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Callus multiplication of Anthurium andraeanum Lind. in liquid media[1]

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Summary

The growth of callus tissue from adult *Anthurium andraeanum* Lind. plants was studied in liquid media. The best medium for growth was a modification of Murashige and Skoog's medium. On this medium genotype A 42-3 reached a fresh weight multiplication rate of 30.7 when grown for 5 weeks at a rotation speed of 120 rev/min, at 25 °C and in continuous darkness. The fresh weight multiplication rate of 6 other genotypes of the same species varied considerably, when grown on the best medium for clone A 42-3.

Introduction

One of the basic requirements for effective propagation of *Anthurium andraeanum* in vitro is a rapid multiplication of callus tissue derived from explants of selected adult genotypes. Methods to obtain callus tissue and to subculture it on solid media were described in a previous paper; in liquid media, however, the fresh weight multiplication rate was rather low, 8–10 in 5 weeks (Pierik et al., 1974). This paper describes how to improve the growth of callus in liquid media by varying the composition of these media.

Materials and methods

Most experiments were done with a callus clone obtained from a single adult plant. This callus clone (A 42-3) originated from an explant out of the spathe which was cultured on a medium as previously described (Pierik et al., 1974). The other callus clones used were obtained from leaves of adult plants. The following liquid medium was used to maintain the callus clones: MS (Murashige & Skoog, 1962) macroelements half strength, MS microelements, glucose 2 %, NaFeEDTA 25 mg/l, MS organic constituents (except Edamin, IAA and kinetin) and PBA [6-(ben-

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zylamino)-9-(2-tetrahydropyranyl)-9H-purine] 1 mg/l. The pH was adjusted to 6.0 before autoclaving.

All experiments were conducted at 25 °C in darkness. Erlenmeyer flasks (300 ml) filled with 100 ml medium, except where indicated, were placed on multi-flask shakers (E. Bühler, model VKS) with rotary motion of 120 rev/min. The basic culture medium (BCM) used in the early experiments was changed in subsequent experiments and is given in Table 1 and Fig. 1. However, the following components of the BCM were identical in all experiments: MS microelements, NaFeEDTA 25 mg/l, MS organic constituents (except Edamin, IAA and kinetin). Each experiment started with a few callus clumps, approximately 0.2 g fresh weight per flask. After 5 weeks of growth, fresh weight was determined and the fresh weight multiplication rate (final fresh weight divided by initial fresh weight) calculated. Values represent the average of 5 flasks.

Results

In the first experiment (Table 1, Exp. 1) callus growth was slightly better in flasks containing 100 or 125 ml medium in comparison to 75 ml. Growth of callus strongly decreased by raising the glucose level from 2 to 4 %. Callus growth was hardly affected by the PBA concentration.

In the second experiment the BCM was changed (see Table 1, Exp. 2) and

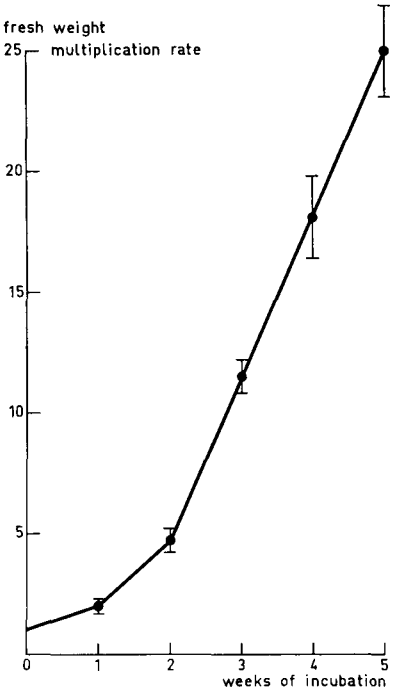

Fig. 1. Time course study of callus fresh weight multiplication (clone A 42-3). BCM: MS macroelements half strength, glucose 2 %, PBA 10^{-7} g/ml, 100 ml per flask.

Table 1. The effect of various factors on the fresh weight multiplication rate of *Anthurium andraeanum* (clone A 42-3) callus tissues in liquid media after 5 weeks.

Exp. No	Growth conditions				Fresh weight multiplication rate
	content flask (ml)	glucose conc. %	MS macro-elements (strength)	PBA conc. (g/ml)	
1.	75	2	1/2	10^{-6}	11.2 ± 0.9
	100	2	1/2	10^{-6}	15.6 ± 0.5
	125	2	1/2	10^{-6}	14.2 ± 0.9
	100	2	1/2	10^{-6}	15.6 ± 0.5
	100	3	1/2	10^{-6}	10.9 ± 0.6
	100	4	1/2	10^{-6}	8.7 ± 0.9
	100	2	1/2	10^{-7}	18.6 ± 1.3
	100	2	1/2	5.10^{-7}	17.6 ± 0.4
	100	2	1/2	10^{-6}	15.6 ± 0.5
2.	100	2	1/4	10^{-7}	13.3 ± 1.3
	100	2	1/2	10^{-7}	25.0 ± 1.9
	100	2	3/4	10^{-7}	27.3 ± 2.8
	100	2	1/1	10^{-7}	30.7 ± 2.6
	100	1	1/2	10^{-7}	18.6 ± 0.5
	100	1½	1/2	10^{-7}	20.6 ± 1.9
	100	2	1/2	10^{-7}	25.0 ± 1.9
	100	2½	1/2	10^{-7}	24.3 ± 2.8
3.	100	2	1/1	0	30.9 ± 0.3
	100	2	1/1	3.10^{-10}	29.9 ± 0.6
	100	2	1/1	3.10^{-9}	29.6 ± 2.6
	100	2	1/1	3.10^{-8}	32.6 ± 2.9
	100	2	1/1	3.10^{-7}	33.1 ± 2.8
	100	2	1/1	3.10^{-6}	31.8 ± 1.7

optimalized according to the results obtained in the first experiment. The best growth was obtained on MS-macroelements full strength and a glucose level of 2–2½ %.

During the second experiment a time course study of callus growth was also made on the medium given in the legend of Fig. 1. This figure shows that the fresh weight multiplication rate is exponential during the first two weeks and is linear after the second week.

In a third experiment the BCM was changed again (Table 1, Exp. 3) and optimalized according to the results obtained in the second experiment. The effect of PBA in a broad concentration range was reexamined and it was shown that callus growth is independent of the PBA concentration.

In a fourth experiment a comparison was made between the callus growth rate of the standard clone A 42-3 and 6 other genotypes. The BCM had the same

composition as in Exp. 3, PBA was added at 10^{-7} g/ml. Fresh multiplication rates obtained after 5 weeks were 30.0 for clone A 42-3 and 6.7, 9.2, 12.3, 21.3, 32.2 and 34.0 for the 6 other genotypes.

Discussion and conclusions

The callus multiplication rate of clone A 42-3 appears to be rather high after 5 weeks. When other genotypes were grown on the best medium for A 42-3, however, striking differences in growth rates become apparent. Two explanations can be given for these differences. The best medium for clone A 42-3 was not optimum for part of the other genotypes or the growth potential of part of the other genotypes is simply lower and cannot be influenced by changing the environment. An extensive study will not be necessary to find media for optimum growth of the weak-growing genotypes.

In these experiments clone A 42-3 appeared to have hardly any cytokinin requirement for callus growth. It is possible, however, that during the first passage from PBA to cytokinin-free media, the endogenous cytokinin content and/or biosynthesis will be high enough to maintain growth, but that cytokinins are required during subsequent subculture of the callus. Therefore, the possible role of cytokinin on callus growth, particularly in successive transfers, should be examined further.

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