THE GROWTH RESPONSES OF EXCISED TOMATO ROOTS TO COCONUT WATER

E. EL HINNAWY and D. STEGWEE

Laboratorium voor Algemene Plantkunde, Plantenfysiologie en Farmacognosie, Universiteit van Amsterdam, Amsterdam.

SUMMARY

The effect of coconut water (CW) and of 2,4-dichlorophenoxyacetic acid (2,4-D), applied singly or in combination, to clonal material of excised roots of tomato cv. 'Moneydor' was investigated. Distinct stimulatory effects on the growth of roots were obtained in both cases. CW showed its highest growth action when applied at low concentrations (0.025% and 0.050% v/v) to roots incubated in darkness. Certain combinations of CW and 2,4-D gave a strikingly high growth rate which exceeded that of the control with or without CW. A combination of 2×10^{-4} µg. ml of 2,4-D with 0.050% (v/v) of CW gave maximum growth stimulation without toxicity to the root tissue.

1. INTRODUCTION

It is now well established that coconut water (CW), the liquid endosperm of *Cocos nucifera* L., acts as a potent stimulus to the growth and development of plant tissues (TULECKE *et al.* 1961). Significant stimulatory effects have been reported for excised embryos (OVERBEEK *et al.* 1941, CUTTER *et al.* 1953, HALPERIN & WETHERELL 1964), detached plants tumours (NICKELL 1950, DUHAMET 1950, DUHAMET & GAUTHERET 1950), excised leaves (MOHAN & WADHI 1965), isolated floral buds (MISRA 1966), and vegetative buds (BILDERBACK *et al.* 1968).

The high nutritional value of CW has led to its widespread use as an adjuvant for many suspension cultures and cultures of parenchymatous tissues (CAPLIN & STEWARD 1948, 1952, RAO & NARAYANASWAMI 1968, NEUMANN & STEWARD 1968, STEWARD *et al.* 1968, BARKER 1969, PATTERSON & CAREW 1969). It is necessary for the growth of many callus tissues and has been shown to enhance the growth of many other types of tissues (STREET 1966). In contrast, isolated root tips of most plant species fail to grow or to develop normally in culture media supplemented with coconut water (BUTCHER & STREET 1964). However, BACHELARD & STOWE (1963) were able to show that CW produced a marked stimulatory effect on excised roots of *Eucalyptus camaldulensis*. This observation promoted the present study of the effect of CW on the growth of excised tomato roots.

2. MATERIALS AND METHODS

Root tip meristems isolated from a tomato root clone kept in continuous culture

were used as experimental material. In order to eliminate genetic differences in the experimental material, a root clone was first established from a root tip of a tomato seedling (*Lycopersicum esculentum* Mill. cv. "Moneydor"). Seeds of this variety obtained from "Vandenberg N.V. – Naaldwijk, Holland" were first surface sterilised by immersion in a 1% aqueous bromine solution for 3 minutes (DAWSON & STREET 1959), then rinsed six times in sterile distilled water and germinated aseptically at 25°C in darkness. A 10 mm long root tip from one seedling was excised and transferred aseptically to an Erlenmeyer flask of 100 ml capacity containing 50 ml of sterile nutrient medium and incubated at 27°C ± 0.5 in darkness to obtain the initial culture of the excised root meristem. By repeated subculturing of tips and sectors at regular intervals, as described by STREET (1966), the clonal material was established and kept in continuous culture. At the time of writing the root clone is in its 150th passage and has been in continuous culture for more than three years. It has provided us with uniform, vigorous root tip material for our experiments.

With minor modifications, the basic nutrient medium of WHITE (1943) modified by STREET & MC GREGOR (1952) was employed. Copper and molybdenum were supplied according to BOLL & STREET (1951) and Fe-EDTA (Ferric ethylenediaminetetraacetic acid) according to SHEAT *et al.* (1959). Sucrose, at a concentration of 1.7% (w/v), was used as the carbon source. The pH of the medium was adjusted to 4.85 before autoclaving at 120°C and a pressure of 1 kg/cm^2 for a period of 6 minutes. The water used for preparing the medium was double distilled in a Jena Duran 50 glass still and the chemicals employed were of Analar grade, B.D.H. All manipulations of root material were performed under strictly aseptic conditions in a cabinet previoulsy sterilized with ultra-violet light. The stock of coconut water used in this investigation was obtained from 70 mature nuts. The CW was heated to boiling point and then filtered. This operation was repeated three times. The filtered CW was stored in test tubes and kept at -20°C.

