major lipids of germinating soya beans was determined so that the data could be interpreted more reliably. The results (Table 3) demonstrated that the fatty acid moieties of these complex lipids contained at least 95% of the label.

The radioactive lipids were separated into three major fractions, triacylglycerols, free fatty acids and phospholipids by TLC. An analysis of the relative proportion of radioactivity in these fractions with germination time is shown in Fig. 2.

Table 3. Distribution of incorporated acetate-[^{14}C] within complex lipids

Complex lipid	^{14}C in fatty acyl groups (%)	Recovery (%)
Triacylglycerol	98 ± 2	93
Phosphatidylethanolamine	100	94
Phosphatidylcholine	94 ± 5	98
Phosphatidylinositol	95 ± 3	96

Figures represent mean ± S.D. (n = 3). Hydrolysis of the acyl groups was made with NaOH–MeOH and the fatty acids separated from H_2O-soluble compounds by two phase partition [3].

A decrease in free fatty acid and an increase in phospholipids is a noticeable feature. The proportions after 48 hr germination are similar to results obtained with slices from developing soya bean cotyledons [18].

The lipids after 24 and 48 hr germination were further fractionated (Table 4). The major lipid fractions (see Tables 1 and 2) contain the majority of the radioactivity but phospholipids have a much higher specific activity due to their lower concentration. Phosphatidylinositol and phosphatidylglycerol were also found in germinating peas to have a high specific radioactivity [3]. The acyl groups of phosphatidylglycerol have been found to have a rapid turnover in other plant systems [21] and the metabolism of phosphatidylinositol may be related to a membrane function such as ion transport [22].

With germinating peas [3], it was found that all the enzymes necessary for fatty acid synthesis in the first 24 hr of germination were present in the dry seed. Protein inhibitors had no effect on acetate-[^{14}C] incorporation. Because soya bean

Table 4. Labelling of complex lipids from acetate-[^{14}C] during germination

Lipid type	% Total lipid (cpm) 24 hr germination (9 experiments)	48 hr germination (8 experiments)	Relative sp. act. at 48 hr
Triacylglycerols	28·2 ± 3·5	34·7 ± 3·2	0·56
Free fatty acids	10·3 ± 3·4	9·1 ± 2·3	1·06
Cardiolipin	3·2 ± 0·6	3·1 ± 1·1	3·54
Phosphatidylethanolamine	8·1 ± 1·6	10·6 ± 2·2	2·12
Phosphatidylglycerol	0·8 ± 0·3	1·7 ± 0·2	3·58
Phosphatidylcholine	22·4 ± 3·1	24·2 ± 1·4	2·11
Phosphatidylinositol	13·1 ± 2·2	13·1 ± 0·8	3·12
Phytoglycolipid	2·9 ± 0·5	2·4 ± 0·7	1·60
Others	11·0 ± 1·9	1·1 ± 0·6	2·20

Relative sp. act. = (% total radioactivity)/(% total lipid). Figures for percentage total lipid represent means ± S.D.

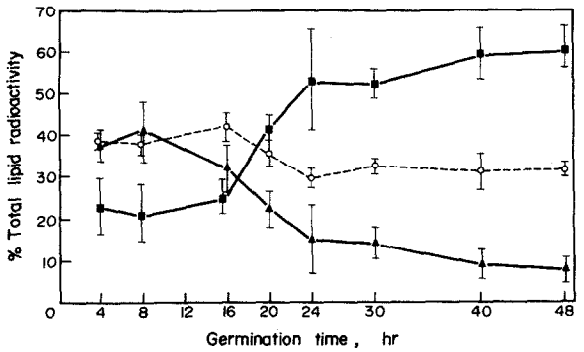

Fig. 2. Distribution of radioactivity incorporated from acetate-[^{14}C] during germination: ■——■ phospholipids; ○——○ triacylglycerol; ▲——▲ free fatty acids.

imbibed water at a much faster rate than pea it was of interest to see whether the inhibitors, chloramphenicol and cycloheximide, would reduce incorporation. Both inhibitors caused some inhibition of total acetate incorporation into lipid (Table 5) but did not affect uptake of isotope by seeds during this germination period. The similarity of the extent of inhibition of total incorporation into lipids was somewhat unexpected since chloramphenicol inhibits chloroplast (protoplast) and mitochondrial protein synthesis [23] and cycloheximide inhibits microsomal protein synthesis [24]. Presumably both sites must be equally important for total lipid synthesis at this stage of germination. Cycloheximide caused a marked reduction in the proportion of label in the free fatty acid fraction. At such a stage in germination it is likely that the most active fatty acid synthesizing enzymes are soluble [20] and, hence, the production of new enzyme is likely to be cycloheximide-sensitive.

