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LIPID METABOLISM IN GERMINATION SEEDS OF *CASSIA*

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The seeds of *Cassia absus, Cassia fistula* and *Cassia occidentalis* were germinated in the dark at 30° and the variations in the lipid class and fatty acid composition in the cotyledons and primary root were determined. Along with losses in dry matter in both the cotyledons and the primary root, the lipid content increased in the cotyledons but were reduced in the root during germination. Lipid content was found to be least in the region of the primary root close to the cotyledons. During germination the relative amounts of the neutral lipids, which were mainly triglycerides, decreased but those of the polar lipids, mainly phosphoglycerides, increased. Similar but more significant changes in the lipid class composition of the primary root were observed, which indicated that the neutral glycerides have a function as a source of reserve energy while the phosphoglycerides and glycolipids play a role as component of the membrane systems in the growing root. Analysis for lipid class pattern in the various regions of the primary root showed that the apical portions had slightly higher proportions of neutral lipids as compared to the other parts. Changes in the distribution of fatty acids were measured during germination in cotyledons and primary roots which did not show any significant variations as compared to the resting seeds. In the primary root, however, relative proportion of saturated fatty acids, mainly $C_{16:0}$ and $C_{18:0}$ increased manifold, while percentage of $C_{18:2}$, the major unsaturated fatty acid, was reduced to about 1/3rd. Percentage of the unsaturated fatty acids was highest in the tip whereas the relative amount of the saturated fatty acids was highest in the regions of the root close to the cotyledons. Metabolic patterns of the Classes and the fatty acids in the cotyledons and the primary root of Cassia have been discussed.

Key words: Lipids, Germination, *Cassia* seeds.

INTRODUCTION

Changes in composition of lipids during germination of a variety of plant seeds have been studied by different workers. Many of these reports have dealth with variation of some specific class of lipids. These changes in the pattern of phospholipids during germination of Wheat [1], Soyabean [2], rice [3], uromyces phaseoli [4], Hazel seeds [5] and different beans [6, 7] have been reported. Reports have also dealt with changes in neutral lipids like triglycerides [8, 9], glycerides and their fatty acids and synthesis of fatty acids during seed germination [10]. There are other studies which have described variation of a greater variety of lipid classes during seed germination [11, 12]. However, none of these studies have presented a complete picture on composition of lipids at different stages of germination. Further more nothing has been reported on lipid metabolism in Cassia seeds during germination.

This investigation, therefore, deals with variation in lipid composition at different stages of germination of the seeds of *C. absus, C. fistula* and *C. occidentalis*. Apart from percentage composition of the lipids, their fatty acid composition was determined at the various stages of the seed germination.

MATERIALS AND METHODS

Plant material. Fresh seeds of *C. absus, C. fistula* and *C. occidentalis* were collected locally from the fields of PCSIR Laboratories Lahore and were air dried and stored. Reagents and standards for thin layer chromatography were prepared by the method of Singleton, Thomas and Ralston [13, 14].

Germination of the seeds. The seeds are obtained above were surface sterilized with a 2.70% aqueous sodium hypochlorite solution, washed with sterilized distilled water. They were soaked in warm water for a few hours and incubated at 30° between the folds of moist filter paper in the dark. At different time intervals the germinated seeds having the same length of the primary root were picked, the primary root and the cotyledon were detached and pooled together. The primary roots of 30 mm length were also cut into 5 segments of equal length by a specially designed cutter and segments of the same region were pooled together.

Extraction of lipids. A known weight of the combined root segments, whole roots of the same length, the cotyledons or the resting seeds were homogenized separately

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each with 20 volume (ml/g) of chloroform/methanol (2:1, v/v) in a homogenizer for 5 minutes and filtered through a filter paper pre-washed with chloroform/methanol (2:1, v/v). Three more washings using the same homogenizer and filter paper were obtained in the same manner. The washings were combined and the non-lipids were removed by the Folch's [15] washing method. After removing the solvent weights of the lipids were determined and their percentages were thus calculated on a dry weight basis. Moisture content in each of the samples was determined by drying a portion to a constant weight at 110°.

Chromatographic analysis. The crude lipids were separated into neutral and polar fractions by silicic acid adsorption column chromatography using hexane/diethyl ether (7:3, v/v) as eluting solvents. The non polar and polar classes of lipids were isolated by thin layer chromatography. Aliquots of the lipids (5 ul of 10% solution) were streaked on activated glass plates (20x20 cm) coated with 0.5 mm thick layer of silica gel G (E. Merck, West Germany). For neutral lipids the solvent system used was hexane/diethyl ether/acetic acid (80:20:2, v/v/v) while polar lipids were separated with chloroform/methanol/ 30% ammonia/water (60:35:5:2.5, v/v/v/v). The various lipid fractions were identified by spraying the plates with the specific reagents and by co-chromatographing the standards prepared as described by Akhtar *et. al.* [16].

For quantitative determination of the lipid classes 5 mg of the lipid (50 ul of a 10% solution was applied in the form of a 10 cm. long band on a 0.5 mm plate. After developing the bands were marked and separated off after spraying the plates with 0.2% 2,7-dichlorofluorescein in methanol and viewing under ultraviolet light.

Gas chromatography. Methyl esters of the fatty acids of each of the lipid class as separated above were prepared by the method of Morrison and Smith [17] and a known amount of $C_{17:0}$ was added to each of the sample as the internal standard. 1.0-1.5 ul of the sample was injected in Pye Unicam 204 Series unit using a glass column (1.5mm x 4mm) packed with 20% PEGS on 80-100 mesh. Column temperature was maintained at 200°. Nitrogen at a flow rate of 40 ml/minute was used as the carrier gas. Detection was made by ionisation detection and the detector was maintained at 250°.

Peak areas were obtained from the product of the retention times and the respective peak heights. The amounts of total fatty acids were determined by comparing the total peak area of all the fatty acids in each fraction with that of the internal standard. By calculating the average molecular weight of the fatty acids, in each of the fractions, the amounts of the various fractions like triacylglycerols, diacylgly-cerols, monoacyl glycerols, free fatty acids, phosphatidyl ethanol amine, phosphatidyl choline etc. and the unknown polar lipid could be calculated. Amount of other components (present only as minute trace, if any) were calculated by difference. Percentages of the various fractions could, therefore, be calculated. Total amount of the neutral and the polar lipids were calculated by adding percentages of the corresponding fractions.

