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Changing root system architecture through inhibition of putrescine and feruloyl putrescine accumulation

Gozal Ben-Hayyim[a,**], Jean-Pierre Damon[a], Josette Martin-Tanguy[b], D. Tepfer[a,*]

[a]*Laboratoire de Biologie de la Rhizosphère, Institut National de la Recherche Agronomique, 78026 Versailles Cedex, France*
[b]*Laboratoire de Physioparasitologie, Station Amélioration des Plantes, INRA, BV 1540, 21034 Dijon Cedex, France*

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Abstract

Plant roots provide anchorage and absorb the water and minerals necessary for photosynthesis in the aerial parts of the plant. Since plants are sessile organisms, their root systems must forage for resources in heterogeneous soils through differential branching and elongation [(1988) Funct. Ecol. 2, 345–351; (1991) Plant Roots: The Hidden Half, pp. 3–25, Marcel Dekker, NY]. Adaptation to drought, for instance, can be facilitated by increased root growth and penetration. Root systems thus develop as a function of environmental variables and the needs of the plant [(1988) Funct. Ecol. 2, 345–351; (1986) Bot. Gaz. 147, 137–147; (1991) Plant Roots: The Hidden Half, pp. 309–330, Marcel Dekker, NY]. We show, in a model system consisting of excised tobacco roots, that both α-DL-difluoromethylornithine (an inhibitor of putrescine biosynthesis) and the *rolA* gene (from the root-inducing transferred DNA of *Agrobacterium rhizogenes*) stimulate overall root growth and cause a conversion in the pattern of root system formation, producing a dominant or 'tap' root. These morphological changes are correlated with a depression in the accumulation of polyamines and their conjugates.

Key words: Root development; Polyamine; DFMO; *rolA*; Ri TL-DNA; *Agrobacterium rhizogenes*

1. Introduction

Although the regulation of pattern formation in root systems is not understood, the Ri TL-DNA (root-inducing, left hand, transferred DNA) from *Agrobacterium rhizogenes* is known to carry genes that induce the formation of genetically transformed roots in dicots, when the bacterium genetically transforms cells in a wound [5–9]. The impetus for the present work was the finding that these changes in the aerial parts can be mimicked by treating whole plants with an inhibitor of putrescine synthesis [11], α-DL-difluoromethylornithine (DFMO), which blocks one of the two pathways that produce putrescine from arginine in higher plants. The first is via ornithine and the second via agmatine. The former is inhibited by DFMO, irreversibly blocking ornithine decarboxylase (ODC). We thus asked whether the inhibition of putrescine biosynthesis by DFMO could alter root system development.

We chose to work with tobacco roots excised from young seedlings because they develop in culture much as they do on whole plants, forming root systems with multiple branches, each showing similar growth. In addition, excised, axenic roots are not influenced by signals and metabolites from the aerial parts of the plant or from rhizosphere microorganisms. Changes in their development thus result from intrinsic biochemical and genetic changes.

2. Materials and methods

Seedlings from self-fertilized, non-transformed and transgenic plants were germinated in vitro. The 35S-*rolA* transformant used in this study was chosen because it was capable of flowering, presenting a relatively attenuated transformed phenotype. Progeny carrying 35S-*rolA* were identified by their phenotype and resistance to 50 mg/l kanamycin. They represented a mixture of hetero- and homozygotes. Roots were excised when plantlets were at the 2–3 cm stage, 2 weeks after sowing seeds. Roots were cultured for 4 weeks in the dark in liquid MS medium [11], in which the total nitrogen was reduced by a factor of 5. Growth was assessed by measuring root length, branching and by determining fresh and dry weights. Polyamines and their conjugates were assayed using published methods [12].

The *rolA* coding sequence was excised from *Eco*RI fragment 15 from the Ri TL-DNA from *A. rhizogenes* [13]. Details of gene construction are given elsewhere (Michael et al., submitted). Briefly, the ORF 10 coding sequence was prepared by cloning the *Eco*RI–*Pst*I fragment containing ORF 10 [14] into pUC19. After digestion with *Bal*31 to reduce the 5′ leader to 22 bp, the ORF was fused to the CaMV 35S promoter carrying a doubled enhancer and then transferred into pBIN19 [15], which served in plant transformation via *A. tumefaciens* LBA4404, using standard methods for inoculating tobacco leaf fragments [16].

* Corresponding author. Fax: (33) (1) 30 21 38 35.

