Crop genotype and a novel symbiotic fungus influences the root endophytic colonization potential of plant growth promoting rhizobacteria

Geeta Singh¹, N. Singh² and T.S. Marwaha¹

¹Division of Microbiology, Indian Agricultural Research Institute, New Delhi-110012, India

²National Bureau of Plant Genetic Resources, New Delhi-110012

ABSTRACT

Effect of plant genotype on the root endophytic colonization ability of a plant growth promoting rhizobacteria (PGPR), *Pseudomonas striata* was undertaken in this study. Use of a lac-Z tagged *P. striata* strain showed that, it can exist as an endophyte and the plant genotype determines the performance of the inoculated PGPR. The cultivars of *Zea mays* L. (maize) and *Vigna radiata* L. (mung bean) tested showed differential affinity to the PGPR (*P. striata*) as reflected by a significant variation in the root endophytic colonization ability of *P. striata*. Coinoculation with a novel symbiotic fungus *Piriformospora indica* was found to stimulate endophytic colonization of *P. striata* in both maize and mungbean. The root exudates of maize and mungbean cultivars showed variations in the total sugar and amino acid contents. However, no consistent relationship was recorded between the concentrations of these metabolites and endophytic colonization of the added PGPR. [**Physiol. Mol. Biol. Plants** 2009; 15(1) : 87-92] E-mail : geetasingh63@yahoo.co.in

Key words : Endophytic colonization, Genotype, Root exudates

Abbreviations : Plant growth promoting rhizobacteria (PGPR), *Pseudomonas striata* (*P. striata*), *Piriformospora indica* (*P. indica*), Maize (*Zea mays* L.), Mung bean (*Vigna radiata* L.)

INTRODUCTION

Rhizosphere microorganisms increase the ability of plants to acquire nutrients from the soil by either increasing the extent of root system (e.g. fungal hyphae) or solubilizing macronutrients such as phosphorus or sulfur (Bucio *et al.*, 2007; Smith and Read, 1997). An axenically cultivable mycorrhiza-like fungus, *Piriformospora indica* significantly improves the growth and biomass of different plants (Varma *et al.*, 1999). Plant growth promoting rhizobacteria (PGPR) are bacteria capable of promoting plant growth by colonizing the plant root (Benizri *et al.*, 2001). A number of studies show synergistic effect of plant growth promoting rhizobacteria and symbiotic fungi (Kohler *et al.*, 2007). Root colonization (endophytic & exophytic) is a pre requisite to derive benefits from a PGPR (Chabot *et al.*, 1996; Singh and Mukerji, 2006).

The plant-microbe interrelationship is a result of a variety of interactions taking place at molecular level between the participating system (Steinkellner *et al.*, 2007). The root zone (rhizosphere) is regarded as a “hot spot” for microbial colonization (endo and exo) and activity (Walker *et al.*, 2003). This is attributed to the availability of nutrients to microbes through the root exudates (Duineveld *et al.*, 2001; Bais *et al.*, 2006). Plants and crops are crucial factors for the supply of energy and carbon to the microbial community by producing root exudates. Plants can alter the composition of soil microbial communities. For example, the community composition of fluorescent *Pseudomonads* in the rhizosphere of flax (*Linum usitatissimum* L., cv. Opaline) and tomato (*Lycopersicon esculentum* Mill., cv. H63-5) differed from that found in crop free soil (Nehl *et al.*, 1997). Similarly, Germida *et al.* (1998) found that the rhizosphere communities of wheat (*Triticum aestivum*) and canola (*Brassica juncea*) grown at the same field site differed in its compositions. Thus, it appears that the plant species influences the abundance

Correspondence and reprint requests : Geeta Singh

and diversity of the soil microbial communities colonizing their roots (Bosco *et al.*, 2006).

Effect of different cultivars (genotypes) of a given crop on the performance of the added microbial inoculant, is however, not clear. The present study describes that variations exist between cultivars of *Zea mays* L. and *Vigna radiata* L. towards their affinity to endophytic colonization of lac Z-tagged *Pseudomonas striata* (*P. striata*) alone or in combination with a novel symbiotic plant growth promoting fungus, *Piriformospora indica*. The biochemical basis of these variations has also been explored.

MATERIALS AND METHODS

Plant Material

Seeds of *Zea mays* L. cvs. PRO 311, Him 129, Seedtech, Bio 9681, Mahikanchan, Kiran and *Vigna radiata* L. cvs Pusa-Vishal, P-Ratna, P-9531, P-105, PS-16, representing contrasting agronomic traits were procured from the Division of Genetics, IARI, New Delhi, India.