The fatty acids of the major lipid classes were analysed by GLC (Table 6). It is noteworthy that the triglycerides contain a much higher linoleic acid content than the phospholipids. The fatty acid content of triacylglycerol in the Fiskeby variety is very similar to that of other soya beans [7,25]. During the early stages of germination, there did not appear to be any dramatic changes in the fatty acid composition of individual lipids. Similar results were observed with germinating hazel seeds [15]. The fatty acids of phosphatidylcholine can be compared with data obtained by other workers. There is a higher content of oleic acid and lower content of linoleic acid when compared to the phospholipid in developing soya bean seeds [18]. The oleic acid content is similar to mature soya bean [26] but in that case the linoleic acid content was 48%. Other fatty acids were similar. Phosphatidylethanolamine and phosphatidylinositol from developing soya bean var. Chippewa 64 both contained more palmitic acid and less oleic acid [18] than in the Fiskeby variety. The data for phosphatidylinositol is very similar to that obtained when the phospholipid is prepared from commercial soya bean phospholipids (Asolectin) [26]. It is noteworthy that phosphatidylinositol frequently contains a higher proportion of palmitic acid than other phospholipids [3,9,27,28] and this can be seen again in Table 6. Overall, the fatty acid composition of the complex lipids of the Fiskeby variety are similar to those of other soya beans. The variations noted are probably due to genetic differences though it is possible that temperature differences during seed maturation may play a part. In the latter case, however, one would not have expected the triacylglycerol fatty acids to be consistent with those of other soya bean preparations. Either genetic or environmental differences have been found to produce quite large changes (40–90%) in individual major fatty acids of soya bean [10].

The distribution of newly synthesized fatty acids within lipid classes is shown in Table 7. Most of the radioactivity after 24 hr of germination is accounted for by palmitic, stearic and oleic acids. These were found to be the principle labelled acids in a number of germinating seeds [3,29]. Triacylglycerol contained the highest proportion of oleic acid and phosphatidylinositol

Table 5. Effect of cycloheximide and chloramphenicol on lipid labelling during germination

Lipid	% Control (cpm) Chloramphenicol (50 μg/ml)	Cycloheximide (10 μg/ml)
Triacylglycerol	72 ± 3	62 ± 2
Free fatty acids	66 ± 2	23 ± 5
Phosphatidylethanolamine	89 ± 3	93 ± 10
Phosphatidylcholine	89 ± 6	90 ± 10
Phosphatidylinositol	48 ± 4	77 ± 11
Others	55 ± 8	76 ± 4
Total	66·1 ± 2·2	69·5 ± 9·6

Mean ± s.d. (three experiments). Germination for 24 hr.

Table 6. Fatty acid composition of major seed lipids

	Germination time (hr)	Fatty acid (% total) 12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	Others	No. of experiments
Triacylglycerol	24	tr	0·2	tr	9·1	tr	3·2	21·7	55·4	10·0	0·4	4
	48	0·2	0·2	tr	11·6	0·5	1·7	20·9	54·9	9·5	0·5	2
Free fatty acids	24	0·5	1·7	2·2	26·0	2·1	8·1	22·8	33·8	0·7	2·1	2
	48	0·1	2·7	1·9	26·0	2·7	6·9	18·3	34·6	6·8	tr	2
Phosphatidylethanolamine	24	0·6	1·3	0·3	21·8	1·1	5·6	19·0	40·2	4·7	5·4	4
	48	0·2	1·2	0·5	19·9	0·1	2·4	16·4	49·6	7·8	1·9	3
Phosphatidylcholine	24	0·4	1·3	1·4	23·1	4·3	7·2	16·3	35·8	4·2	6·0	4
	48	1·0	3·0	2·4	25·8	2·2	3·7	18·8	35·3	2·3	5·4	3
Phosphatidylinositol	24	0·5	0·8	0·4	29·9	0·2	5·8	16·6	40·1	4·7	1·6	2
	48	0·2	3·9	1·1	32·0	nd	2·7	13·5	40·0	6·3	0·3	3
Phytoglycolipid	24	2·9	3·2	7·9	17·7	9·0	6·1	22·0	8·3	7·6	15·3	4
	48	2·1	5·8	9·4	18·7	4·6	6·3	22·6	19·2	2·4	8·9	3

Trace = <0·2%; nd = not detected.

the highest proportion of palmitic acid. Phytoglycolipid only contained ^{14}C-labelled saturated acids. In spite of the fact that linoleic acid is the major fatty acid of soya bean oil, it is only poorly synthesized from acetate-[^{14}C]. Presumably the linoleic acid esterified in phospholipids is derived, at these early stages of germination, from the triacylglycerols which are being catabolized. Even in maturing soya beans, where linoleic acid is being synthesized at high rates it is often difficult to show good labelling in isolated preparations [18]. This possibly indicates that the desaturase involved is labile [30] and perhaps accounts for disappearance of its activity in the mature seed [20]. With safflower (*Carthamus tinctorius*) the oleyl-CoA desaturase is only present during a short period of seed maturation [20,31]. The desaturation reaction involved in the production of oleic acid, on the other hand, has been demonstrated in a number of *in vitro* preparations from maturing soya beans [18,32]. The relative stability of this desaturase probably accounts for its high activity in the germinating seed [33 and Table 7].

