LIPID COMPOSITION IN FAST AND SLOW GERMINATING SUNFLOWER (*HELIANTHUS ANNUUS* L.) SEEDS

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Summary. The physiochemical characteristics and lipid composition in fast and slow germinating sunflower (*Helianthus annuus* L.) seeds were studied. The quantity of phospholipids, glycolipids and sterols in cotyledons and embryonic axes in fast germinating seeds (FG - embryo emerged within 24 h after sowing) increased progressively between the 1st - 6th days after sowing (DAS) compared with the slow growing seeds (SG - embryo emerged after the 4th DAS). The fatty acid composition in cotyledons of FG seeds showed increased levels of palmitic and oleic acids 6 and 8 DAS, while a decline in palmitic and stearic acids as well as accumulation of oleic and linoleic acids were observed in SG seeds. However, higher content of linolenic acid was found in the embryonic axes of FG seeds compared with SG seeds 6 and 8 DAS. The FG seeds located in the peripheral whorls of the inflorescence, with high quantity of storage lipids, contributed to the enhanced rate of germination compared with SG seeds located in the central whorls.

***Key words*:** cotyledon, embryonic axes, germination, sunflower, lipid composition seeds.

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Abbreviations: DW - dry weight, FG - fast germinating seeds, FW - fresh weight, DAS - days after sowing, FFA - free fatty acids, GL - glycolipids, PL - phospholipids, SG - slow germinating seeds, ST - sterols.

INTRODUCTION

Sunflower is one of the major oil seed crops grown all over the world. The accumulation of lipids in seeds is greatly influenced by unhampered supply of photoassimilates from the source (foliage) to the sink (seeds) located in the inflorescence, which is a disc-shaped head or capitulum (Steer et al., 1988). The seeds in the capitulum are arranged in spiral whorls and mature progressively from the periphery to the center. Consequently, the development of seeds at each spiral whorl of the inflorescence takes place under varying environmental conditions. The seeds in the peripheral positions showed a double sigmoid pattern of dry matter and lipid accumulation in comparison to those in the middle and central positions of the sunflower head (Munshi et al., 2003). Thus, it is quite probable that variations in the seeds due to their position in the whorls of the sunflower head can affect their germination potential and viability. Therefore, some of the seeds germinate very fast while others give a poor response, which could be due to a number of factors including the pattern of seed filling on different whorls of the sunflower head and utilization of lipids and reserve proteins of the cotyledons by the embryo for its growth during seed germination. Likewise lipid composition in fast and slow germinating sunflower seeds will provide useful information about the membrane turnover during germination as well as the growth and development of plants and this was the aim of the present study.

MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L., Cv. PSFA 118) seeds were procured from the Oilseed Section, Department of Plant Breeding, Punjab Agricultural University, Ludhiana. The germination percentage was studied at monthly intervals from August till January. Dry sound seeds were selected, washed

with tap water and then surface sterilized by soaking in 0.1% mercuric chloride for 5 min, washed thrice with distilled water and imbibed for 3 h before sowing. The seeds were sown in moistened sterilized sand in Petri dishes placed in a BOD incubator at 25 ± 1 °C. The emergence of embryos after 24 h of sowing, henceforth denoted as 'fast germinating' (FG) seeds, were separated and put in another Petri dish while those emerged on day 4, denoted as 'slow germinating' (SG) seeds were retained for further work and all others emerged on days 2 and 3 were discarded. Samples of germinating seeds were collected on days 1 and 2 after sowing (DAS) in FG seed category, and on days 4, 6, 8 and 10 from both FG and SG categories. Seedlings were washed with distilled water, blotted with filter paper and then dissected in to cotyledons and embryonic axis on ice. A weighed quantity of the tissues (1.0 g) was kept for dry matter determination in an oven at 60 °C for 48 h to a constant weight (Munshi et al., 1990). After drying, the tissues were placed in the desiccators before the final weighing.

