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# In vitro morphogenesis from excised leaf explants of *Digitalis obscura* L.

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ABSTRACT

The morphogenic capacity of *Digitalis obscura* leaf explants cultured in vitro has been studied, noting factors promoting the differentiation of roots, buds and shoots as well as those promoting callus proliferation. Complete plant regeneration was obtained only by first culturing the leaf explants in a medium with NAA and BA to induce formation of buds, and subsequently transferring them to a medium without growth regulators to achieve the further development of shoots.

*Abbreviations*: BA, benzyladenine; 2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, indoleacetic acid; NAA, naphthaleneacetic acid.

## INTRODUCTION

Plant regeneration from leaf discs has been previously described noting specific differences in hormone levels necessary to induce the organogenesis (Dunwell 1981).
It was observed that occasionally there was a reduction in the life cycle of shoots obtained by in vitro culture of leaves (Bhatt et al. 1979). This fact is potentially important as it may facilitate genetic studies such as those of somatic cell selection in species producing economically important secondary products.
To date, morphogenesis from leaf explants of *Digitalis*, an important genus as a source of cardiac glycosides, has only been studied in *D. lanata* (Hagimori et al. 1980) and *D. purpurea* (Hagimori et al. 1980; Rucker et al. 1981; Rucker 1982). In previous work we have determined factors that control the organogenesis from several explants of *Digitalis obscura* L., a species endemic to Spain (Pérez-Bermúdez et al. 1983). We will now report culture requirements promoting plant regeneration from leaf segments in this species.

## MATERIAL AND METHODS

Leaf segments from thirty day old seedlings obtained in sterile conditions were cultured in diverse media containing the Murashige and Skoog (1962) nutrients, sucrose 3% and different concentrations of auxins (IAA, NAA or 2,4-D) added alone or in combination with the cytokinin BA. The pH of the media was adjusted to 5,8. Agar (0,7%) was added prior to autoclaving. The cultures were maintained sixty days at 25±1°C with a light period of sixteen hours. All data are averages of two experiments with sixteen replications each. Relative callus growth was estimated by a visual rating, where 0 represents no growth and 3 maximal growth.
In preliminary experiments it was found that morphogenic responses did not vary when explants were placed with the adaxial or abaxial surface to the medium.

## RESULTS AND DISCUSSION

*Callus Induction*. The synthetic auxins 2,4-D or NAA (both at concentrations equal or greater than 0,5 ppm) promoted callus formation. The IAA by itself produced insignificant results, however, with the inclusion of BA callus induction was observed.
It is noteworthy that the BA showed a clear sinergism with the auxins, not only in the callus induction but also in their growth (Table 1).
*Rhizogenesis*. In the addition of IAA, or of NAA, there was the promotion of root differentiation from leaf segments of *D. obscura*, the optimal concentration being 1 ppm in both cases. On the contrary, none of the concentrations of 2,4-D tried promoted root formation. (Table 1).
The addition of BA to the different media supplemented with auxins inhibited or decreased the rhizogenetic response previously indicated (Table 1)
*Bud and Shoot Formation*. Bud differentiation was obtained only in the presence of NAA and BA. Other combinations such as IAA/BA or 2,4-D/BA did not promote caulogenesis.
The equal concentration of both growth regulators or the slight increase in BA (auxin/cytokinin ratios in the range of 0,1 to 1) provided the best results, whereas the increase of the auxin concentration with respect to the amount of cytokinin inhibited the caulogenetic response (Table 2)
Explants having buds were transferred into a culture medium without growth regulators. Sub

Table 1. Effects of different growth regulators on callus induction and rhizogenesis in *D. obscura* leaf cultures.

