Theor. Appl. Genet. 60, 345-347 (1981)

Plantlet Regeneration from Mesophyll Protoplasts of *Digitalis lanata* Ehrh.*

Xiang-hui, Li
Institute of Genetics, Academia Sinica, Beijing (China)

Summary. Protoplasts were isolated from the mesophyll of *Digitalis lanata* enzymatically and cultured in a liquid regeneration medium (D2a). Protoplast division occurred at a rate of approximately 30%. Mature cell colonies were transferred onto agar medium (D2b) where they developed into cell clusters with a diameter of about 4-5 mm. After transfer onto MS medium, these calli differentiated leaves and shoots which could be rooted on MS medium containing a low hormone concentration.

Key words: Protoplast – Cell division – Regeneration – *Digitalis lanata*

Introduction

Regeneration of plant protoplasts into plants is still limited to few species, most of them solanaceous species (Vasil and Vasil 1980). This report describes the successful isolation and regeneration of mesophyll protoplasts from *Digitalis lanata* Ehrh. into plants. *Digitalis lanata* and *Digitalis purpurea* are important cardiac glycoside-synthesizing plant species (Reinhard and Alfermann 1980; Stohs 1980): this is strictly limited to the leaves. Therefore, in calli induced from leaves of *Digitalis purpurea* only very small amounts of cardiac glycosides can be detected (Hirotani and Furuya 1977). It has been shown that callus cultures of *Digitalis purpurea* possess the potential for plant regeneration in which cardiac glycoside synthesis is restored (Hirotani and Furuya 1977). Protoplasts as a single cell system and their regeneration to plants can be used for plant improvement via spontaneous or induced mutagenesis or via somatic hybridization (Schieder and Vasil 1980). Recently, attempts have been started, using *Digitalis purpurea*, to select cell lines possessing a higher level of cardiac glycosides after protoplast regeneration. However, plant regeneration was not communicated (Diettrich and Luckner 1980). Plants regenerated from protoplasts of *Digitalis lanata* may provide a better opportunity to select lines with a higher level of cardiac glycosides than other cell lines.

* The main part of this work was carried out in the Max-Planck-Institut für Züchtungsforschung, Cologne (FRG)

Materials and Methods

Seeds of *Digitalis lanata* obtained from the Institute of Pharmacy of the Chinese Academy of Medical Sciences in Beijing were sown into soil and the developed seedlings were allowed to develope into plants in the greenhouse. For protoplast isolation fully expanded leaves of 2-5 weeks old plants were taken and surface sterilized with 0.1% $HgCl_2$ and 0.1% SDS (sodiumdodecylsulfat) for 5 min under low vacuum and subsequently rinsed 3 times with filter sterilized water. The ventral epidermis was removed by peeling and the peeled leaves were incubated in an enzyme solution consisting

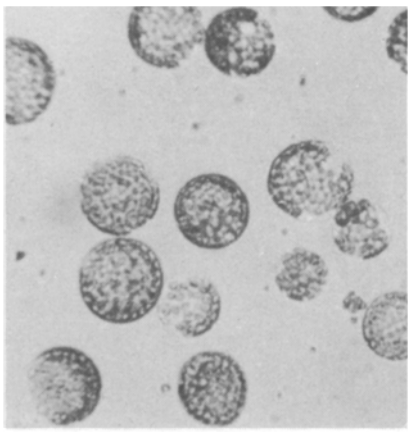
Table 1. Media for protoplast culture

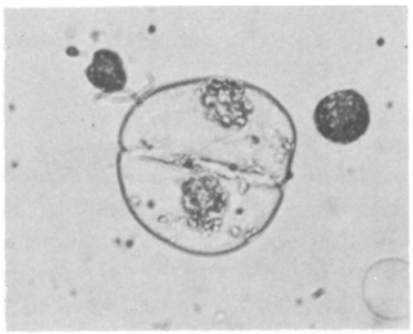
Mineral salts	(mg/l)	Organic components (mg/l)	D2a	D2b
NH_4NO_3	270	m-inositol	100	100
KNO_3	1480	Nicotinic acid	4.0	4.0
$CaCl_2 \cdot 2H_2O$	900	Thiamine-HCl	4.0	4.0
$MgSO_4 \cdot 7H_2O$	900	Glycine	1.4	1.4
KH_2PO_4	80	Pyridoxine-HCl	0.7	0.7
$FeSO_4 \cdot 7H_2O$	27.8	Folic acid	0.4	0.4
Na-EDTA	37.3	Biotin	0.04	0.04
H_3BO_3	2.0	NAA	1.5	1.5
$MnSO_4 \cdot 4H_2O$	5.0	6-BAP	0.6	0.6
$ZnSO_4 \cdot 4H_2O$	1.5	Coconut milk	5%	5%
KI	0.25	2,4,5-T	0.5	0.5
$Na_2MoO_4 \cdot 2H_2O$	0.10	Glucose	0.4M	–
$CuSO_4 \cdot 5H_2O$	0.015	Sucrose	0.05M	0.06M
$CoCl_2 \cdot 6H_2O$	0.010	Agar	–	4000

