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LIPID ACCUMULATION IN DEVELOPING SOYBEAN: INFLUENCE OF SEED POSITION ON STEM AXIS

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Summary. A proper understanding of various biochemical aspects of lipid metabolism during seed development is important as a guide for bringing changes in agricultural practices. Both oils and proteins are subjected to positional effect. This paper reports lipid accumulation in developing soybean seedlings as influenced by their position on the stem axis. The dry matter content of the seeds and pod walls increased 28 days after flowering (DAF) up to maturity at both basal and apical positions. Accumulation of seed lipids was rapid from 42 to 56 DAF at the basal position and from 35 to 56 DAF at the apical position. Maximum incorporation of 1-^{14}C-acetate into lipids was observed at 42 DAF in seeds at both basal and apical positions. The content of starch, total soluble sugars and reducing sugars in seeds as well as pod wall was almost similar in both positions but on mg seed^{-1} basis, these parameters showed higher values at the apical position. Membrane lipids decreased with seed development and triglycerides showed an increasing trend from 28 to 63 DAF in the seeds at both positions. The1-^{14}C-incorporation into polar lipids was maximum at 28 DAF which decreased with seed development at both nodal positions. The proportion of 18:2 was higher and 18:3 lower at apical as compared to basal position. The rate of oil filling was higher in basal nodes in comparison to the apical portion of the soybean plant with no appreciable difference in the membrane lipid composition. However, oil quality attributes changed with seed position on the stem axis.

Key words: Soybean (*Glycine max* L.); lipid accumulation; carbohydrates; fatty acids.

Abbreviations: DAF – days after flowering; GL – glycolipids; PL – phospholipids.

INTRODUCTION

Soybean (*Glycine max* L. Merr) is a high value legume having distinction of providing a preponderance of world's protein and oil trade and thus it has become one of most valuable cultivated crops. Mature soybean seeds contain 35-50% protein, 15-25% good quality edible oil and appreciable amounts of minerals and

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vitamins carrying multiple importance in food, feed and industrial uses. Lipids accumulate as triacylglycerides that are found in oil storage bodies surrounded by the protein oleasin or occasionally as oil droplets in the cytosol. Predominant fatty acids in triacylglycerides are palmitate (16:0), stearate (18:1), linoleate (18:2) and linolenate (18:3). In soybean, cell division in the seed is completed at an early stage of development (20-25 DAF) while the embryo is still quite small (Goldberg et al., 1981). The major increase in seed size which occurs between 25 to 60 days after flowering (DAF) is brought about through enlargement of pre-existing cells. The majority of oil, protein and carbohydrate synthesis and storage occur during this period by simultaneous partitioning of the photosynthates among those three major reserves (Ohlrogge and Kuo, 1984). Bils and Howell (1963) reported that by 26 DAF starch, lipid and protein bodies were present in the cytosol of soybean cotyledons. As the seed developed, the cells of the cotyledons became packed with the lipids, protein and starch bodies. However, the starch bodies disappeared just prior to maturation. Developing soybean seeds contained 5% oil at 25 DAF (Rubel et al., 1972). The oil percentage increased slightly to around 20% by 40 DAF and remained essentially constant during the remaining period of seed development. Wilson and Rinne (1974) reported variations in the accumulation of different phospholipid fractions in the developing soybean seeds but percentage of individual phospholipid species was not significant between any two varieties at a given time period. There was a rapid incorporation of C^{14} acetate or pyruvate into the phospholipids fraction. The oil content and fatty acid composition of the oilseeds are modified by the duration of seed development (Sauer et al., 1992). The composition of the seed depends also on the genetic and environmental conditions as well as the maturity of the seed. In soybean, the seeds mature progressively from the basal portion of the plant to the apical part. Consequently, the development of seeds in each node position takes place under varying environmental conditions, which are expected to have a direct effect on seed quality (Maestri et al., 1998) as in mustard (Munshi and Kochhar, 1994; Munshi and Kumari, 1994), sunflower (Munshi et al., 2003) and soybean (Bennett et al., 2003; Guleria et al., 2007). It is expected that the ontogeny of seed development is regulated by the position of seeds on the stem axis and thus might be mediated by influencing the metabolic contributions by the pod wall towards oil filling in the developing soybean seeds. A search of literature did not reveal the accumulation of lipids in developing soybean seeds as influenced by their position on the stem axis and this paper reports on this aspect.

