Archives of Phytopathology and Plant Protection

ISSN: 0323-5408 (Print) 1477-2906 (Online) Journal homepage: http://www.tandfonline.com/loi/gapp20

Plant growth promoting rhizobacteria (PGPR)-mediated root proliferation in black pepper (*Piper nigrum* L.) as evidenced through GS Root software

Diby Paul & Y. R. Sarma

Archives of Phytopathology and Plant Protection
August 2006; 39(4): 311 – 314

Taylor & Francis
Taylor & Francis Group

Plant growth promoting rhizhobacteria (PGPR)-mediated root proliferation in black pepper (*Piper nigrum* L.) as evidenced through GS Root® software

DIBY PAUL & Y. R. SARMA

Indian Institute of Spices Research, Calicut, Kerala, India

(Received 28 April 2005)

Abstract
Pseudomonas fluorescens strains which are proven biocontrol agents in black pepper against foot rot (caused by *Phytophthora capsici*) were also found to enhance root proliferation and fibre root production. Experiments conducted in the greenhouse with five efficient strains of *P. fluorescens* (IISR-6, IISR-8, IISR-11, IISR-13 and IISR-51) showed that the bacterial strains could significantly increase the root biomass of the plants (30 – 135%). Parameters for total root length, root area and root tips were estimated by scanning the entire root system and analysis through GS Root® software (PP systems, Winterstreet, USA). All the strains increased the root length in the treated plants (12 – 127%), the highest being with IISR-6, which was on a par with IISR-11 and IISR-51. A similar trend was observed with the total root area after bacterization (43 – 200%). *The P. fluorescens* treated plants had a higher number of feeder roots as evidenced by the increased number of root tips (82 – 137%). The enhanced growth parameters upon root bacterization could be corroborated with the production of the plant growth hormones IAA & GA by the bacterial strains and their P-solubilization potential.

Keywords: Pseudomonas fluorescens, *black pepper, root proliferation, plant growth hormones*

Introduction

Black pepper, known as the 'King of Spices', is an important export-oriented crop. It is inevitable that fewer pesticides will be used in the future and that greater emphasis will be directed towards disease management using rhizosphere microorganisms (Sarma 2003). Fluorescent pseudomonads are some of the effective candidates for biological control of soil borne plant pathogens owing to their rhizosphere competence (Kloepper & Schroth 1981). These bacteria are termed as Plant Growth Promoting Rhizobacteria (PGPRs) because of their ability to improve plant growth through suppression of deleterious root colonizing microorganisms and by production of plant growth regulators such as gibberellins, cytokinins and indole acetic acid (Suslow & Schroth 1982).

Lynch and Whipps (1991) proved plant growth promotion by rhizobacteria due to chemical and physical stimulation of plant roots resulting in more rapid emergence, higher chlorophyll level and increased stature. Fluorescent pseudomonads produce plant growth regulators and

Correspondence: Diby Paul, Division of Microbiology, M S Swaminathan Research Foundation, 3rd Cross Street, Taramani Institutional Area, Chennai-600113, India. Tel: +91 44 55299024. Fax: +91 44 22541319. E-mail: dibypaul@yahoo.co.in

ISSN 0323-5408 print/ISSN 1477-2906 online
DOI: 10.1080/03235400500301190

even suppress deleterious soil-borne pathogens (Dubeikovsky et al. 1993). Liu et al. (2000) observed longer shoots/roots in plants treated with ACC (1-amino cyclopropane, 1-carboxylic acid) deaminase produced by PGPR. PGPR can synthesize low molecular weight siderophores which can solubilize and sequester iron from the soil and then provide it to the plant cells. Subsequently PGPR can synthesize several different phytohormones, notably Indole Acetic Acid (IAA) which enhance plant growth at various stages by facilitating the solubilization of minerals such as phosphorus from the soil (which then becomes more readily available for plant growth). And some enzymes that modulate plant growth and development (Brown 1981). The current study dealt with rhizobacteria-mediated root proliferation in black pepper.

Materials and methods

Microrganisms and the plant materials used

Five strains of *Pseudomonas fluorescens* that were previously found to be efficient in suppressing root rot (caused by *Phytophthora capsici*) and enhance the growth of black pepper were selected for the study. The planting material of black pepper (*cv. Karimunda*) was obtained from the nursery of IISR, Calicut. Each treatment had five replicates.

