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SHORT COMMUNICATIONS

Mycorrhizal colonization and phosphorus uptake in presence of PGPRs along with nematode infection

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Abstract
Arbuscular mycorrhiza (AM), plant growth promoting rhizobacteria (PGPRs) and root-knot nematode survive in the rhizosphere and perform the same niche. AM and PGPRs play positive role in roots while nematode oppose it. Nematode damages the root system, which reduces the nutrient uptake specially phosphorus (P) while AM help to uptake. PGPRs are another symbiotic micro-organism helpful to mycorrhizal survival and colonization. AM and PGPRs can defeat the soil borne diseases like root-knot disease which lead to increasing mycorrhizal colonization and nutrient uptake. On this basis current work emphasizes the effects on mycorrhizal colonization and P uptake in presence of PGPRs along with root-knot nematode infection in tomato plants.

Keywords Colonization · Mycorrhiza · Nematode · PGPRs · Phosphorus

1 Introduction

The use of beneficial rhizosphere microorganisms as bioinoculants is very useful to agricultural soils due to the lack of negative effects on the cultivable lands (Sharma et al. 2017; Martınez-Medina et al. 2017; Contreras-Cornejo et al. 2018). Among the bioinoculants arbuscular mycorrhiza (AM) and plant growth promoting rhizobacteria (PGPRs) are most important which play vital role in fields and enhance plant growth and productivity. On the other hand, root-knot nematode (*Meloidogyne* spp.) is very critical pest in agriculture which reduces plant growth by reducing nutrients uptake and it can be defeated only by the symbiotic bioinoculants (Trudgill and Blok 2001; Sharma and Sharma 2017a). The most common nematode affected crops are; horticultural (banana, almond, grape, passion fruit, peach, plum, zinger, etc.), vegetable (tomato, mung bean, French bean, beetroot, capsicum, carrot, cucumber, melon, eggplant, okra, potato onion, etc.), field crops (rice, wheat, clover, chickpea, lucerne, lupin, pigeon pea, soybean, sugarcane, tea, tobacco, etc.), ornamental (carnation, chrysanthemum, dahlia, gerbera, protea, rose, etc.) and many other crops (Sharma et al. 2018). *Rhizophagus irregularis*, is a highly symbiotic mycorrhizal species which is associated with all the plant species and confers resistance to many soil-borne plant pathogens including bacteria, fungi and nematodes (Elsen et al. 2008; Sharma and Sharma 2015a, b; Sharma and Sharma 2017a). Similar to mycorrhiza, plant growth-promoting rhizobacteria (PGPR) including Pseudomonads have great potential to antagonize soil-borne pathogens in different ways (Sharma et al. 2017; Sharma and Sharma 2017b). The PGPRs help to mycorrhiza for establishing their mycelia inside the plant roots which can enhances root colonization. Both the symbionts help to each other for their growth and establishment through using soil resources specially nutrients uptake those have law mobility in the soil. On this bases current study evaluates the mycorrhizal colonization and phosphorus uptake in presence of PGPRs along with nematode infection.

2 Materials and methods

AM (*Rhizophagus irregularis*), two PGPRs (*Pseudomonas jessenii* strain R62 and *P. synxantha* strain R81), root-knot nematode (*Meloidogyne incognita*) and tomato (*Solanum lycopersicum* cv. PT-3) plants were used as biological materials. The experience was performed according to previous study (Sharma and Sharma 2017a). Briefly, two nurseries (one with and other without mycorrhiza) prepared in pure sterilized river bad sand. For inoculation of mycorrhiza, a culture with 50

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spores/g of soil was added to mycorrhizal nursery during seeds sowing. After four leaves stage the plants were transplanted in sterilized mixture of sand and soil followed by two ml containing both PGPRs with 10^7 CFU ml^{-1} in the plant roots. 500 freshly hatched *M. incognita* second-stage juveniles (J2) were provided to roots after 10 days of transplanting. Whole experiment was conducted under glass house conditions at an ambient temperature (23–28 °C), photo period (16/8 h day/night cycle) and relative humidity (60%). Total eight treatments with three replicates were performed and all pots were placed in a completely randomized design. Mycorrhizal percent root colonization and phosphorus (P) uptake were observed 10, 20 and 30 days after inoculations (DAI) of J2. Mycorrhizal percentage colonization were determined by Phillips and Hayman (1970) method by using the following formula-

$$\text{Percentage root colonization} = \frac{\text{No.of infected root pieces}}{\text{Total no.of roots pieces}} \times 100$$

Phosphorus has been estimated by vanadomolybdate reagent method. Briefly, vandate solution (1.25 g ammonium vandate +300 ml boiling distilled water) added to the molybdate solution (22.5 g ammonium molybdate +400 ml distilled water) and cooled than 250 ml of concentrate HNO_3 was added and diluted to 1 l. Plant digest was taken in place of stock solution and the calibration curve standard to P determinations was performed by using concentrations from 0 to 6 μg of KH_2PO_4 with intervals of one μg and it had a $R^2 = 0.99$. P concentration was calculated by using the following equation-

$$\text{P\%} = \text{Sample conc.(ppm)} \times \frac{1}{\text{Wt.of sample(gm)}} \times \frac{100}{\text{aliquot (ml)}} \times \frac{\text{Final vol (ml)}}{10000}$$

Statistically, data were presented in mean values ± SE using three replicates ($n = 3$), one factorial analysis of variance (ANOVA) and least significant differences at $p < 0.05$.