For lipid analysis, fresh tissue (0.5 to 1.0 g), was crushed and boiled in isopropanol to inactivate phospholipases, homogenized in 20 volumes (w/v) of chloroform-methanol (2:1, v/v) and stored at 0-4 °C. The extraction of lipids was done by the method of Folch et al. (1957). A suitable aliquot of lipid extract (1-2 ml) was evaporated to dryness to determine the lipid content by weighing. The lipids were separated into different classes. The fatty acid composition and physicochemical characteristics were determined according to Munshi et al. (1990). All analyses were carried out in triplicate and statistical analysis of the data was performed according to factorial experiment in randomized block design.

RESULTS AND DISCUSSION

The design of our experiment aiming to study the changes in FG and SG sunflower seeds showed a 3-d delay in emergence of the embryo in the SG seeds compared with the FG seeds. Hence, any biochemical parameter including lipids, related to reserve mobilization would be similarly delayed. The differences in reserve mobilization were due not only to initial delay, but also persisted over time. An initial scanning of seeds for germination potential over a period of time during storage revealed a decrease from

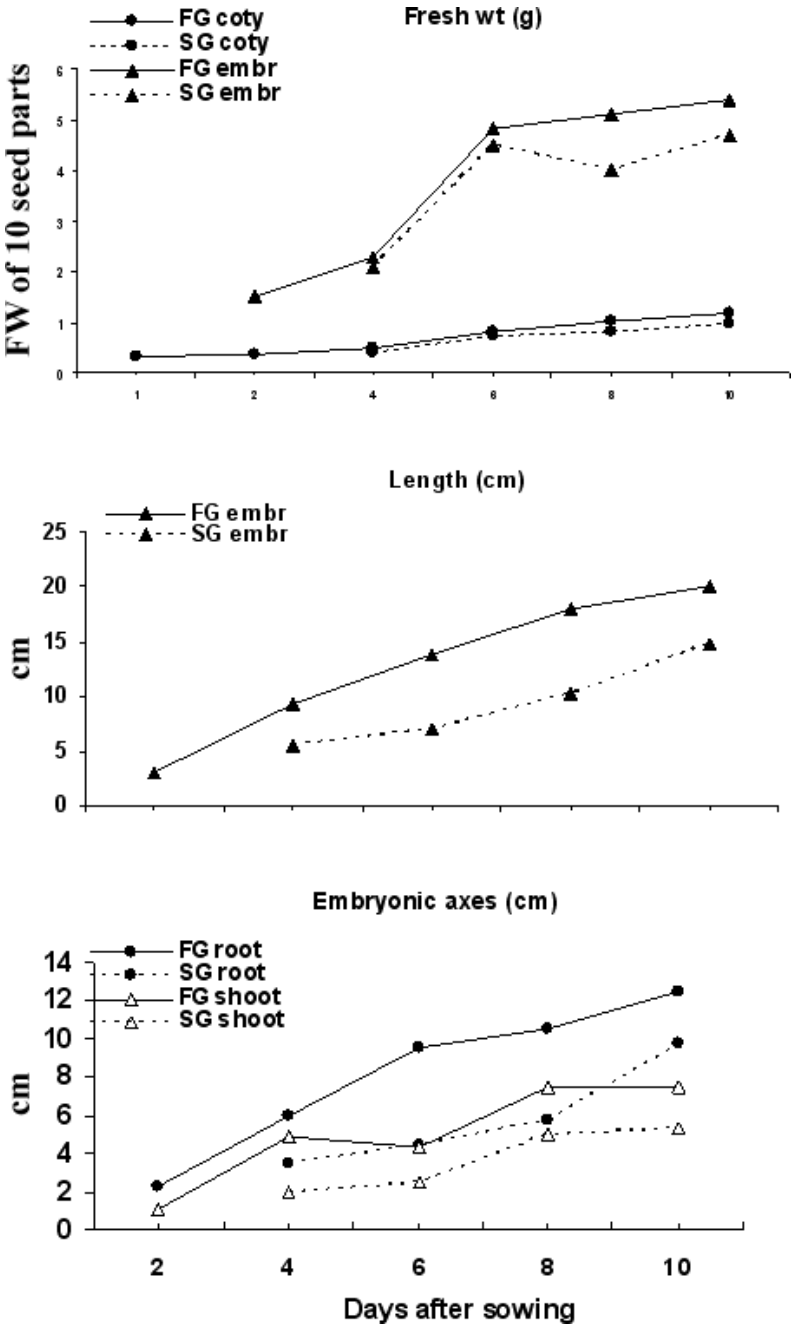

Fig.1. Physical characteristics of embryonic axes of fast and slow germinating sunflower seeds.

82 % in August to 62 % in January next year (data not shown). It was further observed that some seeds germinated in a few hours while others took a few days. Therefore, a study on the growth of embryos and analysis of lipid composition of cotyledons and embryonic axis over a period of 10 days was conducted.

