Biological Sciences Section

Pak j. sci. ind. res., vol.37, no.5, May 1994

STUDIES ON GERMINATING *CARUM CAPTICUM* SEED LIPIDS

IJAZ AHMAD, M.Y. RAIE AND M. WAHEED AKHTAR

PCSIR Laboratories Complex, Lahore-54600, Pakistan

(Received August 27, 1992; revised November 18, 1993)

Studies have been carried out on germinated seeds of *Carum capticum* for the determination of different lipid classes, (8.5 -15.0% and 12.4 - 18.2%) in cotyledons and roots respectively by the application of thin layer chromatography. Each lipid class was hydrolysed, methylated and purified consequently to find out the fatty acid composition ranging $C_{12:0}$ - $C_{20:0}$ by the use of gas liquid chromatography.

Key words: *Carum* seeds, Germination, Lipids, Fatty acids.

Introduction

Pakistan being an agricultural country has tremendous potential of different plants. The number of species of Umbelliferae family among the plant kingdom all over the world is over 3000 whereas 174 species of this family are present in Pakistan [1]. People have been using various species of Umbelliferae family in cosmetics industry, as a medicine [2] in addition to its utilization in food and feed.

An effort is made in the present paper to study the changes in each class of lipid during germination. The pick and choose process is applied for primary roots and cotyledons to extract lipid out of these material and for further studies. The studies on germination seeds help to understand the process of lipid synthesis and the natural pathway as regards to the formation and changes of fatty acids prior to the formation of mature seeds containing almost the same lipids and fatty acids.

Experimental

Germination. The seeds (50 g) of *Carum capticum* after soaking in distilled water for 2 hrs were spread evenly on a wet piece of sack fitted in an iron tray of dimension 15" x 12" x 3". The seeds after covering with a wet sack are placed in an incubator [3] at 30 + 0.5°. The watering process is carried out by spraying 50 ml of water on sack pieces after every 12 hrs within 24 hrs. The seedlings were picked up, and classified into cotyledons and primary roots after 120, 168 and 216 hrs. from the sowing period having root length 10, 20 and 30 mm respectively. These were dried in an oven at 105° for further studies.

Extraction of lipids. The finely crushed cotyledons (0.5g) at root length of 10, 20 and 30 mm were stirred for 1/2 hr. with the solvent mixture [4] (30 ml) of chloroform and methanol (2:1 v/v). The supernatant was separated by centrifugation and the experiment was repeated thrice with solvent mixture (20 ml) to recover the maximum lipids. The combined supernatants were used for the removal of non-lipids [5]. However, cotyledons of the above mentioned root lengths containing lipids 26, 15 and 9 mg respectively whereas 9 , 6 and 3 mg of lipids have been found out in case of roots on dry basis under the same conditions.

Thin-layer chromatography. The qualitative as well as quantitative analysis of lipids of cotyledons and of primary roots was carried out by using 0.25nm thick chromatoplates and 100 ml developing mixture of hexane: ether: acetic acid (40:10:1 v/v) for neutral lipids in both cases. The polar lipids of cotyledons and of primary roots were separated by using solvent mixture chloroform: methanol: 30% ammonium hydroxide: water (24:14:2:1 v/v) [6]. The specific locating reagents [7] were used for the identification of polar and neutral lipids.

Identification of fatty acids. The fatty acid composition of polar/non polar lipids of cotyledons and of primary roots were found out by methylating each lipid fraction with boron trifluoride/methanol reagent [8]. The methyl esters after purification by thin-layer chromatography were identified by using gas liquid chromatography (Pye Unicam 204 series). The column (1.5 m x 4 mm) prepared by using diethylene glycol succinate 10% on diatomite "C" (80-100 mesh) was used for identification of fatty acids produced from polar and non polar lipid classes of cotyledons and of primary roots. The column temperature was 200° and nitrogen was used as a carrier gas at the rate of 40 ml/minutes. The margaric acid used as an internal standard [9] and the percentage of each fatty acid was determined on the basis of the peak area of margaric acid.

Resuls and Discussions

The germination of seeds was accomplished under the controlled conditions [3] i.e. at 33 ± 0.5° in an incubator to carry out studies on lipids. The cotyledons and roots at length of 10 , 20 and 30 mm were dried, powdered and lipids were extracted with chloroform and methanol mixture and non lipids were removed by Folch washing technique [5]. The

TABLE 1. LIPID, DRY WEIGHT AND MOISTURE CONTENTS OF COTYLEDONS AND ROOTS AT DIFFERENT GERMINATION STAGE.

