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FATTY ACIDS IN CALLUS CULTURES: STAGE OF REVERSAL IN THE PROPORTION OF UNSATURATED TO SATURATED ACIDS AND OF CHANGE IN MAJOR COMPONENTS*

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Key Word Index—*Cucumis melo*, Curcurbitaceae, cotyledon, callus, fatty acids

Abstract—The lipids of the cotyledon of *Cucumis melo* contain a large proportion of unsaturated fatty acids with linoleic acid as the major component, whereas those of cotyledon callus show a marked reduction in linoleic acid, an increase in linolenic acid and a predominance of palmitic acid which results in an increase in total saturated acids The fatty acid compositions of total lipids in the cotyledons at different stages of seedling development, excised cotyledon tissue at different stages of callus initiation and in isolated callus show that the observed changes manifested in the established callus occur in the newly formed meristimatic cells as a result of the action of growth substances used for callus initiation

INTRODUCTION

In a previous study, it was shown that the fatty acid compositions of the cotyledon and its callus of each of six plants of the family Cucurbitaceae differed in the proportion of total unsaturated to saturated fatty acids and in the major constituent of each of the two groups of acids [1] In the study, cotyledon segments devoid of growing axis, obtained from aseptically germinated seedlings were used for callus initiation and the excised callus was cultured for six transfers prior to analysis [1]

In this investigation, the fatty acid compositions of cotyledon of *Cucumis melo* var *utilissimus* Duthie and Fuller during different stages of seedling development, and of callus initiation and growth in culture were measured to determine the point at which the fatty acid composition of the callus changes

RESULTS

Palmitic (16 0), stearic (18 0), oleic (18 1), linoleic (18 2) and linolenic (18 3) were the main fatty acids in all the samples (Tables 1 and 2) In the cotyledon at different stages of seedling development (a–c), the percentage of total unsaturated fatty acid was high and linoleic acid was the major component The excised cotyledon segment on transfer to growth medium enlarged three to four times and at this stage(d), the proportion of unsaturated fatty acid decreased with a corresponding increase in palmitic acid With the appearance of callus (stage e) on the enlarged cotyledon segment, there was further drop in total unsaturated fatty acids However, its relative proportion was still higher than that of the saturated acids Linoleic acid remained more or less constant during the stages b–e In the excised callus cultures of the second passage (stage f), the proportion of saturated fatty acids was higher than that of the unsaturated ones and this was due to an increase in the relative proportion of palmitic acid It was accompanied with a marked reduction in linoleic and an increase in linolenic acid The pattern of fatty acid composition observed in the second passage remained more or less the same up to 20 passages of callus growth (Table 2) The presence of a small amount of arachidic acid (20 0) was detected from the second passage onwards whereas behenic acid (22 0) was present only after the 15th passage onwards

DISCUSSION

During germination the mobilization of stored lipid is essential to provide energy and carbon skeletons for the developing embryonic axis The results of studies on fatty acid metabolism of germinating seedlings [2–4] show similarities in the relative proportions of fatty acids in cotyledons during different stages of germination This constancy is explained on the basis of non-selective utilization of the components by the embryonic axis [2, 3] In the present study also, a similar constancy in the distribution pattern of fatty acids has been observed

The growth substances present in the culture medium induce divisions in the cotyledon cells which become meristematic or embryonic in nature and with repeated divisions, the callus is produced In the present study, the excised cotyledon segment taken for callus formation was devoid of any portion of the developing shoot axis The progressive increase in palmitic acid content during the callus-forming stages (d and e) suggest that under the influence of growth substances, the cells of the cotyledon not only undergo division but that the newly formed meristematic cells either synthesize an increased proportion of saturated acids or exhibit a reduction in the synthesis of unsaturated ones This is supported from the studies on germinating pea seeds, where the embryonic axis synthesized saturated acids, palmitic and stearic, but

*Part 2 in the series "Fatty Acids in Callus Cultures" For part 1 see ref [1]

*Deceased 16 January 1983

Table 1 Constituent fatty acids of total lipids of cotyledons during different stages of development and callus formation (expressed as %)

	Developmental and callus stages*					
	Cotyledon			Excised cotyledon		Callus
Fatty acid	(a)	(b)	(c)	(d)	(e)	(f)†
16 0	13 25 ± 0 38	12 88 ± 0 55	14 62 ± 1 14	21 12 ± 1 12	31 68 ± 1 92	56 63 ± 1 43
16 1	—	—	1 40 ± 0 14	1 01 ± 0 13	—	—
18 0	6 14 ± 0 42	15 42 ± 1 16	10 30 ± 1 23	12 59 ± 1 26	11 22 ± 1 44	10 16 ± 0 99
18 1	19 53 ± 1 27	20 33 ± 0 80	23 52 ± 1 27	18 95 ± 1 45	9 02 ± 0 85	5 33 ± 0 45
18 2	61 08 ± 0 51	48 80 ± 0 51	46 18 ± 0 65	41 08 ± 0 23	43 56 ± 0 54	5 47 ± 0 41
18 3	—	2 57 ± 0 25	3 98 ± 0 04	5 25 ± 0 25	4 52 ± 0 16	17 52 ± 0 26
20 0	—	—	—	—	—	4 89 ± 0 14
Total unsaturated fatty acids	80 61 ± 1 27	71 70 ± 0 82	75 08 ± 0 65	66 29 ± 1 21	57 09 ± 1 23	28 32 ± 1 10
Total saturated fatty acids	19 39 ± 0 20	28 30 ± 1 37	24 92 ± 1 78	33 71 ± 2 19	42 91 ± 2 67	71 68 ± 0 65

