CHANGES IN DIFFERENT LIPID CONTENTS IN THE COTYLEDON AND EMBRYO OF GERMINATING SUNFLOWER (*HELIANTHUS ANNUUS* L.) SEEDS

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A literature survey showed that studies on sunflower seeds are far from adequate. Excepting for a few studies on its cultivation practices and breeding very little attention is paid on the physiological and biochemical aspects of the germination of this crop seed. The present investigation was undertaken to determine the qualitative and quantitative changes of different lipids in the cotyledon as well as the embryo of sunflower seed at different stages of germination and to determine their possible roles in the overall germinating process.

The breakdown of the cotyledon lipids in germinating seeds is to provide energy and raw materials needed for the growth and development of different embryonic parts and the biosynthesis of new organelles and cell structures[1–5].

'Peredovik' (EC 68415) variety obtained from the Seed Farm of this University was used for this study. The seeds, after harvest, were dried in the sun. The seeds were found most viable in a month or two after harvest and therefore the experiment was carried out during this period.

The healthy seeds were soaked in sterile glass-distilled water for 6 h and allowed to germinate on the surface of wet Whatman No. 1 filter paper spread on a petri dish of 15 cm diameter. About 30–35 seeds were taken in each petri dish and allowed to germinate in a BOD incubator in the dark at 30°C. The seeds were removed from incubator at intervals of 24, 48, 72, 96 and 120 h of germination and the decoated seeds were dissected into the cotyledon and the embryo parts. The dissected parts were then wiped out of any adhering water and dried in an oven at 45–50°C. The dried materials were then ground in a sieve grinder at 40 mesh. These ground materials, packed in sealed polythene packets, were stored in desiccator at 4 to 5°C. When required, the dry powdered materials were used for analysis.

Germinating seeds of *Helianthus annuus* L. were analysed for total lipid as well as its major fractions at different stages of germination. The cotyledon and the embryonic parts were analysed separately every 24 h of germination for 5 days. The total lipid extracted from each of the cotyledon and embryo was fractionated in a silicic acid column into three major fractions, viz. (i) neutral lipids obtained by elution with chloroform; (ii) glycolipids obtained by elution with acetone, and (iii) phospholipids obtained by elution with methanol. The total lipid was determined[6] and the fractionation of total lipid studied by column chromatography[7].

The changes in the content of total lipid, neutral lipid, glycolipid and phospholipid in the cotyledon and the embryo of germinating seeds of *Helianthus annuus* L. are represented in tables 1 and 2. In both cotyledon and embryo, the total lipid is found to decrease with the increase of hours of germination, the per cent decrease being much higher in cotyledon (> 50%) than in the embryo (< 30%). On fractionation of the lipid, it is found that in both cotyledon and embryo and at all the hours of germination most of the total lipid occurs as neutral lipid, least as phospholipid and a quantity midway between these two values occurs glycolipid.

Table 1 *Total, neutral, glyco and phospholipid content of cotyledon of germinating Helianthus annuus L. seed*

Hours of germination	Total lipid	Neutral lipid	Glyco-lipid	Phospho-lipid
0	37.8 ± 0.081	33.0 ± 0.182	3.5 ± 0.081	0.45 ± 0.014
24	30.7 ± 0.182 (−18.8)	25.0 ± 0.141 (−24.3)	4.4 ± 0.081 (25.7)	0.55 ± 0.008 (22.2)
48	26.2 ± 0.081 (−30.7)	20.0 ± 0.182 (−39.4)	4.9 ± 0.000 (40.0)	0.60 ± 0.018 (33.3)
72	22.5 ± 0.141 (−40.5)	16.0 ± 0.141 (−51.5)	5.3 ± 0.115 (51.4)	0.65 ± 0.018 (44.4)
96	20.0 ± 0.081 (−47.1)	13.0 ± 0.182 (−60.6)	5.8 ± 0.182 (65.7)	0.69 ± 0.011 (53.3)
120	18.1 ± 0.216 (−52.4)	10.0 ± 0.115 (−69.7)	6.2 ± 0.081 (77.1)	0.73 ± 0.014 (62.2)

Data are expressed in g/100 g of ground-dried material. Each value is mean ± S.E. of 4 observations. Values in parentheses represent per cent deviation over control.

