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Morphogenetic Effects of Endomycorrhiza Formation on the Root System of *Calluna vulgaris* (L.) Hull

G. BERTA[1], V. GIANINAZZI-PEARSON[2], G. GAY[3] and G. TORRI[1]

[1] *Dipartimento di Biologia Vegetale dell'Università*
Viale Mattioli 25, 10125 Torino, Italy
[2] *Station d'Amélioration des Plantes, INRA, BV 1540, 21034, Dijon Cedex, France*
[3] *Université Claude Bernard, Lyon I, Departement de Biologie Vègètale, 43, Bd du 11 Novembre 69622 Villeurbanne Cedex, France*
Tel. [1] *011 6699884* [2] *80 693146* [3] *7 8898124 Telex* [2] *INRADIJ 350507 F*

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Abstract

Root development of *Calluna vulgaris* (L.) Hull inoculated with *Pezizella ericae* Read has been studied under simulated soil conditions. Modifications in root morphogenesis of the host plant only occur with the establishment of the mycorrhizal infection. Under similar conditions the ericoid endomycorrhizal fungus shows an IAA synthesizing activity, in the presence of tryptophan. The possibility that the mycorrhizal effect on root development may be hormonal in origin is discussed.

Keywords: endomycorrhizae, calluna, root morphogenesis, hormones

1. Introduction

In a previous study it was shown that the pattern of root development in mycorrhizal seedlings of *Calluna vulgaris* (L.) Hull differs from that of non-mycorrhizal ones, and it was suggested that these differences may be of hormonal origin (Berta and Gianinazzi-Pearson, 1986). Mycorrhizal infection, however, was already established in the plants examined and it was therefore not possible to determine whether such effects resulted from mycorrhizal formation or whether they were linked to an eventual activity of the

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fungus in the rooting medium (hormonal, detoxification...). Preliminary work by Gay and Debaud (1986) indicated that ericoid endomycorrhizal fungi can synthesize indole-3-acetic acid (IAA) when cultured on a mineral medium supplemented with tryptophan. In this paper we present evidence that under simulated soil conditions modifications in root morphogenesis of the host plant only occur with establishment of the mycorrhizal infection, and that under similar conditions ericoid endomycorrhizal fungi can have an indole-3-acetic acid (IAA) synthesizing activity.

2. Materials and Methods

Morphogenetic observations on root systems

C. vulgaris (L.) Hull seeds were aseptically germinated and grown for 6 weeks on water-agar (0.75%) to which sterile heathland soil had been added, as described by Pearson and Read (1973). Half of the tubes were inoculated with a suspension of a macerated culture of *Pezizella ericae* Read (Read, 1974). Plants were placed in a growth cabinet (day-night temperature 20/15°C, irradiance 30 J $m-2s^{-1}$, 16 hr day) and at weekly intervals, whole root systems of 6 randomly chosen inoculated and uninoculated seedlings were excised and fixed in 3% glutaraldehyde-cacodylate buffer (0.1 M, pH 7.2). Shoot fresh weight of individual seedlings was recorded. One root system from each sample was embedded in Durcupan ACM and root tip morphology examined in median longitudinal sections after staining with 1% toluidine blue. Remaining root systems were stained in 0.1% lactic acid-cotton blue and each root tip was examined microscopically to determine whether it was active, semiactive or inactive. Active root tips were stained strongly, inactive apices were not stained and semiactive ones were intermediate (Figs. 1,2). Numbers and length of primary roots and hair roots were estimated microscopically, using an ocular micrometer.

Estimation of IAA-synthesizing activity of P. ericae in pure culture

Culture conditions

Mycelium was grown in Petri dishes for 15 days, in the absence of the host plant, on the sterile soil-water agar medium used for mycorrhizal synthesis. This medium was either unsupplemented or supplemented with 2.78 mM glucose and covered with a sterile cellophane sheet to prevent the mycelium from growing down into the agar medium (Gay and Debaud, 1987). Each dish was inoculated with a 3 mm disc of inoculum cut from the margin of 2 week old cultures developing on the N_2P_3 agar medium described by Gay

(1986). Cultures were incubated at 22 ± 1°C, in the dark.