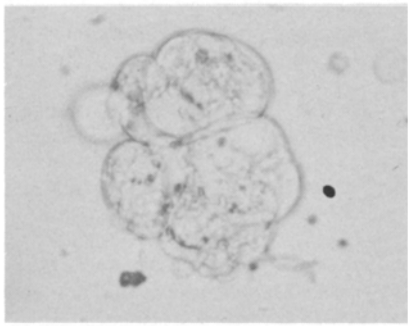
pH = 5.8

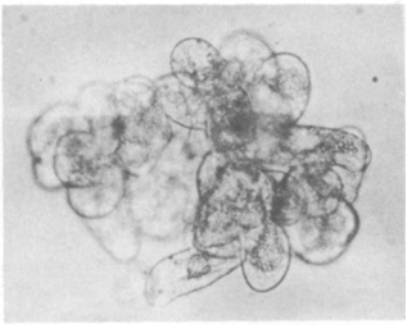
0040-5752/81/0060/0345/$ 1.00

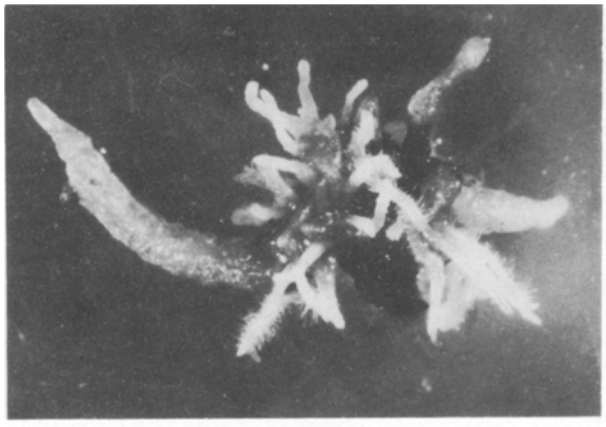
of 1% cellulase Onozuka R-10, 0.2% macerozyme R-10 (both Kinki Yakult Mfg. Co. Ltd., Japan), 10mM $CaCl_2 \cdot 2H_2O$, 0.7mM KH_2PO_4 and 0.55 mannitol. The pH of 5.7 was adjusted with KOH. After incubation for 3-4h at 24-25°C, the enzyme mixture containing protoplasts and debris was filtered through a stainless steel filter of 120μm mesh. The filtrate was centrifugated and the pelled resuspended in 0.6M sucrose. Centrifugation (100 g) for 5min left the healthy protoplasts floating in the supernatant. The supernatant containing the protoplasts was diluted 1:10 with the modified protoplast culture medium D2a (Table 1) according to Li et al. (1980) and centrifuged and additional wash the protoplasts were suspended in the protoplast culture medium up to a density of 1-2 × 10^4/ml and plated in plastic petri dishes. The dishes were placed in a growth chamber adjusted to 26-27°C and to a permanent illumination of 1000-2000 lux. Cell clusters which developed in the protoplast regeneration medium after 15 days of culture were transferred to the agar medium D2b (Table 1) and cultured at 25-27°C and ca. 3000 lux 16h per day. Calli which were grown to a size of about 4-5mm were removed and transferred onto MS agar medium (Murashige and Skoog 1962) containing 7% coconut milk and 0.2mg/l

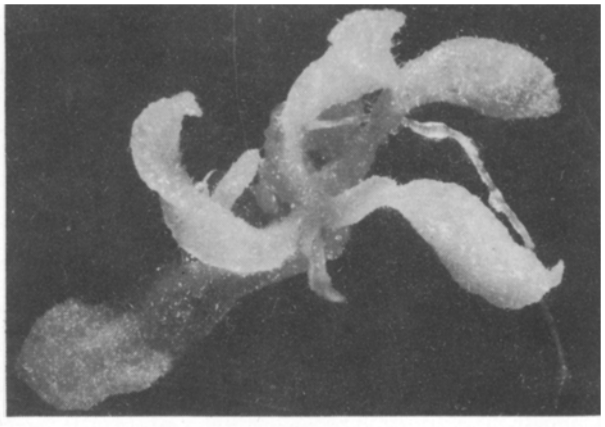

1


2


3


4


5


6

Figs. 1-4. 1 Protoplasts isolated from mesophyll of *Digitalis lanata* E; **2** mitosis in cell regenerated on the D2a medium; **3** ceil cluster formed after two weeks in culture; **4** callus from the cultured protoplasts; **Figs. 5-6.5** Regenerated plantlets with leaves and rootlets differentiated from cultured protoplasts; **6** young regenerated plant of *Digitalis lanata*