RESULTS AND DISCUSSION

Lipid and moisture contents. Total lipid and moisture contents in the primary roots and cotyledons of *C. absus; C. fistula* and *C. occidentalis* at different stages are given in Tables 1-4. The moisture content increased from 18.71-37.61% in *C. absus*; 21.50%-51.85% in *C. fistula* and 13.85-30.06% in *C. occidentalis* as the root length increased from 5mm to 30mm. The lipid content on dry weight basis decreased rapidly with increase in the length of primary root showing that there was a rapid utilisation of lipids. In the cotyledons as well moisture content increased during the course of seed germination but it was not as significant as in the case of roots. Lipid content in cotyledons on dry wt. basis showed an increase from 1.50-3.34% in *C. absus*; 1.25-2.80% in *C. fistula* and 1.27-3.22% in *C. occidentalis* as the germination proceeded. A continuous decrease in the weight of dry matter and lipid content of whole seedlings of soyabean has been reported [18]. In another study the lipid content decreased slightly in cotyledons and increased in hypocotyl of soyabean [19]. This study, however, showed a rapid utilisation of lipids in the primary root as germination proceeded. Increased level of lipid on dry basis in cotyledons during the course of the germination could be due to their biosynthesis which might also be associated with preferential utilisation of non-lipid components.

Lipid and moisture contents in different parts of the primary root which had grown to a length of 30 mm were determined. As given in Table 4 the growing tips contained highest lipid percentage on dry weight basis. The lipid percentage decreased in the parts of root towards the base. The moisture contents increased from the tip to the base of the primary root. A lower level of lipid in the regions of the primary root close to the cotyledons suggested that this was the site of more active metabolism.

Lipid composition in cotyledons. Composition of the lipids in the cotyledons of *C. absus; C. fistula* and *C. occidentalis* at different stages of germination and resting seeds are shown in Fig. 1-3. Their quantitative analysis was determined by Column, Thin-layer and Gas chromatography. Triacyl glycerol, like in the resting seeds, were present in high amounts at all the stages of germination but their amounts decreased as germination proceeded. Free fatty acids showed an increase with the start of germination and their percentages remained almost constant at later stages of germination. In *C. absus* and *C. occidentalis* percentages of sn 1,3-and sn 1,2-diacylglycerols decreased during germination but in *C. fistula* the percentage of sn 1,2-acylglycerol was increased. The amounts of*1* and *2* monoacylglycerols decreased in *C. fistula* and *C. occidentalis* but in

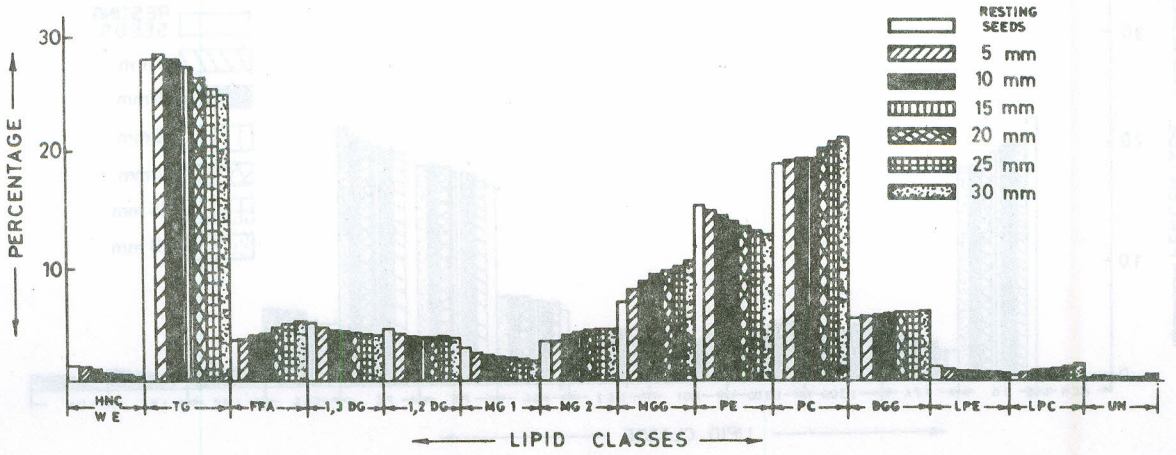

Fig. 1. Percentage of the various fractions in lipid extracts of cotyledons of *Cassia absus* after germinating the seeds to different length of primary root.

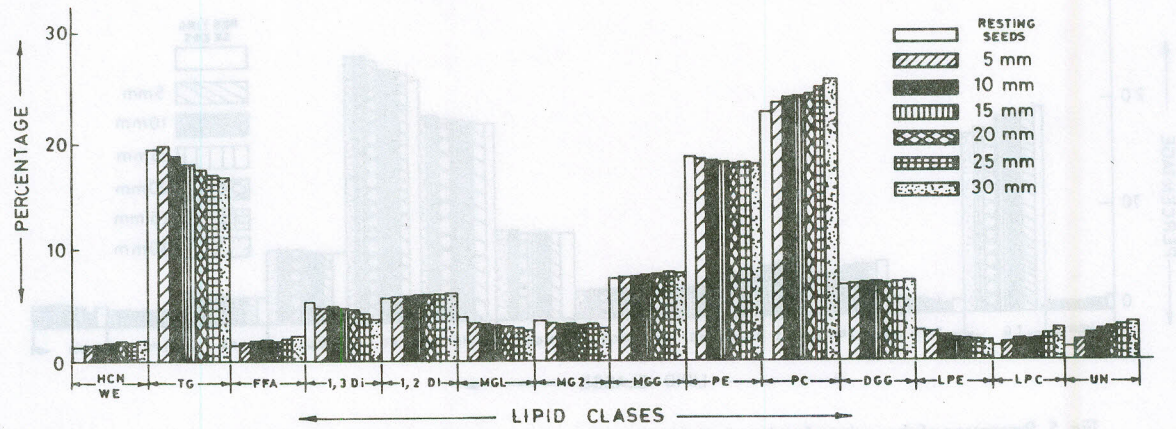

Fig. 2. Percentage of the various fractions in lipid extracts of cotyledons of *Cassia fistula* after germinating the seeds of different length of primary root.