MATERIALS AND METHODS

Soybean seeds, (*Glycine Max* L. Cv. SL-525) were procured from the oilseed section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Soybean plants were grown in the field by keeping 30 cm distances between rows and 15 cm between plants. The crop was raised by following the recommended practices of this University. All chemicals and solvents used were of Analar purity.

Sodium (1-^{14}C) - acetate (56.2 mCi mol^{-1}) was procured from BARC, Bombay.

Pod samples were collected at weekly intervals after flowering from basal (4-7th) and apical (17-20th) positions. The pods were packed in polythene bags and immediately kept in an ice box and brought to the laboratory. Seeds and pod walls were immediately separated and subjected to biochemical analysis/incubation on the same day. For dry matter determination, a weighed quantity of tissue was oven dried at 60°C for 48 h to constant weight. After drying, these were immediately placed in a desiccator before determination of the final weight. A portion of the tissue was crushed and boiled in 1-2 ml of isopropanol to inactivate phospholipases and homogenized in 20 vol (w/v) of chloroform and methanol (2:1, v/v) and stored at 0-4°C until used. The extraction of lipids was done by the method of Folch et al. (1957). A suitable aliquot of the lipid extract was evaporated to dryness to determine the weight of the lipid content. For carbohydrate analysis, a portion of the tissue was put in 10 volumes (w/v) of boiling 80% ethanol and stored at 4°C until required for analysis. The methods used for the estimation of soluble sugars, starch, lipid content, different lipid classes and fatty acid composition were the same as given in Munshi et al.(1990) .

The batches of tissue slices (1mm thick) of 1 g seeds were incubated in 0.1 M potassium phosphate buffer, pH 7.4 and 5µCi 1-^{14}C-sodium acetate in a final volume of 2 ml. The incubation was done at 25°C for 6 h with continuous shaking in SEW metabolic shaker (Dubnoff type). The reaction was stopped by removing the tissue, washing it with distilled water to remove any adhering radioactivity, boiled in 2 ml of isopropanol for 1 min and transferred to a chloroform:methanol mixture (2:1, v/v) for lipid extraction. The method for lipid extraction and the measurement of radioactivity were the same as described by Munshi et al (1990). There were three replications with duplicate observations for each replication and the data were analyzed by using ANOVA test for determining the critical differences between different stages and means were compared by Student's *t*-test at the 5% level of significance.

RESULTS AND DISCUSSION

The flowering in soybean was initiated from the first node near the ground (basal position) and proceeded upward towards to the upper portion of the plant (apical positions) within a period of 10 days. Consequently, the pods developed on these positions and the seeds were laid inside the pods after 20-25 days. The difference between the seed formation inside the pod from the basal to the apical part of the plant was about 7-8 days. Therefore, the seed development on the soybean plant was slightly indeterminate type that expectedly brought about variability in the seed development. The morphological characteristics of pods and seeds were similar to that reported earlier (Wiebold, 2003).

The dry matter content of the soybean seeds increased progressively from 28 to 63 DAF followed by a spurt increase up to maturity at both basal and apical positions of the stem axis (Table 1). The rate of dry matter accumulation was

Table 1. Dry matter and lipid content of soybean seeds and pod wall at basal and apical positions on the stem axis.

DAF	Dry matter [g kg^{-1} FW]				Lipid [g kg^{-1} DW]			
	Seeds		Pod walls		Seeds		Pod walls	
	Basal	Apical	Basal	Apical	Basal	Apical	Basal	Apical
28	154±5	159±7	195±4	211±5	116±5	95±3*	76±3	46±7
35	209±4	232±6*	199±3	214±4*	120±3	91±5*	65±9	38±5
42	256±7	291±9*	209±3	216±4	129±4	102±5*	53±7	23±7
49	307±2	319±3*	238±3	219±6*	163±8	137±2*	46±5	36±2
56	339±5	367±7*	236±3	228±4	184±4	179±3	48±5	39±4
63	356±4	374±3*	238±4	239±3	202±5	191±2	62±7	50±3
M	957±5	960±7	884±3	899±5	–	–	–	–
CD (P<0.05)	34	32	28	37	27	29	13	12

Values are means ±SE, n=6; *Statistically significant at P≤0.05.

