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A morphogenetic role for ethylene in hypocotyl cultures of *Digitalis obscura* L.

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ABSTRACT

The effect of exogenously applied ethylene on organogenesis in *Digitalis obscura* L. hypocotyls cultured in vitro was studied. Interactions of this gas with other growth regulators was also tested. Ethylene by itself only promoted root formation. Shoot regeneration was obtained in presence of indoleacetic acid and kinetin. The addition of ethylene (10 ppm) increased the caulogenetic action of this medium; higher concentrations than 10 ppm reduced this response. Kinetin alone did not promote organogenesis and nullified the promotive effect of ethylene on rhizogenesis.

Abbreviations: BM, basal medium; IAA, indoleacetic acid; Kn, kinetin.

INTRODUCTION

Evidences that low concentrations of ethylene may be required for modulating normal plant growth and development (4), have increased works concerning ethylene effects on cells and tissues grown in culture (1,2,3, 9). However, the obtained results do not make clear the role of ethylene on organogenesis since the effects show a wide range of variability depending on species, tissue in culture, presence of other growth regulators, etc.

Recently we have demonstrated that almost every part of *Digitalis obscura* L. seedlings has a high morphogenetic potential (7,8). From this data hypocotyl cultures were selected to study the effects of exogenously supplied ethylene on organogenesis. The possibility of an interaction of this hormone with other plant growth regulators (IAA and Kn) was also tested.

MATERIALS AND METHODS

Digitalis obscura L. seedlings were grown from seeds cultured under sterile conditions (16 hours photoperiod at 26° C). Normal-grown hypocotyls of 25-day-old seedlings were selected for the experiments.

Hypocotyl segments were cultured on a basal medium (BM) containing Murashige and Skoog nutrients (6), 3% sucrose and 0.8% agar (Difco-Bacto); pH=5.8. Medium had been autoclaved at 120° C for 20 minutes.

Ethylene action was studied on cultures containing BM, or BM supplemented with Kn (2 ppm) or IAA/Kn (0.5/2 ppm). Ethylene (1,5, 10,50 and 100 ppm) was injected into tubes sealed with rubber serum caps as described by Mingo-Castel et al. (5). Two controls were tested: open controls with caps allowing gas interchange, and closed controls sealed and provided with ethylene traps (0.2M $Hg(ClO_4)_2$ in 2M $HClO_4$).

Cultures were maintained for 16 hours in light at 26° C being observed weekly. Final data are average of three experiments with 24 replications each.

RESULTS AND DISCUSSION

Effects of Ethylene on Root Regeneration.

The effect of exogenous ethylene on root formation in *D. obscura* hypocotyl segments is shown in figure 1. Ethylene concentration ranging from 1 to 50 ppm lead to root formation, being optimal at 10 ppm. Removal of the gas by using $Hg(ClO_4)_2$ strongly decreased rooting percentage. From these data we suggest that ethylene could act as endogenous promoter of root regeneration in *D. obscura* hypocotyls segments cultured in vitro.

These results corroborate those obtained by Cornejo-Martín et al. (2) working with rice callus, but do not those reported by Coleman et al. (1) who concluded, from studies on root regeneration in tomato leaf dics, that ethylene was not a rooting hormone per se.

The ethylene-induced rooting was nullified by Kn. Furthermore, the presence of ethylene increased the well known antagonism IAA-Kn in relation to rhizogenesis (Table 1); thus adventitious root formation was strongly inhibited by all ethylene concentrations. This inhibition affected both, rooting explants percentage and root density.

Effects of Ethylene on Shoot Formation.

Ethylene did not promote shoot regeneration in hypocotyls cultured under conditions

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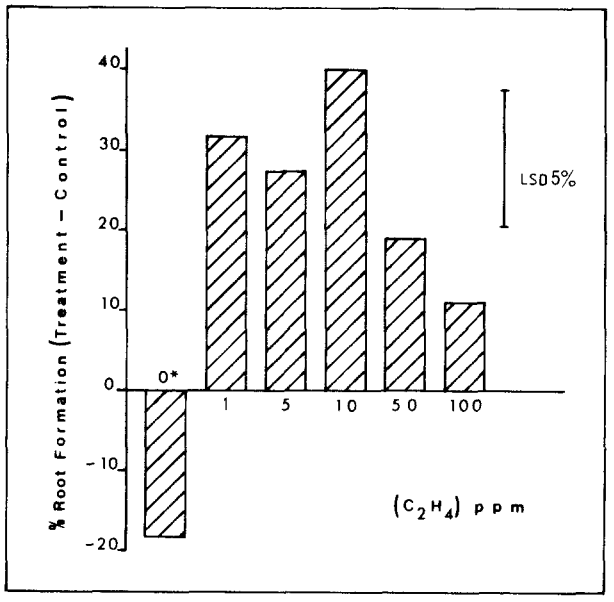