The linear growth of roots, fresh weight and dry weight of 15 replicates of each treatment were recorded after a growth period of 9 days at 27°C ± 0.5. Each experiment was repeated four times and the mean values taken as criteria of growth. To eliminate possible bias, measurements were recorded without knowing to which experimental group the specimens belonged.

Root cultures incubated in continuous light received 500 Lux from daylight fluorescent tubes. Cultures incubated in darkness were wrapped in aluminium foil and interspersed among the group receiving continuous light. Roots of average size and shape were selected for photographs.

3. RESULTS

BACHELARD & STOWE (1963) found that CW (at a concentration of 14% in the medium) was the only one of a number of substances tested for stimulating the growth of excised *Eucalyptus camaldulensis* roots. In a preliminary experiment with excised tomato roots the present authors found that a concentration of

Table 1. Effects of CW at different concentrations, on the growth of excised tomato roots in 1.7% sucrose medium in darkness or in continuous light of 500 Lux.

	Dark					Light				
CW per cent (v/v)	LA	NL	LL	FW	DW	LA	NL	LL	FW	DW
0	168±10[1]	53±3	454±13	51	4.4	204±8	40±2	350±13	46	3.7
0.025	195±5	66±4	639±15	65	5.6	140±7	31±2	322±9	31	3.4
0.050	250±7	70±4	723±10	69	6.0	150±8	35±3	340±12	32	3.1
0.100	150±8	42±5	394±14	38	3.1	130±10	20±2	172±14	29	2.1
0.250	122±5	19±3	204±15	18	1.1	95±7	10±3	110±8	11	0.8
0.500	91±3	6±0.2	83±7	8	0.8	88±8	4±0.2	60±5	7	0.5
1.000	87±6	2±0.1	6±0.2	4	0.3	43±4	0	0	2	0.1

LA = Length of main axis per root in mm.
NL = Number of laterals initiated along the main axis per root.
LL = Total length of laterals per root in mm.
FW = Fresh weight mg per root
DW = Dry weight mg per root.
[1] mean values with standard error (n = 60).

Table 2. Effect of 2,4-D, supplied alone or in combination with CW at 0.050% (v/v) on the growth of excised tomato roots in 1.7% sucrose medium in darkness.

	No coconut water			Coconut water at 0.050 per cent						
Measurements	control	2,4-D µg/ml		control	2,4-D µg/ml					
		2×10^{-4}	4×10^{-3}		4×10^{-5}	1×10^{-4}	2×10^{-4}	4×10^{-4}	1×10^{-3}	2×10^{-3}	4×10^{-3}
LA[1]	160±5	140±8	129±4	175±5	155±5	176±4	189±3	181±2	187±5	165±6	156±4
NL	44±4	32±3	20±5	50±2	45±2	52±2	62±2	59±3	57±2	53±2	49±2
LL	460±12	370±14	300±16	516±8	403±10	489±13	688±15	670±9	656±12	608±15	566±18

[1] For abbreviations see table 1.