Root length (mm)	Contyledons Wt. (g)	Dry wt.	Moisture	Moisture (%)	Lipids	Lipids (%)	Root Wt. (g)	Dry wt.	Moisture	Moisture (%)	Lipid	Lipids (%)
10	1.32	0.46	0.86	65.2	0.070	15.0	1.729	0.229	1.5	68	0.042	18.2
20	1.32	0.36	0.96	72.7	0.040	11.0	1.796	0.196	1.6	73	0.028	14.4
30	1.22	0.26	0.96	78.7	0.022	8.5	1.589	0.089	1.5	78	0.011	12.4

TABLE 2. PERCENTAGE OF VARIOUS FRACTIONS IN LIPID EXTRACTS OF COTYLEDONS AND PRIMARY ROOT AFTER GERMINATING THE SEEDS TO DIFFERENT LENGTHS OF PRIMARY ROOT.

Lipids		Percentage of different lipids present in cotyledons at different root length 10 mm	20 mm	30 mm	Percentage of different lipids present in primary root at different root length 10 mm	20 mm	30 mm
Sterol esters	(SE)	2.7	4.1	5.0	3.5	7.1	8.0
Triglycerides	(TG)	45.6	30.7	19.0	43.0	30.4	18.2
Free fatty acids	(FFA)	13.6	25.1	31.0	5.6	5.0	4.6
1:3-Diglycerides	(1:3 DG)	5.1	4.5	4.0	3.5	2.9	2.1
1:2-Diglycerides	(1:2 DG)	6.7	6.6	7.0	2.8	2.1	1.1
Glycolipids	(GL)	7.5	8.0	9.0	10.9	14.9	9.8
2-Monoglycerides	(2-MG)	4.1	4.4	4.8	6.0	7.1	8.8
1-Monoglycerides	(1-MG)	3.0	3.2	3.0	2.2	3.0	4.0
Phosphatidyl ethanol amine	(PE)	4.8	4.9	5.2	4.0	5.1	9.9
Phosphatidyl choline	(PC)	3.2	4.0	5.0	3.0	4.0	8.8
Lyso phosphatidyl ethanol amine	(LPE)	0.9	1.2	1.5	2.1	3.6	9.0
Phosphatidyl inositol	(PI)	1.7	2.3	3.0	5.8	8.8	9.9

lipids fractions in cotyledons under the above mentioned lengths are 15, 11 and 8.5% respectively whereas in primary roots the results are 18.2, 14.4 and 12.4% respectively. During germination, the percentage of moisture increases in cotyledons and primary roots but the percentage of dry weight and of lipids in both cases decrease (Table 1). It may be interpreted that decrease in lipids may be due to the utilization of lipids as energy source during the process of germination. There is no evidence for the work on germination of *Carum capticum* seeds on revealing of literature. However, the presented studies are fully supported by previous workers who conducted their experiments on the germinated soybean seeds [10] and their work gives weight to our findings, that is the increase of moisture and decrease of dry weight and lipids in cotyledons and primary roots at lengths of 10, 20 and 30 mm respectively.

The different solvent systems were used for the identification and separation of polar as well as non-polar lipids in cotyledons and primary roots at root lengths of 10, 20 and 30 mm. The qualitative methodology was used for the identification of lipids prior to their quantification. The locating reagents like antimony trichloride molybdenum blue, Dragen dorff and ninhydrin were used for the identification of sterols and sterol esters, phospholipids with the indication of orange red, blue, brick red and violet colours respectively [7]. The different neutral as well as polar lipids are differentiated by their comparative R_f values [6]. The solvent system hexane ether and acetic acid was used for the quantitative separation of sterol esters, triglycerides, 1:3 diglycerides, 1:2 diglycerides, free fatty acids, 2 monoglycerides and 1 monoglycerides and the solvent system chloroform, methanol, ammonium hydroxide and water was used for the separation of phosphatidyl choline, phosphatidyl ethanolamine, lysophosphatidyl ethanolamine and phosphatidyl inositol by thin layer chromatography.

Efforts have been made first time in Pakistan for the classification of polar and neutral lipids of germinated *Carum capticum* seeds. Keeping in view the biological transformation of neutral lipids, it has been observed that triglycerides are reduced to partial glycerides, which later on, are converted into free fatty acids. The lipolytic enzyme plays a vital role for the hydrolysis of triglycerides and with a result mono and diglycerides and eventually fatty acids are produced. These lipids in different forms are also used as a source of energy for the nourishment of the plant. The different

TABLE 3. FATTY ACID (%) IN COTYLEDON LIPIDS OF THE FOLLOWING ROOT LENGTHS.