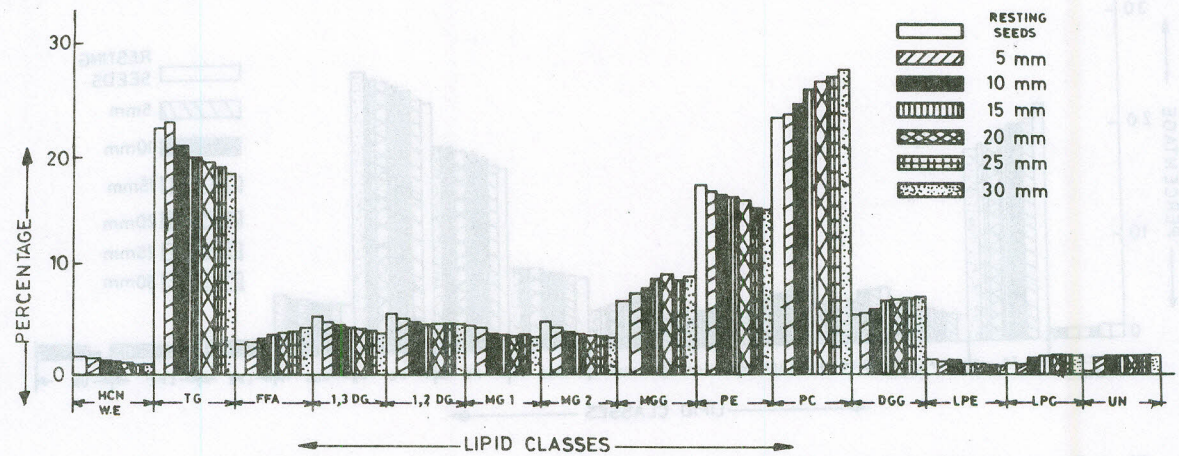

Fig. 3. Percentage of the various fractions in lipid extracts of cotyledons of *Cassia occidentalis* after germinating the seeds to different lengths of primary root.

HCN: Hydrocarbon; WE: Wax Ester; TG: Triacyl Glycerol; FFA: Free Fatty Acid; 1,3DG:1, 3-Diacyl Glycerol; 1-2-DG: 1, 2-Diacyl Glycerol; MG1: Monoacyl Glycerol 1 MG2; Monoacyl Glycerol; MGG: Monogalactosyl Glyceride; PE: Phosphatidyl-Ethanol Amine; PC: Phosphatidyl Choline; DGG: Digalactosyl Glyceride; LPE: Lyso Phosphatidyl-Ethanol Amine; LPC: Lysophosphatidyl Choline; UN: Unknown.

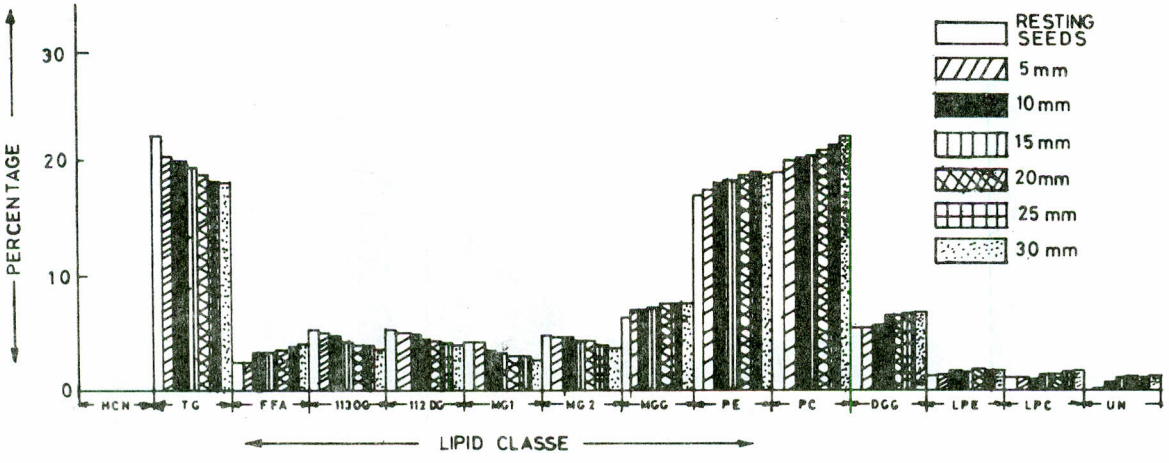

Fig. 4. Percentage of the various fractions in lipid extracts of primary root of *Cassia absus* grown for different lengths.

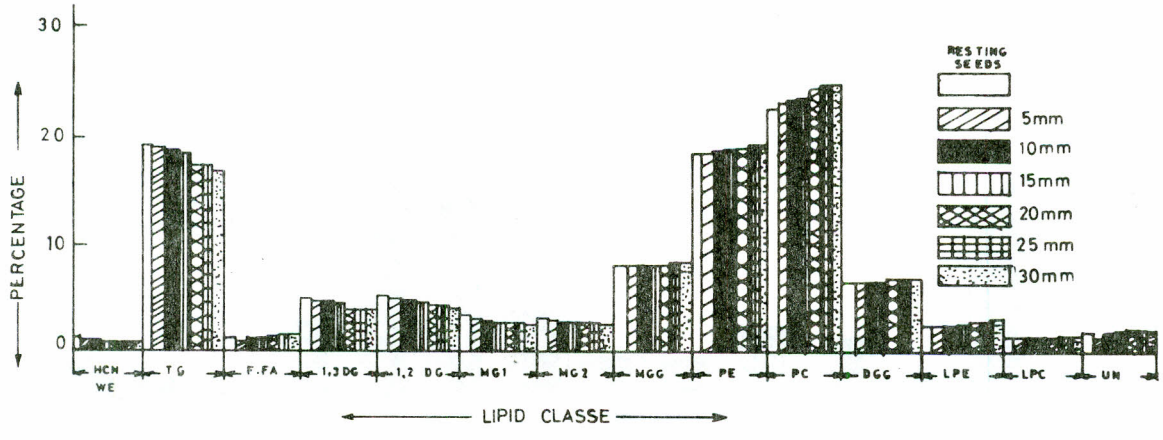

Fig. 5. Percentage of the various fractions in lipid extracts of primary root of *Cassia fistula* grown for different lengths.