The physical characteristics such as fresh weight, the length of embryonic axis, root and shoot displayed higher values in the FG than in the SG seeds at different periods after sowing (Fig. 1), which could be due to variability of seed filling during seed development influenced by their whorl position on the sunflower head (Munshi et al., 2003). Our earlier results showed that seeds located in the peripheral whorls accumulated higher amounts of lipids and soluble sugars (Munshi et al., 2003), and were resistant to the peroxidative damage caused to the seeds during storage (Sung, 1996). The seeds located in the peripheral whorls germinated faster in comparison to seeds in the central whorls which showed slow germination rate. The higher dry matter and lipid content in the FG cotyledons before germination (Fig. 2) was due to enhanced seed/oil filling during seed development in comparison to the SG counterparts possibly derived from peripheral and central whorls. Data obtained in our laboratory showed also that the germination percentage and the growth of seedlings was faster in seeds located in the peripheral whorls than those located in the central whorls of the sunflower head (data not shown).

The higher dry matter content in cotyledons of 10 seeds up to 6 DAS (Fig. 2) was due to biosynthetic activities of lipids and starch occurring in the FG seeds in comparison to the SG seeds even though the rate of decrease in dry matter expressed on a 100g fresh weight basis was higher in the former case from the 1st to the 10th DAS. The faster decrease in lipid content in the cotyledons of the FG seeds in comparison to the SG seeds was due to their increased mobilization for providing energy and carbon skeletons to the growing embryos (Arribere et al., 1994). The quantity of lipids (Fig. 2) and triacylglycerols (Table 1) in the SG cotyledons indicated their lower level of mobilization and slower utilization by the embryonic axes in comparison to the FG seeds. The higher amount of lipids in the embryonic axes of 10 FG seeds on the 8th and the 10th DAS was due to enhanced fatty acid synthesis (Bhatia et al.,1978). Liu and Brown (1996) emphasized that germinating

