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FATTY ACIDS OF CALLUS TISSUES OF SIX SPECIES OF CUCURBITACEAE*

TAPATI HALDER and V. N. GADGIL

Tissue Culture Laboratory, Bose Institute, 93/1, Acharya Prafulla Chandra Road, Calcutta 700 009, India

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Key Word Index—*Cucumis melo* var. *utilissimus*; *C. melo*; *C. sativus*; *Citrullus vulgaris*; *Momordica charantia*; *Luffa acutangula*; Cucurbitaceae; cotyledons; cotyledon callus; fatty acids.

Abstract—A comparative study was made of the fatty acid composition of the total lipids extracted from the cotyledons and the callus cultures derived from cotyledon segments of six species of Cucurbitaceae. Conditions for callus induction and growth of cultures were identical. The difference between the two systems was in the reversal of the ratio of total unsaturated to saturated acids in all callus cultures. In callus cultures, instead of linoleic, linolenic was the major unsaturated fatty acid. In *Momordica charantia*, α-elaeostearic acid present in the cotyledon was not detected in callus and oleic acid was the major unsaturated fatty acid.

INTRODUCTION

In recent years the study of lipids in plant tissue culture has attracted much attention for academic as well as applied interest. Some of the studies clearly show that fatty acid composition is influenced by the chemical and physical properties of the medium in which the cells are cultured [1]. We isolated and cultured callus tissue derived from cotyledons of six species of Cucurbitaceae under identical conditions to compare the relative distribution pattern of fatty acids in the total lipid. The corresponding cotyledons of the various plants were also analysed.

*Part 1 in a series "Fatty acids in callus cultures".

RESULTS AND DISCUSSION

Total lipid content of cotyledons varied from 21.6 to 30.3% and of callus tisues from 2.3 to 3.9% on a dry wt basis (Table 1). Visual observation of TLC analyses of total lipid revealed that in all the tissue cultures, the spots for steryl esters and sterols were most intense and of triacylglycerols the least, whereas in cotyledons the reverse was the case. GC analysis of the fatty acids derived from the total lipids of cotyledons of the six species and callus cultures derived there from is given in Table 2. Palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) were the main acids. In the cotyledons of all species, the proportion of unsaturated fatty acids was more than that of saturated and the major acid was

Table 1. Character and total lipid content of callus tissues and cotyledons

Plant material†		Colour	Texture	GI*	Total lipid(%)
Cucumis melo var. *utilissimus*	Cot†				28.0
	Cal‡	Yellowish	Loose	7.14	3.7
Cucumis melo	Cot				
	Cal	Whitish	Loose	9.23	21.9
					3.9
Cucumis sativus	Cot				
	Cal	Yellow	Loose	6.84	30.3
					2.8
Citrullus vulgaris	Cot				
	Cal	Whitish	Loose	11.04	25.9
					2.3
Momordica charantia	Cot				24.7
	Cal	Brownish	Compact	8.71	3.2
Luffa acutangula	Cot				21.6
	Cal	White	Compact	12.80	2.6

*Growth index = $\frac{\text{Final fr. wt}}{\text{Initial fr. wt.}}$

†Cot, cotyledon; Cal, callus of cotyledon.

Table 2. Gas chromatographic analysis of the constituent fatty acids of total lipids from cotyledon and callus derived from cotyledon of six Cucurbits (data are expressed in relative percent w/w)

Fatty acids	Cmu		Cs		Cm		Cv		Mc		La	
	Cot	Cal	Cot	Cal	Cot	Cal	Cot	Cal	Cot	Cal	Cot	Cal
12:0	—	0.88	—	1.13	—	2.09	—	0.92	—	—	—	4.54
14:0	—	1.20	—	1.92	—	2.77	—	1.42	—	—	—	6.94
15:0	—	1.12	—	3.34	—	—	—	1.25	—	—	—	—
15:1	—	—	—	—	—	—	—	1.96	—	—	—	—
16:0	13.25	53.23	21.32	50.47	12.20	50.11	20.63	50.79	2.71	51.95	21.23	53.61
16:1	—	—	—	—	—	—	—	—	—	—	—	—
17:0	—	—	—	—	—	—	—	—	—	—	—	—
17:1	—	—	—	—	—	—	—	—	—	—	—	—
18:0	6.14	5.53	5.91	2.88	1.01	5.61	5.91	9.92	24.18	12.12	10.14	6.11
18:1	19.53	6.85	24.10	5.52	14.13	4.05	24.10	8.59	—	15.58	28.42	3.33
18:2	61.08	5.61	48.67	3.51	72.67	9.51	48.67	5.33	7.22	10.39	40.21	8.43
18:3	—	20.05	—	31.22	—	21.99	—	19.82	—	9.96	—	17.04
20:0	—	5.53	—	—	—	1.02	—	—	—	—	—	—
22:0	—	—	—	—	—	—	—	—	—	—	—	—
18:3 Conj.	—	—	—	—	—	—	—	—	65.89	—	—	—
Total unsaturated	80.61	32.51	72.77	40.25	86.80	35.55	74.13	35.70	73.11	35.93	68.63	28.80
Total saturated	19.39	67.49	27.23	59.74	13.21	64.46	25.79	64.30	26.89	64.07	31.37	71.20

Cmu, Cucumis melo var. *utilissimus*; *Cs, Cucumis sativus*; *Cm, Cucumis melo*; *Cv, Citrullus vulgaris*; *Mc, Momordica charantia*; *La, Luffa acutangula*; Cot, Cotyledon; Cal, Callus of cotyledon.

linoleic in all except *M. charantia*, where it was α-elaeostearic acid (18:3 conj.). Identity of this acid was confirmed from the UV spectrum (261.5, 271 and 281 nm) of the whole oil. Furthermore in *M. charantia*, 18:0 was the major saturated fatty acid instead of palmitic. Although there was similarity in the ratio of unsaturated to saturated fatty acids between the cotyledons of different species, the relative values of individual fatty acids varied.