Lipids	10 mm								20 mm								30 mm							
	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0
SE	9.0	13.31	16.9	5.75	39.47	15.57	-	-	8.68	13.53	18.17	5.94	35.85	14.83	-	-	8.62	13.6	18.38	6.0	38.7	14.7	-	-
TG	2.93	4.85	10.9	6.0	58.01	8.23	5.15	3.93	2.84	6.32	12.23	7.29	54.6	7.62	4.89	4.21	2.8	6.48	12.43	7.4	54.22	7.57	4.8	4.3
FFA	3.9	9.57	15.36	6.92	41.97	12.29	4.14	5.85	3.68	10.62	16.43	7.41	41.32	11.2	3.15	6.19	3.6	10.7	16.6	7.5	41.2	11.0	3.1	6.3
1:3DG	-	10.54	17.36	8.5	50.81	12.79	-	-	-	11.27	17.7	8.4	50.33	12.3	-	-	-	11.3	17.5	9.0	50.2	12.0	-	-
1:2DG	3.92	6.9	9.14	6.0	49.51	11.53	5.5	7.5	3.98	7.13	10.04	6.78	48.67	10.36	5.2	7.84	4.0	7.19	10.17	6.9	48.5	10.14	5.1	8.0
GL	-	6.1	13.1	6.1	49.2	12.2	5.2	8.1	-	7.38	14.48	8.07	46.53	11.71	3.23	8.6	-	7.5	14.6	8.3	46.3	11.6	3.0	8.64
2-MG	2.0	5.1	10.97	6.71	50.21	9.1	5.97	9.94	2.0	6.4	11.77	6.50	49.76	8.18	5.24	10.15	2.0	6.6	11.7	6.7	49.7	8.01	5.15	10.14
1-MG	4.46	8.21	15.88	8.71	38.23	12.71	5.55	6.25	4.16	9.14	16.35	9.48	38.06	12.48	4.26	6.07	4.14	9.2	16.49	9.6	35.0	12.44	4.13	6.0
PE	9.15	13.84	21.88	7.99	28.01	15.72	3.41	-	8.89	13.74	22.62	8.55	27.16	15.93	3.11	-	8.85	13.7	22.73	8.64	27.08	16.0	3.0	-
PC	9.45	15.28	20.8	9.59	21.47	13.87	4.74	4.8	9.19	15.22	21.78	9.9	20.49	13.97	4.35	5.1	9.1	15.2	22.0	10.0	20.4	14.0	4.2	5.1
LPE	9.0	18.0	18.5	7.28	35.11	12.11	-	-	7.3	18.87	18.88	7.82	35.03	12.1	-	-	7.0	19.0	19.0	8.0	35.0	12.0	-	-
PI	11.00	17.91	18.58	5.13	35.38	12.0	-	-	10.6	18.29	19.7	5.88	34.18	11.35	-	-	10.5	18.37	19.79	6.0	34.11	11.23	-	-

TABLE 4. FATTY ACID (%) IN PRIMARY ROOT LIPIDS OF THE FOLLOWING ROOT LENGTHS.

Lipids	10 mm								20 mm								30 mm							
	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0
SE	9.0	12.40	16.0	6.0	40.4	18.2	-	-	8.22	13.73	18.36	6.83	38.39	14.47	-	-	8.1	13.8	18.5	6.9	38.3	14.4	-	-
TG	2.75	2.8	10.1	5.0	60.5	8.7	5.7	4.45	2.56	6.61	12.78	7.56	53.89	7.29	4.52	4.79	2.5	6.75	13.0	7.7	53.5	7.3	4.35	4.9
FFA	3.7	10.0	15.9	7.0	41.2	12.2	4.0	6.0	3.47	10.75	16.63	7.66	41.12	11.19	2.96	6.22	3.45	10.9	16.8	7.73	41.0	11.0	2.82	6.3
1:3DG	-	10.6	17.0	8.9	51.0	12.5	-	-	-	10.52	18.07	8.97	50.22	12.22	-	-	-	10.56	18.14	9.05	50.12	12.13	-	-
1:2DG	3.55	6.5	8.6	6.0	49.4	12.6	5.55	7.8	3.26	7.63	11.16	6.52	47.83	9.88	5.7	8.02	3.1	7.89	11.46	6.83	47.81	9.81	5.0	8.1
GL	-	5.40	12.3	4.65	51.0	12.85	6.0	7.8	-	7.85	14.66	8.27	46.22	11.5	3.05	8.41	-	8.0	14.8	8.5	46.0	11.4	2.8	8.5
2-MG	2.0	4.75	9.6	7.1	50.6	10.0	6.6	9.35	1.82	8.08	11.81	6.78	49.39	7.99	5.05	9.08	1.8	8.3	12.1	6.8	49.2	7.9	4.9	9.0
1-MG	5.1	6.7	15.8	9.0	38.0	13.0	5.85	6.55	3.72	9.27	16.52	9.48	37.86	12.31	4.26	6.58	3.6	9.4	16.7	9.6	37.8	12.2	4.1	6.6
PE	9.3	13.8	21.75	8.6	27.2	15.35	4.0	-	8.38	14.0	22.63	8.87	26.99	15.45	3.68	-	8.5	14.1	22.8	9.0	26.8	15.3	3.5	-
PC	8.95	15.0	20.6	9.75	21.65	14.1	4.8	5.15	8.38	15.27	21.96	10.08	20.43	13.93	4.17	5.33	8.8	15.3	22.1	10.2	20.3	13.9	4.0	5.4
LPE	7.95	18.6	18.6	7.1	34.9	12.85	-	-	7.01	19.05	18.97	8.1	34.83	12.04	-	-	6.7	19.2	19.1	8.3	34.8	11.9	-	-
PI	10.6	17.5	19.36	5.67	34.9	11.97	-	-	9.48	18.52	19.51	6.64	34.46	11.39	-	-	10.0	18.5	19.7	6.49	34.3	11.01	-	-

