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FATTY ACIDS IN CALLUS CULTURES: INFLUENCE OF GROWTH FACTORS ON FATTY ACID COMPOSITION OF TOTAL LIPIDS IN CALLUS CELLS*

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Key Word Index—*Cucumis melo*, *C sativus*, Cucurbitaceae, cotyledon callus, agar culture, fatty acids, growth factors, α-naphthalene acetic acid, kinetin, 6-benzyl amino purine, indole 3-butyric acid, coconut water

Abstract—Callus tissues were derived from cotyledon segments of *Cucumis melo* and *Cucumis sativus* Four combinations of growth factors, i e, naphthalene acetic acid (NAA) plus coconut water (CW), NAA plus kinetin, NAA plus 6-benzylaminopurine (BAP) and indole 3-butyric acid plus BAP, were incorporated in the medium for callus initiation as well as for growth of excised callus in culture for six passages The proportion of total saturated to unsaturated acids and the ratio of linoleic to linolenic acid was influenced by the change in the type of auxin and cytokinin in the combinations used A many fold increase of myristic acid was recorded for the indole 3-butyric acid plus BAP combination

INTRODUCTION

The fatty acid compositions of the total lipids in callus cells of six plants of Cucurbitaceae were determined previously in this laboratory [1] The tissues were derived from cotyledon segments which were devoid of growing axis and which were obtained from aseptically germinated seedlings The fatty acid compositions of the callus cultures differed from that of the cotyledons in the proportion of unsaturated to saturated acids and in the changes in the types of major constituent fatty acids [1] It was demonstrated that growth factors not only induced meristematic activity to form new cells, but also brought about a change in fatty acid metabolism in such cells [2]

In the present study, we have examined the influence of different growth factors incorporated in the medium used for callus initiation and growth on the fatty acid compositions of cotyledonary calli of *Cucumis melo* L var *utilissimus* Duthie and Fuller and *C sativus* L

RESULTS

The main fatty acids in calli of both the materials, grown in the presence of four combinations of growth substances were myristic (14 0), palmitic (16 0), stearic (18 0), oleic (18 1), linoleic (18 2) and linolenic (18 3) (Table 1) Arachidic acid (20 0) was detected only in *C melo* callus grown in the presence of NAA + coconut water, while tridecanoic acid (13 1) was found in *C sativus* callus grown in the presence of IBA + BAP The relative proportions of total saturated to unsaturated fatty acids and of individual components varied considerably in both the tissues for all four growth factor combinations In media containing coconut water, the proportion of saturated fatty acids was higher than that of unsaturated ones and the concentration of linolenic acid was 2–4 times than that of linoleic acid Replacement of coconut water by kinetin resulted in an increase of linolenic acid and a decrease in total saturated acids Addition of another cytokinin, BAP, in place of kinetin, increased the palmitic acid content thus increasing the proportion of total saturated fatty acids The concentration of linolenic acid was drastically reduced When IBA instead of NAA was used in combination with BAP, there was a further increase in the proportion of total saturated acids, but, most interestingly this was mainly due to an increase in myristic and not palmitic acid as found with NAA + BAP In both the materials, changes in growth factor combinations brought about more or less similar changes in the distribution patterns of the fatty acids

Variations in texture, colour, growth and total lipid content of the two types of callus (Table 2) were not related to fatty acid composition (Table 1)

DISCUSSION

Cultural conditions such as temperature, light, carbon and nitrogen source have been reported to influence the fatty acid compositions of callus cells [3] In the data compiled by Hilditch and Williams [4] on natural fats, a considerable number of references are cited which clearly show that climatic conditions, namely, light and temperature, have a profound influence on fatty acid composition even of the same variety of plant seed The variation in lipid pattern may be the result of a direct effect of these factors on enzyme activities and it was also suggested that these factors may indirectly affect the composition by influencing the types and amounts of growth substances [5] Callus cultures provide a useful model system with which to study the influence of growth substances, since

* Part 3 in the series "Fatty Acids in Callus Cultures" For part 2 see ref [2]

† Deceased 16 January 1983

Abbreviations BAP, 6-benzylaminopurine, CW, coconut water, IBA, indole 3-butyric acid, Kn, kinetin, NAA, naphthalene acetic acid

Table 1 Constituent fatty acids of total lipid of callus cultures (expressed as %)

