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BRIEF COMMUNICATION

Control of Root and Shoot Formation and Production of Trees from Poplar Callus

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Abstract. The technique of trees production from the undifferentiated poplar callus tissue is described. The best root formation was observed on the modified WOLTER and SKOOG medium when NAA in concentration 0.2 to 0.4 mg l^{-1} was used as an auxin and cytokinins were omitted. The induction of leafy shoots from the undifferentiated callus was the most effective on the modified LINSMAIER and SKOOG medium in the absence of auxin and with 0.15 to 0.70 mg l^{-1} of BAP. The best development of roots at the basal end of excised shoots was achieved when shoots were transferred into the sterile mixture of perlit and sand (3 : 1, v/v) containing a modified WOLTER and SKOOG medium.

The induction of roots and shoots from an undifferentiated callus tissue of forest trees has not yet been well explored and understood. In this paper the control of shoot and root formation and a regeneration of trees from callus tissues of poplar (*Populus euroamericana* (DODE) GUINIER cv. *robusta*) is described. We have also achieved a regeneration of trees from callus tissues of *Populus nigra* L. var. *typica* SCHNEIDER, *Populus tremula* L. and *Populus canescens* SMITH.

Cambium tissue from one year old terminal branches and shoot tips 0.5 mm long were used for initiation of the callus tissue. Firm green callus was initiated in February 1971 and subcultured every 3 to 4 weeks on chemically defined media and on supplemented with coconut milk. WOLTER and SKOOG (1966) medium and LINSMAIER and SKOOG (1965) medium supported well the formation and the growth of poplar callus tissue. From chemically defined media the best growth of the undifferentiated poplar callus tissue was obtained on the modified Linsmaier and Skoog medium, where the α-naphthaleneacetic acid (NAA) was used as an auxin (2 mg l^{-1}), 6-benzylaminopurine (BAP) as a cytokinin (1 mg l^{-1}), and *L*-arginine (100 mg l^{-1}) as an organic N source (CHALUPA and DURZAN 1973). 0.7 % Difco Bacto agar was used to solidify the medium. Undifferentiated callus tissues were grown either in a growth cabinet (EF-7H of the Controlled Environments Ltd.) under continuous light (fluorescent and incadescent light, 10 000 lx) at temperature 25 ± 0.5 °C, or in a thermostat under total darkness. The callus grown under

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continuous light was dark green, in darkness white. In both cases the growth of callus tissues was rapid, a firm callus was formed and was subcultered every 3 to 4 weeks.

After half a year of the culture of the undifferentiated callus tissue, the effect of various concentrations of an auxin (NAA) and a cytokinin (BAP) on the formation of roots and shoots was tested. The modified WOLTER and SKOOG (1966) medium (myo-inositol 100 mg l^{-1}, thiamine HCl 0.1 mg