Microbial Inoculation

The culture of *P. striata* used in the present investigation was obtained from the Division of Microbiology, Indian Agricultural Research Institute (IARI), New Delhi-12, India. *Escherichia coli* (S 17-1) containing plasmid pSUPTn:B-20 for transferring Tn5-lacZ marker to *Pseudomonas striata* was obtained from Dr. R. Simon, Fakultät für Biologie, University of Bielefeld, Germany. Genetic tagging of *P. striata* with Tn5-lacZ markers was done by using plasmid pSUPTn:B-20 of *E. coli* (S17-1) (Simon *et al.*, 1983) following the procedure described by Miller (1972). The lac Z tagged culture of *P. striata* was identified as blue colonies on tryptone yeast extract (TY) medium amended with kanamycin and nalidixic acid @ 50 µg ml⁻¹. The TY medium (Kan⁵⁰ NaI⁵⁰) was coated with a mixture containing 40 µl/ml of a stock solution of X-Gal (5 Bromo-4 Chloro-3 indolyl-B-D galactoside) and 4 µl of a stock solution of IPTG (isopropyl-thio-B-D galactoside). Presence of Lac Z marker in *P. striata* was reconfirmed by appearance of the blue colonies on Pikovskaya's medium (1948) amended with Kanamycin and nalidixic acid @ 50 µg ml⁻¹ each.

Piriformospora indica, an axenically cultivable mycorrhiza-like (patented) fungus was provided by Prof. Ajit Kumar Varma; School of Life Sciences, Jawaharlal Nehru University, (JNU) New Delhi, India. The fungus was maintained on modified *Aspergillus* medium (Kaefer, 1977).

Experimental Treatments

The seeds were surface sterilized with 4 % sodium hypochlorite (v/v) for 15 min., followed by 70 % ethanol (v/v) for 30 sec, rinsed 8-10 times with sterile distilled water and inoculated as per the treatments listed below. A total of 400 seeds in four replicates were sown per variety for each treatment.

- (i) No microbial inoculation – control.
- (ii) Lac-Z tagged *Pseudomonas striata* broth culture having 6.2 x 10⁶ cfu/ml.
- (iii) *Pseudomonas striata* and *P. indica* agar disc, fortified with actively growing fungal propagules (hyphae and spores).

Endophytic counts of *Pseudomonas striata*

The surface sterilized and inoculated seeds under different treatments were germinated on water-agar (0.7 %) under aseptic conditions. Germination percentage and length of the radicle was recorded (data not given). To detect the endophytic colonization by the Lac- Z tagged *P. striata* strain, the radicles (0.5 g dry wt.) were aseptically removed from seeds, surface sterilized with NaOCl (1.05 % v/v) in Phosphate Buffer Saline (PBS) and placed on a rotary shaker (200 rpm) at 22 °C for 10 min. Radicles were rinsed four times with sterile PBS and 0.1 ml of the final wash was diluted with 9.9 ml of 1/10th strength nutrient broth to check for contamination (McInroy and Kloepper, 1995). The radicles were then triturated with sterile rods in cavity slide containing 1 ml of sterile PBS. The root-PBS slurry/mixture was diluted with 9.0 ml of sterile PBS, subjected to dilution series and plated on TY plates fortified with Kan⁵⁰ NaI⁵⁰. The number of blue coloured colonies were recorded in each treatment.

Root exudates were collected by germinating the seed in deionized water (20 ml) and were concentrated by lyophilization to one tenth the volume. The amino acid content was estimated by Ninhydrin method (Moore and Stein, 1948) and the non-reducing sugar content by following the protocol given by Miller (1972).

RESULTS

The results presented in tables 1 and 2 revealed a significant promotion of the endophytic colonization of *P. striata* in the roots of different maize and mungbean varieties by the symbiotic fungus *P. indica*. The effect

Table 1. Endophytic counts (cfu/ml) of *Pseudomonas striata* in the axenically grown radicles of different maize (*Zea mays* L.) varieties. Endophytic counts of lac-Z tagged *Pseudomonas striata* as individual or as combined inoculation (*Pseudomonas striata* + *Piriformospora indica*) was obtained on TY medium fortified with Kan⁵⁰ Nal⁵⁰. The blue coloured colonies were counted as described in the Materials and Methods. The results are presented as colony forming units (cfu) per ml. Each ml represents 0.5g dry weight of the root tissue. The values are average of four replicates.

Treatment/ variety	Maize varieties						
	Control	PRO-311	Him-129	Seedtech	Bio-9681	Mahikanchan	Kiran
Ps	0.00	66.667	47.500	45.000	30.000	38.333	25.000
Ps+Pi	0.00	81.000	105.333	24.333	25.333	123.667	32.000
Mean	0.00	73.833	76.416	34.666	27.666	81.000	28.500
	T	V	TXV				
SE+	4.5238	8.4633	11.9690				
CD at 5%	12.7549	23.8624	33.7467				

of combined inoculation (Ps + Pi) was significantly better than single inoculation with *P. striata* alone in both the crops tested. Different cultivars of Maize and mungbean exhibited variation in their affinity to the endophytic presence of *Pseudomonas* sp. in their respective roots. Among the six genotypes (cultivars) of maize tested, PRO-311, Him 129 and Mahikanchan were statistically identical and significantly better than Seedtech, Bio 9681 and Kiran.