**On leave from the The Volcani Center Institute of Horticulture, PO Box 6, Bet-Dagan, 50-250, Israel.

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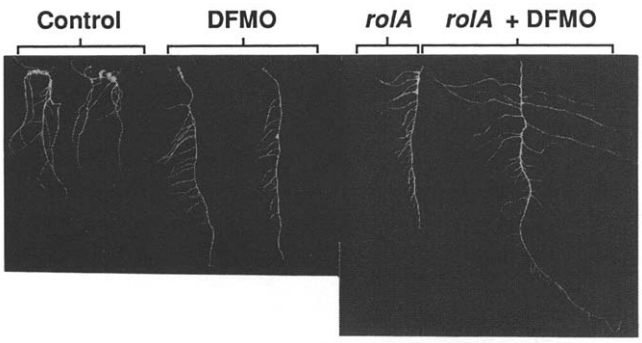

Fig. 1. Effects of DFMO and 35S-*rolA* on the architecture of excised tobacco root systems. Root systems containing no foreign genes or containing 35S-*rolA*, after 4 weeks of growth in vitro, with or without DFMO (1 mM).

3. Results and discussion

In this simplified model for root system development, DFMO (1 mM) caused a fundamental change in root system architecture, converting the untreated system, consisting of less than 10 roots of similar length, into one with a dominant 'tap' root from which numerous laterals formed. These laterals elongated only to a limited extent (Fig. 1). Overall root system length thus increased (Fig. 2A), as did the number of laterals (Fig. 1 and 2B) and overall growth, measured by the accumulation of biomass (Fig. 2D). These changes were annulled when 1 mM putrescine was incuded in the DFMO treatment (Fig. 2D and results not shown), indicating that the effects of DFMO on development were indeed due to putrescine limitation.

In order to determine the nature and the extent of DFMO action, we examined the polyamine content of the roots from these experiments. DFMO decreased free and conjugated putrescine in excised roots by a factor of three, and exogenous putrescine prevented this effect (Table 1). Titres of other amines, spermidine, diaminopropane (not shown) and tyramine, were not changed, but the putrescine conjugate, feruloyl putrescine, was reduced approximately ten fold (Table 1). As expected, the tyramine conjugate, feruloyl tyramine, remained unchanged. The conversion in root system architecture due to DFMO was not a general stress response, since mannitol (included in the medium at 100 mM) decreased general growth but did not mimic or prevent the effects of DFMO on growth and polyamine metabolism (data not shown).

Previous work showed that polyamine accumulation could be reduced genetically by introducing the Ri TL-DNA (or genes there from) into the plant genome [17–21]. Furthermore, the phenotypic changes associated with these genes in the aerial parts were mimicked by treatment of whole plants with DFMO [12]. We therefore examined the effects of one gene, *rolA* (ORF 10) from the Ri TL-DNA on root architecture in vitro. Effects associated with *rolA* on aerial plant parts include leaf wrinkling, reduced stature and sterility [19,22–24]. Under the control of the doubly enhanced CaMV 35S promoter, *rolA* was associated with a pattern of growth (Fig. 1, 2C) in excised roots similar to that produced by DFMO (Fig. 1, 2A,B), i.e. a system consisting of a long principal root with restricted elongation of abundant laterals. It was also associated with increased biomass accumulation (Fig. 2D), and treating roots transformed by 35S-*rolA* with DFMO resulted in the addition of the two effects (Fig. 2D), stimulating overall growth 3.5 fold.

The polyamide profiles in roots excised from seedlings transformed by *rolA* were similar to those in non-transformed, excised roots treated with DFMO, showing a decrease in the level of free putrescine and feruloyl putrescine (Table 1). Again the levels of other amines did not change. When roots containing 35S-*rolA* were treated with DFMO, the additive effects seen on growth and architecture were reflected in further reduction in putrescine and feruloyl putrescine (Table 1).

In summary, both DFMO and *rolA* caused a change in the pattern of growth of excised tobacco roots, which was associated with a reduction in the titers of putrescine and feruloyl putrescine, while other amines remained unchanged. Furthermore, the effects of these chemical and genetic agents were additive. We therefore propose that putrescine and feruloyl putrescine participate in regulating root system development. Since this conversion in architecture took place in axenic, excised roots, it must have been an intrinsic process and not the result of changes in metabolism caused by microorganisms or by the aerial parts of the plant.

We have described here the conversion of a superficial root system to one having a tap root with restricted elongation of laterals. Such a change in architecture is one of the ways that plants adapt to drought stress. To date, genes from the Ri TL-DNA have been shown to have effects on root system architecture that are the opposite of those that would aid in drought resistance; they

Table 1
Titres (μM/g fresh weight) of polyamines and their conjugates in excised roots treated with DFMO or transformed with 35S-*rolA*

Treatment	Put	Spd	Tyr	Put conj*	Tyr conj**
Control	1.81	0.21	1.53	4.28	1.10
DFMO	0.56	0.23	1.44	0.38	1.16
DFMO + Put	4.10	0.23	1.48	4.75	1.16
35S-*rolA*	0.45	0.20	1.20	0.72	1.22
35S-*rolA* + DFMO	0.21	0.21	1.13	0.07	1.21

Standard deviations were approximately 15% of the means. Put, putrescine; Spd, spermidine; Tyr, tyramine; conj., conjugates. See [11] for methods. Each point represents 15–30 roots.
*95% feruloyl putrescine.
**95% feruloyl tyramine.