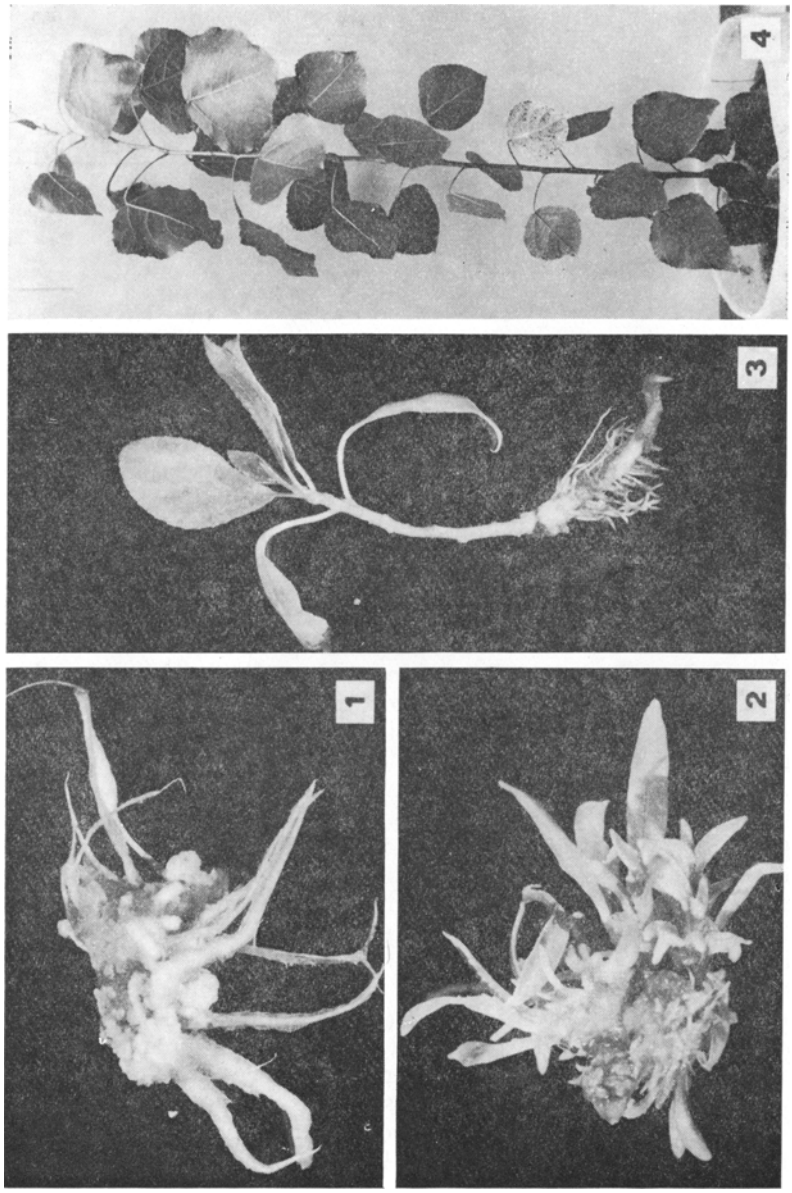

Fig. 1. The formation of roots from a callus tissue (age of the culture 6 weeks)
Fig. 2. The induction of shoots from a callus tissue (age of the culture 4 weeks)
Fig. 3. Shoot rooted in a sterile mixture of perlit and sand containing modified WOLTER and SKOOG medium
Fig. 4. Tree regenerated from a callus tissue (height 82 cm)

l^{-1}, nicotinic acid 0.5 mg l^{-1}, agar 0.7 %) and modified LINSMAIER and SKOOG (1965) medium (*L*-arginine 100 mg l^{-1}, agar 0.7 %) was used. Undifferentiated green callus tissues placed on agar plates were grown in a growth cabinet at 25 °C, illuminance 10 000 lx and 16 h light. After 10 to 14 days shoots and roots started to develop from the undifferentiated callus on both

TABLE 1

EFFECT OF α-NAPHTHALENEACETIC ACID (NAA) ON THE FORMATION OF ROOTS (MODIFIED WOLTER AND SKOOG MEDIUM WITHOUT CYTOKININ, FOR EACH NAA CONCENTRATION 50 CALLUSES WERE USED)

NAA [mg l^{-1}]	Callus with roots [%]	Number of roots per callus
0.0	16	2.1 ± 1.3
0.2	76	4.6 ± 1.8
0.4	84	5.2 ± 2.1
0.8	64	3.4 ± 1.5
1.0	62	2.8 ± 1.3
2.0	18	1.6 ± 0.7
5.0	2	1.0 ± 0.0
10.0	2	1.0 ± 0.0

media. On media without BAP and supplemented with NAA, the formation of roots was induced, on media without NAA and supplemented with various concentration of BAP, the formation of leafy shoots was initiated. On media lacking both auxin and cytokinin, the formation of shoots was not observed, in few cases short roots were formed (Table 1). The development and growth of roots was better on the WOLTER and SKOOG medium where NAA was used as an auxin, than on the LINSMAIER and SKOOG medium. On the other hand, the LINSMAIER and SKOOG medium supplemented with various concentrations of BAP, supported better the initiation and growth of leafy shoots.

Various concentrations of NAA supported initiation and growth of roots quite differently. In the tested range the optimum concentration for formation of roots was usually 0.2 to 0.4 mg l^{-1} of NAA (Table 1). The roots started to develop from the undifferentiated callus after 10 to 14 d and after 4 to 6 weeks were several centimeters long (Fig. 1). The roots grew more rapidly on the surface or above the agar medium, than inside the agar medium. The roots growing inside of the agar medium were without hairs. On media lacking cytokinin and supplemented with NAA, only the formation of roots was observed, in no cases the formation of shoots.

On nutrient agar media lacking auxin and supplemented with BAP, the formation of leafy shoots was induced (Table 2, Fig. 2). Even very low concentration of BAP (0.05 mg l^{-1}) induced formation of shoots. With the increasing concentration of BAP, the number of calluses with shoots increased as well as the number of shoots per callus (Table 2). The best concentration of BAP for the formation of leafy shoots on the modified LINSMAIER and SKOOG medium was 0.15 to 0.70 mg l^{-1}. On media lacking auxin and

supplemented with cytokinin, only the formation of shoots was observed, if callus was grown under high illuminances. On the other hand, the initiation of roots instead of shoots was observed from the undifferentiated callus grown in total darkness on the LINSMAIER and SKOOG medium with low content of BAP (0.05 to 0.10 mg l^{-1}). On the LINSMAIER and SKOOG medium

TABLE 2

EFFECT OF 6-BENZYLAMINOPURINE (BAP) ON THE FORMATION OF SHOOTS (MODIFIED LINSMAIER AND SKOOG MEDIUM WITHOUT AUXIN, FOR EACH BAP CONCENTRATION 50 CALLUSES WERE USED)

BAP [mg l^{-1}]	Callus with shoots [%]	Number of shoots per callus
0.00	0	—
0.05	64	3.4 ± 1.6
0.15	82	4.8 ± 2.1
0.30	86	6.4 ± 2.6
0.70	80	6.9 ± 2.7
2.00	72	6.0 ± 3.0
5.00	72	6.8 ± 3.5

with 1 mg l^{-1} of NAA and 1 mg l^{-1} of BAP, the formation of shoots from the callus grown under high light intensity was observed in some cases, the formation of roots from the callus grown under total darkness.