Figure 1. Effect of ethylene on root formation in *Digitalis obscura* hypocotyls cultured on a medium without growth regulators. 0* means cultures with C_2H_4 traps. Values represent differences in root formation percentage between ethylene treated hypocotyls and the control. LSD 5%= 17.1%

such as BM or BM supplemented with Kn. Nevertheless, the ethylene modified the caulogenetic responses induced by 0.5 ppm IAA and 2ppm Kn (Table 2). Low concentrations did not show significant differences with the open control; however, 10 ppm significantly promoted shoot regeneration. Ethylene concentrations higher than 10 ppm strongly inhibited the normal shoot development. Hypocotyl cultures with gas traps also showed lower percentage of shoot regeneration.

Table 1. Effect of ethylene on rhizogenesis in *Digitalis obscura* hypocotyls cultured on a medium with IAA/Kn (0.5/2 ppm). 0* means cultures with C_2H_4 traps.

C_2H_4 (ppm)	% Explants with roots	Number of roots per explant
Control	47.5	1.4 a
0*	19.6	0.6 bc
1	20.8	0.6 bc
5	27.8	0.8 b
10	27.1	0.8 b
50	16.7	0.5 c
100	12.5	0.4 c

Values followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Table 2. Effect of ethylene on caulogenesis in *Digitalis obscura* hypocotyls cultured on a medium with IAA/Kn (0.5/2 ppm). 0* means cultures with C_2H_4 traps.

C_2H_4 (ppm)	Number of shoots obtained	% Shoot regeneration
Control	3.9	16.4 b
0*	2.5	10.7 c
1	3.5	14.6 bc
5	4.7	19.4 b
10	6.0	25.0 a
50	1.0	4.2 d
100	0.5	2.1 d

Values followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Huxter et al.(3), working with tobacco callus grown in a medium containing IAA and Kn, found that low concentrations of ethylene or ethrel speed up the rate of primordia emergence, although they did not find differences in the final number of shoots developed. On the other hand, Cornejo-Martín et al. (2) showed that ethylene promotes shoot regeneration from rice callus cultured in a medium without growth regulators, which otherwise would not support it. Then, ethylene action appears depending not only on the presence of other growth regulators, but also on the plant species used.

Our results lead us to propose that ethylene is involved in the control of shoots development from *D. obscura* hypocotyls. Although ethylene by itself does not promote shoot induction, it could act as a modulator of the promotive effect of IAA and Kn.

As Lieberman(4) proposed, ethylene may be a modulator of the action of plant hormones in growth and development, and conversely other hormones may modulate the action of ethylene in ripening, aging and senescence.

Finally, it is worth noting that ethylene did not affect the callus formation induced by IAA and Kn. Neither Kn nor ethylene by themselves or in combination promoted callus induction. We have noted that necrosis and malformations of the tissues appeared when the ethylene concentration in the culture atmosphere was higher than 10 ppm.

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REFERENCES

1 Coleman WK, Huxter TJ, Reid DM, Thorpe TA (1980) Physiol. Plant. 48:519-525.

2 Cornejo-Martín MJ, Mingo-Castel AM, Primo-Millo E (1979) Z. Pflanzenphysiol. 94:117-123.
3 Huxter TJ, Thorpe TA, Reid DM (1981) Physiol. Plant. 53:319-326.
4 Lieberman M (1979) Ann. Rev. Plant Physiol. 30:533-591.
5 Mingo-Castel AM, Negm FB, Smith OE (1974) 53:798-801.
6 Murashige T, Skoog F (1962) Physiol. Plant. 15:473-497.
7 Pérez-Bermúdez P, Cornejo MJ, Segura J (1983) Plant Sci. Lett. 30:77-82.
8 Pérez-Bermúdez P, Brisa MC, Cornejo MJ, Segura J (1984) Plant Cell Reports 3:8-9.
9 Zobel RW, Roberts LW (1978) Can. J. Bot. 56:987-990.