Fatty acids	*C melo* NAA+CW	NAA+Kn	NAA+BAP	IBA+BAP	*C sativus* NAA+CW	NAA+Kn	NAA+BAP	IBA+BAP
12 0	0 9±0 58	0 8±0 17	0 9±0 10	2 1±0 12	0 7±0 12	1 7±0 05	0 7±0 05	2 1±0 11
12 1	Tr	0 1±0 02	0 1±0 02	Tr	Tr	Tr	Tr	Tr
13 0	1 6±0 18	1 9±0 15	1 1±0 14	2 3±0 22	1 7±0 12	1 5±0 06	1 3±0 06	0 5±0 10
13 1	—	—	—	—	—	—	—	3 7±0 17
14 0	3 2±0 23	8 2±0 36	10 5±0 20	15 6±0 36	4 2±0 23	10 6±0 18	12 1±0 20	19 5±0 58
16 0	41 2±0 86	36 6±0 45	43 4±0 75	47 8±0 53	43 0±0 19	33 7±0 15	46 1±0 20	49 8±0 43
18 0	6 2±0 42	3 2±0 33	2 5±0 25	2 4±0 24	7 3±0 28	5 4±0 05	3 1±0 14	4 6±0 15
18 1	3 3±0 18	4 2±0 27	6 1±0 16	5 4±0 37	8 0±0 26	6 1±0 23	4 3±0 23	7 9±0 32
18 2	7 7±0 47	6 0±0 40	8 7±0 09	3 3±0 20	7 4±0 30	3 0±0 27	6 5±0 17	2 2±0 14
18 3	32 8±0 33	39 0±0 30	26 6±0 43	21 1±0 41	27 5±0 31	38 0±0 23	25 9±0 12	9 7±0 12
20 0	3 0±0 32	—	—	—	—	—	—	—
Saturated fatty acid	56 1±0 27	50 7±0 16	58 4±0 45	70 2±0 47	56 9±0 45	52 9±0 17	63 3±0 43	76 5±0 23
Unsaturated fatty acids	43 8±0 31	49 3±0 37	41 5±0 21	29 8±0 17	42 9±0 42	47 1±0 34	36 7±0 25	23 5±0 19

Tr, trace < 0 1%, Mean ± s e of nine GLC analyses from three experiments, each of which used 10 g of tissue

Table 2 Character and total lipid content of callus tissues

Character	*C melo* NAA+CW	NAA+Kn	NAA+BAP	IBA+BAP	*C sativus* NAA+CW	NAA+Kn	NAA+BAP	IBA+BAP
Colour	Yellowish	Yellowish	Yellowish	Yellow	Yellowish	Whitish	Whitish	Brownish
Texture	Loose	Compact	Compact	Loosely compact	Loose	Loosely compact	Loose	Loose
*GI	9 21±0 17	7 78±0 20	8 32±0 15	9 09±0 21	7 30±0 50	10 47±0 12	8 56±0 19	7 57±0 28
†Total lipid (%)	4 15±0 13	3 87±0 20	3 70±0 22	4 31±0 34	3 32±0 20	3 30±0 25	3 50±0 34	2 77±0 26

*GI (Growth Index) = $\frac{\text{final fr wt}}{\text{initial fr wt}}$ (mean ± s e of 15 replicates of tissue)

†Total lipid (%) on dry wt basis (mean ± s e of five sets, each of 10 g tissue)

these factors are the most important and essential constituents of the culture medium That growth substance combinations influence fatty acid composition of callus cells is confirmed in our present study The presence of kinetin enhanced desaturation, whilst another cytokinin, BAP, produced the opposite effect Addition of the auxin IBA in combination with BAP, further increased saturation but unlike NAA, the increase in total saturated acids was accounted for by myristic acid and not by palmitic acid These effects can be explained in two ways Firstly, the growth regulators may directly or indirectly affect specific steps in the biosynthesis of fatty acids Secondly, particular combinations of growth substances may favour divisions and increase cell population with genetic capacity to synthesize particular lipid patterns, since callus cultures are normally composed of genetically heterogenous cell populations If the latter explanation is established then the technique may be profitably applied to the isolation of clones containing increased quantities of desired fatty acids

EXPERIMENTAL

Tissue culture Seeds of *C melo* and *C sativus*, purchased from Sutton & Co, Calcutta, were asceptically germinated on an agar–sucrose medium (0 5% Difco-Bacto Agar + 2% sucrose) Cotyledons were dissected out discarding the quarter portion attached to the growing axis and the resulting segments were planted on the basal medium of Murashige and Skoog [6] supplemented with (a) 1 mg/l NAA + 15% (v/v) CW, (b) 1 mg/l NAA + 1 mg/l Kn, (c) 1 mg/l NAA + 1 mg/l BAP, (d) 1 mg/l IBA + 1 mg/l BAP

All the media containing 2% sucrose were adjusted at pH 5 6 and solidified with 0 7% Difco-Bacto agar Initiated callus was removed from the cotyledon segments and transferred to a fresh medium of the same composition in each case and subcultured for six passages of 45 days each, in the dark at 25 ± 1° Twenty replicates of tissue were maintained for each treatment and all the experiments were repeated × 3

Lipid analysis This was performed as described in refs [1, 2]

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REFERENCES

1 Halder, T and Gadgil, V N (1983) *Phytochemistry* **22**, 1965

2 Halder, T and Gadgil, V N (1984) *Phytochemistry* **23**, 47

3 Radwan, S S and Mangold, H K (1980) in *Advances in Biochemical Engineering* (Filchter, A, ed) Vol 16, p 109 Springer, Berlin

4 Hilditch, T P and Williams, P N (1964) *The Chemical Constitution of Natural Fats*, 4th edn, p 226 Chapman & Hall, London

5 Galston, A W and Davis, P J (1969) *Science* **163**, 1288

6 Murashige, T and Skoog, F (1962) *Physiol Plant* **15**, 473