Efficacy of rhizobacteria in root initiation and proliferation in black pepper

The strains of *Pseudomonas* were evaluated in the greenhouse for root initiation and proliferation in black pepper plants. Two node cuttings of black pepper were prepared and dipped in bacterial suspension (10^8 cells ml^{-1}) for 30 min. These cuttings were planted in sterile coir compost and the bags were drenched with bacterial suspension (Log-8 cfu ml^{-1}). These cuttings were uprooted after 60 days and thorough examination of the root system took place. Root length, total number of roots, the total area of roots and the root biomass were estimated after scanning and analysis using the software GS-Root® (PP Systems, Winter Street, USA) and compared with that of the untreated plants. The values obtained were the mean of five replicates and means were separated by Duncans' Multiple Range Test.

Production of IAA and GA by the Pseudomonas *strains*

In order to find out the mechanism by which the *Pseudomonas* strains enhance the growth in black pepper, the strains were tested for the production of IAA and Gibberellic Acid (GA), which are growth-promoting hormones in plants. The assay was performed as per the protocol of Hasan (2002).

Results and discussion

Beneficial rhizobacteria trigger different developmental responses at the organogenesis, cell specifications and elongation levels. These responses involve multiple signaling processes in the plant including hormonal pathways, which may be elicited by various bacterial mechanisms and molecular interactions with the root cells.

The bacterial strains significantly increased the root biomass of the plants by 30 – 135% (Table I). The highest increase among the bacterial strains was noticed with IISR-51. All other strains increased the root length in the treated plants (12 – 127%), the highest being with IISR-6, which was on a par with IISR-11 and IISR-51 (Table I). A similar trend was observed with the total root area after bacterization (43 – 200%) (Table I). The PGPR-treated plants

Table I. Different root-growth parameters of plants under treatment.

P. fluorescens strains	Root biomass (g)	Total root length (cm)	Total root area (mm^2)	Total number of roots
IISR-6	46.95^{ab}	1048.046^{a}	2410.088^{a}	506.7^{a}
IISR-8	48.39^{ab}	0754.308^{bc}	1349.492^{bcd}	415.9^{ab}
IISR-11	47.40^{ab}	0962.685^{ab}	2004.929^{ab}	480.9^{a}
IISR-13	33.06^{bc}	0518.401^{cd}	1155.100^{cd}	389.8^{ab}
IISR-51	59.63^{a}	0917.521^{ab}	1668.761^{bc}	490.2^{a}
Control	25.35^{c}	0461.294^{d}	0803.061^{d}	213.9^{b}

Note: Treatments showing a letter in common in the superscripts, do not differ significantly according to Duncan's Multiple Range Test (DMRT) at $P = 0.05$.

had a higher number of feeder roots as shown by the increased number of root tips in the treated plants (82 – 137%) (Table I). IISR-6, IISR-11 and IISR-51 were equally efficient in enhancing the number of roots in black pepper.

These beneficiary attributes can be corroborated with the hormonal and nutritional factors by which the rhizobacteria influence the plant. Plant growth regulators viz. IAA and GA are produced by these strains, as detected in chromatographic studies. The TLC plate was observed in an Alpha Imager-Image Analysis system and in a UV-trans illuminator. GA showed greenish fluorescens under UV light. IAA showed violet red colour in visible light and orange in UV light. GA was detected with all the strains and IAA, with strains, IISR-6, IISR-11, IISR-13 and IISR-51. These strains also solubilized complex forms of P in the soil thus making it available to the plant. The intake of other minerals such as N and P was also found to be more with *P. fluorescens* treated black pepper plants (Diby Paul et al. 2003). These factors not only stimulated the root for higher absorption of nutrients and minerals but also improved root health.

Most root-promoting bacteria synthesize IAA and this has been clearly demonstrated in many cropping systems. While low levels of IAA stimulate root elongation, high levels of bacterial IAA, whether from IAA over producing mutants or strains that naturally secrete high levels or from high-density inocula, stimulate the formation of lateral and adventitious roots (Barbieri & Galli 1993).