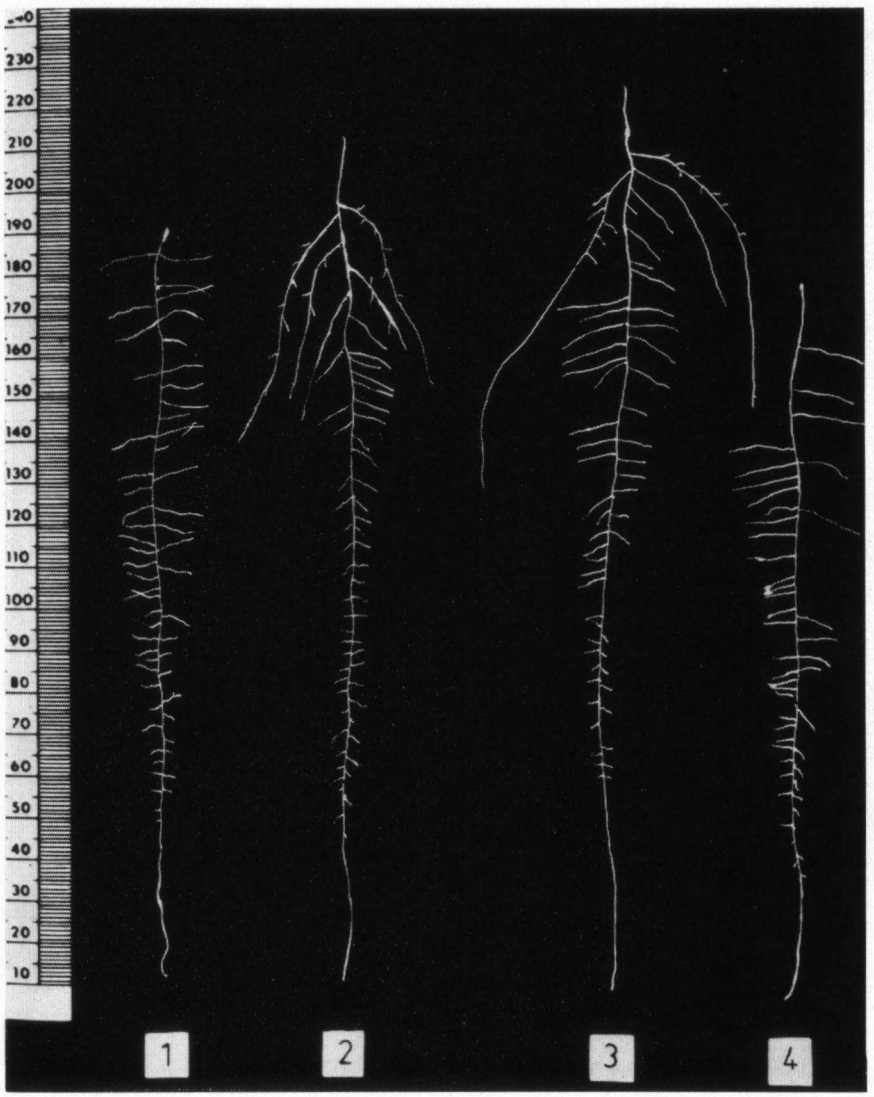

Fig. 1. Effects of low concentrations of CW on the growth of excised tomato roots.
1. Basic medium without CW (control).
2. Basic medium + 0.025% CW.
3. Basic medium + 0.050% CW.
4. Basic medium + 0.100% CW.
The scale on the left is subdivided in millimeters.

14% (v/v) of CW in the nutrient medium was completely inhibitory. The inhibition became progressively less when the concentration was lowered to 1% (unpublished data). In the light of these findings concentrations of less than 1% were used in subsequent experiments. The effects of 6 low concentrations of CW on the growth of isolated tomato root meristems were investigated. Root cultures were incubated either in darkness or in continuous light. The results are recorded in *table 1*. It is evident that CW supplied to root cultures at low concentrations caused a marked stimulation of growth when the roots were incubated in darkness. At the two lower concentrations (0.025% and 0.050%) the linear growth of the main root axes was highly stimulated. Laterals initiated along the main axes increased in number and in total length (see *fig. 1*). The fresh and dry weight of roots were greatly increased. At higher concentrations, however, there was a marked inhibition of growth which became progressively more intense when CW exceeded 0.050% in the medium. The marked and persistent stimulation of root growth shown by low concentrations of CW in cultures incubated in darkness was not observed in cultures kept in continuous light. The data in *table 1* indicate that continuous light of about 500 Lux intensity enhanced the extension growth of main root axes. However, the inclusion of CW in the medium of cultures incubated in continuous light inhibited the growth

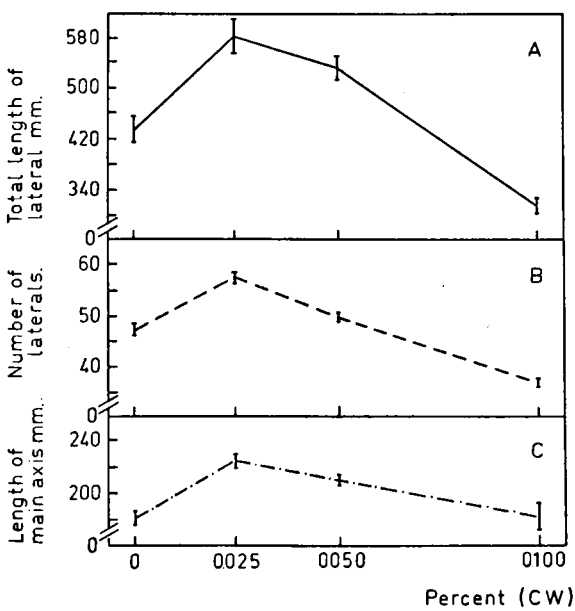

Fig. 2. Influence of low concentrations of CW on the growth of excised tomato roots. The media containing CW were subjected to continuous light of 500 Lux intensity for 9 days before use.
A – Total length of laterals per root in mm.
B – Number of laterals per root.
C – Length of main axis per root in mm.

stimulating effect of light on the main root axes. Moreover, under these conditions CW failed to produce the growth stimulation which it had shown at low concentrations in darkness. The inhibitory effect of CW in light and its stimulatory effect in darkness suggested that this complex nutrient mixture might be light sensitive and that the growth factor(s) (POLLARD *et al.* 1961) was not stable at the light intensity used. In order to test the validity of this interpretation the media containing CW at the three lowest concentrations (0.025%, 0.050% and 0.100%) and the controls were first subjected to continuous light of the same intensity for a period of 9 days before used in cultures incubated in darkness. The data assembled in *fig. 2* show the effect of pre-treated CW on the linear growth of excised tomato roots. Evidently the two lowest concentrations (0.025% and 0.050%) continued to produce marked stimulation, with maximum growth values at a concentration of 0.025% (v/v).