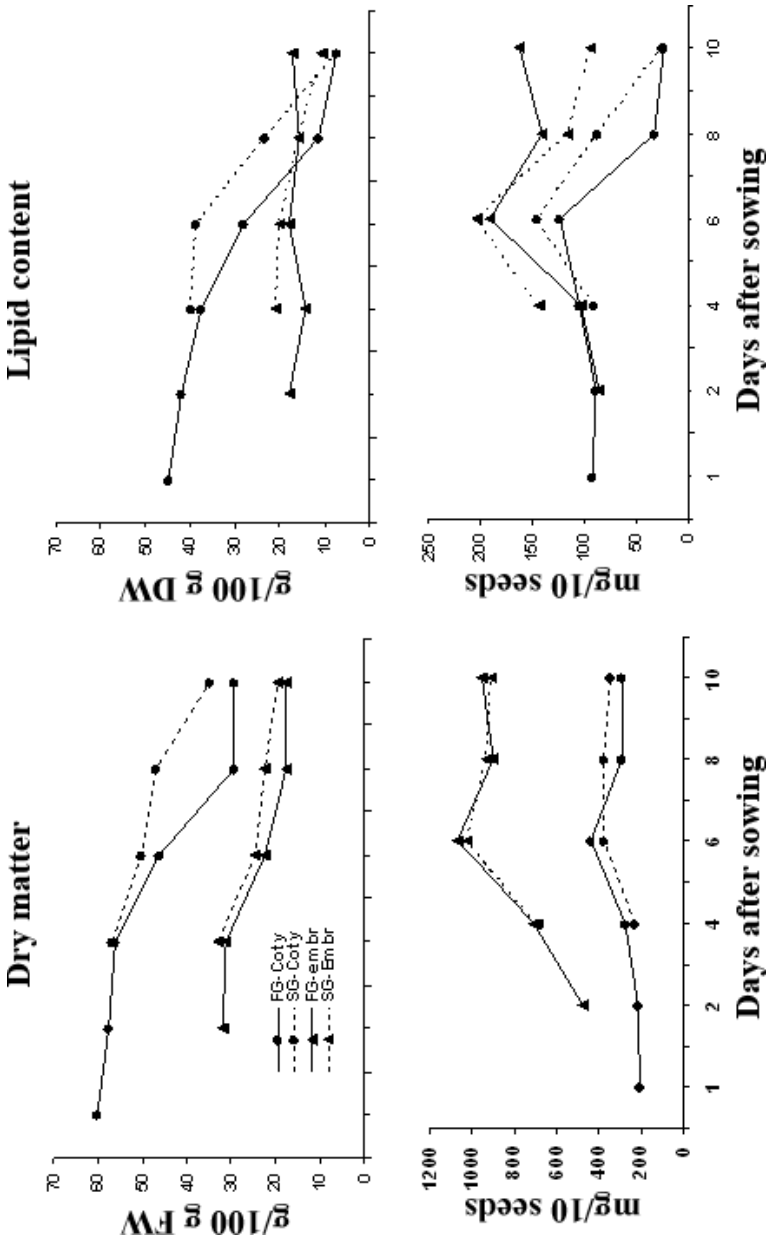

Fig.2. Dry matter and lipid content in cotyledons and embryonic axes of fast and slow germinating sunflower seeds.

Table 1. Composition of lipids in cotyledons and embryonic axes of fast and slow germinating sunflower seeds.
CD (P<0.05) – critical difference at 5 % level of significance; Mean ± SE.

