A Comparative Fatty Acid Profile of Seeds Rich in Oleic and Linoleic Acid with Corresponding Calli

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A comparative study was made of the fatty acid composition of the total lipid extracted from cotyledons and the corresponding calli of five species of *Solanum* and two varieties of *Carthamus tinctorius*. The investigation revealed a closer resemblance in the fatty acid composition of callus cells than that of the cotyledons of different species of *Solanum;* in *C. tinctorius* fatty acid composition of calli was influenced by the character of the original material from which calli were derived.

In recent years the study of lipids in plant tissue cultures has attracted much attention for academic as well as applied interest (1,2), as fatty acid distribution varies in the same variety of seeds/plants in response to different environmental conditions that prevail during seed growth (3). However, the distribution pattern is more or less fixed in tissue culture of the same species, if the tissues are isolated and cultured in identical conditions regardless of the geographic distribution of the mother plant and climatic conditions. Cultures are available for study throughout the year and can be grown practically indefinitely, whereas intact plants/seeds are available only during the appropriate season. So, for certain lipid research, cell culture methods may be more suitable than seeds/plants.

Some earlier studies in plant cell cultures clearly showed that fatty acid composition was influenced by chemical and physical properties of the medium in which cells were cultured (4,5). The distribution pattern of fatty acids in cucurbit callus cultures was influenced by the character of the species under identical conditions of isolation and culture (6). Whether this idea is applicable to other families was the main object of the present study. Hence, a comparative study was made of the fatty acid composition of total lipid extracted from cotyledons and their corresponding calli.

EXPERIMENTAL

Materials. Seeds of *Carthamus tinctorius* L. (F_1-N62.8 × NS133), linoleic rich (CL), and *C. tinctorius* L. (F_1-partial hull), oleic rich (CO), were obtained from the Nimbekar Agriculture Research Institute, Phaltan—415523, India; seeds of *Solanum* Sp., viz., *S. khasianum* C. B. Clarke emend Sen Gupta and *S. trilobatum* L., linoleic rich, and *S. melongena* L. and *S. sisymbriifolium* Lamk, oleic rich, were obtained from the Botanical Survey of India, Howrah. Diploid *S. nigrum* L. seeds, linoleic rich, were obtained from the Botany Department of Nagpur University.

Tissue culture. Seeds of all the species were soaked in water for two hr. After removing the hard seed coats, cotyledons of each species were sterilized with 0.1% $HgCl_2$ for four min and rinsed several times with sterile distilled water; 25% of the cotyledon attached to the embryonic axis was discarded and the remaining portion was aseptically transplanted onto basal medium of Murashige and Skoog (7) containing 1.0 mg/l 2,4-dichlorophenoxy acetic acid and 15% v/v coconut water as growth regulator with 2.0% sucrose and 0.75% Difco bacto agar for solidification. The medium was adjusted to pH 5.6 before autoclaving. Calli were separated from cotyledon segments after initiation and transplanted to fresh medium. All the calli were subcultured after 30 days (1 passage) in dark at 25 ± 1 C with a relative humidity of 65–70%. Twenty replicates were maintained for each material. Callus induction and culture conditions were kept identical in all cases.

Lipid analysis. Reference compounds were purchased from Nu-Chek Prep Inc., Elysian, Minnesota. Solvents used were of analytical grade and distilled before use. All manipulations were carried out at 20–24 C under nitrogen atmosphere as far as feasible. Callus tissues were harvested for lipid analysis at the end of the sixth passage of growth. For cotyledon analysis, seeds were soaked in water and, after removal of the seed coat, 25% of the cotyledon attached to the embryonic axis was removed and the remaining portion was taken for fatty acid analysis of cotyledons.

Total lipids of cotyledons and the corresponding calli of all the species were extracted (8,9) and purified (10) following established procedure.

A measured portion of individual extract was used for estimating total lipid gravimetrically, and the remaining portion was subjected to methanolysis (11). The purification of Me-esters followed by their gas chromatographic analysis was performed as described elsewhere (12). All the experiments were repeated in triplicate.

