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Difluoromethylornithine counteracts effects of auxins and inhibitors of polar auxin transport on plant development

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Abstract

Difluoromethylornithine (DFMO) counteracted several processes that are promoted or inhibited by auxins or inhibitors of polar auxin transport: inhibition of asymmetric gene expression in carrot, stimulation of gametic embryogenesis in *Brassica*, inhibition of root elongation in tobacco, inhibition of the development of lateral roots in pea and adventitious roots in apple, and inhibition of floral bud formation in *Arabidopsis*.

Additional key words: adventitious roots, embryogenesis, floral buds, lateral roots, root elongation.

Introduction

Indole-3-acetic acid (IAA), the most common naturally occurring auxin, regulates the development of polarity in plant cells and tissues (*e.g.* Warren Wilson and Warren Wilson 1993 and references therein). In somatic embryogenesis in carrot, exogenously supplied auxins and inhibitors of polar auxin transport inhibit the formation of embryos from proembryogenic masses, presumably by disrupting an internal IAA gradient necessary for polarized growth (Michalczuk *et al.* 1992). Difluoromethylornithine (DFMO) counteracts the inhibition by 2,4-dichlorophenoxyacetic acid (2,4-D) (Robie and Minocha 1989) and other auxins and inhibitors of polar auxin transport but not by the antiauxin 2-(*p*-chlorophenoxy)-isobutyric acid

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Abbreviations: CPIB - 2-(*p*-chlorophenoxy)-isobutyric acid (also abbreviated PCIB); 2,4-D - 2,4-dichlorophenoxyacetic acid; DFMO - D,L-α-difluoromethylornithine; HFCA - 9-hydroxyfluorene-9-carboxylic acid; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; NPA - N-1-naphthylphthalamic acid; TIBA - 2,3,5-triiodobenzoic acid.

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(CPIB) (Nissen and Minocha 1993), again indicating the requirement for an internal IAA gradient.

In the present study it was examined whether the effect of auxins and inhibitors of polar auxin transport is counteracted by DFMO in other systems. This may yield information on the requirement for polar auxin transport and internal auxin gradients in plant development and, presumably, on the as yet puzzling mechanism of action of DFMO in counteracting effects of auxins and inhibitors of polar auxin transport.

Materials and methods

Experimental solutions and media: All experimental compounds were added in the form of filter-sterilized stock solutions. The solutions and media were not changed in the course of the experiments. DFMO was as indicated used at 1 or 5 mM, *i.e.* at concentrations somewhat suboptimal for somatic embryogenesis in carrot (Nissen and Minocha 1993), except for the experiments on floral bud formation in *Arabidopsis* where concentrations higher than 0.1 mM proved inhibitory.

Asymmetric gene expression in carrot: The embryogenic carrot (*Daucus carota* L.) line 58-1-5 transformed with the T-DNA of the plasmid pGUS1B1 (Mattsson *et al.* 1992) was obtained from P. Engström. The culture was grown and the experiments were performed essentially as described by Nissen and Minocha (1993). The GUS assay was as described by Mattsson *et al.* (1992). The experiments were counted "blind" to avoid any bias in the scoring of the globular embryos as polarly or uniformly stained.

Gametic embryogenesis in *Brassica*: Plants of rape seedling (*Brassica napus* L. cv. Topas) were grown in a climate-controlled room under a 16-h photoperiod of approximately 200 μmol m^{-2} s^{-1} of white fluorescent light at 14 °C in the light and 10 °C in the dark. Buds were harvested when the microspores were at the late uninuclear stage. The isolation and culture of the microspores were essentially as described by Hansen and Svinnset (1993).

Root elongation in tobacco: Tobacco (*Nicotiana tabacum* L. cv. W38) seeds were germinated on filter paper wetted with the treatment solutions and were incubated for 9 d at 24 °C and a 16-h photoperiod of approximately 90 μmol m^{-2} s^{-1} of white fluorescent light.

Development of lateral roots in pea and tobacco and adventitious roots in apple: Pea (*Pisum sativum* L. cv. Alaska) seeds were sown in vermiculite essentially as described by Hinchee and Rost (1992). Seedlings with roots about 2.5 cm long were placed in 1.5-cm^3 Eppendorf tubes containing 1 cm^3 of experimental solution and incubated in the dark at 24 °C. The number of lateral roots > 1 mm were counted after 3 d.

