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The effect of polyamines on endomycorrhizal infection of wild-type *Pisum sativum*, cv. Frisson (nod $^+$myc $^+$) and two mutants (nod $^-$myc $^+$ and nod $^-$myc $^-$)

Abstract The effect of four polyamines, putrescine, cadaverine, spermidine and spermine, on arbuscular mycorrhizal (AM) infection by *Glomus intraradices* was tested on *Pisum sativum*, cv. Frisson (nod $^+$myc $^+$) and two isogenic mutants of this cultivar, P56 (nod $^-$myc $^+$) and P2 (nod $^-$myc $^-$). Polyamines were applied at 0 and 5.10^{-4} M as soil drenches. Endomycorrhizal infection parameters were measured 3 weeks after inoculation. Polyamine treatment significantly increased the frequency of mycorrhizal infection in the myc $^+$ pea lines (cv. Frisson and P56) and the number of appressoria formed in the myc $^-$ line (P2). A positive correlation was found between polyamine chain length and their stimulation of fungal development. Results are discussed in relation to the possibility that polyamines may act as regulatory factors in plant-AM fungus interactions.

Key words Polyamines · Endomycorrhizal infection *Glomus intraradices* · *Pisum sativum*

Introduction

Polyamines, such as putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm), are cationic molecules which are widely distributed in all living cells. They have been shown to be involved in several aspects of plant growth and differentiation (Tabor and Tabor 1984), and are essential for many important cellular functions. It is known that particularly Spd and Spm interact directly with anionic cellular components such as membranes (Frydman et al. 1984) and nucleic acids (Frydman et al. 1990).

Put, Spd and Spm are linked by a common biosynthetic pathway which originates by the decarboxylation of either ornithine or arginine to give Put through the action of ornithine decarboxylase or arginine decarboxylase, respectively. Put is then aminopropylated to Spd and Spm (Tabor and Tabor 1984). Cad is not as widely distributed as Put and is mainly found in the leguminosae (Smith 1975) and in the flower of *Arum* lilies (Smith and Meeuse 1966). It originates in the decarboxylation of lysine through the action of lysine decarboxylase (Bagni 1989).

The role of free or bound polyamines in various processes, particularly in host-parasite interactions (Martin and Martin-Tanguy 1981; Bellés et al. 1991), has prompted us to evaluate their possible implication in a symbiotic association, the arbuscular mycorrhiza (AM). Although this symbiosis is widely distributed among endomycorrhizal fungi and higher plant roots, the biochemical mechanisms responsible for infection establishment and development are still unknown. Investigations into these can be greatly aided by the use of plants mutated for their ability to form AM. Recently, Duc et al. (1989) and Gianinazzi-Pearson et al. (1991) reported that some nonnodulating (nod $^-$) pea mutants were incapable of forming endomycorrhiza (myc $^-$), the fungal symbiont only reaching the morphological stage of appressorium differentiation at the root surface.

In the present study, we report the presence of the free polyamines Put, Cad, Spd, and Spm in roots of pea (*Pisum sativum*) wild type cv. Frisson (nod $^+$myc $^+$) and two isogenic mutants of this cultivar, P56 (nod $^-$myc $^+$) and P2 (nod $^-$myc $^-$). The effect of these four polyamines on *Glomus intraradices* development in roots of the three pea lines was also tested.

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Materials and methods

AM inoculum

A soil-based inoculum was used in the experiments. It was prepared from *Allium porrum* L. roots grown in a gamma-irradiated

(10 KGy) clay loam soil, pH 8.0 (Epoisses soil) and inoculated with *G. intraradices* Schenck and Smith (LPA 8). After 3 months, mycorrhizal roots and soil were mixed together to give the soil-based inoculum containing infected roots, hyphae and spores.

Plant material and growth conditions

Seeds of *P. sativum* L. cv. Frisson (nod^+myc^+) and P56 (nod^-myc^+) and P2 (nod^-myc^-) mutants were surface sterilized in 95% alcohol for 20 min and germinated in vermiculite in the dark at 25° C. Roots of some seedlings were harvested after 4 days and used for free polyamine analysis. The remaining plants were transplanted into pots containing 200 g of a soil-based inoculum, irradiated Epoisses soil and perlite mix (2/1/1, v/v/v). Plants were grown for 3 weeks in a constant environment room (16 h, 20° C, 80% RH, 320 $\mu E\ m^{-2}\ s^{-1}$). The protocol was: one plant/pot, five replicates and daily drenching of soil with solutions of polyamine (Put, Cad, Spd or Spm) at 0 or 5.10^{-4} M.

