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Changes in Total Lipids, Their Components and Lipases during Seed Germination in Soybean

BALWANT SINGH*, P S SUKHIJA and I S BHATIA
Department of Biochemistry, Punjab Agricultural University, Ludhiana 141004

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The total lipid content of the soybean cotyledons declined during growth. Both the polar and non-polar lipids are degraded, but the non-polar lipids are utilised preferentially over polar lipids. The free fatty acid levels increased marginally at early stages and thereafter remained constant. The GLC analysis of total lipids of cotyledons at different stages of seedling growth showed an even distribution of fatty acids in different classes of lipids which are degraded. The changes in acid and alkaline lipases have also been analysed during seedling growth. The present study revealed characteristic profiles of lipase activity and lipid components.

Key Words: Lipids, Lipases, Soybean, Germination

Introduction

The major source of energy for germination and seedling growth of the fatty seeds is the fat reserve in cotyledons. During growth of the seedlings this reserve is depleted (Ching 1963, Bhatia et al. 1974 and Marriott & Northcote 1975). These fats are degraded to provide energy and precursors for structural components to the developing embryo. The first step in degradation of lipids is the action of lipases. In plants two types of lipases, acid and alkaline, are reported (Muto & Beevers 1974, Marriott & Northcote 1975). Surprisingly, no much information is available on lipases during germination and seedling growth. The present study was undertaken to correlate the changes in total lipids, polar lipids, non-polar lipids, free fatty acids, fatty acid composition of total lipids and acid as well as alkaline lipases of soybean cotyledons during seedling growth.

Materials and Methods

Soybean (*Glycine max* L., Variety Bragg) seeds were procured from the Plant Breeding Department of Punjab Agricultural University, Ludhiana. For germination, the seeds were surface-sterilised with 0.1% mercuric

***Present Address*: Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, 6701 Szeged, P. O. Box 521, Hungary**

chloride, washed and sown in Petri plates (50×20 mm) lined with filter paper saturated with distilled water at 25±1°C in BOD incubator. The samples were collected at desired time, washed with distilled water and cotyledons were separated for further analysis.

The total lipids were extracted and purified by the method of Folch et al. (1957). For fractionation of total lipids into polar and non-polar classes, solvent partition method of Nicholas (1964) was used. Free fatty acid content was estimated by the method of Mahadevan et al. (1969). The fatty acid analysis was done by GLC. Methyl esters were prepared by the method of Luddy et al. (1968) and analysed using AIMIL, GLC apparatus fitted with a copper column (10′×1/8″) packed with 20% diethylene glycol succinate (DEGS) on 60×80 mesh chromosorb-W.

Identification of the peaks was made by comparison of their retention time with those of standard fatty acids. Relative percentages of fatty acids were calculated directly from the peak areas.

For extraction of lipases, the cotyledons were partially freed from fat with acetone. One g of fat-free cotyledons was ground in 20 ml of 0.05M tris HCl buffer *p*H8.6/0.05M sodium acetate buffer *p*H 5.0 for alkaline/acid lipase. The homogenate was centrifuged for 20 min at 15000g and clear supernatant was used as enzyme preparation. The pellet which contained 5±1% of the total acid lipase activity and 15±1% of total alkaline lipase activity, at all stages of germination, was discarded.

The lipases were assayed according to the method of Nachlos and Saligman (1949) as described by Marriott and Northcote (1975) with some modifications. The reaction mixture in a final volume of 1.25 ml contained: 50μmoles sodium acetate buffer (*p*H 5.0), 50 μ moles tris HCl buffer (*p*H 8.6) together with 0.1 μmole β-naphthyl propionate as substrate and 2.5 μmoles EDTA. Required amount of β-naphthyl propionate in methanol was dried in the reaction tube and buffer & EDTA were added before warming at 60°C. The contents were shaken thoroughly and cooled. The reaction was started by adding 0.1 ml of enzyme extract (0.45–0.50 mg protein) and allowed to proceed at 37°C for 15 min. It was stopped by adding 2 ml of post-coupling solution (4% SDS and 0.2% fast red TR in 0.05 M acetate buffer *p*H 5.0). Released β-napthol coupled to fast red TR was determined colorimetrically at 495 nm.

Protein content was estimated by the method of Lowry et al. (1951).

Results and Discussion

Changes in Lipids: (table 1) During first 3 days after imbibition, the decrease in lipid content of cotyledons was very small, as the activation or *de novo* synthesis of enzymes responsible for degradation of lipids required some time. Bhatia et al. (1974) working on barseem seed have also reported a little change in total oil content during first 24 hr of germination. The decrease in total lipids per day was marked between 3 to 11 days after imbibition. Thereafter, the rate of lipid depletion largely decreased perhaps due

Table 1 *Changes in the lipid content of soybean seedling cotyledons*

Period of incubation (days)	Total lipids (%*)	Total lipids (%**)	Polar lipids (%**)	Non polar lipids (%**)	Free fatty acids (%***)
0	31.7	31.70	2.40	29.30	0.89
1	31.7	31.70	2.40	29.30	1.10
3	30.6	29.07	2.30	26.71	1.40
5	27.1	23.98	2.18	21.83	1.98
7	23.6	17.86	2.02	15.81	1.96
9	20.3	13.64	1.84	11.85	2.03
11	17.4	10.77	1.64	9.17	1.96
13	15.8	9.32	1.49	7.86	1.64