faster in the seeds from the apical in comparison to the basal position and the values of dry matter were significantly higher in the apical position at all stages of seed development (Table 1). The dry matter content in pod wall increased rather slowly from 28 DAF up to maturity at both nodal positions. The dry matter accumulation was less in the pod wall at the apical in comparison to the basal position. The increase in dry matter during seed development is largely due to oil and protein accumulation as reported by other authors (Haberle et al., 1981). The data on dry matter indicated that the photoassimilates were channelized mainly towards the seed with very less retention in the pod wall and this mobilization of photoassimilates was higher at the apical in comparison to the basal position. The lipid accumulation was initially low and it started rapidly from 42 to 56 DAF followed by a slow increase at the basal position (Table 1). However, the period of rapid accumulation was from 35 to 49 DAF in seeds located at the apical position. The lipid content was higher in seeds from basal as compared to apical position. This might be due to a higher proportion of large seeds present at apical as compared to basal position (Guleria et al. 2008). This pattern of lipid content was similar to Collins and Cartter (1956) and Bennett et al. (2003) and these differences were attributed to environmental factors (Maestri et al., 1998). The increase in sulphur has been shown to enhance the lipid synthesis in seeds of peanut (Sukhija et al., 1987) and Indian mustard (Munshi et al., 1990). Thus, the proportionately higher availability of sulphur in relation to nitrogen in the basal region of the plant from the nutrient assimilation or low nitrogen-sulphur ratio would increase the lipid content while the high nitrogen:sulphur ratio in the upper part of the plant (Bennett et al., 2003) would

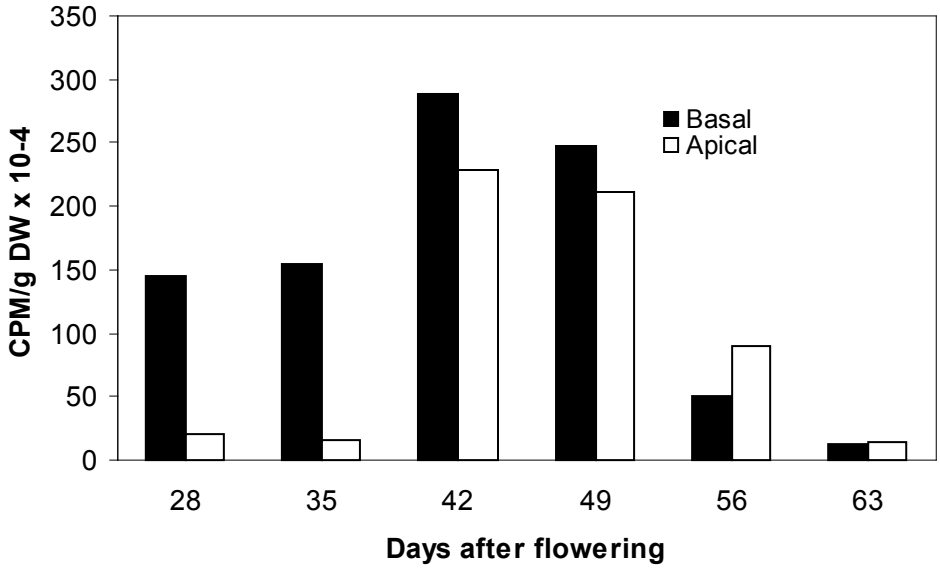

Fig 1. Incorporation of 1-^{14}C-acetate into lipids of developing soybean seeds as influenced by basal and apical positions on the stem axis. Critical difference ($p<0.05$): Basal=14.3; Apical=11.4.

increase the accumulation of protein, more specifically β-conglycinin (Sunarpi and Anderson, 1997). The lipid content in the pod wall was less as compared to seeds and it did not show any pattern of accumulation. The lipid content of pod wall was higher in seeds from basal as compared to apical position. These studies indicated that the period of rapid oil filling in both positions commenced at similar conditions of environmental temperature and moisture as the seeds at the apical position started their development after one week of the basal position. Consequently, the rapid oil filling in the soybean seeds started at 42 DAF in the basal nodes and 35 DAF in the apical nodes. A similar type of position wise variation has been shown in Indian mustard, sunflower (Munshi et al., 2003; Bayder and Erbas, 2005) and soybean (Guleria et al., 2007). The maximum incorporation of 1-^{14}C-acetate into lipids was observed at 42 DAF in seeds from both positions (Fig. 1) followed by a decline up to 63 DAF. These observations confirm previous findings on other oilseeds (Munshi et al., 1983; Simcox et al., 1979). The low rate of oil accumulation at early stages has been attributed to reduced availability of metabolites like ATP, NADPH and glycerol for lipid biosynthesis (Adams et al., 1980). In order to assess the contribution of carbohydrates towards the synthesis of lipids, the tissue was analyzed for starch and soluble sugars. The starch content increased in seeds as well as pod wall steeply from 28 to 56 DAF followed by a decrease to 63 DAF (Fig 2). The starch content in seeds from basal and apical nodes was almost similar and so was the pattern of accumulation. The total soluble sugars increased from 28 to 63 DAF in seed as well as pod wall at basal and apical positions of the stem axis (Fig. 3). The reducing sugar content in the seed was similar to starch at

