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The effect of polyamines and propamocarb on the growth and yield of tomato (*Lycopersicon esculentum* Mill.) plants grown in a low P tropical soil inoculated with a vesicular-arbuscular mycorrhizal fungus

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Abstract

An experiment was designed to examine the effect of two types of polyamines, cadaverine (CAD) 0.5μM and putrescine (PUT) 0.5μM, and also a fungicide, propamocarb (PRO) 640ppm, on the growth and yield of tomato (*Lycopersicon esculentum* Mill.) plants grown in a low P tropical soil inoculated with a vesicular-arbuscular mycorrhizal (VAM) fungus, *Glomus caledonium* (Nicol. & Gerd.) Trappe and Gerdeman.

Change in root colonization levels with time and biomass accumulation was recorded. Shoot P content, fruit yield and fruit quality were also quantified. The polyamine and PRO treatments showed significantly higher levels of root colonization at the 4th and 7th weeks after germination. Higher biomass weight and fruit yield was recorded for the polyamine and PRO treatments as compared to the control. Fruit quality for the PRO treatment was also significantly higher compared to the control plants. There was, however, no significant difference in shoot P content for all the treatments.

We propose that the stimulation of spore germination and hyphal elongation of VAM fungi by the exogenously applied polyamines and PRO leads to accelerated root colonization of candidate host plants. These plants benefit from the symbiotic relationship for a slightly longer time as demonstrated by the improved performance of polyamine and PRO treated plants.

Key Words: cadaverine, *Glomus caledonium*, propamocarb, putrescine, tomato

Introduction

Polyamines are low molecular weight organic nitrogen compounds that are present in living cells as cationic molecules. They serve many functions in plants, either in free or bound form. They play a role

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in several aspects of plant growth and differentiation (Tabor and Tabor, 1984), and they have attracted attention as secondary messengers and in the protection of membranes (Evans and Malmberg, 1989). Polyamines have also been implicated in the molecular signaling events in plant pathogen interactions. For example, a marked decrease in the putrescine content of *Gyanura aurantica* and *Lycopersicon esculentum* leaves in plants infected with citrus exorcortis viroid has been reported (Bellês et al., 1991). It has also been reported that several inhibitors of polyamine biosynthesis reduce mycelial growth of the fungus *Ophiostoma ulmi* and that the action of inhibitors can be reversed by the addition of polyamines (Biondi et al., 1993).

The four polyamines, PUT, CAD, spermidine and spermine, applied on *Pisum sativum* L. cv. Frisson plants (nod^+ myc^+) and two isogenic mutants P56 (nod^- myc^+) and P2 (nod^- myc^-) inoculated with a VAM fungus *Glomus intraradices* had a stimulating effect on mycorrhizal infection frequency of the two myc^+ lines. The proportion of roots with appresoria development on the root surface of the myc^- line also increased (El Ghachtouli et al., 1995).

Propamocarb is a soil applied systemic carbamate fungicide that is used in fruits and vegetables. It is active by root uptake against phythium and pytopthora (Cremlyn, 1991). Applied in low concentrations, it has been reported to stimulate extensive root colonization of trifoliate orange seedlings by a VAM fungus, *Gigaspora ramisporophora*, leading to an increase in the production of both fresh and dry biomass (Ishii et al., 1996b).

It has been amply demonstrated that mycorrhizal as opposed to non-mycorrhizal plants absorb P and other elements more efficiently in environments where these elements are limiting. They also demonstrate to some extent, a higher tolerance to a variety of environmental stresses. It is in such circumstances that mycorrhizal plants exercise an advantage over non-mycorrhizal ones (Sreeramulu and Bagyaraj, 1985; Mosse, 1981).

Thus, the objective of this experiment was to examine the effect of the two polyamines PUT and CAD and a fungicide PRO on the rate and degree of VAM formation in tomato roots, and the effect this has on the growth rate, yield and fruit quality of the tomato plants.