RESULTS AND DISCUSSION

The fatty acid compositions of total lipids of seeds of five plants of the present study have not been reported previously. Determination of composition of these has been carried out for the first time. For *S. khasianum* (13) and *S. trilobatum* (14) fatty acid composition has been reported; present results follow closely with the reported values of these species. Fatty acid composition of callus tissues of all the species were analyzed for the first time.

The analyses showed (Table 1) that in all the species the total lipid levels in cotyledons, which represent stored lipids, were high compared to their respective calli. The latter were uniformly low and possibly represent the levels in parenchymal cells of the callus.

The data showed the presence of myristic, palmitic, stearic, oleic, linoleic and linolenic acid in all the cotyledons of *Solanum* sp., whereas myristic, stearic and linolenic acids were totally absent in cotyledons of both the varieties of *C. tinctorius*. Lauric acid was detected in trace amounts in *S. nigrum, S. khasianum, S. melongena, S. sisymbriifolium,* and palmitoleic acid in *S. khasianum, S. melongena, S. sisymbriifolium* and *S. trilobatum,* but not in *S. nigrum.* Neither lauric nor palmitoleic acid was

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detected in the cotyledons of either of the varieties of *C. tinctorius*. Palmitic acid was the major component of the saturated acids in all the *Solanum* sp., but the percentage varied from 12.4% *(S. nigrum)* to 35.1% *(S. melongena)*. Palmitic acid was the only saturated acid detected in cotyledons of both *C. tinctorius* varieties, with little variation from 5.8% (CO) to 6.8% (CL). Variation also has been recorded in the contents of other saturated acids among the *Solanum* sp.: myristic from 0.5% (*S. khasianum* and *S. trilobatum*) to 1.5% *(S. sisymbriifolium)* and stearic from 2.5% *(S. nigrum)* to 6.2% *(S. khasianum)*. 1.2% Arachidic and 0.3% behenic acid were recorded only in *S. trilobatum* cotyledons. In the case of unsaturated fatty acids, the palmitoleic acid content varied only from 1.4% *(S. trilobatum)* to 2.2% *(S. sisymbriifolium)*, but wide variation was revealed in oleic (14.4% to 42.1%) and linoleic acids (14.5% to 60.4%). The lowest oleic acid content was detected in *S. khasianum* and the highest in *S. sisymbriifolium*, but linoleic acid was lowest in *S. sisymbriifolium* and highest in *S. nigrum*. Linolenic acid was detected at 3.4% in *S. nigrum* and trace amounts in other species, except *S. trilobatum*. In *C. tinctorius* (CO) cotyledons, oleic acid was very high (77%), but linoleic acid was 17.2%. In *C. tinctorius* (CL), the percentage of oleic acid was 19.6% and linoleic was 73.6%. The proportion of total unsaturated fatty acid was more than that

TABLE 1

GC Analysis of the Constituent Fatty Acids of Total Lipids from Cotyledons and Their Respective Calli[a]

	Plant species					
	S. nigrum		*S. khasianum*		*S. trilobatum*	
Fatty acids	Cot	Cal	Cot	Cal	Cot	Cal
12:0	tr	—	tr	tr	—	1.6 ± 0.1
14:0	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0	1.8 ± 0.1	0.5 ± 0.1	1.8 ± 0.1
14:1	—	0.2 ± 0	0.3 ± 0	—	—	0.7 ± 0
16:0	12.4 ± 0.4	31.5 ± 0.3	21.2 ± 0.1	28.3 ± 0.3	15.6 ± 0.5	26.6 ± 0.4
16:1	—	0.3 ± 0	1.8 ± 0.1	2.2 ± 0.1	1.4 ± 0.3	2.5 ± 0.3
18:0	2.5 ± 0.2	5.2 ± 0.2	6.2 ± 0.1	5.1 ± 0.1	3.9 ± 0.1	7.1 ± 0.1
18:1	20.5 ± 0.7	7.5 ± 0.1	14.4 ± 0.2	8.6 ± 0.4	22.5 ± 0.2	14.8 ± 0.4
18:2	60.4 ± 0.5	51.0 ± 0.1	55.6 ± 0.1	47.5 ± 0.2	54.6 ± 0.3	38.4 ± 0.3
18:3	3.4 ± 0.2	2.2 ± 0.1	tr	4.3 ± 0.1	—	2.5 ± 0.1
20:0	—	1.2 ± 0.1	—	2.2 ± 0.2	1.2 ± 0.1	2.0 ± 0.1
22:0	—	—	—	—	0.3 ± 0	—
Total unsaturated	84.3 ± 1.0	61.4 ± 0.7	72.1 ± 0.1	62.6 ± 0.5	78.5 ± 0.3	60.9 ± 0.1
Total saturated	15.6 ± 0.3	38.6 ± 0.4	27.9 ± 0	37.4 ± 0.5	21.5 ± 0.3	39.1 ± 0.4
Total lipid (% dry wt)	12.3 ± 0.3	1.7 ± 0.1	14.6 ± 0.3	2.1 ± 0.1	16.1 ± 0.1	2.3 ± 0.2

