

ポプラカルスからの茎葉分化に対する培地の無機成分の効果

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短 報

Effects of Inorganic Elements in the Medium on Shoot Differentiation from *Populus Callus**

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I. Introduction

The composition of culture medium is a key factor in the culture of tissues. The culture condition favouring callus growth may not always be suitable for shoot or plantlet differentiation.

Populus plants are known to give rise to shoots or plantlets in somatic callus culture (1~11). Several media developed by various workers to suit regeneration consist of balanced mixtures of micro- and macronutrient elements: namely, vitamins, a carbon source, organic growth factors, a nitrogen source and plant growth regulators.

This paper deals with the effects of inorganic elements within the medium on shoot differentiation from callus of *Populus*.

The ploidy of the differentiated shoots was not investigated.

II. Materials and Methods

Pieces 10 cm long were cut from newly elongated branches of 2-year-old cuttings of *Populus × euramericana* cv. I-214 in October, 1974. These pieces were sterilized with 70 percent ethyl alcohol and then rinsed twice with sterile distilled water. These sterilized branch pieces were peeled aseptically with a surgical knife which had been rinsed in 2 percent liquid sodium carbonate. The cambial part of the peeled sections was cut into pieces 5 mm square and inoculated aseptically in 18 mm × 180 mm test tubes containing 20 ml of the modified MURASHIGE and SKOOG's (1962) medium (modified MS medium, basal medium shown in Table 1).

The calluses which developed on the basal medium were then subcultured on the same kind of medium twice, 4 weeks apart.

Four weeks after the last subculture, calluses were inoculated in tubes containing media with various concentrations of N, P and K by adjusting the amounts of KCl, NaH₂PO₄ and NH₄NO₃. One piece of callus, with an average fresh weight of 50 mg, was inoculated in each tube. Ten pieces of calluses were prepared for each treatment. The calluses inoculated in the tubes were subjected continually to a temperature of 25°C and a

fluorescent illumination of about 4,000 lx for 16 hr daily throughout the culture period.

III. Results and Discussions

The cambial pieces of *Populus* developed large amounts of greenish callus when cultured on the basal medium. When these calluses were transferred to the media having varying amounts of KCl, NaH₂PO₄ and NH₄NO₃ 4 weeks after the last subculture, greenish shoots appeared on the slowly growing brown callus in 4 weeks (Fig. 2). The

Table 1. Composition of media used in this study

Component	Basal medium* (ppm)	Medium for shoot formation (ppm)		
		(L)	(M)	(H)
NH ₄ NO ₃	1,650	88	568	2,370
NaH ₂ PO ₄ ·2H ₂ O	—	9	45	170
KCl	—	—	229	1,087
KH ₂ PO ₄	170	—	—	—
KNO ₃	1,900	—	78.7	—
KI	0.83	—	0.83	—
CaCl ₂ ·2H ₂ O	440	—	642	—
MgSO ₄ ·7H ₂ O	370	—	1,562	—
Na ₂ -EDTA**	37.3	—	37.3	—
FeSO ₄ ·7H ₂ O	27.8	—	27.8	—
H ₃ BO ₃	6.2	—	6.2	—
MnSO ₄ ·4H ₂ O	22.3	—	22.3	—
ZnSO ₄ ·7H ₂ O	8.6	—	8.6	—
Na ₂ MoO ₄ ·2H ₂ O	0.25	—	0.25	—
CuSO ₄ ·5H ₂ O	0.025	—	0.025	—
CoCl ₂ ·6H ₂ O	0.025	—	0.025	—
Thiamine HCl	0.4	—	0.4	—
Inositol	40	—	40	—
Niacin	1.5	—	1.5	—
Pyridoxine HCl	0.4	—	0.4	—
L-glycine	0.2	—	0.2	—
L-tyrosine	—	—	32	—
2,4-dichlorophenoxyacetic acid (2,4-D)	0.04	—	0.04	—
6-benzylaminopurine(BAP)	0.20	—	0.50	—
Biotin	—	—	0.5	—
Sucrose	30,000	—	30,000	—
Agar	10,000	—	10,000	—

Note: pH was adjusted to 5.8 with NaOH or HCl prior to addition of agar and before autoclaving.