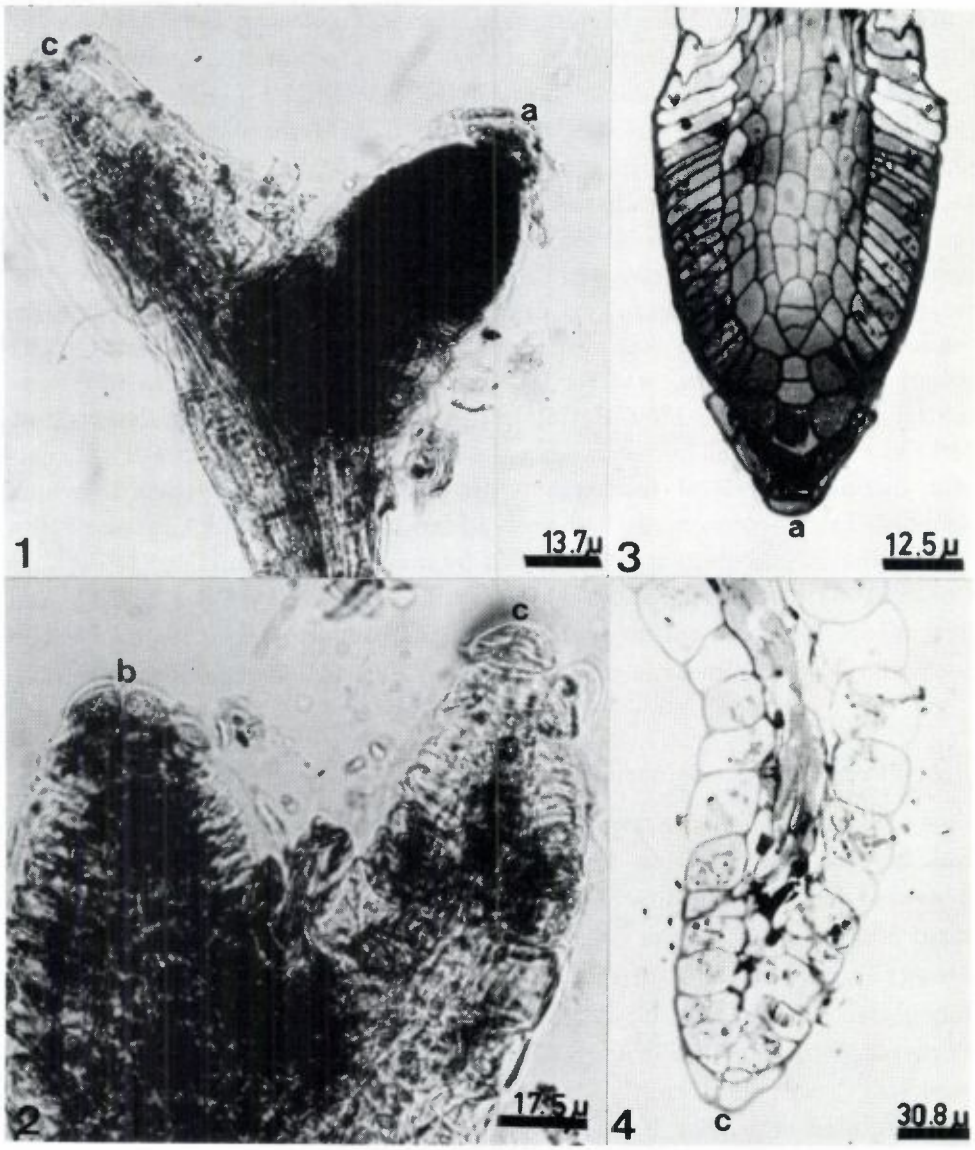
In vivo IAA synthesizing activity of the mycelia