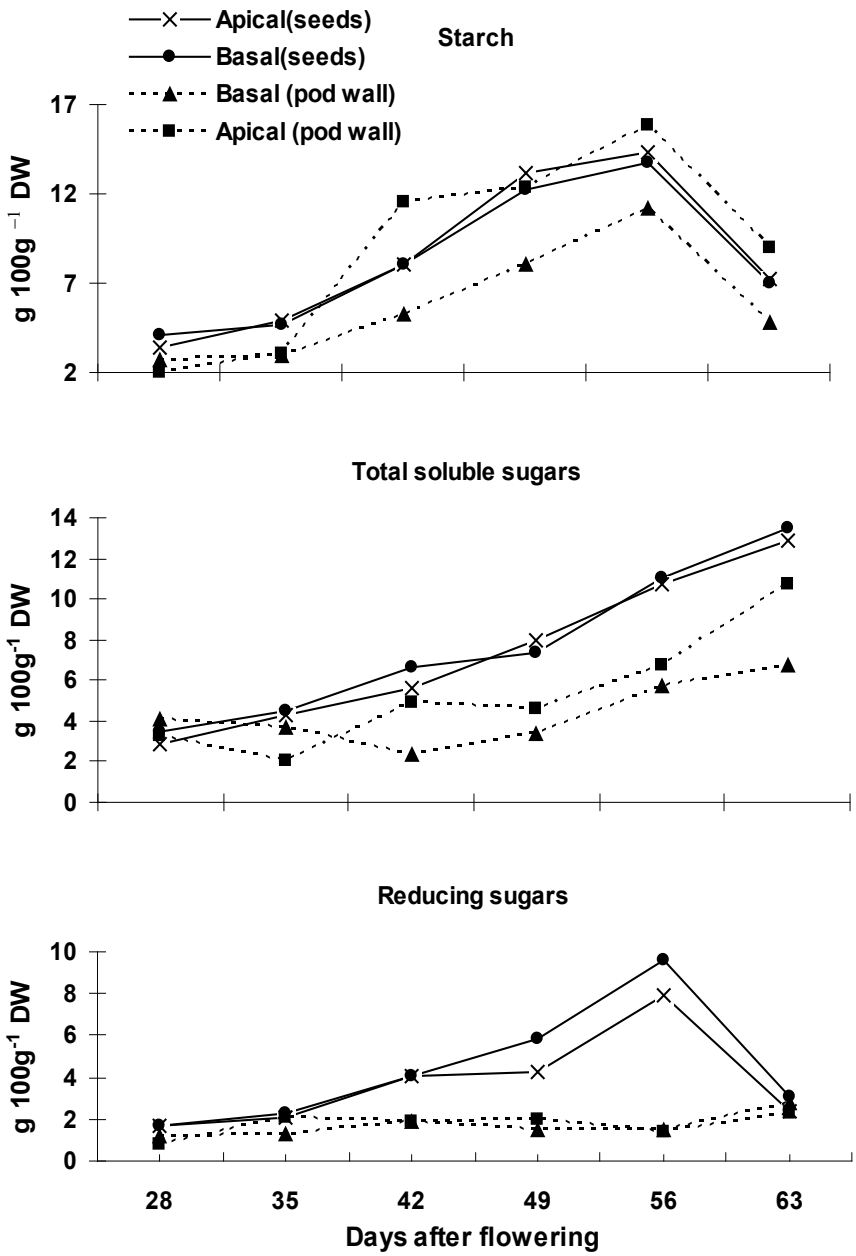

Fig 2. Carbohydrate composition of developing soybean seeds and pod wall as influenced by basal and apical positions on the stem axis.

different stages of seed development and the pod wall did not retain reducing sugars content at both positions. The patterns of accumulation of starch and total soluble sugars were similar to those reported earlier in soybean (Adams et al., 1980). However, this trend was in contrast to that reported in other oilseeds like Indian mustard (Munshi et al., 1990), sunflower (Luthra et al., 1991) and groundnut (Sukhija et al., 1987) which described accumulation of starch at the initial stages of seed development to act as a transient reserve material to be used