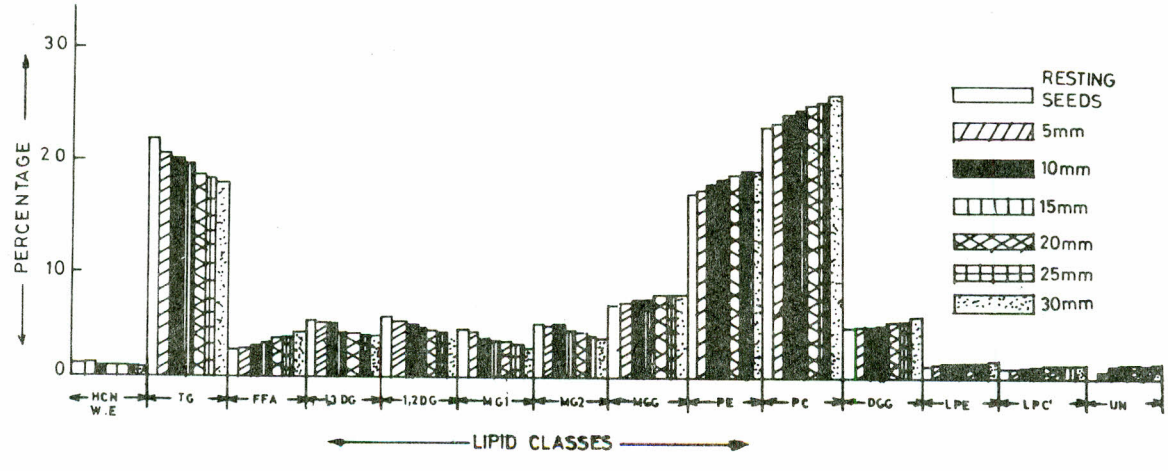

Fig. 6. Percentages of the various fractions in lipid extracts of primary root of *Cassia occidentalis* grown for different lengths.

HCN: Hydrocarbon; WE: Wax Ester; TG: Triacyl Glycerol; FFA: Free Fatty Acid; 1,3DG:1, 3-Diacyl Glycerol; 1-2-DG: 1, 2-Diacyl Glycerol; MG1: Monoacyl Glycerol 1 MG2; Monoacyl Glycerol; MGG: Monogalactosyl Glyceride; PE: Phosphatidyl-Ethanol Amine; PC: Phosphatidyl Choline; DGG: Digalactosyl Glyceride; LPE: Lyso Phosphatidyl-Ethanol Amine; LPC: Lysophosphatidyl Choline; UN: Unknown.

C.absus the amount of 2-monoacyl glycerols increased as the germination proceeded. Hydrocarbon-wax-ester percentages showed a decrease in the cotyledons of *C. absus* and *C. occidentalis* but in *C. fistula* their amounts increased during the germination period. Monogalactosyl and diagalactosyl glycerides were present in low amounts in the resting seeds and showed increase in the cotyledons. Along with germination the percentages of phosphatidyl choline, which is the major polar lipid, increased but that of phosphatidyl ethanol amine decreased with germination. Lyso phosphatidyl ethanolamine were present in low amounts in the resting seeds but increased gradually as the germination proceeded. The amounts of Lysophosphatidyl choline, which was present in small amounts in the resting seeds were, however, increased in the cotyledons.

Tables 1-3 show proportions between the total neutral and the total polar lipids of the cotyledons when the seeds were germinated to different root lengths. The proportion in cotyledons changed from 0.96-0.80 in *C. absus*, 0.67-0.55 in *C. fistula* and 0.86-0.63 in *C. occidentalis* when the length of their primary roots increased from 5mm to 30 mm.

An increase in the phospholipids content in the cotyledons of hazel seed [5] and mung bean [6, 7] has been reported. However, a marked decrease of phosphatidyl choline and phosphatidyl ethanol amine in the cotyledons of germinating soyabean was observed [11].

Although the relative percentage of neutral lipids in the cotyledons of *C. absus, C. fistula* and *C. occidentalis* dropped with germination, their absolute amount did not decrease as the over all lipid content increased in the cotyledons. This might suggest a simultaneous breakdown as well as synthesis of the neutral lipids, mainly triglycerides, as has been observed previously in the seedlings of flax

Table 1. Lipids and moisture contents of primary roots and cotyledons at different germinating stages of *Cassia absus*.

	Cotyledons					Roots				
Root length (mm)	Moisture (%)	Lipid (%)	Neutral lipid (%)	Polar lipid (%)	Neutral lipid / Polar lipid	Moisture (%)	Lipid (%)	Neutral lipid (%)	Polar lipid (%)	Neutral lipid / Polar lipid
Resting Seeds	9.50	4.80	49.89	50.11	0.99	9.90	4.80	49.89	50.11	0.99
5	10.80	1.50	49.04	51.04	0.96	18.71	3.90	48.46	51.44	0.94
10	11.91	1.75	48.56	51.26	0.94	20.35	3.00	46.73	52.21	0.88
15	12.16	2.64	38.03	51.82	0.92	26.65	2.75	44.41	54.75	0.81
20	12.39	2.89	47.36	52.06	0.90	30.31	2.41	43.20	55.97	0.77
25	12.65	3.16	46.89	52.53	0.89	32.75	2.08	42.33	56.79	0.74
30	13.11	3.34	45.49	54.51	0.83	37.61	1.58	41.21	57.87	0.71

Table 2. Lipids and moisture contents of primary roots and cotyledons at different germinating stages of *Cassia fistula*

	Cotyledons					Roots				
Root length (mm)	Moisture (%)	Lipid (%)	Neutral lipid (%)	Polar lipid (%)	Neutral lipid / Polar lipid	Moisture (%)	Lipid lipid (%)	Neutral lipid (%)	Polar lipid (%)	Neutral / Polar l ipid
Resting seeds	5.5	3.0	39.30	58.72	0.67	5.5	3.0	39.30	59.72	0.65
5	15.5	1.15	38.48	52.91	0.64	21.5	1.4	38.19	59.91	0.63
10	16.34	1.83	37.58	60.21	0.62	31.10	1.00	37.43	60.57	0.61
14	17.66	2.33	37.01	60.49	0.61	36.45	0.65	36.88	60.98	0.60
20	18.06	2.61	36.33	61.01	0.59	40.60	0.45	35.58	61.42	0.57
25	19.75	2.73	35.76	61.82	0.57	42.70	0.36	34.95	62.78	0.55
30	19.99	2.80	34.57	62.10	0.55	51.85	0.20	33.97	63.73	0.53