| AUXINS | (ppm) | BA (ppm) | CALLUS* GROWTH | %RHIZOGENIC EXPLANTS |
|---|---|---|---|---|
| | 0 | 0 | 0 | 0 |
| | 0 | 0.5 | 0 | 0 |
| | 0 | 1 | 0 | 0 |
| NAA | 0.1 | 0 | 0 | 0 |
| | 0.1 | 0.1 | 1 | 6 |
| | 0.1 | 1 | 1 | 0 |
| | 0.1 | 2 | 1 | 0 |
| | 0.5 | 2 | 2 | 21 |
| | 1 | 0 | 1 | 83 |
| | 1 | 0.1 | 1 | 54 |
| | 1 | 1 | 1 | 11 |
| | 1 | 2 | 2 | 3 |
| IAA | 0.1 | 0 | 0 | 44 |
| | 0.1 | 0.2 | 1 | 0 |
| | 0.1 | 1 | 1 | 0 |
| | 0.1 | 2 | 1 | 0 |
| | 1 | 0 | 0 | 56 |
| | 1 | 1 | 1 | 0 |
| | 1 | 2 | 2 | 0 |
| 2,4-D | 0.1 | 0 | 0 | 0 |
| | 0.1 | 0.1 | 1 | 0 |
| | 0.1 | 1 | 1 | 0 |
| | 0.5 | 0 | 1 | 0 |
| | 0.5 | 0.5 | 1 | 0 |
| | 0.5 | 1 | 1 | 0 |
| | 1 | 0 | 1 | 0 |
| | 1 | 0.1 | 1 | 0 |
| | 1 | 0.5 | 1 | 0 |
| | 1 | 1 | 1 | 0 |

* 0: no growth; 1: minimal growth; 2: intermediate growth

sequently, approximately 50% of the transferred buds developed shoots.
In those cases in which shoot regeneration was not accompanied by root formation, they were transferred into a medium containing IAA in a concentration of 0,5 ppm to achieve root development.
Factors regulating organogenesis in *D. obscura* leaf explants seem to be similar to those found in *D. purpurea* (Rucker et al. 1976, 1981; Rucker 1982). Rhizogenesis was promoted by the auxins IAA or NAA ($10^{-5}$-$10^{-6}$ M), this range of concentrations is practically the same required in *D. obscura* leaf cultures. In the same way, the authors observed that the presence of cytokinins inhibited the root formation.

Table 2. Effects of NAA and BA on caulogenesis in *D. obscura* leaf cultures.

| NAA (ppm) | BA (ppm) | %caulogenic explants | differentiated buds/developed shoots |
|---|---|---|---|
| 0 | 0 | 0 | 0/0 |
| 0 | 0.5 | 0 | 0/0 |
| 0 | 1 | 0 | 0/0 |
| 0.1 | 0 | 0 | 0/0 |
| 0.1 | 0.1 | 17 | 2/1 |
| 0.1 | 1 | 11 | 7/3 |
| 0.1 | 2 | 3 | 2/0 |
| 0.5 | 2 | 17 | 24/11 |
| 1 | 0 | 0 | 0/0 |
| 1 | 0.1 | 0 | 0/0 |
| 1 | 1 | 14 | 8/4 |
| 1 | 2 | 23 | 30/15 |

On the other hand, bud differentiation in *D. purpurea* leaves was limited to media containing IAA and the cytokinins BA or kinetin, the optimal combination being $10^{-6}$ M IAA/$10^{-5}$ M kinetin.
A comparison between these results and those previously obtained (Pérez-Bermúdez et al. 1983), leads to the conclussion that all *D. obscura* explants tested show a similar morphogenetic pattern. However, leaf explants were less sensitive to growth regulators, being as their responses were lower than those obtained in root, hypocotyl and cotyledon explants.

REFERENCES

Bhatt PN, Bhatt DP, Sussex IM (1979) Z Pflanzenphysiol 95: 355-362
Dunwell JM (1981) J Exp Bot 32: 789-800
Hagimori M, Matsumoto T, Kisaki T (1980) Plant Cell Physiol 21: 1391-1404
Pérez-Bermúdez P, Cornejo MJ, Segura J (1983) Plant Sci Lett 30: 77-82
Rucker W (1982) Z Pflanzenphysiol 107: 141-151
Rucker W, Jentzsch K, Wichtl M (1976) Z Pflanzenphysiol 80: 323-335
Rucker W, Jentzsch K, Wichtl M (1981) Z Pflanzenphysiol 102: 207-220