*Percent of seedling cotyledons dry weight
**Percent of ungerminated seed cotyledons dry weight
***Percent of total oil

to utilization of lipid reserves. The polar lipid content which formed 2.4% of ungerminated seed cotyledons, decreased to 1.49% during 13 days seedling growth. McKersie et al. (1978) and Gilkes et al. (1979) also observed a similar decline in phospho lipids in cotyledons of *P. vulgaris* and mung been seedlings respectively. The non-polar component of lipids showed a large decrease from 29.3–7.8% during 13 days of seedling growth. The rate of non-polar fraction utilization decreased in the later stage of seedling growth (after 9 days) as compared to early stage, while reverse was the case for polar lipids. This clearly indicates that the nonpolar lipids are preferentially utilized over polar lipids. This is in accordance with the known role of triglycerides which are potential sources of cell energy.

The free fatty acid content, which constituted a small fraction of total lipids of cotyledons of ungerminated seeds (0.89%), showed a marginal increase during early period of seedling growth (up to 5 days). The fatty acids are liberated mainly from triglycerides by the action of lipases and are ultimately utilized by enzymes of β-oxidation and glyoxylate cycle. Since the free fatty acid level remained constant after 5 days, they were perhaps used at the same rate at which they were released.

Fatty acid composition (table 2): At all stages per cent amount of saturated fatty acids was lower as compared to that of unsaturated ones. Among the saturated fatty acids, palmitic acid was about 4 times more than stearic acid. Among the unsaturated, palmitoleic acid was present in very small amounts (<0.4%) while linoleic acid was predominant (more than 40%) at all stages. During seedling growth, no significant change in fatty acid composition was observed, except the palmitic acid content which decreased during first 5 days and remained constant thereafter. Thus the fatty acids are utilized non-preferentially during seedling growth. Further, the nonpolar lipids are utilized at fast rate during early stages and polar lipids during later stages (table 1). Therefore, it is suggested that different fatty acids might be evenly distributed among polar and nonpolar lipids. These results are contrary to those of Ganeiva et al. (1973) and Sukhija and Bhatia (1972) who reported the utilization of unsaturated fatty acids to a greater extent in cotton and taramira seeds respectively. But the present results support Zimmerman and Klosterman (1965) who

Table 2 *Fatty acid composition of lipids of soybean seedling cotyledons*

Period of incubation (days)	16:0	18:0	Total Saturated	Fatty acids (Relative %)				Total Unsaturated
				16:1	18:1	18:2	18-3	
0	19.81	4.16	23.97	0.26	31.25	43.23	4.17	78.81
1	18.88	4.62	23.50	0.31	30.33	42.28	4.28	77.20
3	18.08	4.59	22.67	0.24	31.12	40.89	5.28	77.53
5	16.21	4.19	20.40	0.36	33.02	43.93	4.29	81.60
7	16.85	4.71	21.56	0.32	30.97	42.79	3.86	77.84
9	16.40	5.02	21.42	0.29	31.14	42.27	4.38	78.08
11	15.93	4.64	20.57	0.31	32.01	43.51	4.98	80.81
13	16.19	4.54	20.73	0.33	31.86	43.57	4.33	80.19

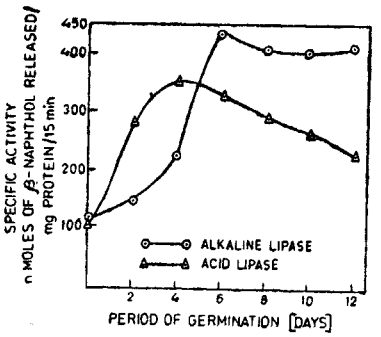

Figure 1 Changes in acid and alkaline lipase activity during germination of soybean

reported a non-preferential utilization of fatty acids in germinating flax seeds.

Both acid and alkaline lipase activities were detected in extracts of soybean seedling cotyledons (figure 1). The acid lipase was most active during the early period of seedling growth (on 4th day) and then declined. On the contrary the alkaline lipase was most active on 6th day and remained constant onwards. This pattern of lipases activity is similar to that in castor bean endosperms (Muto & Beevers 1974 and Marriott & Northcote 1975). The decline in acid lipase activity after 4 days can be due to block in its synthesis or presence of some inhibitory substance(s) or both. The appearance of alkaline lipase in later stage is understandable as they are located chiefly in glyoxysomes (Muto & Beevers 1974) which show a phenomenal increase in their number during germination (Doig et al. 1975). Surprisingly the lipase activity was also found in cotyledons of dormant seeds. The present study is in agreement with the findings of Marriott and Northcote (1975), who reported such an activity in dormant caster been endosperm. To explain the absence of triglycerides hydrolysis they postulated that either the enzyme was not in contact with its substrate or its action was blocked by some localized inhibitor.

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