	Plant species							
	S. melongena		*S. sisymbriifolium*		*C. tinctorius* (CL)		*C. tinctorius* (CO)	
Fatty acids	Cot	Cal	Cot	Cal	Cot	Cal	Cot	Cal
12:0	tr	0.4 ± 0	tr	tr	—	2.6 ± 0.2	—	2.1 ± 0.1
14:0	1.4 ± 0.1	0.5 ± 0	1.5 ± 0.2	0.4 ± 0	—	3.8 ± 0.2	—	2.5 ± 0.2
14:1	—	0.2 ± 0	1.0 ± 0.1	—	—	—	—	—
16:0	35.1 ± 0.4	29.5 ± 0.2	33.8 ± 0.3	28.2 ± 0.4	6.8 ± 0.2	22.9 ± 0.1	5.8 ± 0.1	25.2 ± 0.1
16:1	2.1 ± 0.2	1.3 ± 0.1	2.2 ± 0.2	1.0 ± 0.1	—	1.5 ± 0.1	—	2.6 ± 0.2
18:0	3.5 ± 0.3	7.1 ± 0.4	5.0 ± 0.2	5.9 ± 0.3	—	4.0 ± 0.1	—	2.4 ± 0.2
18:1	39.4 ± 0.2	8.5 ± 0.2	42.1 ± 0.4	19.5 ± 0.2	19.6 ± 0.2	12.8 ± 0.2	77.0 ± 0.2	39.6 ± 0.2
18:2	18.5 ± 0.2	44.1 ± 0.2	14.5 ± 0.3	44.6 ± 0.5	73.6 ± 0.3	44.3 ± 0.2	17.2 ± 0.2	16.6 ± 0.4
18:3	tr	6.0 ± 0.2	tr	8.3 ± 0.2	—	5.3 ± 0.1	—	7.5 ± 0.4
20:0	—	1.4 ± 0.1	—	2.3 ± 0.2	—	—	—	—
22:0	—	—	—	—	—	2.9 ± 0.2	—	1.6 ± 0.2
Total unsaturated	60.0 ± 0.6	61.2 ± 0.2	59.7 ± 0.6	63.4 ± 0.3	93.2 ± 0.1	63.9 ± 0.2	94.3 ± 0.2	66.3 ± 0.2
Total saturated	40.0 ± 0.3	38.8 ± 0.5	40.3 ± 0.6	36.6 ± 0.4	6.8 ± 0.2	36.1 ± 0.3	5.8 ± 0.1	33.7 ± 0.4
Total lipid (% dry wt)	16.5 ± 0.2	2.2 ± 0.1	16.3 ± 0.5	2.2 ± 0.1	36.3 ± 0.2	3.5 ± 0.2	44.2 ± 0.2	4.7 ± 0.1

[a]Data are expressed in relative percent w/w.