3 Results

3.1 Mycorrhizal colonization

Mycorrhizal colonization was confirmed at each observation. Initially during transplanting 69.33% mycorrhizal root colonization were recorded. After that mycorrhizal percentage colonization were increased from initial (10 DAI) to final (30 DAI) level in each mycorrhizal treatment (Table 1). Maximum increase in mycorrhizal percentage was observed in mycorrhiza and PGPRs (MP) treated followed by mycorrhiza (M) alone treated plants. In nematode infected plants mycorrhizal percentage colonization were also increased from initial to final level where PGPRs treated nematode infected plants (MPN) performed better over mycorrhiza treated (MN) plants.

Table 1 Mycorrhizal percentage colonization at 10, 20 and 30 days after inoculation (DAI) of nematode on tomato plant roots. M = Mycorrhiza, N = Nematode, P = PGPRs, (MN, MN & MPN = treatments in combination)

Treatments	Mycorrhizal Percentage Colonization (%)		
	10 DAI	20 DAI	30 DAI
M	73.33	84.00	86.67
MP	73.33	85.33	89.33
MN	70.76	74.67	78.67
MPN	72.00	77.33	81.33

3.2 Phosphorus estimation

Phosphorus uptake level was gradually decreased from initial (10 DAI) to final observations (30 DAI). In nematode infected plants phosphorus uptake level was highly decreased at 30 DAI. At 10 DAI, the maximum uptake level was recorded in mycorrhiza and PGPRs inoculated plants as compared with infected plants. At 20 DAI, MPN and MN plants showed significantly high uptake (28.03 and 26.81% respectively) of phosphorus over nematode (N) infected plants. At 30 DAI, the uptake level was significantly decreased in nematode infected plants. 45.20%, 43.27%, 38.02 and 41.03% phosphorus uptake reduction recorded in nematode infected plants when compared with the MPN, MN, PN and uninoculated (C) plants, respectively (Table 2).

4 Discussion

Several bioinoculants have been applied in field or greenhouse to improve plant growth and health with a positive effect on

Table 2 Effect on Phosphorus uptake on tomato plants at 10, 20 and 30 days after inoculation (DAI) of nematode on tomato plant roots

Treatments	Phosphorus (P) uptake (%)		
	10 DAI	20 DAI	30 DAI
C	0.35 ± 0.014^{ab}	0.33 ± 0.014^{b}	0.29 ± 0.012^{b}
M	0.51 ± 0.010^{d}	0.48 ± 0.010^{e}	0.44 ± 0.012^{cd}
P	0.46 ± 0.010^{c}	0.44 ± 0.011^{d}	0.41 ± 0.012^{c}
MP	0.54 ± 0.019^{d}	0.52 ± 0.019^{e}	0.46 ± 0.008^{d}
N	0.32 ± 0.013^{a}	0.27 ± 0.011^{a}	0.17 ± 0.009^{a}
MN	0.39 ± 0.011^{b}	0.37 ± 0.010^{c}	0.30 ± 0.013^{b}
PN	0.36 ± 0.016^{ab}	0.33 ± 0.016^{bc}	0.28 ± 0.011^{b}
MPN	0.40 ± 0.012^{b}	0.37 ± 0.009^{c}	0.31 ± 0.011^{b}

All the parameters based on three replicates ($n = 3$). C = Control, N = Nematode, P = PGPRs, M = Mycorrhiza, (MP, MN, PN & MPN = treatments in combination). Different letters denote significant differences ($P < 0.05$) among treatments and control. Data with same letter in superscript does not differ significantly

disease control. In this study, we found positive effects on mycorrhizal colonization and phosphorus uptake since 10 DAI to 30 DAI, it is due to the combine effects of PGPRs and mycorrhiza on plants. PGPRs help to mycorrhiza for colonization in rhizosphere and promote fungal spore germination, tube elongation and hypha density (Andreucci et al. 1999; Ravnskov and Jakobsen 1999). Similarly, many of the previous studies confirmed that mycorrhiza and bacteria could interact positively to improve plant growth and nutrition (Siddiqui and Akhtar 2009; Zhang et al. 2012; Flor-Peregrin et al. 2014). Phosphorus (P) is an essential nutrient for plant growth, development, and reproduction that forms part of key molecules such as nucleic acids, phospholipids, ATP, and other biologically active compounds. After nitrogen, P is considered to be the second most important nutrient limiting agricultural production (Lopez-Bucio et al. 2000). In the present study, P uptake was reduced as the plant grew older, similar results were reported in *Citrus limonia* when treated with *Glomus intraradices* (Nogueira and Cardoso 2006). P in soil affects mycorrhizal infection during the early stages of root colonization, when primary infection established (Miranda and Harris 1994; Nogueira and Cardoso 2006). Jaizme-Vega et al. (2006) recorded higher increased of P content in *Meloidogyne* infected papaya plants when treated with co-inoculation of *G. mosseae* and PGPR; similar results were recorded in combined co-inoculation of *Azospirillum* and *Azotobacter* along with nitrogen and sesame cake treated tomato plants (Tiyagi et al. 2015). Hence, PGPRs lead to enhance mycorrhizal colonization which help to AM for phosphorus (P) uptake even in the nematode infections.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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