Table 3. Lipids and moisture contents of primary roots and cotyledons at different germinating stages of *Cassia occidentalis*

	Cotyledons					Roots				
Root length (mm)	Moisture (%)	Lipid (%)	Neutral lipid (%)	Polar lipid (%)	Neutral lipid / Polar lipid	Moisture (%)	Lipid (%)	Neutral lipid (%)	Polar lipid (%)	Neutral lipid / Polar lipid
Resting seeds	7.5	3.50	46.44	53.56	0.86	7.50	3.50	46.44	53.56	0.86
5	10.20	1.27	44.30	54.59	0.81	13.85	1.25	43.86	55.90	0.78
10	11.30	55	41.97	54.93	0.76	18.90	1.00	42.51	56.41	0.75
15	11.66	1.3	40.65	56.51	0.72	20.21	0.85	40.93	57.88	0.70
20	12.21	2.35	0.60	57.25	0.69	23.61	0.51	39.39	58.31	0.67
25	12.65	2.93	39.01	58.02	0.67	26.05	0.35	38.51	60.45	0.63
30	13.55	3.22	39.98	59.65	0.63	30.06	0.15	37.53	61.18	0.61

[20]. A faster rate of metabolism of non-polar lipids in cotyledons of soyabeans has also been reported [11].

Lipid composition of primary root. The various lipid classes detected in the lipids obtained from primary roots of different length and the resting seeds of *C. absus; C. fistula* and *C. occidentalis* are shown in Fig. 4-6. Their quantitative analysis by Combined Thinlayer and Gas Chromatography showed that in general, like in the case of cotyledons, the percentage of polar lipids increased in the roots but the increase was more significant in this case. Percentages of triacylglycerols, free fatty acids, diacyl glycerols, monoacyl glycerol and hydrocarbon-wax-ester decreased in the primary root of 5 mm length as compared to those in the resting seeds. Amounts of all these lipids decreased gradually in the primary root alongwith the increase in its length. Free fatty acid increased from 3.72%-4.04% in *C. absus*; 1.39%-1.66% in *C. fistula* and 2.54%-4.16% in *C. occidentalis* as the primary root length increased from 5mm to 30 mm. Changes in the pattern of polar lipids were even more significant. Quantities of monogalactosyl glycerides, phosphatidyl ethanol amine, phosphatidyl choline, digalactosyl glyceride, Lysophosphatidyl ethanol amine and lysophosphatidyl choline were higher in the primary root as compared to those in the resting seeds and along with increase in the root length their quantities showed further increase.

Proportions between the total neutral and the total polar lipids in the resting seeds changed from 0.99-0.71 in *C. absus*; 0.65 to 0.53 in *C. fistula* and 0.86 to 0.61 in *C. occidentalis*.

An increase in the relative amounts of free fatty acid, phosphoglycerides, monogalactosyl glyceride and digalactosyl glyceride and a decrease in neutral glycerides and hydrocarbon-wax-ester along with increase in root length was associated with an overall depletion of the lipid content, as shown above. Decreased levels of neutral glycerides, accompanied with higher amounts of free fatty acids, suggested a higher level of lipolytic activity in the primary root. These results like those in the cotyledons suggest that the neutral lipids, which were mainly triacyl glycerols, function as the source of reserve energy and the polar lipids, which were mainly phosphoglycerides, have function as components of the membrane systems of the growing root.

Lipids in different parts of the primary root. Lipids of different parts of the primary roots which had grown upto a length of 30mm were analysed. The various lipid classes detected are shown in Fig. 7-9.

Quantitative analyses showed that amongst the neutral lipids triacylglycerol were present in highest and almost constant amount from apical portion to the base of the primary root. Free fatty acids were present in significant amount in the apical portions, but lower in the rest of the portions. Percentage of triacylglycerol remained high and nearly constant in all the portions of the roots. percentages of monogalactosyl glyceride, digalactosyl glyceride, phosphatidyl ethanol amine, phosphatidyl choline, lysophosphatidyl ethanol amine and lysophosphatidyl choline

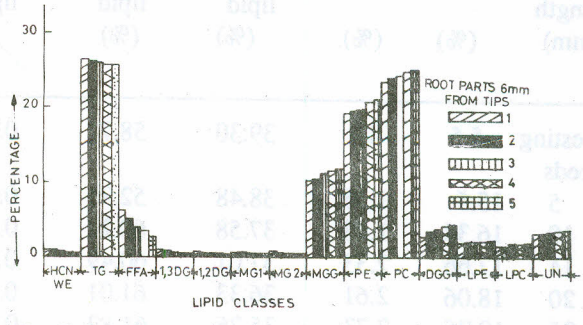

Fig. 7. Percentages of various fractions in the lipid extracts from different parts of the primary root of *Cassia absus*.

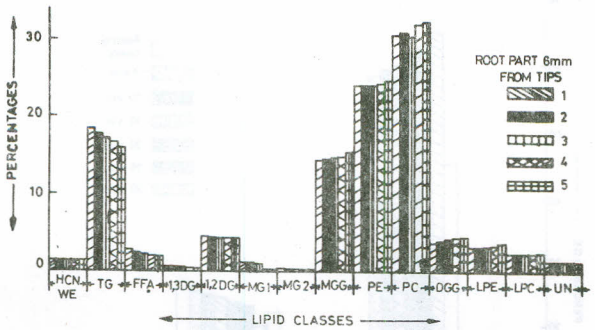

Fig. 8. Percentages of various fractions in the lipid extract from different parts of the primary root of *Cassia fistula*.

