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# Rapid Clonal Multiplication of *Digitalis lanata* in Tissue Culture

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ABSTRACT

Shoot cultures of *Digitalis lanata* have been established by inoculating the shoot tip of seedlings germinated in aseptic culture, or of field-grown plants, onto Linsmaier and Skoog's RM medium supplemented with 1 mg 6-benzylaminopurine and 0.1 mg indolacetic acid $l^{-1}$. On this medium formation of up to 30 new axillary shoots could be induced. Shoots could be grown into functional plants after root induction on a medium containing reduced amounts (one-fifth of normal) of nitrogen and indolebutyric acid (0.5 mg $l^{-1}$).

## INTRODUCTION

*Digitalis lanata* plants are the source of the important cardiac glycosides used in medicine. Tissue culture of this species has been described and its potential for glycoside production and biotransformation studied (for review c.f. Reinhard and Alfermann 1980).

In this paper we describe a procedure for the rapid clonal propagation of *Digitalis lanata*. The method is based on repeated induction of shoots from axillary buds in sterile culture and reconstitution of functional plants by rooting the shoots. Similar methods have been described for a number of species (Vasil 1980).

## MATERIALS AND METHODS

For sterilization seeds were wetted with 70% ethanol, soaked in 0.5% sodium hypochlorite (3 min), rinsed in sterile distilled water (5x) and germinated on basal tissue culture medium. Shoot tips (30 mm long, covered with four to five leaves) taken from field plants during winter or spring were sterilized by the same procedure. The leaves were then removed and the tip (3-5 mm) used for inoculating the cultures.

The basal medium contained the Linsmaier and Skoog's RM (1965) salts plus 1 mg thiamine and 3% sucrose $l^{-1}$. For shoot and root induction the basal medium was supplemented with cytokinins and/or auxins. The composition of the P basal medium was the same as that of the RM basal medium except that the concentration of $KNO_3$ and $NH_4NO_3$ was reduced to one-fifth. The cultures were incubated at 28°C in low light (1500 lux provided by Airam Floralux fluorescent tubes; illumination for 16 h a day).

## RESULTS AND DISCUSSION

Shoot Multiplication. Shoots grown from shoot tips of field-grown plants or from seedlings germinated in aseptic cultures formed either no axillary shoots or at most two to four branches during a six-week culture period on basal medium.

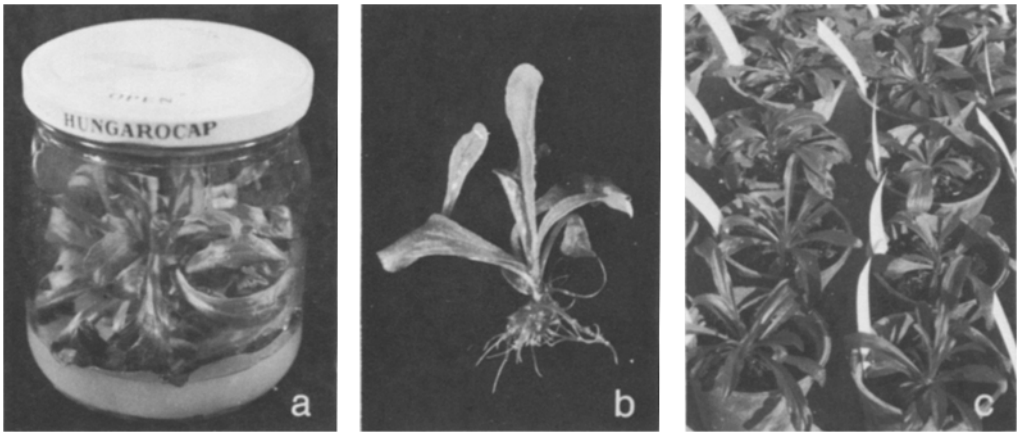


Fig. 1. A six-week old culture in the shoot multiplication phase (a), a plantlet ready for potting in the greenhouse (b) and plants after two months in the greenhouse (c)

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In order to increase the number of shoots developing from the axillary buds the RM medium was supplemented with the cytokinin 6-benzylaminopurine (0; 0.01; 0.1; 1.0; 5.0 mg $ml^{-1}$) in combination with the auxins indole-3-acetic acid or 1-naphtaleneacetic acid (0; 0.01; 0.1; 1.0 mg $l^{-1}$) or 2,4-dichlorophenoxy-acetic acid (0; 0.01; 0.1 mg $l^{-1}$). Benzyladenine (1 mg $l^{-1}$) in combination with low auxin concentrations (0.01; 0.1 mg $l^{-1}$), was an efficient inducer of shoot multiplication. Out of the auxins tested indole-3-acetic acid (0.1 mg $l^{-1}$) was selected for routine use. With this medium (benzylaminopurine 1 mg $l^{-1}$, indole-3-acetic acid 0.1 mg $l^{-1}$) formation of 15-30 new shoots could be induced on a single shoot during a six-week period (Fig. 1a). Clones have now been maintained on this medium for two years.

Rooting of Shoots. Roots developed only on a small proportion (Table 1) of shoots transferred from the shoot induction medium into the auxin and cytokinin free basal RM medium. The proportion of root-forming shoots could be increased by decreasing the concentration of salts and including the auxin indolebutyric acid in the medium (Table 1). On most of the shoots inoculated onto the P medium containing indolebutyric acid roots appeared in two to three weeks. Rooting under these conditions was 100% when the incubation time was extended (4-5 weeks). Indolebutyric acid treatment was not needed for root induction during the entire culture period. Efficient (100%) rooting was obtained if the shoots were transferred onto an auxin-free medium after the initial (7-10 d) induction period. A plantlet ready for potting is shown (Fig. 1b).

Table 1. Effect of culture medium on root induction[a]

| Medium | % shoots forming roots | |
|---|---|---|
| | - IBA | + IBA (0.5 mg $l^{-1}$) |
| RM | 25 | - |
| RM (salts diluted 2x) | 48 | 85 |
| P | 54 | 84 |

[a]Scored after three weeks. In two experiments, altogether 150-200 shoots tested

Transfer of Plants to Greenhouse. Most of the plants (85-98%) survived the transfer if the roots were induced on hormone-free medium, or the shoots were transferred to hormone-free medium after indolebutyric acid treatment. Plants cultured on indolebutyric acid during the entire rooting period were more susceptible to disease and only a smaller proportion (10-60%) survived the adaptation period. A plant derived from micropropagation is shown on Fig. 1c.

The method described makes feasible maintenance of *Digitalis lanata* clones in sterile culture and establishment of large uniform populations of heterozygous plants.

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