Table 2 *Total, neutral, glyco and phospholipid content of embryo of germinating Helianthus annuus L. seed*

Hours of germination	Total lipid	Neutral lipid	Glyco-lipid	Phospho-lipid
0	1 800 ± 0 014	1.450 ± 0 018	0.300 ± 0.018	0.030 ± 0.001
24	1.630 ± 0.018 (− 9.5)	1.200 ± 0.008 (−17.3)	0.370 ± 0.008 (23.3)	0.036 ± 0.001 (20.0)
48	1.590 ± 0.008 (−11.7)	1.100 ± 0.014 (−24.2)	0.430 ± 0.018 (43.3)	0.041 ± 0.001 (36 6)
72	1.420 ± 0.014 (−21.1)	0.890 ± 0.018 (−38.6)	0.460 ± 0.014 (53.3)	0.043 ± 0.001 (43.3)
96	1.390 ± 0.014 (−22 8)	0.840 ± 0.014 (−42.0)	0.490 ± 0.014 (63.3)	0 047 ± 0.001 (56.6)
120	1.330 ± 0 014 (−26.1)	0.770 ± 0.016 (−46.9)	0.510 ± 0.018 (70.0)	0.049 ± 0.001 (63 3)

Data are expressed in g/100 g of ground-dried material. Each value is mean ± S.E. of 4 observations. Values in parentheses represent per cent deviation over control.

As regards the change of the individual fractions, the neutral lipids in both embryo and cotyledon decrease with increase of hours of germination, while both glycolipids and phospholipids increase with increase of hours of germination. The decrease of neutral lipid in cotyledons is comparatively higher (about 69%) than that in the embryo (about 50%). The increase in glycolipids in the cotyledon is about 80% and in embryo about 70%. The increase in phospholipids in cotyledon and embryo is almost the same. The percentages of distribution of the total lipid into the three fractions are found to change during the period of germination. The proportion of neutral lipid, glycolipid and phospholipid in the cotyledon at '0' hour of germination is about 74:8:1, which gradually changes to 13:9:1 at '120' hours. This proportion in embryo, however, is 15:10:1 at '0' hour and remains the same at '120' hours.

In the sunflower seeds, a very high percentage of the reserve materials in cotyledon is fat. Germination being basically a growth process of the embryo at the cost of hydrolysis of the materials reserved in cotyledon, the total lipid content falls appreciably in cotyledon with increase of the germination period. Marriott *et al*[8] found that the total lipid content of castor bean endosperm decreases rapidly by ten days of germination. In contrast, the phospholipid content increased during this period reaching a maximum after 5 to 6 days of germination. Harwood[9] examined the lipid content and its changes during the first ten days of germination in soybean. Triacyl glycerol, the principal storage of lipid, was reduced on germination and this was accompanied by rise in phospholipid content. Therefore the products formed as a result of hydrolysis of the lipids in cotyledon are presumed to be transported to different growing parts of the embryo. The quantitative changes of major lipid classes during germination of sunflower seeds (tables 1 and 2) indicate that triglycerides (neutral-lipid) are oxidised as a result of which its concentration decreases with increase of hours of germination in both cotyledon and embryo, the rate of utilization being comparatively higher in the cotyledon. The results show that phospholipids and glycolipids are synthesized in both cotyledon and embryo, presumably for the new cellular and organellar membranes in the seedling. The utilization of the triglycerides leads to the formation of ATP molecules which are used for various anabolic reactions occurring in the growing embryo[10]. The total lipid content in embryo, being very small, the major part of energy in the form of ATP is supposed to be provided at least initially from the triglyceride oxidation in cotyledon.

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