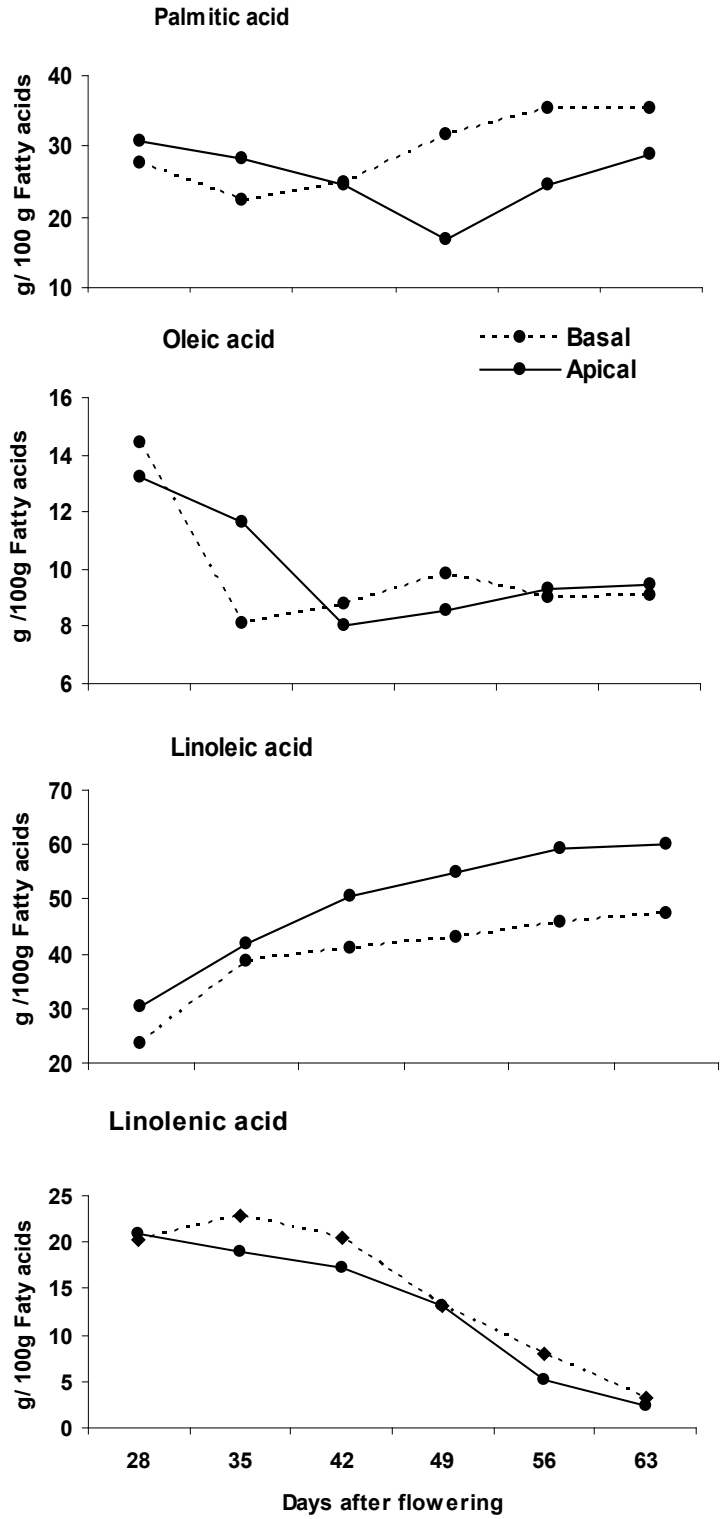

Fig 3. Fatty acid composition of developing soybean seeds as influenced by basal and apical positions on the stem axis.