The results of experiments in which 2,4-dichlorophenoxyacetic acid (2,4-D) was supplied singly or in combination with CW are shown in *table 2*. With 2,4-D alone growth was retarded at both concentrations tested (2×10^{-4} and 4×10^{-3} μg. ml.) while, as found before, CW alone produced marked stimulation of growth. When 2,4-D and CW were supplied together, the growth rate was intermediate between those produced by either substance alone. In proper combination, however, the two substances gave a strikingly high growth rate which exceeded that of the control with or without CW. It would appear, therefore, that CW relieves the retarding effect of 2,4-D on the growth processes of tomato roots. The extent to which CW and 2,4-D are each involved in stimulating the growth of excised tomato roots depends on their relative concentrations in the nutrient medium.

4. DISCUSSION

The clone of tomato roots (*Lycopersicum esculentum* Mill. cv. "Moneydor") kept in continuous culture for three years in our laboratory represents a successful culture of a new variety which can be added to the list (BUTCHER & STREET 1964) of nine tomato varieties whose isolated roots can be grown continuously in sterile culture. Roots attain maximum growth when sucrose is supplied at a concentration of 1.7% (w/v) to the nutrient medium. The average growth rate of roots over a period of two years, expressed as extension growth of main axes, is 18 mm per day.

The results recorded in *table 1* show clearly the growth promoting effect of CW when supplied to the isolated root tip meristems of this clone. Of the 6 different concentrations of CW tested only very low levels (0.025% and 0.050% v/v) showed persistent growth stimulation as reflected by the extension growth of main root axes and of the laterals along the proximal part of the roots *(fig. 1)*. The growth stimulating effect of CW occurs only when excised roots are incubated in darkness. Continuous light of approximately 500 Lux obtained from daylight fluorescent tubes retards the initiation and extension growth of laterals and lowers the fresh and dry weights of roots. Other examples of root inhibition

by light have been reported by BURSTRÖM (1959, 1960, 1961) and by others. The inhibition of wheat roots by fluorescent light of 2500 Lux has been attributed to some inhibiting substance formed in the roots (BURSTRÖM 1960). However, the main root axes of our clonal material were either not affected or showed little stimulation when the roots were incubated in continuous light. Very low light intensities (4–79 Lux) obtained from a tungsten filament lamp have been reported by STREET (1953) as stimulating the main axes of excised tomato roots. Light stimulation of wheat roots has also been reported by STREET *et al.* (1961) and by SCOTT *et al.* (1961). The results in *fig. 2* show that fluorescent light of 500 Lux has no destructive effect on the growth stimulating factor(s) (POLLARD *et al.* 1961) in CW. The light stability of the CW growth factor(s) is a characteristic feature which can be added to the other properties of CW reported by MAUNEY *et al.* (1952). The retardation in growth of roots supplied with CW and incubated in continuous light may be attributed to a substance(s) synthesised in the root tissues. In the presence of CW growth factor(s) this substance(s) causes unbalanced chemical regulatory control over root growth. However, roots supplied with CW but incubated in darkness do not synthesise this substance, or at least only in very small amounts, with the result that the growth promoting effect of CW is not antagonised.

The growth enhancing interaction of CW with 2,4-D supplied together to excised tomato roots is clearly demonstrated in *table 2*. It would seem that 2,4-D interacts with that part of the CW system which stimulates growth by cell division and thereafter 2,4-D separately and in combination with CW has supplementary effects on cell enlargement. It is also clear that there are certain combinations of CW and 2,4-D which keep these two aspects of root growth in balance. The optimum concentrations at which stimulation of growth takes place without toxicity to the root tissues are 2×10^{-4} μg/ml of 2,4-D and 0.050% (v/v) of CW in the nutrient medium. The interaction of the synthetic auxin 2,4-D with the whole of the CW stimulus, which is made up from its different growth regulating moieties, indicates that root growth is a metabolic system which is activated by auxins (THIMANN 1951). Hence, through the interaction of cell division factors in CW with their inhibitors and synergists, a balanced regulatory control of root growth is achieved (STEWARD 1968). Since excised roots of other plants react differently to CW and to its combination with 2,4-D this problem clearly requires further investigation.

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