At the end of the culture period, mycelium was collected and it's IAA synthesizing activity determined by measuring IAA production after incubation, for 1, 4 or 6 days in the dark, in sterile 10 mM MOPS (3-(N-morpholino) propane sulfonic acid), pH 6.0, containing 10 mM filter-sterilized tryptophan. Mycelia incubated in the absence of tryptophan served as controls. IAA released into the incubation medium was identified by HPLC as described by Rouillon et al. (1986). Ten ml of incubation medium were adjusted to pH 3.0 and extracted 3 times with 7.5 ml ethyl acetate. The extract was then evaporated to dryness under vacuum at 30 ± 1°C, subsequently solubilized in 300 μl of acetonitrile and analysed by HPLC. The column (4 mm×30 cm) was RP 18 and the solvent system was 25% acetonitrile, 74.9% H_2O, 1% CH_3COOH. Indole compounds were detected at 280 nm and identified by comparing the elution pattern from the HPLC column during analysis of an extract with that of the same extract to which standard indole compounds had been added (Gay, 1987). IAA released into the incubation medium was quantified by a colorimetric method (Pilet and Collet, 1962) using the Salkowski reagent, modified by Pilet (1957), and previously used with ectomycorrhizal fungi (Rouillon et al., 1986). Protein content of mycelium was estimated according to Lowry et al. (1951).