for the synthesis of lipids in the developing seeds. Soybean is categorized as oilseed due to the presence of more than 20 % lipids in the seeds and a phase of rapid oil filling was similar to that of other oilseeds. Starch seems to perform an additional role in soybean at early phases of seed development as there is a considerable demand of the soybean plant for carbohydrates to support nitrogen fixation (Ryle et al., 1979). Rubel et al. (1972) reported that starch was utilized for the synthesis of 70 % of its total protein and oil found in mature soybean seed. Total soluble sugars are partitioned towards raffinose and stachyose during early phases of seed development also in addition to starch accumulation (Adams et al., 1980; Lowell and Kuo, 1989). Phospholipids (PL) content in the soybean seeds at the initial stages of seed development was higher and decreased significantly with the period of seed development whereas PL of pod wall did not change significantly with the development period. The proportion of PL in seeds/pod wall did not show significant variation in soybean seeds located at both basal and apical positions. Glycolipids (GL) content decreased significantly with the period of seed development at the basal position. Seeds from apical position showed a non-significant decrease during the development period. In the pod wall, a significant increase in glycolipids content could be noticed up to 56 DAF at both basal and apical positions (Table 2). GL content was significantly higher in pod wall from the apical as compared to the basal positions at all stages of development (Table 2). The free fatty acids content was too small to account for any difference between developmental stages and the position of seeds on the stem axis. Sterol content of seeds/pod wall from both positions decreased from 35 to 63 DAF and the sterol content of pod wall from apical positions was significantly lower as compared to basal positions. The content of triglycerides, which was calculated by subtracting the sum of phospholipids, glycolipids, sterols and free fatty acids from 100, showed an increasing trend from 28 to 63 DAF. Triglycerides content of seeds was significantly higher in seeds from apical position up to 42 DAF. Total glycerides did not change significantly in pod wall (Table 2). The incorporation of 1-^{14}C-acetate into different lipid classes in soybean seeds from apical and basal positions showed that polar lipids which are mainly membrane lipids constituting phospholipids and glycolipids, were synthesized maximum at 28 DAF and their proportion decreased while that of neutral lipids consisting of partial glycerides, sterols, triglycerides, sterol esters and hydrocarbons, increased with the period of seed development up to 63 DAF (Table 3). This was expected since polar lipids are important components of cell membranes and hence are synthesized to prepare the cell for lipid synthesis at a later stage. Wilson and Rinne (1974) reported that in immature soybean cotyledons incubated with ^{14}C-acetate, there was a rapid incorporation into the PL fraction. Triglycerides showed a slow but steady increase (about 25%) after 42 DAF. Data on different lipid classes have indicated that seeds at initial stages synthesize membrane lipids containing phospholipids, glycolipids and sterols. Besides their utility for membrane

Table 2. Lipid composition (g kg^{-1} lipids) of soybean seeds and pod wall at basal and apical positions on the stem axis.

DAF	Seeds		Pod walls	
	Basal	Apical	Basal	Apical
	Phospholipids			
28	54.7±1.2	55.6±2.0	26.7±1.8	30.5±2.5
35	49.5±3.1	46.7±2.5	30.1±3.1	29.0±1.9
42	46.7±5.0	44.8±2.6	30.5±5.0	24.3±2.7
49	36.1±3.2	32.4±3.1	28.3 ±1.7	24.3±1.5
56	28.6±2.5	24.5±2.7	26.3±2.1	22.9±1.3
63	21.2±3.4	25.2±2.5	22.3±1.5	16.6±2.1
CD (P<0.05)	4.5	3.4	3.2	3.8
	Glycolipids			
28	41.8±1.5	23.8±3.5*	8.8±2.0	20.9±1.3*
35	42.8±3.2	30.5±2.8*	16.9±1.1	27.7±2.1*
42	36.8±2.6	29.0±3.1	18.1±1.2	33.5± 3.1*
49	31.9±2.7	26.9± 1.6	20.0±1.6	38.9±2.5*
56	20.1±1.5	25.6±4.5	19.5±1.3	29.1±2.4*
63	16.5±0.09	20.6±2.3	14.5±0.9	27.6±2.1*
CD (P<0.05)	2.8	2.6	2.3	5.1
	Sterols			
28	23.8±1.3	27.5±1.8	29.5±0.8	22.5±2.2*
35	33.0±1.9	37.8±2.5	29.0±1.6	20.0±1.3*
42	32.2±2.8	27.7±2.2	27.0±1.2	22.9±0.8*
49	21.5±1.9	16.9±1.3	22.5±0.6	19.0±0.5*
56	21.5±2.0	14.7±1.8	24.4±1.0	12.8±0.7*
63	16.6±2.0	12.1±0.8	13.4±0.5	11.2±0.3*
CD (P<0.05)	2.2	3.1	1.8	2.6
	Triglycerides			
28	877±1.5	884±0.9*	921±1.2	921±1.3
35	879±5.0	898±3.3*	924±3.8	930±1.5
42	891±6.0	899±5.0	926±4.6	934±2.5
49	910±3.1	914±5.0	925±3.0	937±3.8
56	929±5.1	933±2.2	928±1.7	949±4.0*
63	945±2.1	942±4.2	948±5.0	958±1.5
CD (P<0.05)	34	38	15	13

DAF: Days after flowering; Values are means ±SE, n=6; *Statistically significant at P≤0.05.