For the development of lateral roots, tobacco seeds were germinated and incubated as described above but for 16 d.

The apple (*Malus* × *domestica* Borkh. cv. Jork 9) stem disc system was as described by Welander and Pawlicki (1993). The slices were incubated with 25 μM indole-3-butyric acid (IBA) in the dark for 1 d and then transferred to the light and the experimental media for 7 d before rooting was determined.

Floral bud formation in *Arabidopsis*: *Arabidopsis thaliana* ecotype Columbia seeds were surface sterilized and sown on agar in Magenta boxes using the medium given by Okada *et al.* (1991). The plants were grown at 24 °C and a 16-h photoperiod of approximately 90 μmol m^{-2} s^{-1} of white fluorescent light for about 2 months.

Results

Asymmetric gene expression in carrot: In a carrot line transformed with the T-DNA gene 5 promoter fused with GUS (Mattsson *et al.* 1992), the percentage of polarly stained globular embryos slightly decreased in the presence of IAA or markedly decreased in the presence of 2,4-D (Fig. 1). This decrease was counteracted by DFMO. DFMO itself caused a small but consistent increase in the percentage of polarly stained embryos (Fig. 1 and several unpublished experiments). This effect cannot, however, account for the marked reversal of the inhibition by IAA and 2,4-D.

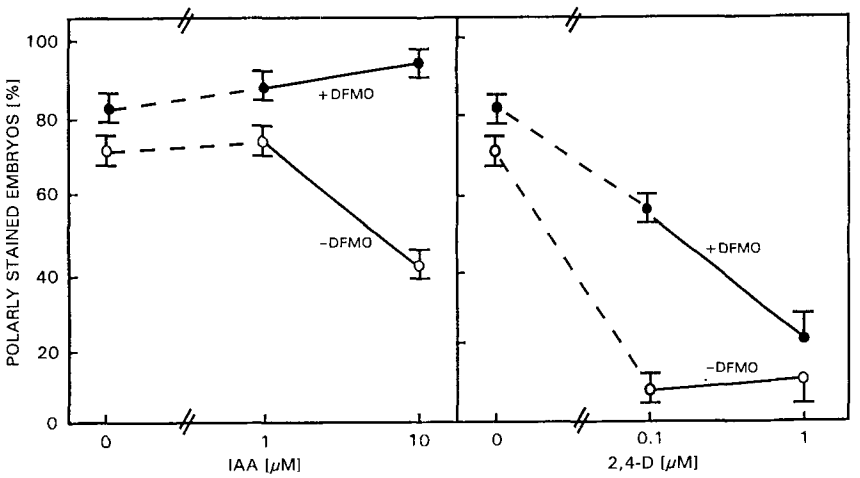

Fig. 1. Effects of IAA, 2,4-D and DFMO (5 mM) on the percentage of polarly stained globular embryos in the transgenic carrot line 58-1-5. Staining determined after 7 d. Means ± SE (n = 8 for control, n = 4 for treatments).

Gametic embryogenesis in *Brassica*: A microspore culture of rape seedling required 30 μM IAA for maximal embryogenesis (Fig. 2). However, embryogenesis at this IAA concentration was abolished when DFMO was also present in the medium. In contrast, DFMO promoted embryogenesis at lower concentrations of IAA where there was little or no embryogenesis.

Root elongation in tobacco: 2,4-D and the polar auxin transport innibitors 2,3,5-triiodobenzoic acid (TIBA), N-1-naphthylphthalamic acid (NPA) and 9-hydroxyfluorene-9-carboxylic acid (HFCA) inhibited growth of the primary root of tobacco. The antiauxin 2-(*p*-chlorophenoxy)-isobutyric acid (CPIB) had no effect (Table 1). There was a statistically significant reversal by DFMO of the inhibition by 2,4-D, NPA and HFCA despite the fact that DFMO by itself did not stimulate root growth. The apparent lack of an effect on the inhibition by TIBA could have been caused by the low inhibition by TIBA in this experiment, in line with more detailed