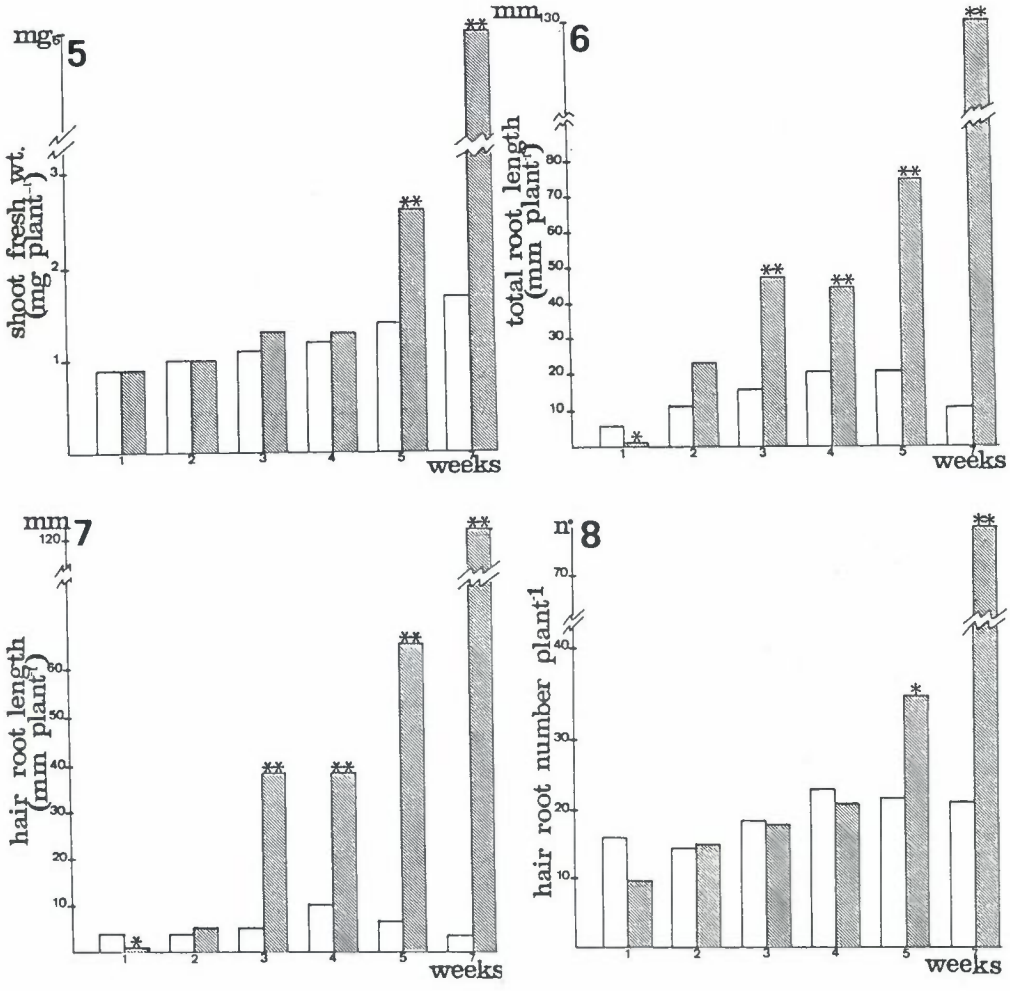
3. Results

Root morphogenesis of inoculated and uninoculated C. vulgaris seedlings

Mycelium of *P. ericae* developed rapidly in the soil-agar rooting medium, but mycorrhizal infection was only observed in inoculated plants after 3 weeks' growth. The mycorrhizal effect on shoot growth was not evident until 2 weeks later, at the fifth harvest, and it increased up to the last harvest (Fig. 5). Changes in root development were already apparent in the inoculated seedlings at the third harvest, coinciding with the establishment of mycorrhizal infection (Figs. 6,7). From the third to the seventh week total root length was significantly greater in inoculated seedlings as compared to uninoculated ones (Fig. 6). This was related to an increase in the length of hair roots in the inoculated plants (Fig. 7) rather than to a change in primary root length, which remained similar in the uninoculated (7.1–8.8 mm plant^{-1}) and inoculated (6.3–9.2 mm plant^{-1}) seedlings throughout the experiment. The number of hair roots (Fig. 8) produced per inoculated plant was signifi-



Figures 1–4. Whole preparations (1,2) and longitudinal sections (3,4) of active (a), semi-active (b) and inactive (c) hair root apices of mycorrhizal *C. vulgaris* stained with cotton blue (1,2) or toluidine blue (3,4).



Figures 5–8. Measurements of shoot (5) and root (6–8) production of *P. ericae* inoculated and uninoculated seedlings of *C. vulgaris*.

Table 1. Hair root production and root apex activity of *P. ericae*- inoculated (I) and uninoculated (NI) seedlings of *C. vulgaris*

Weeks growth	Hair root length		Percentage of apices					
			Active		Semiactive		Inactive	
	Total root length							
	NI	I	NI	I	NI	I	NI	I
1	66.1	74.0	39.8	21.7	25.9	21.6	34.3	56.7
2	28.9	23.0	46.5	46.4	25.7	25.0	27.8	28.6
3	30.1	80.8	41.4	40.6	32.3	20.3*	26.3	39.1
4	47.9	84.8	42.4	42.6	21.8	16.0*	35.8	41.4*
5	30.5	87.9	39.7	45.1	25.9	14.7*	34.2	40.2*
7	32.6	93.7	30.5	33.3	44.5	16.1**	25.0	50.6**

*,**significantly different from control at $P<0.05$ and $P<0.01$ (analysis of variance).

cantly greater than in uninoculated ones at the fifth harvest, and paralleled the increased shoot production in the mycorrhizal seedlings (Fig. 5). This effect of myocrrhizal infection on hair root growth in *C. vulgaris* was also shown by the fact that hair root always formed a greater proportion of the root system in inoculated mycorrhizal seedlings as compared to uninoculated ones (Table 1).

Histological examination of root tips confirmed that those staining strongly with cotton blue had the typical morphology of an active meristem (Figs. 1,3), while completely unstained root tips were parenchymatous (Figs. 2,4) and therefore inactive (D'Amato, 1960). There was no significant effect of *P. ericae* inoculation on the proportion of root apices that were active in the *C. vulgaris* seedlings. However, the percentage of inactive and semiactive apices was modified. The percentage of inactive apices was always greater in roots of inoculated uninfected and infected plants as compared to those of uninoculated plants, and differences were significant from the fourth harvest onwards (Table 1). In contrast, semiactive apices were more numerous in uninoculated than inoculated plants and differences were already significant after 3 weeks.