*(a) Seed, (b) hypocotyl 0 5–1 0 cm, (c) hypocotyl 2–3 cm, (d) excised cotyledon segment enlarged three to four times, prior to callus initiation, after 7–10 days after transplanting in culture medium, (e) cotyledon segment with visible callus about 20 days after transplanting in culture medium, (f) callus after second passage of growth, Mean ± s e of nine GLC analyses from three experiments each of which used 10 g of tissue

†Contained traces (< 0 1 %) of 12 0, 14 0 and 15 0

Table 2 Constituent fatty acids of total lipids of cotyledon callus at different passages of growth (expressed as %)

	Passages*			
Fatty acids	2nd	7th	15th	20th
12 0	Tr	0 60 ± 0 05	Tr	1 01 ± 0 17
14 0	Tr	1 12 ± 0 09	1 17 ± 0 09	0 80 ± 0 04
15 0	Tr	1 01 ± 0 13	0 85 ± 0 05	1 69 ± 0 08
15 1	—	—	—	—
16 0	56 63 ± 1 43	55 48 ± 1 37	51 72 ± 1 81	52 64 ± 1 15
18 0	10 16 ± 0 99	4 79 ± 0 36	7 52 ± 0 97	6 00 ± 0 82
18 1	5 33 ± 0 45	6 04 ± 0 29	6 28 ± 0 21	4 69 ± 0 18
18 2	5 47 ± 0 41	4 85 ± 0 14	5 99 ± 0 1	4 22 ± 0 13
18 3	17 52 ± 0 26	21 61 ± 0 21	22 53 ± 1 05	23 80 ± 0 44
20 0	4 89 ± 0 14	4 49 ± 0 23	3 94 ± 0 1	4 22 ± 0 14
22 0	—	—	Tr	0 93 ± 0 30
Total unsaturated fatty acid	28 32 ± 1 10	32 50 ± 0 09	34 80 ± 1 20	32 71 ± 1 04
Total saturated fatty acid	71 68 ± 0 65	67 49 ± 1 59	65 20 ± 0 86	67 29 ± 1 91

*One passage = 45 days, Tr, trace < (0 1 %), Mean ± s e of nine GLC analyses from three experiments each of which used 10 g of tissue

not the unsaturated ones [3, 4] The occurrence of a high proportion of unsaturated fatty acids during callus stages d and e is due to the composition of the material analysed, i e the cotyledon segments, all the cells of which were not in the process of proliferation, plus the visible callus The reversal in the ratio of the two classes of acids and the change in the major constituent in callus from the second passage can be explained by the fact that the callus inoculum excised from the cotyledon consisted only of newly formed cells, which synthesize large amounts of saturated acids The changed fatty acid metabolism of callus cells resulted in a marked reduction in linoleic acid and a three to four fold increase in linolenic one, which remained constant throughout the culture period Thus, though the reversal in the proportion of unsaturated to saturated fatty acids and the change in the major constituents of the two classes of acids was recorded in the callus grown for six passages in our earlier study [1], these

new experiments demonstrate that the actual change occurs with the initiation of meristematic activity and in the newly formed cells under the action of growth substances in the culture medium The influence of the type of growth substances used for callus induction and growth on constituent fatty acids in callus cells is under investigation [5]

Constancy in the pattern of fatty acid composition during cultural regime of 20 passages has been reported earlier [6] It is suggested that such a result is reproducible provided the passage length and the cultural conditions are not altered As in the present study, long-chain fatty acids have also been detected in other callus cultures [7, 8]

EXPERIMENTAL

Tissue culture The method for isolation and growth of callus was described earlier [1] For callus induction, about three-quarters of a cotyledon was used The remaining quarter with the seedling axis was discarded Fatty acid composition of total lipid was determined at the following stages (a) cotyledons without seed coat and embryo, (b) cotyledon just after germination, (c) cotyledon when the hypocotyl was about 2–3 cm in length, (d) excised and enlarged cotyledon, 7–10 days after transplanting in culture medium, (e) cotyledon with visible callus, (f)–(i) excised callus from cotyledon segment from the 2nd, 7th, 15th and 20th passage of growth respectively (one passage = 45 days) For callus initiation and growth of excised callus, the basal medium of Murashige and Skoog [9] supplemented with 1 0 mg/l NAA and 15% (v/v) coconut water and solidified with 0 75% Difco-bacto agar, was employed Ten replicates of each experimental stage were maintained and the expt was repeated × 3

Analysis Reference compounds were purchased from Sigma Chemical Company, U S A Solvents were of analytical grade and redistilled prior to use All manipulations were carried out in an atmosphere of N_2 as far as feasible The methods for extraction and purification of the total lipid and determination of fatty acid composition by GLC have been described [1] Triplicate analyses of fatty acid ester preparations were performed by GLC

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