Table 3. Relative incorporation (%) of ^{14}C-radioactivity from 1-^{14}C-acetate into polar and non-polar lipids of soybean seeds at basal and apical positions on the stem axis.

Lipid classes	Basal			Apical		
DAF	28	42	63	28	42	63
Polar lipids	77.4	39.5	14.7	74.8	44.4	21.1
Partial glycerides	9.9	17.8	24.4	8.9	12.4	15.2
Sterols	1.7	4.5	7.2	2.7	4.3	4.6
Triglycerides	6.4	25.6	40.6	9.8	28.6	42.5
Sterol esters	5.9	5.2	14.9	1.9	5.5	7.9
Hydrocarbons	0.4	7.3	17.6	1.6	4.7	16.2

synthesis, these lipid fractions are also used for the synthesis of triglycerides (Slack et al., 1985) and a decrease in their content at later stages was a proportional decrease with a concomitant increase in triglycerides content (Munshi et al. 1990). High amounts of PL, GL and sterols at the initial stages of seed development (Table 2) might be due their enhanced synthesis at the cost of the nonpolar lipids for the formation of membranes (Munshi and Sukhija, 1984). The decrease in membrane lipids during seed development might be due to the decreased relative proportion of membranes in the seeds during development (Wang et al., 2006). The lower percentage of membrane lipids in the pod wall (except GL) at all stages of seed development indicated that the membranes of the pod wall were intact throughout the period of seed development (Nes, 1987) as this is the organ which translocates photoassimilates from the leaves to the seeds besides being photosynthetic in nature (Singal et al., 1987). An increase in dry matter and lipids per pod wall was expected since the pod wall acquired a large size, developing maximum photosynthetic surface area to make it an important part for supplying the seeds with photoassimilates (Singal et al., 1987). The increase in 1-^{14}C acetate incorporation (Fig. 1) at 42 DAF also supported the above information. Changes in fatty acids are of special importance to the quality of the oil and 16:0, 18:1, 18:2 and 18:3 are the major fatty acids present in soybean seeds. The fatty acid composition has revealed that proportion of 18:2 increased with the period of seed development with a corresponding decrease in 18:1 and 18:3. However, the values of 18:2 were higher in the seeds at apical in comparison to the basal portion from 42 to 63 DAF. The data on fatty acid composition indicated that the seeds at different positions of the stem axis might show similar compositional pattern and the variation could be due to position as well as different size of the seed. Our study on developing seeds showed the rate of oil filling being higher in the basal nodes in comparison to the apical portion of the plant with no appreciable difference in the membrane lipid composition. However, the oil quality attributes changed with seed position on the stem axis.

REFERENCES

Adams CA, Rinne RW, Fjerstad MC, 1980. Starch deposition and Carbohydrase activities in developing and germinating soybean seeds. Ann Bot, 45: 577–582.

Bayder, H., S., Erbas, 2005. Influence of seed development and seed position on oil, fatty acids and total tocopherol contents in sunflower (*Helianthus annuus* L.). Turk. J. Agric., 29: 179–186.

Bennett, J.O., A.H., Krishnan, W.J., Wiebold, H.B., Krishnan, 2003. Positional effect on protein and oil content and composition of soybeans. J. Agric. Food Chem. 51: 6882–6886.

Bils, R.F., R.W., Howell, 1963. Biochemical and cytological changes in developing soybean cotyledons. Crop Sci., 3: 304–308.

Collins, F.I., J.L., Cartter, 1956. Variability in chemical composition of seed from different portions of the soybean plant. Agron. J., 48: 216–219.

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 .

Goldberg, R.B., G., Hoschek, S.H., Tam, G.S., Ditta, R.W., Breidenbach, 1981. Abundance, diversity and regulation of mRNA sequence sets in soybean embryogenesis. Dev. Biol., 83: 201–217.

Guleria, S., S., Sharma, B.S., Gill, S.K., Munshi, 2008. Distribution and biochemical composition of large and small seeds of soybean (*Glycine max* L.). J. Sci. Food Agric., 88: 269–272.

Guleria, S., S., Sharma, S.K., Munshi, 2007. Compositional changes in soybean (*Glycine max* L.) seeds influenced by their positions on stem axis. J. Food Sci. Technol., 44: 506–509.

Haberle, B., R., Heneberg, J., Momirovic, L., Balint, 1981. Total lipids, fatty acids and total tocopherols in developing soybean seed. Zeitschrift-fur-acker-und-pflanzenbau, 150: 147–159.