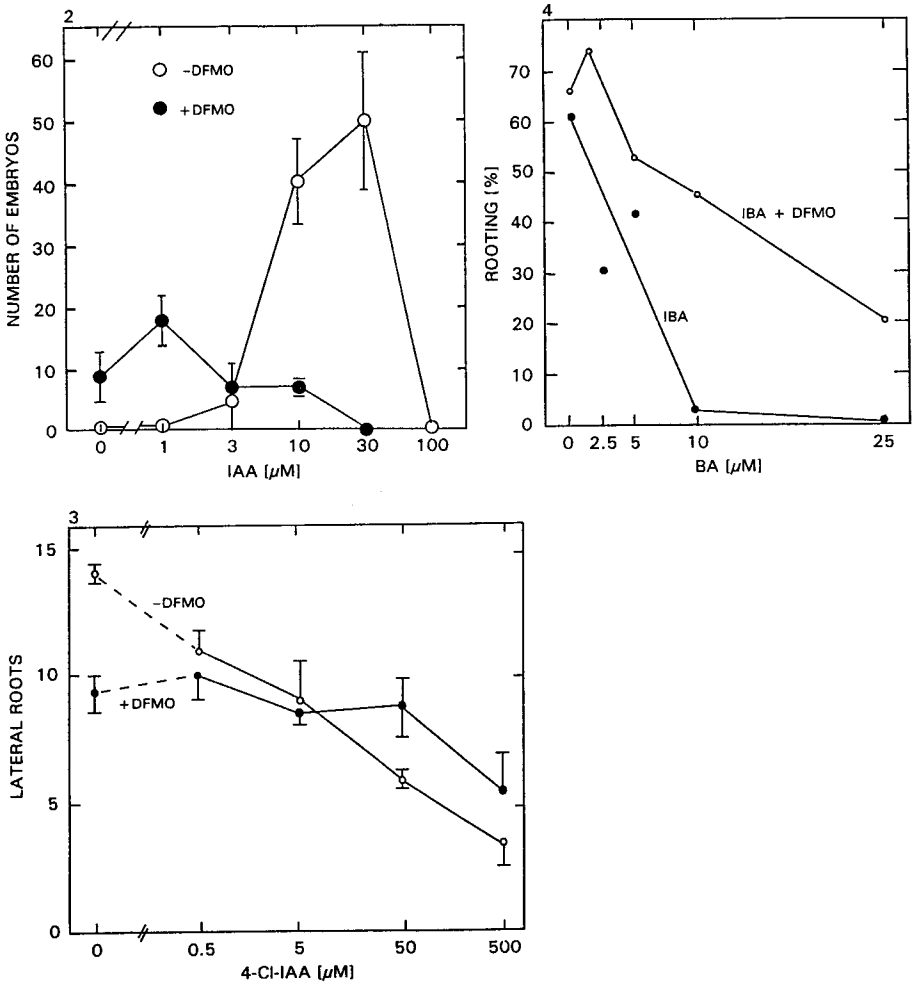

Fig. 2. Effects of IAA and DFMO (5 mM) on embryo formation in a microspore culture of *Brassica napus*. Embryos counted after 17 d. Means ± SE ($n = 3$).
Fig. 3. Effects of 4-Cl-IAA and DFMO (5 mM) on development of lateral roots in pea. Means ± SE ($n = 8$).
Fig. 4. Effects of IBA and DFMO (5 mM) on development of adventitious roots in thin slices of apple stem.

findings for somatic embryogenesis in carrot (Nissen and Minocha 1993). Root growth in the presence of CPIB was, as expected, not affected by DFMO.

Development of lateral roots in pea and tobacco and adventitious roots in apple: Auxins stimulate the initiation of lateral roots but inhibit their development. The inhibition of the development of lateral roots in pea by 4-Cl-IAA was counteracted by DFMO (Fig. 3). The reversal was complete or nearly so for 4-Cl-IAA concentrations up to 50 μM when the one-third inhibition by DFMO in the absence of the auxin is taken into account.

Although the elongation of tobacco roots was not affected by DFMO (Table 1), the development of lateral roots was almost completely inhibited (Table 2). Neither L-ornithine, a precursor of polyamine biosynthesis via the ornithine pathway, nor the phytotropin NPA had any effect.