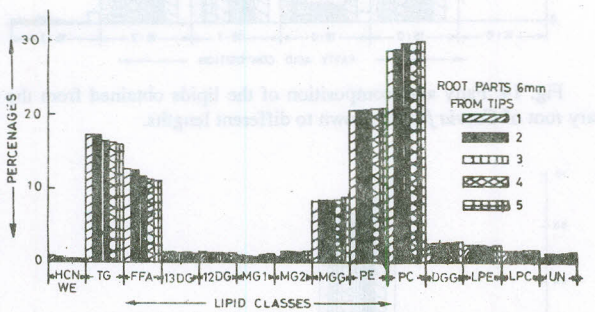

Fig. 9. Percentages of various fractions in the lipid extracts from different parts of the primary root of *Cassia occidentalis*.

HCN: Hydrocarbon; WE: Wax Ester; TG: Triacyl Glycerol; FFA: Free Fatty Acid; 1,3DG:1, 3-Diacyl Glycerol; 1-2-DG: 1, 2-Diacyl Glycerol; MG1: Monoacyl Glycerol 1 MG2; Monoacyl Glycerol; MGG: Monogalactosyl Glyceride; PE: Phosphatidyl-Ethanol Amine; PC: Phosphatidyl Choline; DGG: Digalactosyl Glyceride; LPE: Lyso Phosphatidyl-Ethanol Amine; LPC: Lysophosphatidyl Choline; UN: Unknown.

were lower in the apical portion increasing slightly towards the base of the root. Relative amounts of hydrocarbon wax-ester, sn 1,3-and sn 1,2-diacyl glycerols, *1* and 2 monoacyl glycerols, digalactosyl glyceride and lysophosphatidyl ethanol amine were similar in all the parts. Higher level of free fatty acids in the tip portion of the root suggested increased breakdown of the glycerides due to lipolysis. This indicates that the tip portion of the root is the area of most active growth.

Table 4 shows proportions between total neutral and total polar lipids of different parts of the primary roots. In the apical portions the proportion between the neutral and the polar lipids was 0.63, 0.32 and 0.58 in *C. absus; C. fistula* and *C. occidentalis* respectively and decreased gradually in the portions towards the base and it was 0.45, 0.26 and 0.49 respectively in the portions which were attached to the cotyledons.

Fatty acid composition. Fatty acid compositions of the lipids of cotyledons of *C. absus; C. fistula* and *C. occidentalis* during germination are presented in Fig. 10-12. The major fatty acids present were palmitic, oleic and linoleic. The percentages of $C_{16:0}$ was high in *C. fistula* and *C. occidentalis* and remained around 50.50% at all the stages of germination whereas in *C. absus* the amounts of this acid were low and remained around 30.0%. Its amount increased alongwith germination and was maximum when the length of the primary root reached 30mm. $C_{18:2}$ was present in significantly high amounts in *C. absus* and remained around 50.0% as compared to *C. fistula* and *C. occidentalis* 25.0% and 30.0% at all the stages of germination. $C_{18:0}$ was also present in significant amount and its percentage remained

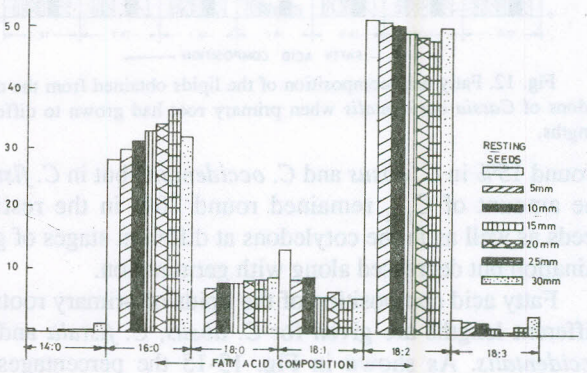

Fig. 10. Fatty acid composition of the lipids obtained from the cotyledons of *Cassia absus* when primary root had grown to different lengths.

Table 4. Moisture and lipid contents in various parts of the roots of *Cassia absus, Cassia fistulla,* and *Cassia occidentalis*

Root Part 6mm each from tip	*Cassia absus* Moisture (%)	Lipid (%)	Neutral lipids (%)	Polar lipids (%)	Neutral lipids / Polar lipids	*Cassia fistula* Moisture (%)	Lipid (%)	Neutral lipids (%)	Polar lipids (%)	Neutral lipids / Polar lipids	*Cassia occidentalis* Moisture (%)	Lipid (%)	Neutral lipids (%)	Polar lipids (%)	Neutral lipids / Polar lipids
1.	20.21	3.25	37.62	59.37	0.63	25.60	1.26	24.22	74.58	0.32	16.31	20.6	36.10	62.20	0.58
2.	25.31	2.98	35.14	61.60	0.57	38.10	0.95	23.40	75.35	0.31	28.0	17.4	35.62	63.26	0.56
3.	34.21	2.11	33.34	62.72	0.53	42.30	0.81	22.30	76.44	0.29	35.0	15.9	34.39	64.43	0.53
4.	38.39	1.79	32.25	64.24	0.50	49.2	0.40	22.15	77.13	0.28	40.0	11.8	33.24	65.55	0.50
5.	41.72	1.23	30.15	66.49	0.45	58.0	0.10	20.28	78.39	0.26	46.0	4.25	32.77	65.98	0.49

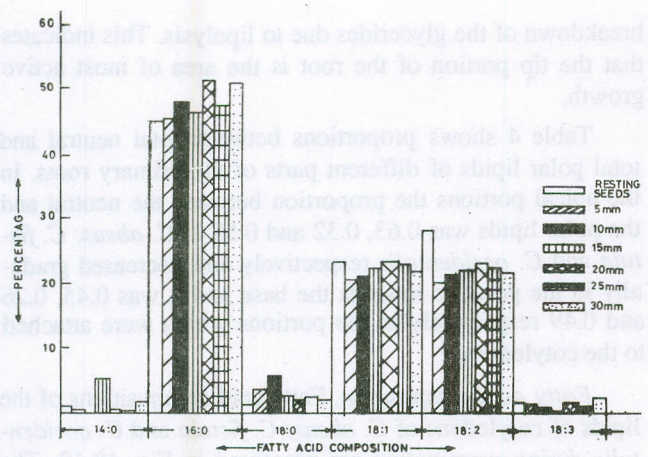

Fig. 11. Fatty acid composition of the lipids obtained from the cotyledons of *Cassia fistula* when primary root had grown to different lengths.

