Effect of polyamines on endomycorrhizal infection of *Pisum sativum* and spore germination of *Glomus mosseae*

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Effect of Polyamines on Endomycorrhizal Infection of *Pisum sativum* and Spore Germination of *Glomus mosseae*

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Summary.- We have identified the following polyamines in root exudates of pea: putrescine, cadaverine, spermidine, spermine, phenylethylamine and 3-hydroxy,4-methoxyphenylethylamine. Putrescine and spermidine had a stimulating effect on *in vitro* spore germination and hyphal growth of *Glomus mosseae*. Putrescine, cadaverine, spermidine and spermine stimulated endomycorrhizal infection of pea *in vivo*. We found a positive correlation between polyamine carbon chain length and the extent of enhancement of the endomycorrhiza development.

Keywords: Pisum sativum, Glomus mosseae, *endomycorrhizal infection, spore germination, hyphal growth*

Introduction

Polyamines are cationic molecules which are widely distributed in all living cells. They have been shown to be involved in a variety of growth and developmental processes in a wide range of organisms, including fungal systems (4, 12). They are also implicated in host-parasite relations (2, 6). Recent investigations from our laboratory on the involvement of polyamines in plant-endomycorrhizal fungi interactions have demonstrated a stimulating effect of polyamines on endomycorrhizal infection by *Glomus intraradices* (3). In this study the presence of polyamines was analysed in root exudates of pea. The effect of some of these compounds was tested on spore germination and hyphal growth of *Glomus mosseae in vitro* and on infection of pea roots by this fungus *in vivo*.

Materials and Methods

Root exudates and polyamine analysis. Seeds of *Pisum sativum* L. cv. Frisson were surface sterilized and grown axenically on a grid in a box containing water in which root exudates were collected. Polyamines in root exudates were analysed by HPLC (11).

Spore germination and hyphal growth. Spores of *Glomus mosseae* (BEG 12) were surface sterilized and germinated on water agar supplemented with putrescine or spermidine at concentrations of 0, 10^{-6}, 10^{-5} and 5.10^{-4} M.

Plant culture and polyamine treatments. *Pisum sativum* L. cv. Frisson was grown in pots containing a soil-based inoculum (*Glomus mosseae*) / Terragreen (calcined clay) mix (1:3) in a constant environment room. Polyamines (putrescine, cadaverine, spermidine or spermine) were applied daily at 0 or 5.10^{-4} M as soil drenches.

Mycorrhizal evaluation. Endomycorrhizal infection was measured after three weeks' growth by frequency (F%), intensity (M%) and arbuscule frequency (A%) (14). Total amount of fungal tissue was revealed by trypan blue staining, living mycelium by succinate dehydrogenase activity and the proportion of active mycorrhizal infection by alkaline phosphatase activity (13).

Statistical analysis. All data were analysed statistically using Newman-Keuls test (P=0.05).

Results

The main free amines identified in root exudates of *P. sativum* cv. Frisson grown in axenic conditions were putrescine, cadaverine, spermidine, spermine, phenylethylamine and 3-hydroxy, 4-methoxyphenylethylamine.

Putrescine and spermidine had stimulatory effects on spore germination and hyphal growth of *G. mosseae* (Figure 1), the most important effects on hyphal growth being observed mainly at 5.10^{-4} M.

All four polyamines enhanced total endomycorrhizal infection of pea cv. Frisson at 5.10^{-4} M (TB; Figure 2). Living and active mycelium indicated, respectively, by succinate dehydrogenase and alkaline phosphatase activities (ALP and SDH, Figure 2) increased proportionally to total endomycorrhizal infection.

A positive correlation was found between polyamine carbon chain length and the extent to which endomycorrhizal infection was stimulated (Figure 3).