percentage of these lipids in cotyledons and primary roots are shown in Table 2.

The sterol esters in cotyledons and primary roots increases in germination and the range of sterol is 2.7 - 8.0% in both cases. The sterol esters play an important role in cell membrane and thus with the increase of root length, the sterol esters are proportionally increased. The highest percentage (8.0%) has been found out at 30 mm of primary roots.

The sterol esters show increase in cotyledons and roots during germination. It might be playing role for new cells building in plants. Same results are also shown by previous workers [11]. In addition to sterol esters, there is also increase in phospholipids and glycolipids in cotyledons as well as in roots. The previous workers also showed similar results by carrying out their experiments on the germination of cotton and *Sterculia foetide* seeds [12,13]. The increase of phospholipids and the glycolipids might be essential as components for the development of mambrane system in the growing phenomenon.

The behaviour of fatty acids either in free or esterified form is the same i.e. the graph of saturated fatty acids is increased whereas the percentage of unsaturated fatty acids is decreased. The overall percentage of unsaturated fatty acids as compared to saturated fatty acids is higher either in the case of cotyledons or roots. The major fatty acids detected are $C_{14:0}$, $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$ usually present in all lipid fractions of cotyledons and roots. The highest percentage among these fatty acids is of $C_{18:1}$. It was also observed by Zimmerman and Kloster [14] during their studies on germinated flax seeds. The percentage increase of saturated fatty acids and decrease of unsaturated fatty acids at root lengths of 10 mm, 20 mm and 30 mm clearly indicates the consumption of unsaturated fatty acids into saturated fatty acids to produce the end product during the process of germination. The importance of triglycerides is ever established due to highest percentage among lipid classes. The metabolic system of the plant for the interconvention of fatty acids is such that the process of hydrogenation in natural phenomenon takes place for the production of saturated fatty acids from the unsaturated fatty acids which is also supported by the previous workers in the field [3,9].

References

1. M.K. Bhatty, Essential Oils of the Plant Family Umbelliferae, Final Report, PCSIR Labs. Lahore (1), (1977), pp.1-480.
2. K.R. Kirtikar and B.D. Basu, *Indian Medicinal Plants*, (Lalit Mohan Basu, Allahbad, India 1935), Vol. II, 2nd ed., pp. 1190-1231.
3. Tehseen Aman, Shafiq Ahmad Khan and Waheed Akhtar, Pak. j. sci. ind. res., **33** (9), 374 (1990).
4. Waheed Akhtar, M. Zafar Iqbal and M. Nadeem Nawazish, Pak. j. sci. ind. res., **32** (3-4), 295 (1980).
5. J. Folch, M. Lee and G.H. Solane-Stanley, J. Biol. Chem., **226,** 497 (1957).
6. M. Yaqub Raie, Ijaz Ahmad, M. Akhtar Javed and Imran Waheed, Proc. Pakistan Acad. Sci., **26** (3), 199 (1989).
7. John M. Lowsenstein, *Methods in Enzymology*, LIPIDS, (Academic Press, 1969), Vol.XIV, pp. 542-548.
8. William R. Morrison and Lloyd M. Smith, J. Lipid Res., **5,** 600 (1964).
9. Shahina Zaka, M. Waheed Akhtar and Shafiq Ahmad Khan, Pak.j.sci.ind.res., **32** (5), 323 (1989).
10. B.E. Brown, E.M. Meade and Jean R. Butterfield, J.Am.Oil Chemist's Soc., **39,** 327 (1962).
11. M.H. Abd-El-Aal, E.H. Rahma, Fd. Chem., **22,** (3), 193 (1986).
12. A.S. El-Nockrashy, H.M. Mostafa, Y. El Shattory and M. H. Abbassy, Nahrung, **18,** (3), 285 (1974).
13. G. Lakshminarayana, N. Gopalakrishnan, T.N.B. Kaimal, J. Oil Technol. Assoc. India, **17/** (2), 33 (1985).
14. D.C. Zimmarman and H.J. Klosterman, J. Am. Oil Chemists Soc., **42** (1), 58 (1965).