Lipid class	Cotyledons				Embryonic axes			
	Fast germinating		Slow germinating		Fast germinating		Slow germinating	
DAS	% of total Lipids	mg 10 Cotyledons^{-1}	% of total Lipids	mg10 Cotyledons^{-1}	% of total Lipids	mg10 Embr. axis^{-1}	% of total Lipids	mg10 Embr axis^{-1}
Triacylglycerol								
1	79.9±0.1	73.3±0.6	-	-	-	-	-	-
2	79.9±0.7	75.0±0.3	-	-	81.5±0.6	70.3±0.5	-	-
4	78.0±0.6	82.1±0.4	76.0±0.5	68.4±1.3	78.7±0.3	80.8±1.2	81.3±0.4	116.6±1.1
6	74.0±0.6	91.0±0.3	77.6±0.3	113.1±0.9	83.1±0.5	158.0±0.8	85.6±0.6	173.3±1.3
8	66.3±0.4	22.1±0.5	79.5±0.4	70.9±1.6	81.4±0.2	114.2±0.9	88.3±0.4	101.9±1.5
10	65.6±0.5	15.7±0.3	69.1±0.6	17.3±0.5	88.0±0.7	142.7±1.2	86.8±0.3	81.6±1.3
CD (P<0.05)	1.5	3.2	1.7	2.8	2.4	5.6	1.9	5.6
Phospholipids								
1	6.5±0.1	6.0±0.2	-	-	-	-	-	-
2	6.9±.0.2	6.4±0.6	-	-	10.6±0.4	9.1±0.4	-	-
4	8.8±0.4	9.2±0.5	8.9±0.1	8.0±0.1	12.1±0.4	12.5±0.3	10.6±0.4	15.1±0.6
6	13.3±0.6	16.3±0.7	11.4±0.7	16.6±0.6	5.6±0.3	10.7±0.3	4.5±0.3	9.1±0.7
8	21.6±0.7	7.2±0.3	10.8±0.5	9.4±0.4	4.5±0.3	6.3±0.5	4.4±0.1	5.1±0.2
10	22.9±1.0	5.5±0.4	20.3±0.7	5.1±0.4	4.6±0.4	7.5±0.6	4.8±0.3	4.5±0.2
CD (P<0.05)	1.4	1.0	1.2	0.8	0.9	1.3	0.4	0.6
Glycolipids								
1	5.1±0.4	4.7±0.4	-	-	-	-	-	-
2	4.9±0.1	4.4±0.1	-	-	4.1±0.1	3.5±0.1	-	-
4	4.8±0.2	4.6±0.2	4.7±0.1	4.2±0.1	4.1±0.6	4.2±0.6	4.2±0.1	6.0±0.1
6	5.0±0.2	5.7±0.2	4.1±0.3	4.9±0.4	4.8±0.4	8.2±0.7	3.9±0.6	7.0±0.3
8	5.4±0.1	1.8±0.1	3.1±0.3	2.7±0.3	5.4±0.4	7.6±0.6	3.7±0.4	4.2±0.4
10	7.6±0.6	1.8±0.2	6.5±0.3	1.6±0.1	6.3±0.3	10.2±0.4	6.3±0.5	5.9±0.3
CD (P<0.05)	0.5	0.3	0.4	0.4	0.6	0.7	0.7	0.7
Sterols								
1	7.3±0.3	6.7±0.3	-	-	-	-	-	-
2	7.0±0.2	6.4±0.1	-	-	2.8±0.1	2.4±0.1	-	-
4	8.1±0.1	8.4±0.1	9.0±0.1	8.1±0.1	3.9±0.1	4.0±0.1	2.6±0.1	3.7±0.1
6	8.9±0.2	10.9±0.2	5.8±0.1	8.4±0.6	6.8±0.3	13.0±0.4	4.8±0.1	9.8±0.1
8	5.8±0.2	1.9±0.1	5.9±0.1	5.1±0.3	7.6±0.4	10.6±0.4	2.9±0.1	3.4±0.1
10	4.6±0.2	1.1±0.1	3.7±0.1	0.9±0.1	1.6±0.1	2.5±0.2	1.7±0.1	1.6±0.1
CD (P<0.05)	0.4	0.4	0.7	1.0	0.3	0.5	0.6	1.0
Free fatty acids								
1	1.2±0.1	1.1±0.1	-	-	-	-	-	-
2	1.0±0.1	0.9±0.1	-	-	0.9±0.1	0.8±0.1	-	-
4	0.9±0.1	0.9±0.1	1.4±0.2	1.3±0.1	1.1±0.1	1.1±0.1	1.3±0.1	1.9±0.1
6	0.7±0.1	0.9±0.16	1.2±0.1	1.7±0.2	0.6±0.1	1.2±0.1	1.1±0.1	2.3±0.1
8	0.6±0.1	0.2±0.01	0.8±0.1	0.7±0.1	0.5±0.08	0.7±0.1	0.7±0.1	0.8±0.04
10	0.4±0.1	0.1±0.01	0.4±0.1	0.1±0.01	0.3±0.01	0.6±0.02	0.50±0.1	0.6±0.01
CD (P<0.05)	0.1	0.1	0.2	0.2	0.4	0.1	0.2	0.2

Table 2. Fatty acid composition in cotyledons and embryonic axes of fast and slow germinating sunflower seeds. 14:0 myristic acid, 16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linolenic acid, 18:3 linolenic acid; Tr-traces; CD ($P<0.05$) – critical difference at 5 % level of significance.