Lowell, C.A., T.M., Kuo, 1989. Oligosaccharide metabolism and accumulation in developing soybean seeds. Crop Sci., 29: 459–465 .

Luthra, R., S.K., Munshi, P.S. Sukhija, 1991. Relationship of carbohydrate metabolism with lipid biosynthesis in developing sunflower (*Helianthus annus* L.) seeds. J. Plant Physiol., 137: 312–318.

Maestri, D.M., D.O., Labuckas, J.M., Meriles, A.L., Lamarque, J.A., Zygadlo, C.A., Guzman, 1998. Seed composition of soybean cultivars in different environmental regions. J. Sci. Food Agric., 77: 494–498.

Munshi, S.K., A., Kochhar, 1994. Carbohydrate metabolism in the siliqua relating to oil filling in mustard seeds. J Agron Crop Sci., 172: 126–136.

Munshi, S.K., A., Kumari, 1994. Physical characteristics of siliqua and lipid composition of seeds located at different positions in mature mustard inflorescence. J. Sci. Food Agric., 64: 289–293.

Munshi, S.K., P.S., Sukhija, 1984. Compositional changes and biosynthesis of lipids in the

developing kernels of almond *(Prunus amygdalus* Batsch) J. Sci. Food Agric., 35: 689–697.

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. Food Agric., 83: 1622–1626.

Munshi, S.K., P.S., Sukhija, I.S., Bhatia, 1983. Lipid biosynthesis in developing kernels of almond (*Prunus amygdalus* Batsch). Phytochemistry, 22: 79–83.

Munshi, S.K., S., Vats, K.S., Dhillon, P.S., Sukhija, 1990. Lipid biosynthesis in seeds of mustard (*Brassica juncea L.*) influenced by zinc and sulphur deficiency. Physiol. Plant., 80: 102–108.

Nes, W.R., Multiple roles for plant sterols. In"The metabolism, structure and function of plant lipids". (eds. Stumpf PK, Mudd JB and Nes WR). 3–11. Plenum Press, New York. (1987).

Ohlrogge, J.B., T.M., Kuo, 1984. Control of lipid synthesis during soybean seed development: enzymic and immunological assay of acyl carrier protein. Plant Physiol., 74: 622–625.

Rubel, A., R.W., Rinne, D.T., Canvin, 1972. Protein, oil and fatty acid in developing soybean seeds. Crop Sci., 12: 739–741.

Ryle, G.J.A., C.E., Powell, A.J., Gordon, 1979. The respiratory costs of nitrogen fixation in soybeans, cowpea and white clover. II. Nitrogen fixation and the respiration of the nodulated root. J. Exp. Bot., 30: 135–153.

Sauer, D.B., C.M., Meronuck, C.M., Chirstensen, 1992. Microflora, in *Storage of Cereal Grains and their products,* Ed. by Sauer DB. 4th edn American Association of Cereal Chemists, Inst Paul, Mn.

Simcox, P.D., W., Garland, V., Deluca, D.T., Canvin, D.T., Dennis, 1979. Respiratory pathways and fat synthesis in the developing castor oilseed. Can. J. Bot., 57: 1008–1014.

Singal, H.R., G., Talwar, A., Dua, R., Singh, 1987. Pod photosynthesis and seed dark CO_2 fixation support oil synthesis in developing *Brassica* seed. J. Biol. Sci., 20: 49–58.

Slack, C.R., P.G., Roughan, J.A., Browse, S.E., Gardner, 1985. Some properties of choline phosphotransferase fron developing safflower cotyledons. Biochem. Biophys. Acta, 83: 438–448.

Sukhija, P.S., V., Randhawa, K.S., Dhillon, S.K., Munshi, 1987. The influence of zinc and sulphur deficiency on oil filling in peanut (*Arachis hypogea* L.) kernels. Plant Soil, 103: 261–267.

Sunarpi, J.W., Anderson, 1997. Effect of nitrogen nutrition on remobilization of protein sulfur in the leaves of vegetative soybean and associated changes in soluble sulfur metabolites. Plant Physiol., 115: 1671–1680.

Wang, L., T., Wang, W.R., Fehr, 2006. Effect of seed development stage on sphingolipid and phospholipids contents in soybean seeds. J. Agric. Food Chem., 54: 7812–7816.

Wiebold, B., 2003. Growth and development of Soybean: Seed-filling. Integrated Pest Crop Management Newsletter.

Wilson, R.F., R.W., Rinne, 1974. Phospholipids in the developing soybean seed. Plant Physiol., 54: 744–747.