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REFERÊNCIA

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CALLUS CULTURES FROM SEEDS AND ANTHERS OF *Sesamum indicum* L.*

L.S.Caldas**
W.R.Sharp***
O.J. Crocomo****

ABSTRACT

Continuously growing *Sesamum* hypocotyl callus cultures were successfully initiated from hypocotyl tissues of seeds cultured on Wetherall's Medium containing 0.5 mg/l 2,4-D and subcultured on Murashige and Skoog (MS) medium containing 0.1 mg/l 2,4-D and 100 mg/l inositol. Both 2,4-D and inositol appear to be essential for maintenance of continous growth. Callus cultures were likewise established from explants of anthers, cotyledon, and hypocotyl on the MS medium with the occurrence of arrested globular structures in some cultures.

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** Departamento de Botânica, Universidade de Brasília.

*** Pioneer Research Laboratory, Campbell Institute for Agricultural Research, Cinnaminson, New Jersey 08077, USA.

**** Departamento de Química, E.S.A."Luiz de Queiroz",USP.

INTRODUCTION

Culture of plant cells and tissues *in vitro* has many advantages for biochemical studies, particularly kinetic studies, since the media may be changed with ease and problems of pools within the organisms and time required for diffusion from the organism are reduced. For these reasons, we have started cultures of cells from seeds and anthers of sesame to continue biochemical studies of potassium deficiency effects on polyamine biosynthesis (CROCOMO *et alii*, 1970; CROCOMO & BASSO, 1974). The present report provides the methods used for establishment of *Sesamum* cell cultures.

MATERIALS AND METHODS

Seeds of *Sesamum indicum* L. were washed with detergent and sterilized in a 5% solution of commercial hypochlorite solution for 30 minutes. After washing in sterile distilled water, several seeds were used to inoculate each tube of medium. At 30°C, the seeds germinated and produced a massive thickening of the hypocotyl within eight days.

Medium 1 (M1), used for starting cultures was that of Wetherall (Table 1) supplemented in some experiments with inositol, kinetin, or arginine. Thereafter, callus was transferred to MS medium (MURASHIGE and SKOOG, 1962) containing (w/v) sucrose, cysteine HCl (10 mg/l), 2,4-D (0.1 mg/l), and kinetin (0.1 mg/l). Other cultures were started on the same medium supplemented with 4% (w/v) glucose, 5 mg/l thimine, 1 mg/l NAA and 0.5 mg/l kinetin. Coconut water (CW) from green coconuts was filtered and added to media in some experiments to give a final concentration of 10% CW before the media was autoclaved. Anthers were excised from unopened buds of sesame which had been pre-disinfected and placed on Murashige and Skoog medium. All cultures were maintained in the laboratory where the ambient temperature varied from 24-28° under cool white lamps.

RESULTS

Sesame seeds placed on M1 germinate at a high frequency, but subsequent root development was inhibited. The root meristem remained as an arrested growing point in the rapidly proliferating callus at the base of the hypocotyl. Shoot development is limited to growth of the hypocotyl and greening of the cotyledons.The cotyledon tissue proliferates and forms callus if subcultured into M1 containing 1.6 or 2.0 mg/1 2,4-D. On MS medium the same general pattern was observed, but the roots after a short interval of arrestment either resumed growth or adventitious root development at which time callus proliferation ceased. Moreover, callus proliferation occurred when isolated explants of hypocotyl or cotyledon were cultured on M1 with further development depending on the subculture medium (callus growth on M1 and root development of MS medium). Callus produced on M1 containing only 2,4-D as a growth regulator ceased to grow after one month. However, growth continued for ca. 8 months for cultures cultured on M1 supplemented with 10 or 50 mg/1 arginine and subcultured onto Medium 1 containing 0.5 mg/1 2,4-D and 100 mg/1 inositol.Continuous callus proliferation occurred in cultures on MS medium supplemented with 0.1 mg/1 2,4-D and 100 mg/1 inositol.

The MS medium also contains 0.1 mg/1 kinetin, but trials with different levels of kinetin (0.01, 0.1, and 0.5 mg/1) added to Medium 1 with inositol and 2,4-D did not produce a marked effect on callus production from seeds. Without kinetin, a very wet-looking, extremely friable callus is formed. Cotyledons which were in contact with medium containing kinetin have a greater increase in mass than those in the absence of kinetin.

Kinetin used in the establishment of callus may affect the subsequent response to other media: when callus formed on M1 without kinetin or with different levels of kinetin was transferred to the MS medium, several tubes underwent a type of differentiation, producing yellow-green globular structures (Fig. 2) and a small amout of dark callus, while others maintained a lighter, rapidly-growing callus.

In still other cases, an orange pigment, reminiscent of the flower color, was seen. To test the potential for subsequent development of these structures, transfers were made to a series of different growth regulators (GR) (MS - GR ± 0.1 mg/l 2,4-D ± CW; MS + 0.5 mg/l 2,4-D ± CW; MS + 5 mg/l 2,4-D ± CW; and MS + 0.5 mg/l 2,4-D + 0.1 mg/l kinetin). Transfers were made every three to four weeks to fresh media of the same composition. Average fresh weights of 22-day old cultures are shown.