*Modified from MURASHIGE and SKOOG's medium (1962)

**Na₂-EDTA: disodium ethylenediaminetetraacetate, dihydrate

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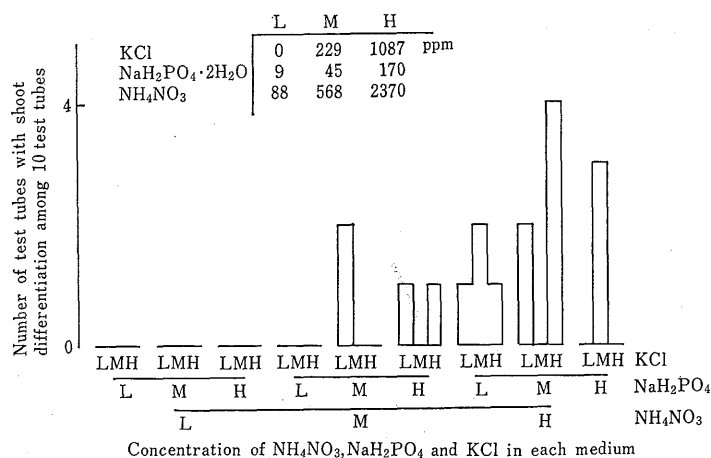


Fig. 1. Effects of three different concentrations of NH₄NO₃, NaH₂PO₄ and KCl in the medium on shoot differentiation

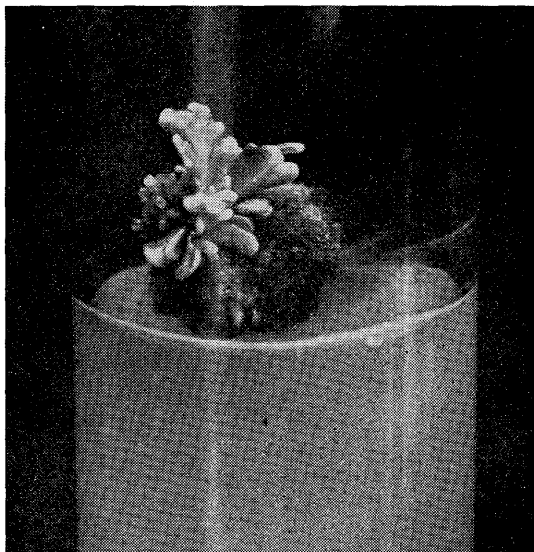


Fig. 2. Shoot derived from callus
Tube diameter: 18 mm

results are shown in Fig. 1. The relationships among various combinations of N, P and K with different concentrations ranging from 0.02 ppm to 2.00 ppm were distinct. A greater amount of shoot growth was induced with media in which the concentration of NH₄NO₃ was set at higher levels (Fig. 1).

These results suggest that, in order to induce embryogenetic response of *Populus* callus, NH₄NO₃ must be present in higher concentrations whereas such compounds as KCl and NaH₂PO₄ with above mentioned concentrations are not necessary.

Little attention has been paid to the effects of N,

P and K levels on shoot formation from callus. The results of the present experiments support the possibility that embryogenesis in callus cultures is controlled by shifts in the ratio of inorganic elements.

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Literature cited

- (1) BAWA, K.S. & STETTLER, R.F.: Organ culture with black cotton-wood: morphogenetic response of female catkin primordia. *Can. J. Bot.* **50**: 1627~1631, 1972
- (2) BERBEE, J. & HILDEBRANT, A.: Induction of callus and trees from stem tip cultures of a hybrid poplar. *In Vitro* **7**: 269, 1972
- (3) CHALUPA, V.: Control of root and shoot formation and production of trees from poplar callus. *Biol. Plant.* **16**: 316~320, 1974
- (4) MATHES, M.C.: The culture of isolated triploid aspen tissue. *For. Sci.* **10**: 35~38, 1964 a
- (5) ———: The *in vitro* formation of plantlets from isolated aspen tissue. *Phyton* **21**: 137~141, 1964 b
- (6) SATO, T.: Callus induction and organ differentiation in another culture of poplars. *J. Jap. For. Soc.* **56**: 55~62, 1974*
- (7) VEVERLOO, C. J.: The formation of adventitious organs. I Cytokinin induced formation of leaves and shoots in callus cultures of *Populus nigra* L. var. *italica*. *Acta Bot. Neerland.* **22**: 390~398, 1973
- (8) WINTON, L. L.: Plantlets from aspen tissue cultures. *Science* **160**: 1234~1235, 1968

- (9) WINTON, L. L.: Shoot and tree production from aspen tissue cultures. *Am. J. Bot.* 57: 904~909, 1970
- (10) ———: Tissue culture propagation of European aspen. *For. Sci.* 17: 348~350, 1971
- (11) ———: Bibliography of somatic callus cultures from

deciduous trees. *Genetics and Physiology Notes* No.17, 19 pp, Inst. of Pap. Chem. Appleton, Wisconsin, 1972

* Japanese with English summary

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