Callus cultures of the cotyledons of all species examined were characterized by a reversal in the proportion of unsaturated to saturated fatty acid because of an increased 16:0 content. Further, in contrast to cotyledons, 18:3 was the major unsaturated fatty acid except in *M. charantia*, where it was 18:1. In the callus of *M. charantia* α-elaeosteric acid was not detected. Arachidic acid (20:0) was detected in small amounts only in *C. melo* var. *utilissimus* and *C. melo*.

Distribution patterns of the fatty acids in the total lipids of the cotyledons of the plants studied were similar and generally similar to reported values [2] with obvious differences in the values for individual components. By GC α-elaeostearic acid did not give a single peak, but two poorly resolved peaks in the 22:0 region, because of *cis–trans* isomerization on the GC column at the operating temperatures used, as reported previously [3]. This uncommon fatty acid has been reported in the seed fat of only two other genera, viz. *Telfairia* and *Triehosanthes* [2, 4] of the Cucurbitaceae. The absence of this acid in the callus of *M. charantia* may be explained on the basis that callus culture is a continuously proliferating system where accumulaion does not occur, possibly because of quick turnover, in contrast to the cotyledon, a storage organ, where accumulation of uncommon fatty acids occurs during seed maturation. In seedling callus of *Artemisia absinthium* [5] and *Brassica napus* [6], epoxy and erucic acids were reported to be absent. In root callus of *Daucus carota* and seedling callus of *Petroselinum hortense* [7], petroselenic acid and, in seedling callus of *Hydnocarpus anthelminthica* [8], cyclopentenyl acids were detected only in trace amounts. However, these acids do occur in considerable amounts in the stem and root. These observations support the suggestion [1] that appropriate conditions for the operation of the enzyme systems involved in biosynthesis of specific compounds in plants may be lacking in tissue culture. The reported occurrence of cyclopropane and cyclopropene acids, 2–3 times greater quantity in callus cultures than in stem and cotyledon of *Malva species* [9], deserves special mention in this connection.

Only one report is available on the comparison of fatty acid composition between callus tissues and cotyledons [10]. In cultures of *Corchorus capsularis*, *C. olitorius* and *Yucca glauca* a higher proportion of saturated fatty acid prevailed but, in contrast to the present observation, 18:1 acid was the major unsaturated fatty acid.

The present investigation has revealed a closer resemblance in the fatty acid composition of callus cultures than that of the cotyledons of different species. *M. charantia* cotyledons differed from those of others and the difference was reflected in its callus. If the result of *M. charantia* is taken into consideration, it may be said that the fatty acid composition of callus is influenced by the character of the original material. On the other hand, if data of other species are taken into account, it may be concluded that if the species in question show close similarity, the callus derived from these will not show marked differences under identical conditions of isolation and culture.

EXPERIMENTAL

Tissue culture. Seeds of *Cucumis melo* var. *utilissimus*. Duthie

and Fuller., *C. sativus* Linn., *Citrullus vulgaris* Schrad., *Momordica charantia* Linn. and *Luffa acutangula* (Linn.) Roxb., obtained from a local nursery, were germinated aseptically in nutrient medium [11] containing sucrose (2%) and agar (0.5%). After germination, 25% of the cotyledon attached to the growing axis was discarded and the remaining piece was transferred to nutrient medium [12] containing NAA (1 mg/l.) and coconut water (15%, v/v) as growth regulators and Difco-bacto agar (0.75%) for solidification. The medium was adjusted to pH 5.6 before autoclaving. The callus initiated was separated from the segment and cultured through successive passages of 45 days each in the medium having the above composition. The cultures were grown in the dark, at 25 ± 2°, with a relative humidity of 65–70%.

Lipid analysis. Reference compounds were purchased from Sigma. All solvents used were of analytical grade and redistilled prior to use. All manipulations were carried out in an atmosphere of N_2 as far as was feasible.

Callus tissues were harvested for analysis at the end of the sixth passage of growth. Cotyledons of soaked seeds, after removing the seed coat and embryo, were used for analysis of fatty acid composition for comparison. The total lipids of cotyledons and callus tissues were extracted [13] and purified [14] following established procedures.

A portion of the extract was used for estimating total lipid, gravimetrically, and the remainder for TLC separation of the lipid components and for GC analysis. TLC separation was carried out on Si gel G with hexane–Et_2O–HOAc (80:20:1.5). An aliquot of the total lipid extract in each case was subjected to methanolysis and the Me esters purified by prep. TLC [15]. GC of the Me esters was carried out on a dual FID instrument using a 1.8 m × 2 mm.id. column, packed with 10% PEGA on 100–120 mesh diatomite CAW. The temp. of the column was 180°. Peaks were identified by comparison of their R_t with those of standards. The relative percentages of the various components were determined from peak areas.

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