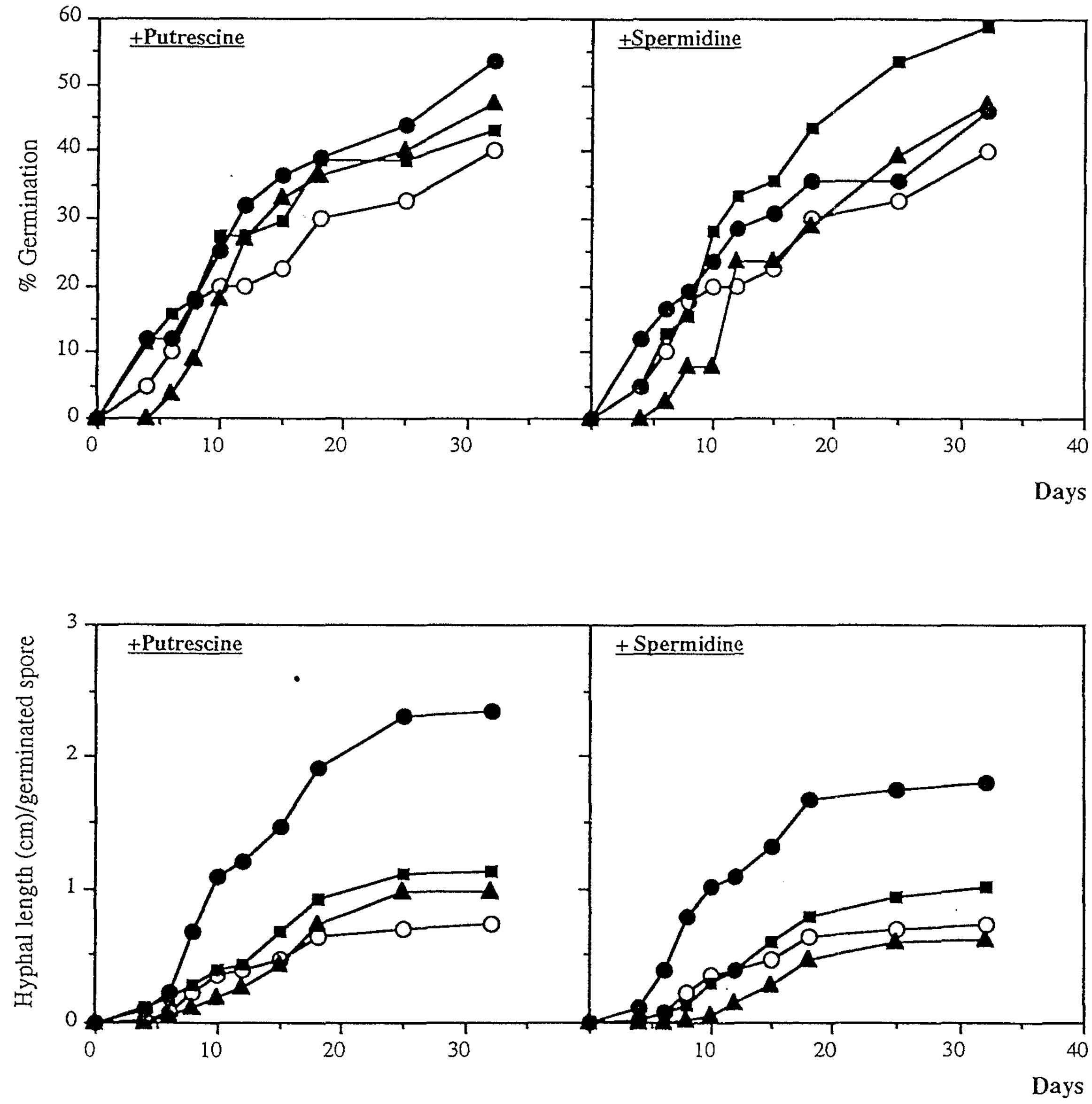
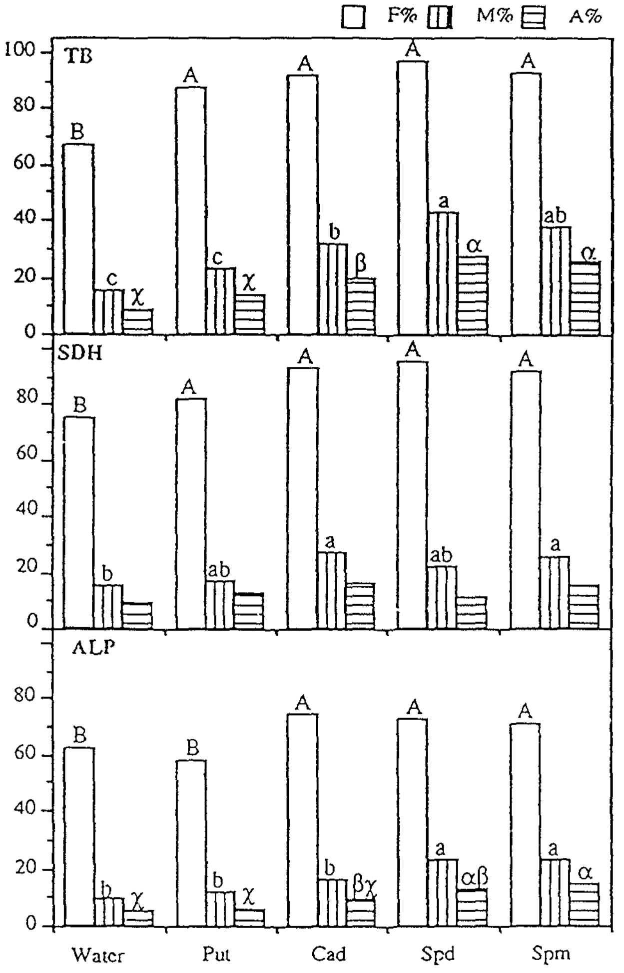


