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CALLUS CULTURE OF AUSTRIAN PINE (*PINUS NIGRA* ARN.) ON DEFINED MEDIUM

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Introduction

It is common knowledge that it is not easy to obtain and subculture tissue culture of Gymnosperms; Austrian pine is no exeption there. Numerous authors have attempted to culture tissue of various *Pinus* species, but only a few had some success. The first to succeed in obtaining such tissue cultures were Loewenberg and Skoog (1952). They obtained callus cultures of *Pinus strobus* on a medium containing unautoclaved malt extract. Attempts by other authors, who cultured tissues of some *Pinus* species, with greater or lesser succes, followed. The culturing was, however, successful only in cases, when the medium was enriched by some undefined natural substances such as the most used coconut milk.

But, it has been shown recently that, on a relatively rich medium with addition of substances with stronger inductive effects, tissue cultures of some *Pinus* species are feasible also on synthetic media. Brown and Lawrence (1968) succeeded to culture callus tissue from *Pinus palustris* on a medium with 2,4-D and kinetin. On a similar medium David (1970) obtained callus cultures with *Pinus pinaster* and Rogozińska (1970) with *Pinus silvestris.*

In the present paper the procedure, by which a callus culture of *Pinus nigra* Arn. was obtained, will be described.

Material and Methods

For the culture of Austrian pine the MS-nutrient-solution (Murashige and Skoog 1962) was used. To this medium sucrose, vitamins (thiamin-HCl, nicotinic acid, pyridoxine-HCl, ascorbic acid, Ca-pantothenate, biotin, B_{12} and meso-inositol), asparagine, kinetin and growth

regulators (IAA, IBA, IPA, NAA, 2,4-D and 2,4,5-T) were added. The medium was solidified by agar. For initial cultures, in which the first callus was obtained from proliferating cells, hypocotyl fragments were used. They were taken from 8—10 days old seedlings grown from sterilized seeds.

The initial cultures as well as the transferred callus were grown in 33 × 160 mm test tubes with 20 ml medium, each with one explant.

The cultures were maintained at a temperature of 26° C in artificial light (fluorescent lamps, 1000—1500 lx, 16 hours illumination daily).

Results

For the culture of Austrian pine it was necessary to systematically teste each component of complete MS-medium as well as each of the components added, to find out which combinations and concentrations would yield best growth.

The results of the work allow the following conclusions:

1. The macro and micro elements in the composition of the MS-solution, gave very good results and therefore they were not changed.

2. A 2% concentration of sucrose proved best. (The increase to 3% did not show any improvement.)

3. Vitamins in combinations: thiamin-HCl (10^{-7}), nicotinic acid ($5 \cdot 10^{-7}$), pyridoxine-HCl (10^{-7}) and meso-inositol (10^{-4}) gave good results. The enrichment of the medium by Ca-pantothenate (10^{-6}), ascorbic acid (10^{-6}), biotin (10^{-6}) and B_{12} (10^{-7}) did not produce any considerable improvement.

4. The addition of asparagine (10^{-4}) gave very good results.

5. Kinetin is not indispensable but it considerably stimulates the rate of cell proliferation of hypocotyls in initial cultures, as well as the rate of growth after the transfer to the fresh medium.

6. Substances with kinetin like effects, as edamin and yeast extract (0,5—3 g/l), did not considerably improve the medium.

7. Among the growth regulators, 2,4-D ($5 \cdot 10^{-6}$) gave the best results in combination with kinetin. This was especially the case in the cell proliferation of hypocotyls in initial cultures, while after the transfer to the fresh medium a similar effect as with 2,4-D was obtained with 2,4,5-T and NAA. The effect of IAA, IBA and IPA was in both cases very weak.

Fig. 1. Initial callus culture (obtained from proliferating hypocotyl fragment).
Sl. 1. Početna kultura kalusa (koji je nastao proliferacijom stanica fragmenata hipokotila).

Fig. 2. Callus tissue culture after the third transfer.
Sl. 2. Presađeni kalus u kulturi nakon trećeg presađivanja.