	Fatty acid composition (%)					
Sample	14:0	16:0	18:0	18:1	18:2	18:3
Cotyledons						
1 DAS Fast	2.4	21.5	22.4	28.9	22.8	2.1
2 DAS Fast	3.1	16.9	20.0	27.8	36.7	1.6
4 DAS Fast	1.7	19.6	15.1	20.0	40.4	5.7
6 DAS Fast	Tr	26.5	14.0	28.3	23.5	5.8
8 DAS Fast	Tr	55.1	Tr	35.0	9.0	Tr
4 DAS Slow	4.6	39.4	32.2	15.8	8.0	Tr
6 DAS Slow	1.45	25.4	28.5	17.3	25.5	1.5
8 DAS Slow	Tr	16.1	13.2	18.0	51.5	1.4
CD ($P<0.05$)	0.4	2.3	1.9	2.1	2.6	0.8
Embryonic axes						
4 DAS Fast	1.6	52.7	16.1	11.7	16.9	1.0
6 DAS Fast	2.2	25.8	Tr	16.4	16.1	39.5
8 DAS Fast	Tr	16.5	Tr	20.3	13.5	49.7
4 DAS Slow	Tr	45.0	15.0	20.7	15.2	1.4
6 DAS Slow	Tr	40.0	Tr	20.0	12.5	27.5
8 DAS Slow	Tr	25.0	Tr	14.2	18.9	42.0
CD ($P<0.05$)	0.2	2.1	1.3	2.0	2.2	2.3

seeds synthesized fatty acids used in the formation of membrane lipids in the growing embryos. The increase in the quantity of phospholipids (PL), glycolipids (GL) and sterols (ST) in cotyledons and embryonic axes of 10 FG seeds from the 1st to the 6th DAS indicated the synthesis of membrane

lipids (Bose et al., 1988; Cantisan et al., 1999) and increased population of glyoxysomes and mitochondria during germination (Ohta et al., 1995) as compared to the SG tissues. The enhanced membrane lipid biosynthesis contributed significantly to cellular development and faster growth of embryonic axes in the FG in comparison to the SG tissues. The enzymes for lipid mobilization, such as lipase (Cantisan et al., 1999) and/or acyl ester hydrolase (Hoppe and Theimer, 1997) were inhibited in the SG seeds due to the presence of inhibitors (Shekhargouda et al., 1996) and/or the peroxide radicles (Sung, 1996) during storage. The higher content of free fatty acids (FFA) in the cotyledons and embryonic axes in the SG seeds (Table 1) could be due to slower acylation reactions for triacylglycerol (TG) biosynthesis in comparison to the FG tissues. Likewise a number of enzymes can control the flux of FFA towards TG biosynthesis (Dahlqvist et al., 2000; Ganger et al., 2001), the activity of which was higher in the FG in comparison to the SG seeds.

The fatty acid composition of cotyledons and embryonic axes (Table 2) indicated that desaturation reactions were slower on the 4th DAS in the SG cotyledons than in the FG cotyledons, and increased on the 6th and the 8th DAS, ultimately slowing down the synthesis of membrane lipids. The embryonic axes also showed accumulation of higher amounts of linolenic acid (18:3) on 6th DAS and a decrease in the content of palmitic acid (16:0) from 4th to 8th DAS in the FG seeds (Table 2). These results indicated that desaturation and elongation of fatty acids was faster in the FG seeds in comparison to the SG seeds. Linolenic acid is particularly required for the synthesis of glycolipids, which are the major components of chloroplast membranes (Ohta et al., 1995; Cantisan et al., 1999).