IAA synthesis by P. ericae

P. ericae did not release detectable amounts of indole compounds when incubated in the absence of tryptophan. When incubated in the presence of

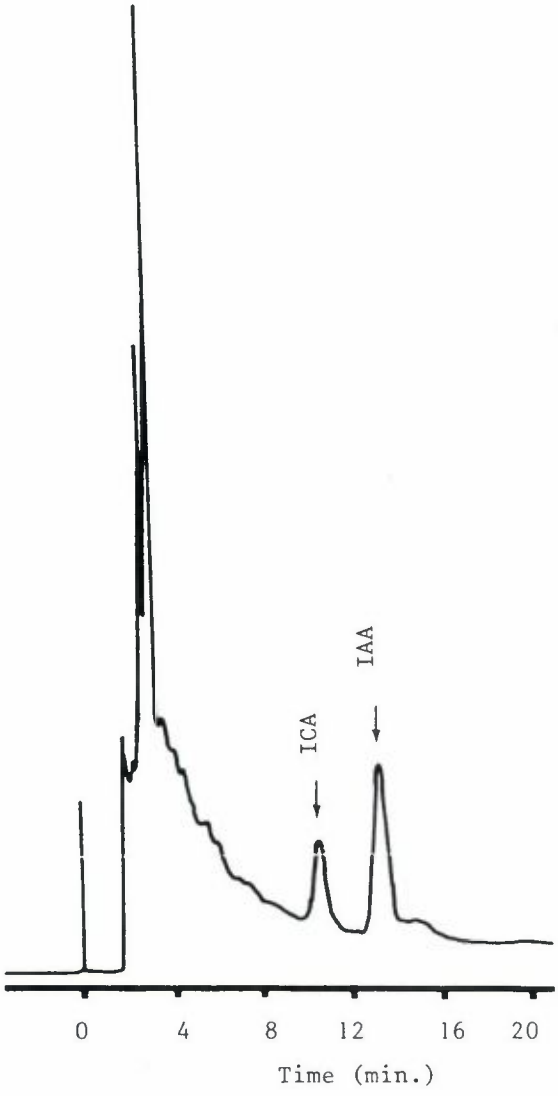


Figure 9. Elution pattern from the HPLC column during analysis in the indole compounds release by *P. ericae* pre-cultured on a soil-water medium and incubated in the presence of 10 mM tryptophan buffered with 10 mM MOPS, pH 6.0. Sample injection: 5 μl, flow rate: 1 ml min^{-1}, detector sensitivity: 0.2.

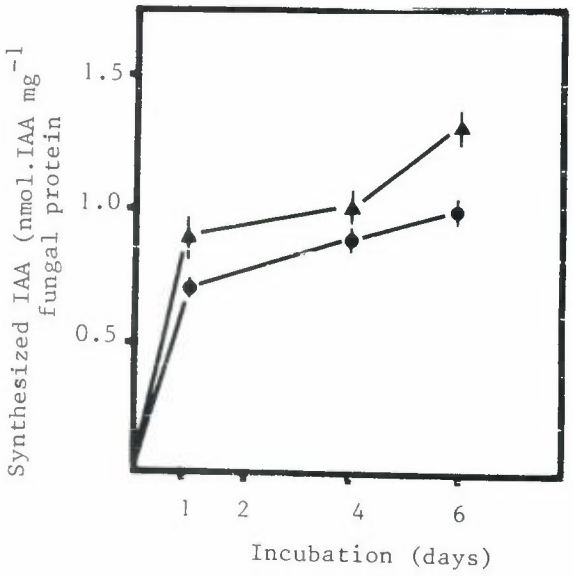


Figure 10. *in vivo* IAA synthesizing activity of mycelium of *P. ericae* pre-cultured on a soil-water agar medium supplemented (▲) or not (•) with 2.78 mM glucose and incubated in the presence of 10 mM tryptophan buffered with 10 mM MOPS, pH 6.0.