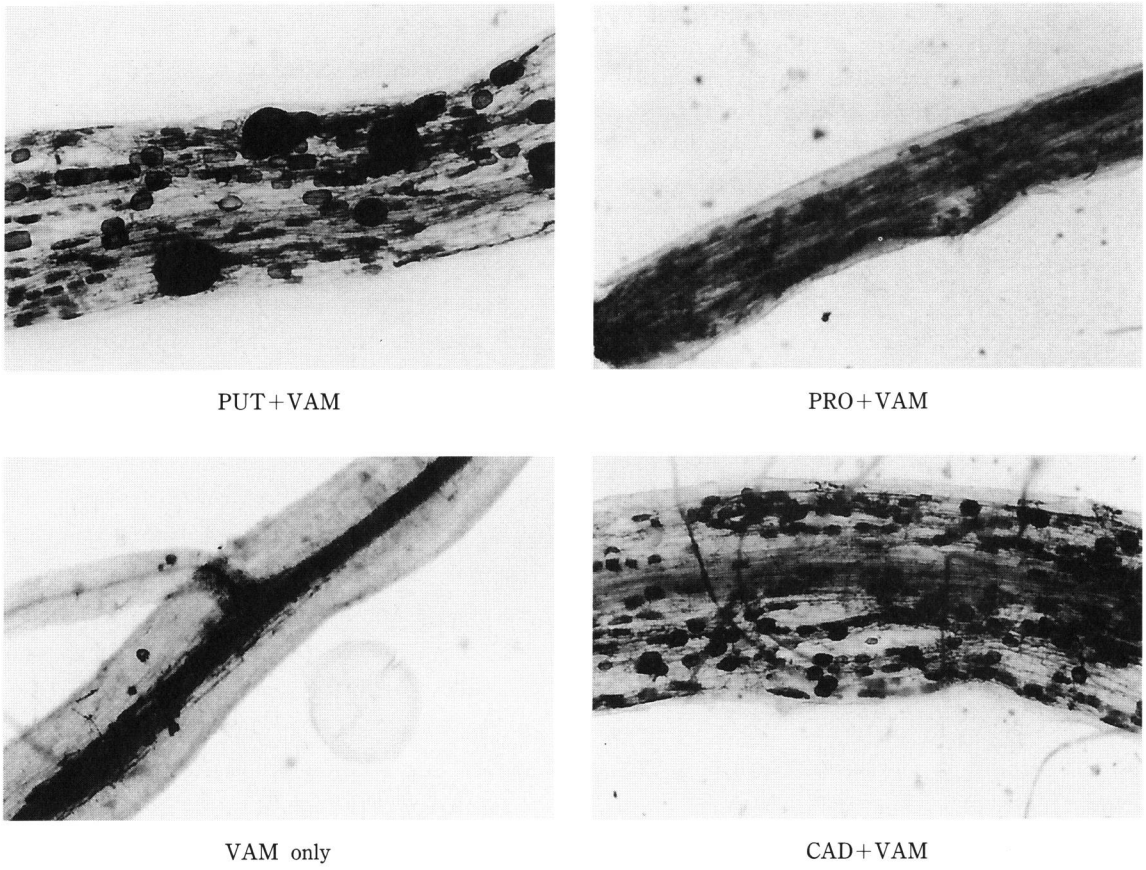
Materials and methods

Certified seed of tomato (*Lycopersicon esculentum* Mill. var. Moneymaker) were direct seeded into plastic pots each containing 3.5kg of a sterilized brown loam soil (1200ppm N, 9ppm P, 431ppm K, pH (H_2O) 6.4 and EC 0.398mS·cm^{-1}). A soil based inoculum containing about 400 spores of *Glomus caledonium* was mixed with the top half layer of the soil before sowing. A week after sowing, a plant per pot was adjusted by thinning, and then 200 ml of 0.5μ M CAD, 0.5μ M PUT and 640ppm PRO were separately treated to the soils weekly for 6 weeks. Control (VAM only) plants received similar volumes of water. There were four treatments (VAM only, CAD+VAM, PUT+VAM and PRO+VAM) each replicated five times. The plants were raised under greenhouse conditions and the pots were uniformly spaced and randomly distributed. No soil amendment was used in this experiment.

To measure levels of infection, root pieces were sampled from all the plants 7 weeks after sowing and infection density examined using trypan blue staining (Brundrett, 1994 and Ishii et al., 1996a). Fruit harvesting started from the 8th week after sowing and lasted for 4 weeks. Fruits were weighed immediately after harvesting and yield per pot was calculated at the end of harvesting. During the harvesting time, 4 fruits were randomly sampled from each treatment and fruit color was measured using a Minolta CR200b color meter. Thirteen weeks after sowing, plant tissue was weighed at harvesting and after oven drying at 70℃ for three days. Tissue and soil analysis was done as according to Okalebo et al. (1993).

Results

There was a significant difference in levels of colonization at the 4th and 7th weeks after germination (Fig. 1). It was noted that infection levels at the 7th week were about twice the levels observed in the 4th week for all the treatments, suggesting that the interaction between plant roots and hyphae continued at about the same rate within this period (Table 1). Biomass weight, both before and after drying was higher in the PUT, CAD and PRO treated plants as compared to the control (Table 2). However, there was no significant difference in the levels of



PUT+VAM　　PRO+VAM

VAM only　　CAD+VAM

Fig.1. Vesicular-arbuscular mycorrhizae (VAM) of tomato plants 7 weeks after inoculation with a VAM fungus, *Glomus caledonium* and treatment with two polyamines, putrescine (PUT) and cadaverine (CAD), and a fungicide, propamocarb (PRO) (Magnification: x100).

Table 1. Effect of polyamines (PUT and CAD) and propamocarb (PRO) on root colonization of tomato plants grown in a low P tropical soil inoculated with a vesicular arbuscular mycorrhizal (VAM) fungus, *Glomus caledonium*. Column values with different letters are significantly different at 5% level using Duncan's multiple range test.