The other evidences include that of *P. putida* GR12-2 cells that produced wild type levels of IAA stimulated the formation of many short adventitious roots on mung bean cuttings and in IAA over producing mutant stimulated the formulation of even more adventitious roots than the wild type strain (Mayak et al. 1997). In contrast, the IAA deficient mutant of *P. putida* GR 12-2 stimulated the formulation of fewer roots than the wild type bacterium, and the roots were generally longer than those induced by the wild type strain. Interaction of adventitious and lateral roots may be mediated by IAA-induced ethylene. An ACC deaminase – negative mutant of *P. putida* GR12-2 which cannot reduce ethylene levels in plants stimulated the formulation of more small adventitious roots than the wild type strain (Mayak et al. 1997). The increase in the number of roots on the cuttings has also been correlated with an increase in ethylene production. The IAA-deficient mutant of *P. putida* GR12-2 may not stimulate the formulation of ACC synthetase and therefore ethylene synthesis in plants. Thus fewer adventitious roots were initiated on the cuttings (Pattern & Glick 2002).

In conclusion, strains of *P. fluorescens* identified as biocontrol agents in black pepper were found to enhance the root initiation and root proliferation imparting better growth. The bacteria bring this about by production of plant-growth promoting hormones and improvement of nutrient uptake by plants.

Acknowledgements

The authors are grateful to the Director of Indian Institute of Spices Research, Calicut and the Head of the Division of Crop Protection for their help and support.

References

Barbieri P, Galli E. 1993. Effect on wheat root development of inoculation with an *Azospirillum brasiliense* with altered indole-3-acetic acid production. Res Microbiol 144:69–75.

Brown ME. 1981. Microbiology of roots infected with the take all fungi (*Gaeumannomyces graminis* tritici) in phased sequence of winter wheat. Soil Biol Biochem 13:285–291.

Diby Paul, Srinivasan V, Anandaraj M, Sarma YR. 2003. *Pseudomonas fluorescens* mediated nutrient flux in the black pepper rhizosphere microcosm and enhanced plant growth. In: Reddy MS, Anandaraj M, Eapen SJ, Sarma YR, Kloepper JW, editors. Abstracts and short papers of the 6th International PGPR workshop. Calicut, India: Indian Institute of Spices Research. pp 18–24.

Dubeikovsky AN, Mordukhova EA, Kochethov VV, Polikarpova FY, Boronin AM. 1993. Growth promotion of black currant soft wood cuttings by recombinant strain, *Pseudomonas fluorescens*, BSP 53a synthesizing an increased amount of indole-3-acetic acid. Soil Biol Biochem 25:1277–1281.

Hasan HSH. 2002. Gibberellin and auxin production by plant root fungi and their biosynthesis under salinity – calcium interaction. Rostlina vyroba 48:101–106.

Kloepper JW, Schroth MN. 1981. Relationship of *in vitro* antibiosis of plant growth promoting rhizobacteria and the displacement of root microflora. Phytopathology 71:1020–1024.

Liu J, Ovakim DH, Charles TC, Glick BR. 2000. An ACC deaminase minus mutant of *Enterobacter cloacae* UW4 no longer promotes root elongation. Curr Microbiol 41:101–105.

Lynch JM, Whipps JM. 1991. Substrate flow in the rhizosphere. In: Keister DL, Cregan PB, editors. The rhizosphere and plant growth. Dordrecht: Kluwer. pp 15–24.

Mayak ST, Tirosh T, Glick BR. 1997. The influence of plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2 on the rooting of mung bean cuttings. In: Ogoshi A, Kobayashi K, Homma Y, Kodama F, Kondo N, Akino S, editors. Plant growth-promoting rhizobacteria; present status and future prospects. Paris: OECD. pp 313–315.

Pattern CL, Glick BR. 2002. Role of *Pseudomonas putida* IAA in development of the host plant root system. Appl Environ Microbiol 68:3795–3801.

Sarma YR. 2003. Global scenario of disease and pest management in black pepper. Int Pepper News Bull July–Dec:69–74.

Suslow TV, Schroth MN. 1982. Rhizobacteria of sugar beets: Effects of seed application and root colonization on yield. Phytopathology 72:199–206.