tryptophan, mycelium pre-cultured on the medium used for mycorrhizal synthesis released indole compounds into the incubation medium. The elution pattern from the HPLC column during the analysis of the indole compounds released by *P. ericae* presented a first peak at 280 nm, showing a retention time of 3 min 12 sec (Fig. 9), which corresponded to residual tryptophan remaining in the thyl acetate fraction during IAA extraction. Elution patterns showed two additional peaks at 280 nm, having retention times respectively of 10 min 42 sec and 13 min 30 sec; these compounds were identified by reference to standard indole compounds as being indole-3-carboxylic acid (ICA) and IAA, respectively. Rouillon et al. (1986) demonstrated that ICA does not react with the Salkowski reagent modified by Pilet (1957), so that this reagent can be considered as specifically revealing IAA released by *P. ericae* in pure culture. IAA release by *P. ericae* when incubated in the presence of 10 mM tryptophan was very rapid for the first day of incubation (0.7–0.9 nmol IAA synthesized 24 hr^{-1} mg^{-1} mycelial protein) and slowed down later (Fig. 10).

The presence of 2.78 mM glucose in the soil-water agar medium used to culture the fungus only slightly affected the IAA synthesizing activity of the mycelium.

4. Discussion

As previously reported (Barta and Gianinazzi-Pearson, 1986), mycorrhizal infection influences the pattern of root development in *C. vulgaris* in axenic culture. The present study clearly shows that changes in hair root production are directly associated with establishment of the mycorrhizal infection and eliminates simple detoxification of the rooting medium by the growing fungus as a satisfactory explanation for the observed morphogenetic effects. Furthermore, the time difference between the early modifications in hair root length, and the appearance of a growth response in the shoot make nutritional effects an unlikely cause. This could, on the contrary, easily explain the later increase in hair root number which coincides with the improved shoot production in the mycorrhizal plants.

The greater percentage of inactive apices observed in roots of mycorrhizal *C. vulgaris* is in agreement with previous observations (Berta and Bonfante-Fasolo, 1983; Berta and Gianinazzi-Pearson, 1986). This phenomenon does not seem to be due to a blocking effect of the fungus on meristem cell division, as suggested to occur in ectomycorrhizal and vesicular-arbuscular endomycorrhizal systems (Harley and Smith, 1983; Berta et al., 1983; Fusconi et al., 1986). In fact, contrary to the situation in the latter where roots become more numerous, branched and shorter with mycorrhizal infection, in *C. vulgaris* both total and hair length plant increase with mycorrhizal establishment, whereas the effect on root number is somewhat reduced. A possible explanation for this root development in *C. vulgaris* could be that the meristematic activity of hair roots is stimulated, so that these reach their maximum length more quickly. This could lead to a more rapid ageing, and inactivation of root apices. Alternatively, or perhaps simultaneously, in mycorrhizal roots there may be synchrony of the apical cells which all stop dividing at the same moment, so that at any one time there are few semiactive apices and a high proportion of inactive ones. In roots of uninoculated *C. vulgaris* seedlings, in contrast, this synchronization may not occur so that cells are in different states of division and differentiation, giving a higher proportion of apices with a semiactive morphology.

Changes in hormone levels have been suggested to be responsible for modifications in root development, and in particular for alterations in the activity

of root apices (Trewavas, 1985). *P. ericae* pre-cultured on the soil-water agar medium used for mycorrhizal synthesis is able to release IAA and ICA, an intermediate of IAA breakdown (Gaspar et al., 1982), when incubated in the presence of tryptophan. Although results recorded under pure culture conditions should be extrapolated with caution to the symbiotic association, it does appear from the present work that *P. ericae* has enzymes which probably enable it to synthesize and release IAA in the symbiotic association. IAA release by the fungal associate in the symbiotic condition is no doubt lower than that recorded under pure culture conditions, especially because of the low tryptophan concentration in root exudates (Bowen, 1969). It should however be emphasized that roots are sensitive to very low auxin concentrations (about 10^{-11}M (Batra et al., 1975) so that a weak but continuous IAA release within host cells might affect root metabolism. The absence of a morphogenetic effect of the fungus before the establishment of the ericoid endomycorrhizal infection can be compared with results recorded for ectomycorrhizal fungi which are also able to release IAA under pure culture conditions (Slankis, 1973; Harley and Smith, 1983; Gay, 1986, 1987; Gay and Debaud, 1987).

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