The present study revealed that the FG sunflower seeds displayed higher values of fresh weight of cotyledons, embryonic axes, and the length of embryonic axes in comparison to the SG seeds. The rate of depletion of lipids was higher in cotyledons but slower in embryonic axes in the FG compared to the SG seeds. The increase in the proportion of membrane lipids and the decrease of FFA coupled with enhanced desaturation and elongation reactions of fatty acids observed in the FG seeds contributed to the increased rate of seed germination and growth of seedlings in comparison to the SG seeds. The results of the present investigation indicate that the FG

seeds filled with enhanced amount of lipids and soluble sugars are located in the peripheral whorls while the position of the SG seeds is in the inner whorls of the sunflower head.

References

Arribere, M.C., N.S. Priolo, N.O. Caffini, 1994.Properties of the lipolytic system responsible for the mobilization of storage lipids in *Helianthus annuus* L. seeds. Phyton-Buenos-Aries, 56, 17-26.

Bhatia, I.S., K.L. Ahuja, P.S. Sukhija, 1978. Changes in the activity of acetyl CoA carboxylase in germinating and ripening sunflower seeds. Physiol. Plant., 44, 141-144.

Bose, G., B.N. Ghosh, P.C. Bose, 1988. Changes in different lipid components in the cotyledon and embryo of germinating sunflower seeds. Curr. Sci., 57**,** 95-98.

Cantisan, S., E.M. Force, R.A. Ortega, R. Garces, 1999. Lipid characterization in vegetable tiissues of high-saturated fatty acid sunflower mutants. J. Agric. Food Chem., 47, 78-82.

Dahlqvist, A., U. Stahl, M. Lenman, A. Banas, M. Lee, L. Sandager, H. Ronne, S. Stymne, 2000. A Phospholipids: diacylglycerol acyl transferase playing a role in the biosynthesis of triacylglycerols and cloning of genes encoding them. Proc Natl Acad Sc. USA 97, 6487-6492. Folch, J., M. Less, G.H. Sloane-Stanley, 1957. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226, 497-509.

Ganger, A., A.A.Karande, R. Rajasekharan, 2001. Isolation and localization of a cytosolic 10S triacylglycerol biosynthetic multienzyme complex from oleaginous yeast. J. Biol. Chem., 276**,** 10290-10298.

Hoppe, A., R. R. Theimer, 1997. Degradation of oil bodies isolated from cotyledons during germination of rapeseed seedlings. J. Plant. Physiol., 151, 471-478.

Liu, K.S., E.A. Brown, 1996**.** Fatty acid composition in newly differentiated tissues of soybean seedlings. J. Agric. Food Chem., 44, 1395-1398.

Munshi, S.K., B. Kaushal, R.K. Bajaj, 2003.Compositional changes in seeds

influenced by their positions in different whorls of mature sunflower head. J. Sci. Fd. Agric., 83, 1622-1626.

Munshi, S.K., S.Vats, K.S. Dhillon, P.S. Sukhija, 1990. Lipid biosynthesis in seeds of mustard (*Brassica juncea*) influenced by zinc and sulphur deficiency. Physiol. Plant., 79, 1-7.

Ohta, H., M. Shimojima, K. Ookata, T. Masuda, Y. Shioi, K. Takamiya, 1995. A close relationship between increases in galactosyltransferase activity and the accumulation of galactolipids during plastid development cucumber seedlings. Plant Physiol., 36, 1115-1120.

Shekhargouda, M., R. P. Katiyar, A. Nagaraja, M.N. Merwada, N.K.Biradarpatil, 1996. NK Effect of seed position in capitulum on seed quality of sunflower Cv EC 48414. Seed Res., 24, 30-33.

Steer, B.T., P.J. Hocking, and A. Low, 1988. Dry matter, minerals and carbohydrates in the capitulum of sunflower (*Helianthus annuss* L.): Effect of competition between seeds and defoliation. Field Crop Res., 18, 71-75.

Sung, J.M., 1996. Lipid peroxidation and peroxide scavenging in soybean seeds during ageing. Physiol. Plant., 97, 85-89.