Treatment	Root colonization (%)	
	After 4 weeks	After 7 weeks.
VAM only	14.8a	26.2a
PUT+VAM	36.4b	76.6b
CAD+VAM	46.2c	78.8b
PRO+VAM	48.1c	89.9c

PUT-putrescine, CAD-cadaverine.

Root colonization(%)=(Root length infected/root length observed)×100.

Table 2. Effect of polyamines (PUT and CAD) and propamocarb (PRO) on biomass yield of tomato plants grown in a low P tropical soil inoculated with a vesicular arbuscular mycorrhizal (VAM) fungus, *Glomus caledonium*. Column values with different letters are significantly different at 5% level using Duncan's multiple range test.

Treatment	Total weight (g)		Root weight (g)	
	Fresh	Dry	Fresh	Dry
VAM only	37.7a	5.4a	19.0a	3.0a
PUT+VAM	71.2b	10.2b	39.3b	5.4b
CAD+VAM	85.8bc	11.8b	34.8b	6.5b
PRO+VAM	100.3c	16.4c	44.0b	6.8b

PUT-putrescine, CAD-cadaverine.

Table 3. Effect of polyamines (PUT and CAD) and propamocarb (PRO) on fruit yield and quality of tomato plants grown in a low P tropical soil inoculated with a vesicular arbuscular mycorrhizal (VAM) fungus, *Glomus caledonium*. Column values with different letters are significantly different at 5% level using Duncan's multiple range test.

Treatment	Fruit color		Yield (g)
	L	a	
VAM only	63.0a	−3.3a	30.4a
PUT+VAM	55.8ab	7.2ab	40.3b
CAD+VAM	58.3ab	4.5ab	68.5c
PRO+VAM	52.1b	17.9b	70.4c

PUT-putrescine, CAD-cadaverine.

shoot P for all the treatments.

Fruit yield for the VAM only treatment was significantly lower compared to all the other treatments (Table 3). In particular, treatment with PRO led to a significant improvement in fruit quality. Fruits from CAD, PRO and PUT treatments also ripened to fresh market quality a day earlier than those from the VAM only treatment.

Discussion

An increase in polyamine levels especially PUT in VAM spores before germination implies that polyamines could be implicated in the induction of dormancy break in the spores (El Ghachtouli et al., 1996). It has also been reported that exogenous application of polyamines stimulates hyphal growth in *Glomus mosseae* and that the endogenous concentration of these compounds may be a limiting factor to hyphal growth (El Ghachtouli et al., 1996).

Polyamines apparently improve the mycelial growth of VAM fungi thereby increasing contact area and frequency with host plant roots. The mechanism by which polyamines affect plant and fungal interactions could be through modifications in host plant physiology. It is possible that by interaction with pectinases, polyamines may regulate adhesion and penetration of the cell wall by a VAM fungus (El Ghachtouli et al., 1995). The stimulatory effect of PRO on VAM fungi is possibly due to increased levels of ethylene production by the plant roots. Ishii et al. (unpublished data) observed that trifoliate orange roots dipped in 800 ppm and 1600 ppm PRO for 30 minutes, respectively released about 9 and 36 times more ethylene than the untreated roots 12 hours later. Very low concentrations of ethylene (around 0.05ppm) markedly enhanced hyphal growth of VAM fungi and VAM development in trifoliate orange roots (Ishii et al., 1996a).

In this experiment, polyamine and PRO treatments improved both the rate of root colonization and plant performance. It seems that the accelerated and more extensive root colonization had a direct impact on the rates of P acquisition by the plants. P is essential for optimal plant growth because much of the energy required for growth is stored chemically in the form of complex organic phosphates. It is then released as required to drive important chemical processes involved in growth (Adams, 1986), and P deficiency leads to a reduction in leaf expansion and leaf surface area (Freedan et al., 1989). Varying levels of chlorophyll content in fruits, a factor related to nutrient acquisition could account for the difference in fruit quality. This is because the production of the normal red color of ripe fruit is due to the destruction of chlorophyll and the accumulation of the carotenoids β-carotene and lycopene as the chloroplasts are transformed into chromoplasts (Grierson and Kader, 1986).

The insignificant difference in shoot P for all treatments at the end of the experiment is explained in part by the differences in yield. This is because a large proportion of the nutrients absorbed by mature tomato plants are found in the fruit (Adams, 1986). It is also possible that the little P in the soil had been depleted at the time the shoots were sampled for P analysis at the end of the experiment.

The role of stimulators in accelerating the symbiotic interaction between host plants and mycorrhizal fungi has not been well studied. In this experiment it is demonstrated that earlier colonization of roots induced by polyamines and PRO is beneficial to the plants. We would like to recommend more research into the possibility of using the stimulators to enhance the inoculum potential of VAM fungi.

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