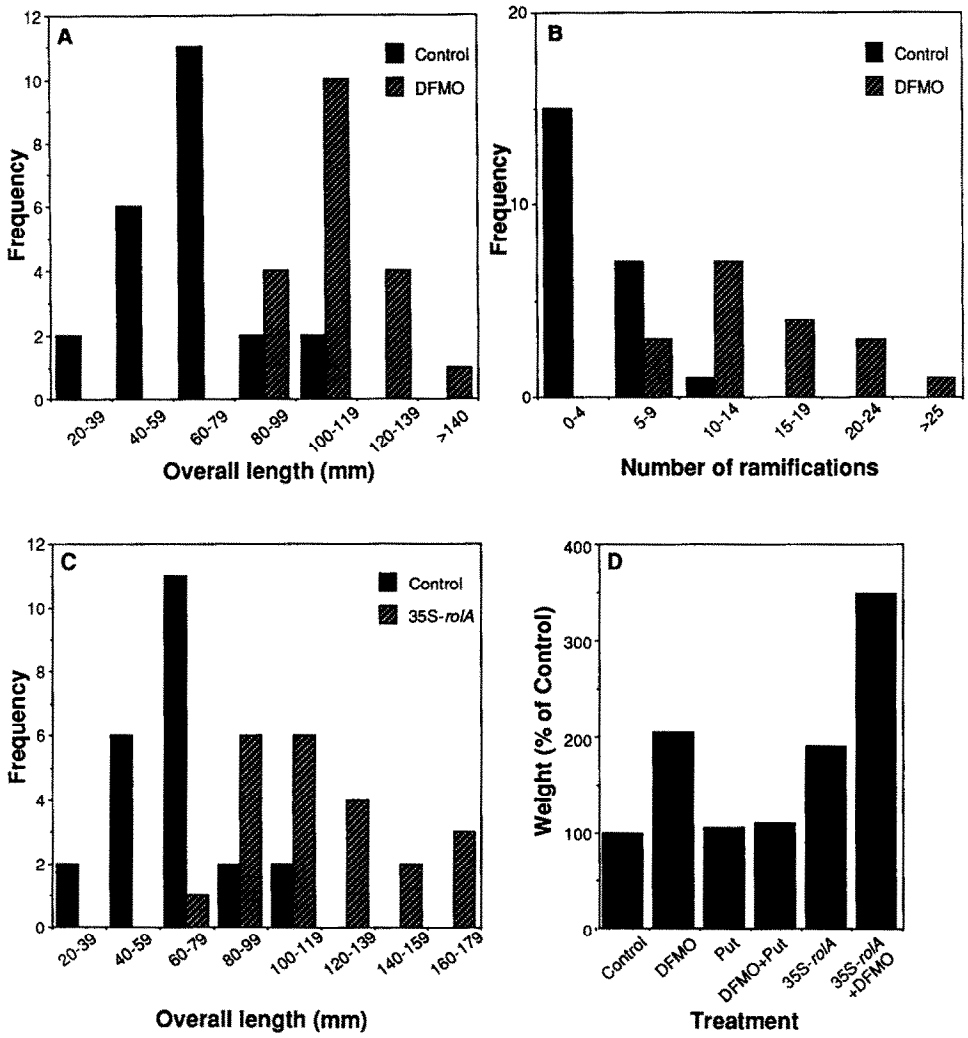

Fig. 2. Quantitative analysis of the architecture and growth of excised root systems treated with DFMO and/or transformed by 35S-*rolA*. (A) Distribution of overall root system length in untreated controls and in roots excised from untransformed plants and treated with DFMO. (B) Distribution of branching frequencies (ramifications per root) in untreated controls and in roots excised from untransformed plants and treated with DFMO. (C) Distribution of root lengths in non-transformed controls and in roots excised from plants transformed by 35S-*rolA*. (D) Effects of DFMO, putrescine (Put) and 35S-*rolA*, on the growth (fresh weight accumulation) of excised roots after 4 weeks in culture. The control (100%) value was 0.107 ± 0.015 g. Each treatment comprised 15–30 excised roots.

cause an increase in lateral production and growth. However, these effects occur in whole plants expressing the foreign genes in their aerial as well as subterranean parts. Using inducible and root-specific promoters, it should be possible to modulate the expression of genes (e.g. *rolA*) that cause changes in polyamine metabolism, and thus produce the changes in intact plants that we have observed in excised roots.

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