Leafy shoots, which after 4 to 8 weeks of growth were several centimetres long, were excised from the callus and placed on the modified WOLTER and SKOOG medium lacking cytokinin and supplemented with 0.4 mg l^{-1} of NAA. In 2 to 4 weeks roots developed at the basal end of shoots, but were usually short. Better development of roots at the basal end of excised shoots was achieved when shoots were transferred into the sterile mixture of perlit and sand (3 : 1 v/v) containing modified WOLTER and SKOOG medium (half concentration of macro and microelements, without cytokinin and with 0.2 mg l^{-1} of NAA and low content of sucrose, 0.2 %). More than 80 % of excised shoots produced in this nutrient mixture long, strong roots in 2 to 3 weeks (Fig. 3). The rooted shoots were transplanted into a mixture of soil, peat and perlit (3 : 1 : 1 v/v/v) and were grown in a growth cabinet under constant temperature 22 ± 0.5 °C, high illuminance 25 000 lx, 16 h light and 70 % relative humidity. The growth of trees under these conditions was rapid and in several months after transplanting trees were several decimetres high (Fig. 4). The comparison of chromosome numbers is carried out.

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BOOK REVIEWS

ELLENBERG, H. (ed.): **Ökosystemforschung.** — Springer-Verlag Berlin, Heidelberg, New York 1973. 280 S., 101 Abb., DM 39,—.

Die Ökosystemforschung gewinnt als Voraussetzung für gesundere Umweltbedingungen auf der Erde sehr an Bedeutung. Vorliegender Band bringt zuerst eine Einführung in die Ziele und den Stand der Ökosystemforschung (von H. ELLENBERG), wo die Grundbegriffe, die verschiedenen Richtungen und die Geschichte der Ökosystemforschung erklärt werden. Im weiteren Abbschnitt werden Beispiele einzelner Ökosysteme durchgesprochen wie ein Hochgebirgsee (R. PECHLANDER u.a., M. TILZER), der Schilfgürtel eines Steppensees (K. BURIAN, H. SIEGHARDT, R. MAIER, G. DRAXLER, M. DOKULIL), marine Ökosysteme (G. RHEINHEIMER), Land-Ökosysteme (M. RUNGE, W. FUNKE, B. ULRICH und R. MAYER) und Land-Ökosysteme im Hochgebirge (W. LARCHER u.a., A. CERNUSCA, W. MOSER, W. BRZOSKA). Es werden die Stoffproduktion und deren Steuerung, Energiebilanz, Gaswechsel, Strahlungsnutzung, Wasserverbrauch, Nährstoffkreislauf, Datengewinnung und -verarbeitung u.v.m. behandelt. Im letzten Abschnitt des Bandes ist Prof. H. ELLENBER bemüht eine Klassifikation der Ökosysteme der Erde nach funktionalen Gesichtspunkten durchzuführen und einen Bestimmungsschlüssel zusammenzustellen; er reiht die im Buch behandelten Ökosysteme in das System ein. Die einzelnen Kapitel des Buches fassen einen Teil der Ergebnisse über Funktionieren und Gesetzmässigkeiten von Ökosystemen zusammen, die im Rahmen des Internationalen Biologischen Programms von deutschen und österreichischen Arbeitsgruppen erlangt und auf den Symposien der Deutschen Botanischen Gesellschaft und der Gesellschaft für Angewandte Botanik in Innsbruck im Jahre 1971 vorgetragen wurden. Um das Buch zu mehr als einer losen Folge verschiedener Beiträge werden zu lassen, wurden Zusammenfassungen, Hinweise auf Institute, Geldgeber, Danksagungen weggelassen und die Beiträge durch Einleitung in die Problematik und abschliessende Klassifikation in ein Ganzes zusammengefügt, das eine wertvolle Einführung in die Ökosystemforschung in der deutschsprachigen Literatur darstellt. Da in der englischen Literatur schon ähnliche Bücher erschienen sind, wurde hier auf englische Zusammenfassungen verzichtet. Das Buch ist rein für den deutschen Sprachraum gedacht, was jedoch eine gewisse unnötige Begrenzung bedeutet. Das Buch erschien in tadellosem Druck in flexiblem Kunststoffeinband, ist reich mit Abbildungen ausgestattet, enthält ein Mitarbeiterverzeichnis und endet mit einem übersichtlichen Sachregister. Jeder Beitrag schliesst mit einem Literaturverzeichnis; in diese haben sich leider z.B. auf Seite 77, 78, 193, 223 Druckfehler in Autorennamen hineingeschlichen.

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