IAA (indolylacetic acid). Roots were induced on calli which subsequently developed leaves and shoots on MS agar medium supplemented with only 0.1mg/l IAA and 0.1mg/l BAP (6-benzylaminopurine).

Results and Discussion

A larger number of healthy protoplasts from young leaves of *Digitalis lanata* were isolated from the enzyme mixture after incubation for 3-4h (Fig. 1). Older leaves gave a very low yield of protoplasts with the same treatment. After 5 days of culture in the protoplast regeneration medium D2a, first divisions could be observed (Fig. 2). After 15 days of culture approximately 30% of the plated protoplasts had started with the first division. Multicellular cell colonies (Fig. 3) were found after 3 weeks. At this time the colonies stained the medium with brownish color which, however, did not prevent further development. The developed cell colonies were transferred 3 weeks after isolation of the protoplasts onto agar medium D2b where they showed a fast growth rate and developed a green color.

Calli showing an approximate size of 4-5mm were transferred onto the modified MS agar medium. (Fig. 4) First leave structures could be observed after 10-16 days of culture on this medium (Fig. 5). The calli which had developed leaves were transferred to MS agar medium containing a lower IAA and BAP concentration in order to facilitate the differentation of roots. With this treatment a large number of roots could be initiated. In two independent regeneration experiments 4 plantlets and 5 calli with roots were obtained (Fig. 6). The limited number of regenerated plantlets from protoplasts of *Digitalis lanata* demonstrates that more work has to be done to optimize the culture conditions for obtaining larger number of plants. However, it has been shown that it is, in general, possible to regenerate plants from a member of the *Scrophulariaceae*, a family from which up to now no successful protoplast regeneration has been reported.

Acknowledgement

This work was supported by Max-Planck-Gesellschaft of F.R.G. The author wishes to thank Dr. O. Schieder and Dr. G. Wenzel for reading the manuscript and for helpful discussions during his stay in Köln. He would also like to thank all his colleagues at the Max-Planck-Institut für Züchtungsforschung for preparing the materials and the photographs.

Literature

Diettrich, B.; Luckner, M. (1980): Isolation and characterization of protoplast derived clones from cell cultures of *Digitalis purpurea*. In: Adv. Protoplast Res. pp. 340-348. Budapest: Academiai Kiado

Hirotani, M.; Furuya, T. (1977): Restoration of cardenolide synthesis in redifferentiated shoots from callus cultures of *Digitalis purpurea*. Phytochemistry 16, 610-611

Li, Hsiang-hui; Yan, Qui-sheng; Huang, Mei-juan; Sun, Yung-ru; Li, Wen-bin (1980): Division of cells regenerated from mesophyll protoplast of wheat. In: Adv. Protoplast Res. pp. 261-267. Budapest: Academiai Kiado

Murashige, T.; Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15, 473-497

Reinhard, E.; Alfermann, A.W. (1980): Biotransformation by plant cell cultures. In: Adv. Biochem. Engineering (ed. Fiechter, A.) pp. 50-84. Berlin, Heidelberg, New York: Springer

Schieder, O.; Vasil, I.K. (1980): Protoplast fusion and somatic hybridization. In: Intern. Rev. Cytol; Acad. Press; (ed. Vasil, I.), New York: Supplement IIB, pp. 21-46

Stohs, S.J. (1980): Metabolism of steroids in plant tissues. In: Adv. Biochem. Engineering (ed. Fiechter, A.), pp. 85-107. Berlin, Heidelberg, New York: Springer

Vasil, I.K.; Vasil, V. (1980): Isolation and Culture of Protoplasts. In: Intern. Rev. Cytol. (supplement IIB. (ed. Vasil, I.), pp. 1-20. New York: Acad. Press

Prof. Dr. Li Xiang-hui
Institute of Genetics
Academia Sinica
Beijing (China)

Received May 7, 1981
Communicated by Hu Han