In two other studies with germinating seeds it was also found that labelled palmitic acid was rapidly transferred into phosphatidylinositol

Table 7. Acetate-[^{14}C] incorporation into fatty acids of soybean lipids during germination

Lipid class	No. of expts.	Fatty acid labelling (% total) 14:0	16:0	16:1	18:0	18:1	18:2	Others
Triacylglycerol	3	tr	18·5 ±0·5	nd	27·2 ±3·3	51·0 ±1·1	3·3 ±3·0	tr
Free fatty acids	2	nd	18·2 ±1·1	nd	41·7 ±5·7	38·1 ±5·6	2·0 ±1·0	tr
Phosphatidylethanolamine	3	0·7 ±0·4	18·5 ±3·2	nd	34·8 ±8·3	37·0 ±1·4	9·0 ±4·5	tr
Phosphatidylcholine	3	0·2 ±0·1	23·8 ±0·9	3·0 ±1·1	26·0 ±1·9	38·6 ±1·4	5·1 ±0·8	3·3 ±1·1
Phosphatidylglycerol	2	nd	23·8 ±2·2	nd	37·2 ±1·9	39·0 ±4·1	nd	tr
Phosphatidylinositol	3	nd	36·1 ±8·5	nd	25·5 ±4·2	38·4 ±4·2	tr	tr
Phytoglycolipid	2	nd	37·0 ±21·0	nd	63·0 ±21·0	nd	tr	tr
Cardiolipin	2	nd	13·0 ±2·5	nd	41·6 ±11·2	43·4 ±5·6	2·0 ±1·1	tr

Figures are mean ± s.d. nd = not detected. tr = trace (<0·2%). Germination for 24 hr.

[3,4]. In common with data from other workers, synthesized fatty acids are transferred to all phospholipids and distribution varies quantitatively rather than qualitatively [3,4,18,27]. It is interesting that no *trans* 3-hexadecenoic acid was detected in phosphatidylglycerol (Table 7) since this acid is localized there only in tissue which is actively photosynthesizing [20,34].

In conclusion the data presented in this paper show that seeds from the soya bean variety Fiskeby V have a similar lipid composition to other varieties. The rapid changes in lipid composition, together with the high rates of fatty acid synthesis and complex lipid acylation, which were observed make this a very suitable seed in which to study the relationship of lipids to the germination process.

EXPERIMENTAL

Seeds. Soya bean seeds variety Fiskeby V were obtained from Thompson and Morgan Ltd., Ipswich. Germination, lipid extraction and methylation procedures were described previously [3].

Lipid separations. Lipids were separated by TLC on Si gel G using Me_2CO–HOAc–H_2O (98:2:1) and $CHCl_3$–MeOH–HOAc–H_2O (170:30:20:7). 2-Dimensional TLC was carried out using Me_2CO–HOAc–H_2O (98:2:1) or Me_2CO–HOAc–H_2O (170:30:20:7) in the first dimension and $CHCl_3$–MeOH–HOAc–H_2O (170:30:20:7) or $CHCl_3$–MeOH–7N NH_4OH (65:35:5) respectively, in the second. Lipids were revealed with I_2 vapour or 0·001% aq Rhodamine 6G (when fatty acids were to be analysed). Lipids were eluted with two changes each of $CHCl_3$–MeOH–HOAc (200:100:1) and $CHCl_3$–MeOH (2:1). Identification of lipids was carried out by previously published methods [3,34].

Fatty acid methyl esters were separated by GLC on 15% DEGS, 20% PEGS and 15% PEGA columns using both isothermal and temp-programmed operation. Identification was based on comparison of retention times with authentic standards on at least two different columns. GC–RC was used to quantitate ^{14}C-fatty acids.

Measurement of radioactivity. Fractions separated by TLC were counted directly using a scintillant consisting of PCS (Amersham-Searle)–H_2O (6:4). This gave very high efficiencies provided that not more than 25 mg Si gel was included/10 ml scintillant. It obviated the difficulties experienced due to differential elution of phospholipids from silica with solvents [35].

Estimations. Complex lipids were quantified using ester [36], phosphorus [37] glycerol [38] and fatty acid estimations. Phosphorus analysis and fatty acid methylation were carried out directly on the lipids in the presence of silica gel and ester and glycerol estimations after elution [38] from the gel.

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