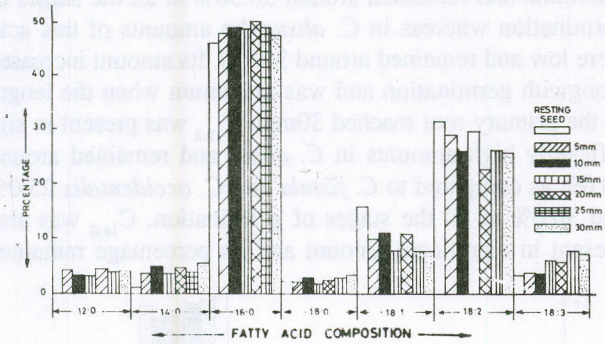

Fig. 12. Fatty acid composition of the lipids obtained from the cotyledons of *Cassia occidentalis* when primary root had grown to different lengths.

around 15% in *C. absus* and *C. occidentalis* but in *C. fistula* the amount of $C_{18:1}$ remained round 20% in the resting seeds as well as in the cotyledons at different stages of germination but decreased along with germination.

Fatty acid composition of the lipids of primary roots of different lengths are given for *C. absus*, *C. fistula* and *C. occidentalis*. As shown in Fig. 13-15 the percentages of

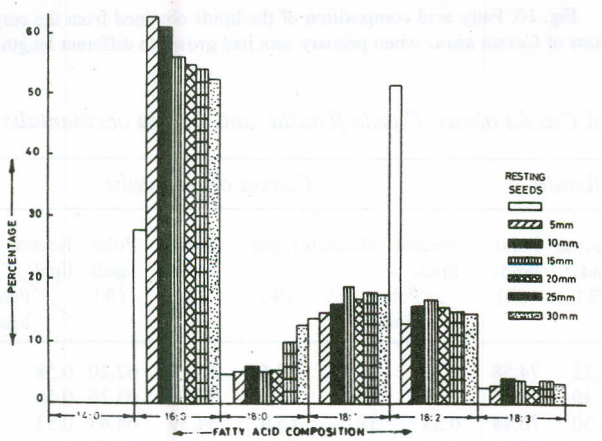

Fig. 13. Fatty acid of the lipids obtained from the primary root of *Cassia absus* grown to different lengths.

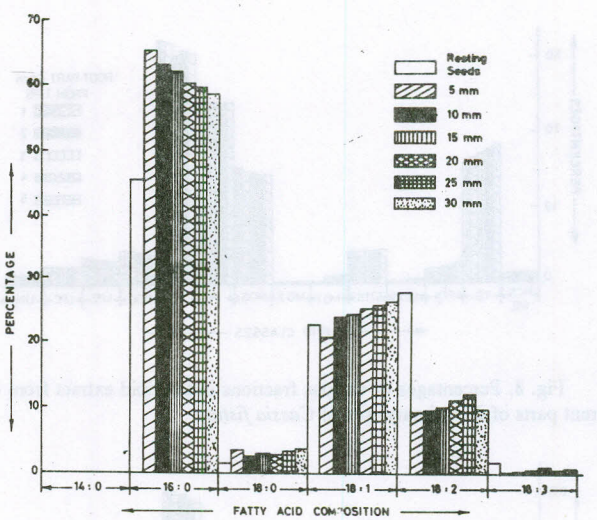

Fig. 14. Fatty acid composition of the lipids obtained from the primary root of *Cassia fistula* grown to different lengths.

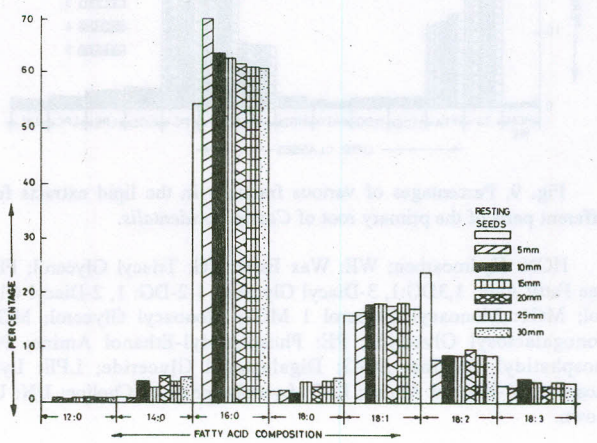

Fig. 15. Fatty acid composition of the lipids obtained from the primary root of *Cassia occidentalis* grown to different lengths.

$C_{16:0}$ increased in the primary root of newly germinated seed as compared to that in the resting seed and cotyledons and it was present in maximum amount (62.70%) in *C. absus*, (65.57%) in *C. fistula* and (70.00%) in *C. occidentalis*. Its quantity gradually decreased with increase in root length and was (52.32%) in *C. absus*; (58.77%) in *C. fistula* and (60.63%) in *C. occidentalis* roots of 30 mm length. In contrast to the resting seeds or the cotyledons the percentages of $C_{18:2}$ decreased manifold in the primary roots and it has more or less constant throughout the period of germination studied. Percentages of $C_{18:0}$ showed a 2-3 fold increase in the primary roots of *C. absus* and *C. occidentalis* whereas in *C.fistula* the amount of $C_{18:0}$ was more or less constant during the period of germination. The percentages of $C_{18:1}$; $C_{18:3}$; $C_{14:0}$ and $C_{12:0}$ did not change much. It is thus clear that, whereas in the cotyledons the changes in fatty acid composition as compared to the resting seeds were not as

significant, there was increase in the percentage of saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) and the relative amount of $C_{18:2}$ decreased.This suggested a preferential utilisation of $C_{18:2}$ in the primary root. Similar results on changes in fatty acid content in the cotyledons and embryo of hazel seeds have been reported [18].