Analysis of polyamines

Amines were extracted according to Flores and Galston (1982). Tissues were ground in 0.1 M HCl and samples were then centrifuged at 20000 *g* for 30 min and the supernatant fraction used for analysis of free polyamines prepared as their dansyl derivatives. These were quantified by fluorescence spectrophotometry and separated by HPLC according to Smith and Davies (1985). Standards (Sigma) were analysed in the same way.

Estimation of root infection

The amount of infection developing in roots was estimated according to Phillips and Hayman (1970) modified by Trouvelot et al. (1986) and expressed as: F% frequency of root samples with fungal infection structures, M% relative colonisation intensity of the whole root system for the myc^+ plants or the intensity of appressoria development for the myc^- plants, a% frequency of arbuscule formation in mycorrhizal portions of the root system. All data were analysed statistically using the Newman-Keuls test ($P=0.05$).

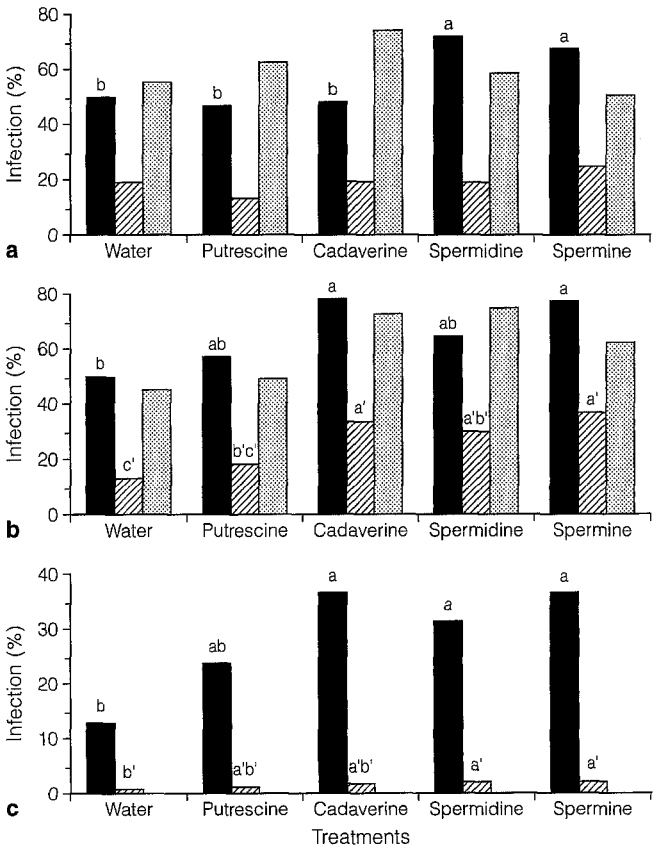

Fig. 1 Endomycorrhizal infection rate at 3 weeks of *Pisum sativum* L. cv Frisson (**a**), P56 (**b**) and P2 (**c**) treated or not with putrescine, cadaverine, sperimidine or spermine. ■ F% frequency of root samples with fungal infection structures, ▨ M% relative colonisation intensity of the whole root system, ⊞ a% frequency of arbuscule formation in mycorrhizal portions of the root system. For the same line, values with different letters are significantly different ($P<0.05$)

Results and discussion

Four free polyamines, Put, Cad, Spd and Spm, were found in the 4-day-old roots of cv. Frisson, P56 and P2 (Table 1). Levels of the four polyamines were not significantly different among the three pea lines. Similar levels have been reported by Shen and Galston (1985) for roots of *P. sativum* cv. Alaska.

When applied to the plants, the four polyamines had a stimulating effect on mycorrhizal infection frequency (F%) of the two myc^+ lines (cv. Frisson and P56) and the proportion of roots with appressoria development on the root surface of the myc^- line (P2) was increased (Fig. 1). The relative infection intensity and the intensity of appressoria formation (M%) for P56 and P2, respectively, were also significantly increased (Fig. 1). The frequency of arbuscules in mycorrhizal portions of the root system (a%) was not significantly affected for cv. Frisson and P56. In the case of P2, the myc^- character was not altered by polyamine treatment, fungal development always being limited to appressorium formation (a% = 0) (Fig. 1). A positive correlation was found between the carbon chain length of the polyamines and their effect on the frequency of roots with fungal infection structures (Fig. 2).

Table 1 Free polyamine content (nmol/g fresh wt.) of 4-day-old pea roots given as the means of three experiments

Pea line	Polyamine			
	Putrescine	Cadaverine	Spermidine	Spermine
cv. Frisson	1588± 48	1868± 62	185±13	46±10
P56	1480± 32	1535±138	135±25	43± 4
P2	1375±155	1546±122	166±20	48± 6

These results suggest that polyamines may have a role in the initial steps of the infection process by *G. intraradices,* since they particularly stimulate infection frequency of the root systems of the different pea lines. Of the different effects that polyamines have been shown to have on fungal and plant physiology, several may have implications for establishment of AM symbiosis.