The inhibition by IBA of root development in thin slices of apple stem was also markedly counteracted by DFMO (Fig. 4).

Table 1. Effects of 2,4-D, the polar auxin transport inhibitors TIBA, NPA and HFCA, the antiauxin CPIB and DFMO (1 mM) on root growth in tobacco. Length of root in mm. Means ± SE of 5 Petri dishes, each with 25 seeds. Significance symbols: n.s. - not significant ($P > 0.10$), (*) - $0.05 < P \leq 0.10$, * - $0.01 < P \leq 0.05$, ** -$0.001 < P \leq 0.01$, *** - $P \leq 0.001$ (first column, significance of difference from control; second column, significance of effect of DFMO).

Treatment	-DFMO	+DFMO
Control	8.18 ± 0.35	7.66 ± 0.29 n.s.
1 μM 2,4-D	5.12 ± 0.19 ***	6.75 ± 0.41 **
30 μM TIBA	7.11 ± 0.14 *	7.20 ± 0.13 n.s.
30 μM NPA	5.46 ± 0.19 ***	6.34 ± 0.25 *
300 μM HFCA	5.70 ± 0.26 ***	6.34 ± 0.22 (*)
300 μM CPIB	8.44 ± 0.15 n.s.	8.31 ± 0.29 n.s.

Table 2. Effects of DFMO, L-ornithine and NPA on development of lateral roots in tobacco. Percentage of plants with lateral roots. Means ± SE of 15 Petri dishes, each with 25 seeds.

Treatment	%
Control	89.6 ± 1.5
1 mM DFMO	8.7 ± 1.8
0.1 mM L-ornithine	89.7 ± 1.7
50 μM NPA	83.1 ± 2.2
50 μM NPA + 1 mM DFMO	1.4 ± 0.6

Floral bud formation in *Arabidopsis*: *Arabidopsis* plants cultured in the presence of phytotropins develop structural abnormalities resembling those of the pin-formed flower mutant *pin* 1, indicating that polar auxin transport is required in the early stages of floral bud formation (Okada *et al.* 1991). The inclusion of DFMO in the culture medium counteracted the formation of pin-type plants caused by NPA

(Table 3). In two of the three experiments in Table 3 the reversal was complete, in the third it was only partial.

Table 3. Effects of NPA (10 μM) and DFMO (0.1 mM) on flower morphology in *Arabidopsis thaliana*. Number of plants with normal and pin-type morphology. Means ± SE of 3 experiments.

	−DFMO		+DFMO	
	normal	pin	normal	pin
−NPA	19 ± 4	0	19 ± 11	0
+NPA	2 ± 1	9 ± 2	10 ± 4	4 ± 4

Discussion

It appears that DFMO is able to reverse widely different effects of auxins and inhibitors of polar auxin transport on plant development, irrespective of whether these effects are negative (Figs 1, 3 and 4, Tables 1 and 2) or positive (Fig. 2). The auxin 2,4-D and the polar auxin transport inhibitor TIBA similarly stimulated the formation of embryogenic tissue in Norway spruce when added to a medium containing benzyladenine. The addition of DFMO to these media abolished the formation of embryogenic tissue (H. Kvaalen, personal communication). The opposing effects of auxin and DFMO are most clearly seen in Fig. 2 where DFMO caused some embryogenesis to occur in the absence of IAA while it was inhibitory in its presence.

These findings for a variety of systems are similar to previous results for somatic embryogenesis in carrot (Nissen and Minocha 1993), namely that DFMO counteracts the effects of auxins and inhibitors of polar auxin transport. I conclude that development requires the generation and maintenance of an internal IAA gradient.

The ability of DFMO to counteract the inhibition by auxins and inhibitors of polar auxin transport appears to be unrelated to its sometimes stimulatory effect in the absence of these compounds. DFMO itself is quite often without effect or even inhibitory (Table 1, Fig. 3, see also Nissen and Minocha 1993). We may here be seeing two quite different effects of DFMO. It appears conceivable that the action of DFMO in reversing the effects of auxins and inhibitors of polar auxin transport is not related to its inhibition of ornithine decarboxylase activity and thus of polyamine biosynthesis via the ornithine pathway.

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