Fatty acid composition of the lipids of the various sections of the primary root of 30mm length, in *C. absus, C. fistula* and *C. occidentalis* are presented in Fig. 16-18. Amounts of $C_{16:0}$ and $C_{18:0}$ were lower in the apical portion

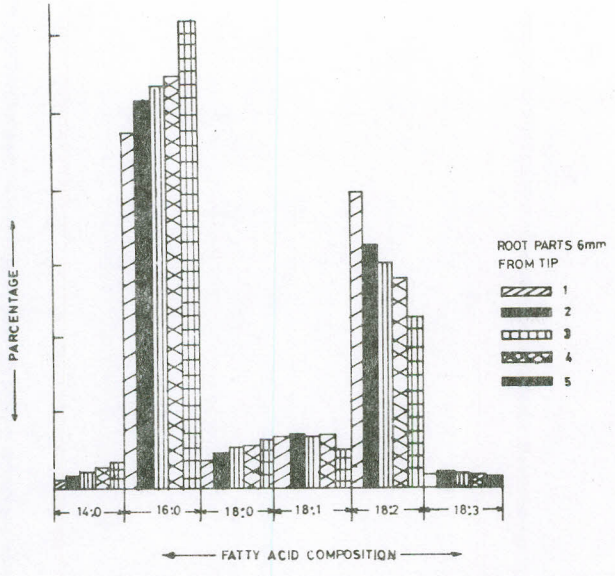

Fig. 16. Fatty acid composition of the lipids obtained from different parts of the primary root of *Cassia absus*.

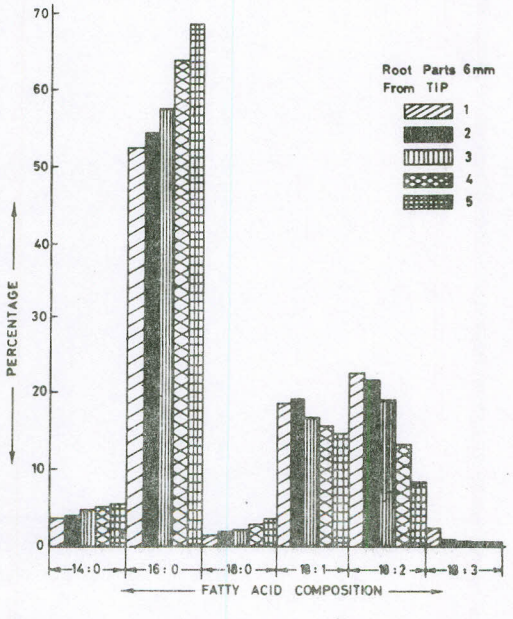

Fig. 17. Fatty acid composition of the lipids obtained from different parts of the primary root of *Cassia fistula*.

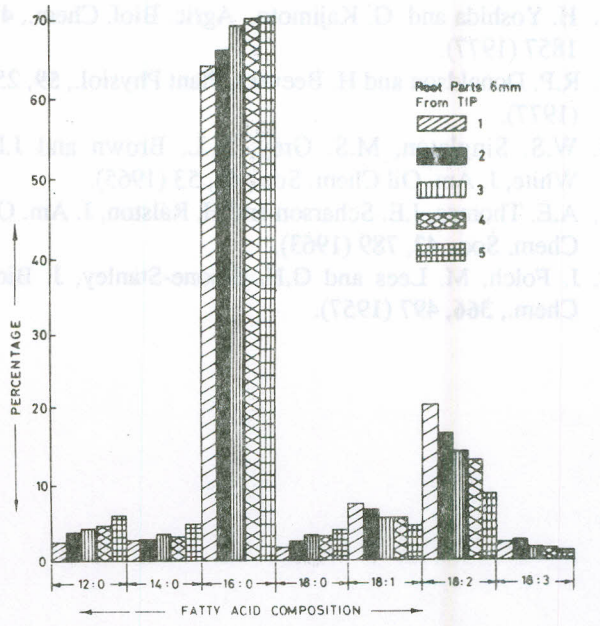

Fig. 18. Fatty acid composition of the lipids obtained from different parts of the primary root of *Cassia occidentalis*.

and increased in the regions towards the cotyledons, the increase being more pronounced in the case of $C_{16:0}$. Percentages of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ were present in greater amounts in the growing tips decreasing gradually towards the cotyledons. $C_{12:0}$ and $C_{14:0}$ were present in low amounts in all portions of the roots. Percentages of Unsaturated fatty acid was highest in the tip, whereas the relative amount of the saturated fatty acid was highest in the regions of root close to the cotyledons.

REFERENCES

1. I.A. Dela Roche, C.J. Andrews and M. Kates, Plant Physiol., **51**, 468 (1973).
2. J.L. Harwood, Phytochemistry, **15**, 1459 (1976).
3. S. Mukherji, B. Dey, A.K. Paul and S.M. Silkar, Physiol Plant, **25**, 94 (1971).
4. R.J. Laugenbach and H.W. Kuoche, Plant Physiol., **48**, 753 (1971)
5. P.R. Shewry, N.J. Pinfield and A.K. Stobart, J. Expt. Bot., **24**, 1100 (1973).
6. M. Katayama and S. Funahashi, J. Biochem (Tokyo), **66**, 479 (1969).
7. C.C. Dogras, D.R. Dilley and R.C. Heiner, Plant Physiol., **60**, 897 (1977).
8. D.L. Laidman, R.J.A. Tavener, Biochem. J., **124**, 4 (1971).
9. R.J.A. Tavener, D.L. Laidman, Biochem. J., **109**, 9 (1968).
10. J.L. Harwood and P.K. Stumpf, Plant Physiol., **46**, 500 (1970).

11. H. Yoshida and G. Kajimoto, Agric. Biol. Chem., **41**, 1857 (1977).
12. R.P. Donaldson and H. Beevers, Plant Physiol., **59**, 259 (1977).
13. W.S. Singleton, M.S. Grey, M.L. Brown and J.L. White, J. Am. Oil Chem. Soc., **42**, 53 (1965).
14. A.E. Thomas, J.E. Scharson and H. Ralston, J. Am. Oil Chem. Soc., **42**, 789 (1963).
15. J. Folch, M. Lees and G.H. Sloone-Stanley, J. Biol. Chem., **366**, 497 (1957).
16. M.W. Akhtar, M.Z. Iqbal and M.N. Nawazish, Pak. J. Sci. ind. res., **32**, 239 (1980).
17. W.R. Morrisen and L.M. Smith, J. Lipid Research, **5**, 600 (1964).
18. P.R. Shery, N.J. Pinfield and A.K. Stobart, Phytochemistry, **11**, 2149 (1972).
19. H. Yoshida and G. Kajimoto, Eiyo To Shokuryo, **28**, 185 (1975).
20. D.C. Zimmerman and J.H. Klosterman, J. Am. Oil Chem. Soc., **42**, 58 (1964).