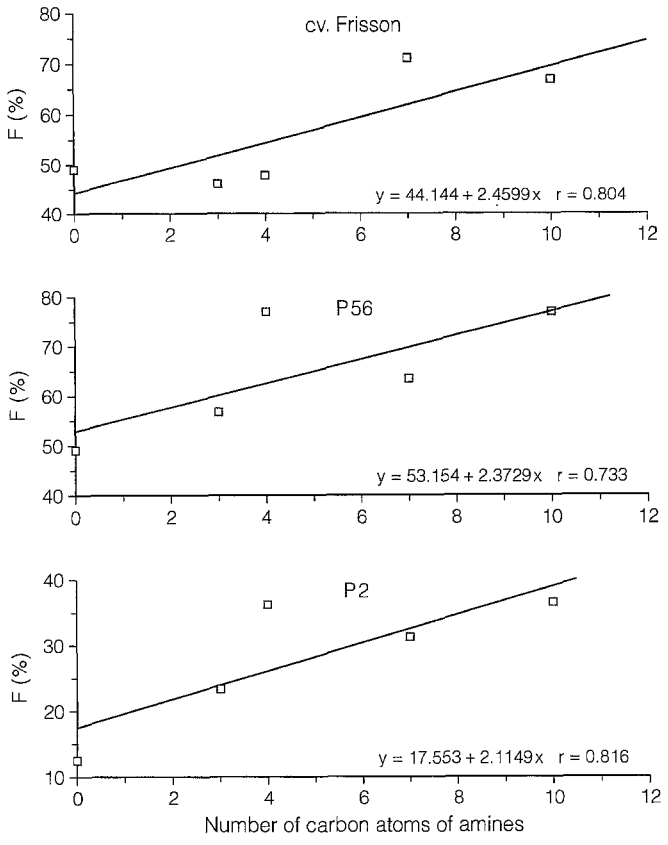

Fig. 2 Correlation between polyamine carbon chain length and mycorrhizal infection frequency in cv. Frisson, P56 and P2. Values represent the means of five replicates

Firstly, polyamines have been suggested to be implicated in molecular signalling events in plant pathogen interactions. Bellés et al. (1991) have reported a marked decrease in the putrescine content of *Gynura aurantiaca* and *Lycopersicon esculentum* leaves in plants infected with citrus exocortis viroid.

Secondly, it has been shown that several inhibitors of polyamine biosynthesis reduce mycelial growth of the fungus *Ophiostoma ulmi* (Buism.) Nannf. (Biondi et al. 1993) and that the action of the inhibitors can be reversed by the addition of polyamines. Polyamines may, therefore, act directly on mycelial growth of an AM fungus, so increasing contact events with host roots. Preliminary investigations indicate that at low concentrations, polyamines stimulate hyphal growth from *Gigaspora rosea* spores whilst some polyamine biosynthesis inhibitors have an inhibiting effect.

Alternatively, polyamines may influence AM fungal development through modifications in host plant physiology. Polyamines interact with the negative charges of pectic substrates, which can condition the binding of the pectin methyl esterase (Charnay et al. 1992). Pectin methyl esterase is a pectinase that has been shown to play a fundamental role in the penetration of many microorganisms into plant cells (Martinez-Molina et al. 1979; Collmer and Keen 1986). There are biochemical and cytological indications that pectinases may be involved in the AM colonisation process (Bonfante-Fasolo et al. 1990; Garcia-Romera et al. 1990; Garcia-Romera et al. 1992). It is possible that, by interacting with pectinases, polyamines may regulate adhesion and/or penetration of the plant cell wall by an AM fungus.

It has also been shown that polyamines can inhibit ethylene production in excised plant tissues (Even-Chen et al. 1982). Ethrel (2-chloroethyl phosphonic acid), a compound that releases ethylene, negatively affects AM infection in soybean (Morandi 1989) and in *Medicago sativa* and *Triticum vulgare* (Azcon-Aguilar et al. 1981). Thus a further mechanism by which polyamines may stimulate initial AM infection events could be inhibition of ethylene production by roots.

Finally, it has been demonstrated that carrot cell walls preferentially adsorb large polyamines and that this leads to the release of calcium into the medium (Mariani et al. 1989). Calcium can act as a secondary messenger, for example stimulating 1,3-β-D-glucan synthase in soybean cells (Kauss et al. 1983). Such a mechanism may explain, at least in part, our observation that the largest polyamines are the most effective in favouring mycorrhizal infection.

It is evident that further experiments are necessary to test these different hypotheses.

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