No further development of the globular structures was noted on media without GR (± CW) and callus growth ceased within two months with the exception of a single line transferred from MS + 5 mg/l 2,4-D + CW wich has been growing very well for more than two months. Media with 0.1 mg/l 2,4-D (± CW) maintained good growth of callus and the globular structures were retained by cell lines which had formed them on the original MS medium. Another cell line which did not form such structures has continued to produce only friable callus on the medium without CW, while new formation of globular structures occurred on the medium containing CW. When 2,4-D was present at a concentration of 0.5 mg/l, with and without CW, growth continued at a good level but formation of globular structures was sporadic and they were not maintained. At 5 mg/l 2,4-D, a toxic concentration seems to be reached as all cultures stopped growing within three months.

Anthers were placed directly on the modified MS medium, and after three months, several had developed a large quantity of callus tissue. This tissue has, however, ceased to grow.

DISCUSSION

Callus of *Sesamum* can be produced from cotyledon or hypocotyl explants as well as from seeds germinated directly on the callus-induction medium Seeds of various species of arabidopsis (SHEN-MILLER and SHARP, 1966; SINAPI, BAJAJ and BOPP, 1972) have been used to start cultures of callus, thus,

the method can be considered as widely applicable. The theoretical implication of this phenomenon has not been emphasized, as YEOMAN (1970) state in a review of callus development that callus is produced from a wound. Callus production from an uninjured seed demonstrates that wounding is not a necessary prerequisite for induction. On a callus-inducing medium, callus will be produced, but until the use of seeds, there was no experimental control for testing the influence of wounding.

We have obtained growth of the *Sesamum* callus only on a medium containing both 2,4-D and inositol. However, the specificity of these two requirements was not checked, i.e., no attempt was made to define conditions or treatments in which continued growth was possible in their absence. Some of the rapidly growing callus lines are on media which include CW, a factor which does not facilitate critical biochemical studies. However, at least two rapidly growing lines have been established on completely defined media(0.1 2,4-D, which seems to be the optimal, 0.5 mg/l 2,4-D and 0.5 2,4-D + 0.1 mg/l kinetin). Callus cultures sufficiently friable for growth in liquid media are among these lines and will be preferable for kinetic studies of potassium deficiency in research on putrescine synthesis.

Since the organ or tissue-specificity of putrescine and other polyamine biosyntheses by sesame under conditions of potassium deficiency has not been established, this physiological response may be characteristic of only a specific differentiated cell type or tissue. For this reason, not all callus lines, even those growing on the same culture medium, may demonstrate the desired phenotypic response for polyamine synthesis. The morphologically different strains established on the same medium can be examined for this response, as well as lines on different media. On the different media, even lines which are similar in appearance may prove to be different in biochemical properties, due partly to the past history of the culture, i.e., the media on which it has grown compared to another culture originating from the same common cell linage.

RESUMO

CULTURA *IN VITRO* DE SEMENTES E ANTERAS DE *Sesamum indicum* L.

Sementes de gergelin (*Sesamum indicum* L.) foram cultivadas *in vitro* em meio de cultura de Wetherall contendo 0,5 mg/l de 2,4-D e em seguida transferidas para meio de Murashige e Skoog (MS) contendo 0,1 mg/l de 2,4-D e 100 mg/l de inositol. Ambos, 2,4-D e inositol mostraram-se ser necessários para o desenvolvimento de calos a partir de sementes, do mesmo modo que para o contínuo crescimento dos meios em cultura. Foram também obtidos calos de explantes de anteras, cotiledones e de hipocotilo de *Sesamum* utilizando-se o meio MS com a ocorrência de estruturas globulares.

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Table 1 - Medium for sesame callus (D.J. Wetherall, 1966, Pers. Communication)

Compound	mg/1	mM/1
KNO_3	4000	40
NH_4Cl	540	10
$MgSO_4.7H_2O$	185	0.74
$CaCl_2$	166	1.5
KH_2PO_4	68	0.45
$MnSO_4.H_2O$	7.0	0.04
$ZnSO_4.7H_2O$	4.0	0.01
H_3BO_3	2.4	0.04
$(NH_4)_6Mo_7O_4 .4H_2O$	0.01	1×10^{-6}
KI	0.38	2×10^{-3}
$CuSO_4$	0.01	6×10^{-5}
$FeSO_4.7H_2O$	14.0	0.05
Na_2EDTA	18.6	0.05
thimine HCl	3	
2,4-dichlorophenoxy-acetic acid	0.5	
sucrose	20000	
pH 5.6		

Table 2 - Fresh weights of 22-day old sesame callus grown on MS medium with various hormone combinations. The number in parentheses is the number of samples for each treatment. The inoculum was 0.1-0.2 g. and the hormone concentrations are in mg/l.

Medium	Fresh Weight (g)
MS - hormone + CW	1.5 (3)
MS + 0.1 2,4-D	0.5 (3)
MS + 0.1 2,4-D + CW	0.5 (3)
MS + 0.5 2,4-D + CW	0.8 (5)
MS + 0.5 2,4-D + 0.1 kinetin	1.8 (13)