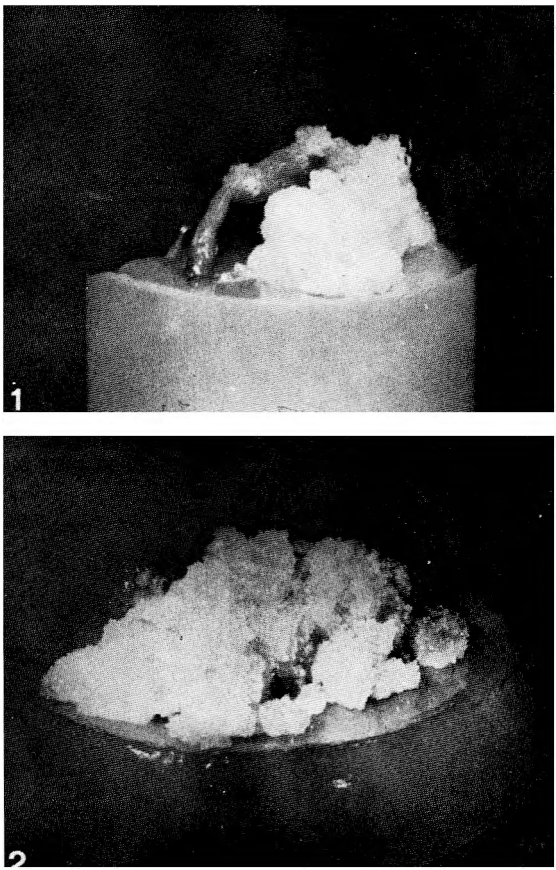

Fig. 1—2. — Sl. 1—2.

8. The most suitable solidity of the medium was achieved by an addition of 1.0—1.2% of agar. It was shown that the compactness of callus tissue was increased by higher agar concentration. As a compact callus tissue is easier to handle higher agar concentrations were prefered.

According to these results the composition of the MS-medium was modified as follows:

Inorganic salts	mg/l	Organic additions	mg/l
NH_4NO_3	1 650	Thiamin-HCl	0,1
KNO_3	1 900	Nicotinic acid	0,5
$CaCl_2 \cdot 2H_2O$	440	Pyridoxine-HCl	0,1
$MgSO_4 \cdot 7H_2O$	370	Meso-inositol	100,0
KH_2PO_4	170	Asparagine	100,0
H_3BO_3	6,2	Kinetin	0,5
$MnSO_4 \cdot 4H_2O$	22,3	2,4 D	5,0
$ZnSO_4 \cdot 4H_2O$	8,6	Sucrose	~ 20 000,0
KI	0,83	Agar	~ 10 000—12 000,0
$Na_2MoO_4 \cdot 2H_2O$	0,25		
$CoCl_2 \cdot 6H_2O$	0,025		
FeEDTA	65,1		

In the initial cultures the period before the cell proliferation did not exceed 4—8 days (Fig. 1). The transferred callus tissue (Fig. 2) grew relatively rapidly and the time of subculturing was between 4 and 7 weeks.

Discussion

The culture of Austrian pine had already been obtained twice before. Bogdanović and Jelinić (1968) obtained a callus culture from cambium on Heller's medium with an addition of IAA, NAA and 2, 4-D. A cambial callus culture of Austrian pine was also grown by Harvey and Grasham (1968) on a rather poor nutrient medium of original composition with the same additional growth substances.

In the present paper the procedure for growing callus cultures of Austrian pine differs from the procedures of the cited author in two points:

a) The callus was grown from proliferating cells of hypocotyl fragments.

b) The MS-medium was adapted as nutrient medium. This medium is characterized by a high content of nitrogen (inorganic as well as organic in reduced form in asparagine). The addition of vitamins, especially of meso-inositol is not less important, while the added 2, 4-D, especially in combination with kinetin, represents a growth regulator with especially strong inductive effectiveness.

Summary

Callus culture of Austrian Pine (*Pinus nigra* Arn.) has been grown on a modified MS-medium for over a year. It was obtained by cell proliferation from hypocotyl fragments and subcultured by eight transfers.

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References

Bogdanović, M. and *Đ. Jelinić*, 1968: Uticaj materija porasta na razvoj početne kulture tkiva stabla Pinus nigra Arn. Zemlj. i biljka *17*, 305—313.

Brown, A. M. and *R. H. Lawrence*, 1968: Culture of Pine callus on a defined medium. Forest Sci. *14*, 62—64.

David, A. M., 1970: Obtention d'une souche de Pinus pinaster Sol. à partir de fragments d'hypocotyls. C. R. Acad. Sc. Paris *271*, 1866—1868.

Harvey, A. E. and *J. L. Grasham*, 1968: Procedures and media for obtaining tissue cultures of 12 conifer species. Can. J. Bot. *47*, 547—549.

Loewenberg, J. R. and *F. Skoog*, 1952: Pine tissue culture. Physiol. Plant. *5*, 33—36.

Murashige, T. and *F. Skoog*, 1962: A revised medium for rapid growth and bioassay with tabacco tissue cultures. Physiol. Plant. *15*, 473—497.

Rogozińska, J. H., 1970: Culture of Scots pine callus and its nutritional requirements. Acta Soc. Bot. Pol. *39*, 151—160.

SADRŽAJ

KULTURA KALUSNOG TKIVA CRNOG BORA (*PINUS NIGRA* ARN.) NA MEDIJU ODREĐENOG SASTAVA

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Kultura kalusnog tkiva crnog bora (*Pinus nigra* Arn.) dobivena je proliferacijom stanica fragmenata hipokotila i održavana kontinuirano kroz godinu dana na modificiranom MS-mediju presađivanjem kroz osam pasaža.

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