Figure 1. Effect of putrescine and spermidine at 0 (—O—), 10^{-6} (—▲—), 10^{-5} (—■—) and 5.10^{-4}M (—●—) on spore germination and hyphal length per germinated spore of *Glomus mosseae*. Values represent the mean of eight replicates and the effect on hyphal growth was significant at 5.10^{-4} of polyamines from fifteen days onwards (P=0.05).



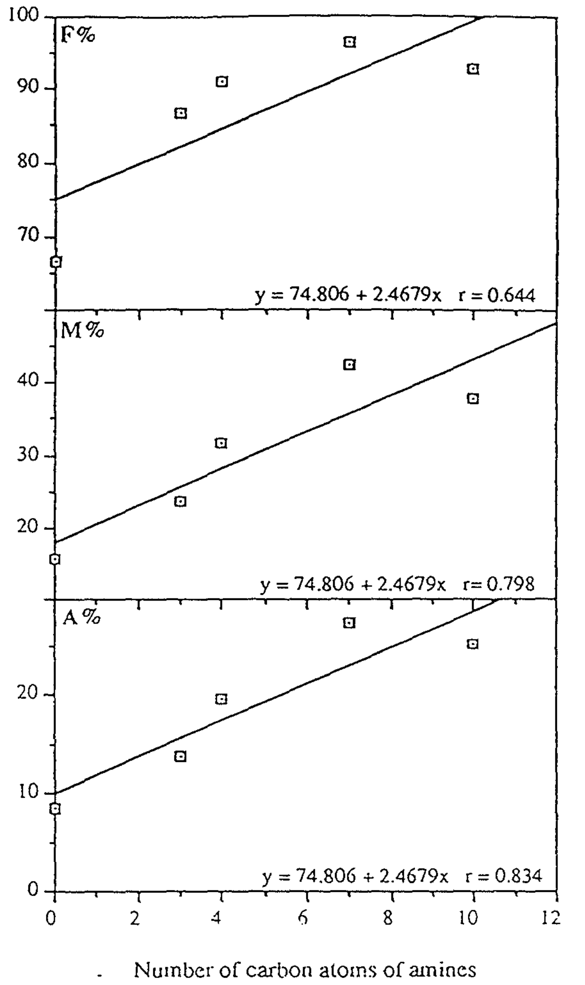


Figure 2. Endomycorrhizal infection of pea cv. Frisson treated with putrescine (Put), cadaveric (Cad), spermidine (Spd) or spermine (Spm) revealed by trypan blue staining (TB), succinate dehydrogenase (SDH) or alkaline phosphatase (ALP) activities. F%: infection frequency; M%: endomycorrhizal intensity; A%; arbuscule frequency. Values with different letters are significantly different (P=0.05).

Figure 3. Correlation between polyamine carbon chain length and endomycorrhizal infection by *Glomus mosseae*. Values represent the mean of five replicates and r is significant at P=0.005).

Discussion and Conclusion

Our findings of a positive effect of polyamines on *in vivo* endomycorrhizal infection of pea roots by *Glomus mosseae* confirm previous results obtained with another fungus, *Glomus intraradices* (3).

Root exudates have been reported to stimulate *in vitro* hyphal growth of certain arbuscular-mycorrhizal (AM) fungi (1, 5, 8) and some root exudate compounds like flavonoids have been found to have a stimulatory effect on spore germination and hyphal growth (5, 7, 9). The fact that polyamines are present in pea root exudates and that they stimulate spore germination and hyphal growth of *G. mosseae* suggests that these compounds, together with flavonoids may be involved in the first molecular events of the infection process. The role of polyamines in growth and development of other fungi is largely documented (10, 15).

Plant-exuded polyamines in the rhizosphere may act as factors which enhance AM fungal development, so favouring cell to cell contact essential to the initiation of the infection process between the AM symbionts.

Acknowledgments: We thank Dr. V. Gianinazzi-Pearson for critically reading the manuscript.

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