

Effect of Coconut Milk Fractions on Growth of Carrot Tissue. (19994)

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(Introduced by J. J. Oleson)

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Since the discovery by van Overbeek *et al.* (1) of the growth promoting action of coconut milk for immature *Detura* embryos this material has been shown to enhance the growth *in vitro* of several plant tissues(2-6). In the present study the effect of coconut milk on the growth *in vitro* of a clone of carrot tissue was investigated and evidence was obtained that at least two fractions of the milk were concerned in the growth of this tissue.

Methods and results. Carrot tissue isolated from a single fragment of the phloem region of one carrot (var. Chantenay) was cultured on a medium containing 2% sucrose, White's mineral solution and 1% agar, to which the solid material from lyophilized coconut milk was added to give a concentration of 7 mg/ml. On this medium, the tissue was grown in darkness at a temperature of 25°C, with transfers every 2 weeks. This tissue was designated Clone II and, being genetically homogeneous, was used in all further experiments. This tissue has been grown in this way for over a year, showing a constant rate of increase of about 10 times the original dry weight during the 2 weeks period. *Coconut milk* pooled from batches of mature coconuts was stirred with Nuchar (C-1000-N)—equal to 2% of the solids of the milk—for 30 minutes. After filtration, the Nuchar cake was eluted 3 times with a chloroform-methanol (5:2) mixture. The combined eluates were evaporated to dryness *in vacuo* and the residue lyophilized. On an equivalent volume basis, the eluate averaged 0.2 mg/ml of solids as compared to 50 mg/ml of solids for the original milk. The original coconut milk and the filtrate and eluate fractions were incorporated in sucrose mineral agar and sterilized by autoclaving (15 lb for 15 min.). After being distributed to 18 x 150 mm pyrex tubes in 10 ml portions, a fragment of Clone II carrot tissue was placed on the surface of the agar in each tube. At the end of a 14 days incubation period in the dark at 25°C, the fragments were removed

TABLE I. Average Fresh Weight of Clone II Carrot Tissue after 14 Days Growth on Media Containing Coconut Milk and Fractions. All concentrations equivalent by volume to that of coconut milk (CM). Average of mean values from 9 assays.

Conc. of CM, mg/ml	Wt of carrot tissue (mg)			
	CM	Nuchar filtrate	Nuchar eluate	Eluate-filtrate
10	323	85	70	—
7	334	84	73	277
5	293	82	66	—
2	136	64	67	—
0	54	—	—	—

TABLE II. Average Growth in 14 Days of Carrot Tissue (Clone II) in the Presence of Filtrate and Eluate Factors from Coconut Milk. (Wet weights in mg.)

Filtrate conc., mg/ml	Eluate conc., mg/ml				
	.5	.1	.05	.01	0
7	414	341	205	168	89
1	206	191	147	123	70
.4	94	78	66	69	66
.0	74	74	56	58	54
.0	74	65	57	57	58

and their wet and dry weights determined. Five replicates for each concentration of fractions tested were used. As dry weights were proportional to wet weights, only wet weights are presented here. Nine separate batches of coconuts were treated in this way and assayed separately. The average weights from the 9 mean values obtained from a total of 1530 cultures are shown in second column of Table I. Agreement of the individual assays was fairly close, the standard error of the means being $\pm 10\%$. The samples assayed were original coconut milk, Nuchar filtrate, chloroform-methanol eluate and combinations of filtrate and eluate; concentrations of eluate and filtrate being equivalent by volume to that of the original coconut milk.

Results. As shown in Table I indicate that the clone of carrot tissue required two factors or groups of factors in coconut milk for its optimal growth response. The Nuchar eluate

TABLE III. Average Growth in 14 Days of Carrot Tissue (Clone II) in the Presence of the OM Filtrate Factor and Indole-3-Acetic Acid. (Wet weights, mg.)

Filtrate conc., mg/ml	IAA conc., mg/ml				
	.1	.01	.001	.0001	0
7	92	219	222	180	122
0	161	136	104	113	71

contains one group of the factors and the filtrate contains the other. Neither fraction alone was able to give the optimum response of the whole coconut milk. Table II shows that over the range of concentrations tested, the maximum response occurred when the filtrate was combined with the eluate in a ratio of 7.0 mg/ml filtrate to 0.5 mg/ml of eluate.

Since Caplin and Steward(5) reported that coconut milk may contain free indoleacetic acid (IAA), which might contribute to the activity of the eluate fraction, this possibility was investigated. The eluate was extracted 3 times with ether at pH 2.8. The ether extract gave no growth promoting response when combined with the filtrate. Moreover, the extract gave a negative response to the Salkowski test for IAA. Although the eluate fraction did not contain free IAA a comparative study was made to learn whether IAA could replace the eluate fraction. Addition of IAA to agar containing the filtrate factor resulted in an enhancement of growth, the maximum effects being at a concentration of 0.001 mg/ml (Table III). IAA without the filtrate factor enhanced the growth of the tissue to a greater extent than did the eluate fraction alone (Table I). The maximum growth enhancement of the carrot tissue by IAA alone was 2-fold, by IAA and filtrate fraction 3-fold, and 7-fold by the coconut milk eluate and filtrate combination. These results

indicate that the activity of the eluate fraction is not due to IAA. Four other clones of carrot tissue were found to respond to the 2 fractions in a manner similar to Clone II. Shanz and Steward(7) have reported recently that 3 crystalline components from coconut milk singly will enhance the growth of carrot tissue only when supplemented with casein hydrolysate. It is possible that the casein hydrolysate exerts an effect similar to that of the coconut milk filtrate.

Summary. Addition of coconut milk (7 mg/ml) to a medium containing 2% sucrose, 1% agar and White's mineral solution supported the growth in darkness of Clone II carrot tissue for over a year through more than 100 transfers. Adsorption of coconut milk on Nuchar followed by elution with chloroform-methanol (5:2) gave two fractions, neither of which would support the growth of this tissue alone, but which, on recombination, had the same activity as the original coconut milk. The effect of the eluate factor could be partially reproduced by indole acetic acid. The filtrate factor could not be replaced by any of the chemically defined substances tested.

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