Cot, cotyledon; cal, callus; tr, trace (<0.1%). Mean ± SE of three sets of experiments each of which used 10 g cotyledon or callus.

of saturated ones in all the species of *Solanum* and the varieties of *C. tinctorius;* however, within *Solanum* sp. there was a wide variation, i.e., 59.7% *(S. sisymbriifolium)* to 84.3% *(S. nigrum).* In the two varieties of *C. tinctorius* practically no variation was recorded, i.e., CL-93.2% and CO-94.3%. The *Solanum* sp. showed wide variation also in total saturated fatty acids content, i.e., 15.6% *(S. nigrum)* to 40% (*S. melongena* and *S. sisymbriifolium*), but not in the varieties of *C. tinctorius,* i.e. CL-6.8%, CO-5.8%.

In all the cotyledonary calli of *Solanum* sp. myristic, palmitic, stearic, oleic, linoleic, linolenic and arachidic acids were detected. Of these, the main components were palmitic, stearic, oleic and linoleic acids. In the calli of *S. nigrum, S. khasianum* and *S. trilobatum,* the palmitic acid content is more than that of their corresponding cotyledons; this tendency does not hold for *S. melongena* and *S. sisymbriifolium.* Little variation appeared in the palmitic acid content in calli of all the *Solanum* sp., i.e. 26.6% *(S. trilobatum)* to 31.5% *(S. nigrum),* and the oleic acid content varied from 5% *(S. khasianum)* to 7.1% (*S. trilobatum* and *S. melongena*). Myristic and arachidic acids were detected in calli of all the *Solanum* sp. as minor saturated fatty acids. Palmitoleic, oleic and linoleic acids were detected in the calli of all the *Solanum* sp. with the following variations: palmitoleic 0.3% *(S. nigrum)* to 2.5% *(S. trilobatum),* oleic 7.5% *(S. nigrum)* to 19.5% *(S. sisymbriifolium)* and linoleic 38.4% *(S. trilobatum)* to 51.0% *(S. nigrum).* The relative amounts of both saturated and unsaturated fatty acids were more or less constant in calli of all the species of *Solanum.* Both the calli of *C. tinctorius* have more palmitic acid, i.e. CL-22.9% and CO-25.2%, in lieu of the 5–6% present in cotyledons. The levels of oleic and linoleic acids were predominant in the respective calli of CO and CL cotyledons. The ratio of total unsaturated to saturated fatty acids was always more than unity in calli of all the *Solanum* sp. and varieties of *C. tinctorius.*

The data on cotyledons of all the *Solanum* sp. showed a greater diversity in their fatty acid distribution. Because there is little information on the genetic control of fatty acid composition in seeds/plants of *Solanum* sp., a thorough study is needed with inbred, more homozygous lines to throw some light on the mode of fatty acid accumulation/distribution in the seeds/plants of *Solanum* sp. as well as their chemotaxonomic significance, if any. The present data on the fatty acid distribution in the cotyledons of *C. tinctorius* varieties demonstrates a consistency, which probably is due to the use of inbred, more homozygous lines as experimental material. On the mode of genetic control of fatty acid accumulation and distribution in the cotyledons of *C. tinctorius,* a considerable volume of information is available (15) which further supports the present explanation.

In general, all the cotyledonary calli data showed a similitude in the amounts of total saturated and unsaturated fatty acid contents, regardless of species or varieties. This probably was due to the fact that the same temperature was used in isolation and for their maintenance, rather than to lack of variation in the species or varieties. Even so, the *C. tinctorius* calli reflected the character of their respective cotyledons on major fatty acid contents. The *Solanum* calli did not respond in the same way but presented a fixed distribution pattern regardless of the character of their mother plants. The biosynthesis of fatty acids in *C. tinctorius* calli is under the direct control of genes therein, but not in the case of *Solanum* calli. One of the reasons may be that the experiment was not done with pure homozygous lines of *Solanum* sp.

Because callus cultures normally are composed of genetically heterogeneous cell populations, it may be possible to regenerate crop plants with specific fatty acid composition by using specific growth factor combinations in the culture medium (5). Isolating single cell clones from *C. tinctorius* calli can be used for the study of its fatty acid biosynthesis and its genetic control, as well as for the selection of clones containing increased quantities of desired fatty acids. However, in case of *Solanum,* similar applications can be hoped for only if the calli were isolated from homozygous lines.

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