A highly significant promotion in endophytic colonization of the added PGPR (*P. striata*) following co-inoculation with *P. indica* was noticed in maize cultivars Him 129 and Mahikanchan. However, the symbiotic fungus did not show similar synergistic effect on maize cultivars Kiran, Bio-9681, Seedtech and PRO-311. The highest endophytic count of *Pseudomonas striata* was recorded in the radicles of Mahikanchan followed by Him 129 and PRO 311 in treatments receiving

Table 2. Endophytic counts (cfu/ml) of *Pseudomonas striata* in the axenically grown radicles of different Mungbean (*Vigna radiata*) varieties. Endophytic counts of lac-Z tagged *Pseudomonas striata* as individual or as combined inoculation (*Pseudomonas striata* + *Piriformospora indica*) was obtained on TY medium fortified with Kan⁵⁰ Nal⁵⁰. The blue colored colonies were counted as described in the Materials and Methods. The results are presented as colony forming units (cfu) per ml. Each ml represents 0.5g dry weight of the root tissue. The values are average of four replicates.

Treatment	Moongbean varieties					
	Control	P-Vishal	P-Ratna	P-9531	P-105	P-16
<i>P. striata</i> (Ps)	0.000	57.500	286.667	328.333	198.333	499.167
<i>P. indica</i> + <i>P. striata</i> (Ps+Pi)	0.000	1488.333	286.667	197.500	168.300	755.000
Mean	0.000	772.916	228.667	262.916	183.316	627.08
	T	V	TXV			
SE+	17.0579	29.5451	41.7830			
CD at 5%	48.2397	83.5535	118.1623			

P. indica as a co-inoculant. The response of the different mung bean cultivars in terms of their affinity to the endophytic occurrence of *Pseudomonas striata* in their root systems was also found to be variable. The cultivars P-Vishal and PS-16 were statistically identical and significantly better than P-Ratna, P-9531 and P-105. The affinity of P-Ratna and P-9531 was similar. Least counts of root endophytic *P. striata* were observed in cultivar P-105. In Mung bean cultivars P-9531 and P-105, co-inoculation with *P. indica* did not promote endophytic counts of *P. striata*. However, a significant promotion of endophytic counts of *P. striata* was recorded in the roots of P-Vishal and PS-16 following co-inoculation with *P. indica*.

The content of amino acid (A.A.) and non-reducing sugars (N.R.) estimated in the lyophilized root exudates of different maize cultivars are presented in table 3. Statistically significant differences were noticed in the quantities of total amino acid and N.R. sugar content in the root exudates of the different maize varieties analyzed. The three varieties namely, PRO-311, Bio-9681 and Kiran showed significantly high amino acid content as compared to Seedtech, Him 129 and Mahikanchan. Whereas the least amount of A.A. was recorded in Seedtech (21.722 µg/ml), which was significantly lesser than Him 129 and Mahikanchan. Highest A.A. and N.R. sugar content in the maize root exudates was recorded in the variety Kiran (77.508 µg/

ml) and Him 129 (172.57 µg/ml) respectively. These metabolites were significantly low in maize variety Seedtech and Mahikanchan. The non-reducing sugar content of PRO 311 and Seedtech was statistically identical and significantly lower than Him 129, Bio 9681 and Kiran. The assay of root exudates of the different mungbean varieties evaluated also showed a wide variation with reference to their amino acid and N.R. sugar content. The highest A.A. content was recorded in mungbean variety P.105 and the highest non-reducing sugar content was found in P-Ratna. The amino acid content of P-9531, P-16 and P-Vishal was statistically identical and significantly lower than P-105. The N.R. sugar content was significantly lower in P-Vishal and P-9531 as compared to P-Ratna. Root exudates of maize var Seedtech and Mahikanchan were relatively poor in their A.A. and N.R. sugar content whereas Bio 9681 and Kiran had relatively high amounts.

DISCUSSION

Root endophytic and exophytic colonisation is a prerequisite to derive benefits from PGPR (Chabot *et al.*, 1996; Bucio *et al.*, 2007). The presence of endophytic microorganisms imparts tolerance to biotic and abiotic stresses. In this study, the use of a genetically tagged (lac-Z) *Pseudomonas striata* strain conclusively demonstrated that this PGPR exhibits exophytic as well

Table 3. The amino acid and non reducing sugar contents in the root exudates of Maize (*Zea mays* L.) were estimated by germinating the seeds in deionized water (20 ml) for one week duration followed by lyophilization to one tenth the volume. The amino acid (AA) and non reducing sugar (N.R. Sugar) contents were expressed as µg/ml of the root exudates.

Variety	PRO-311	Him-129	Seed-tech	Bio-9681	Mahi-kanchan	Kiran	SEm+	CD at 5 %
Amino acid (µg/ml)	76.203	56.670	21.722	71.488	47.473	77.508	4.2487	14.1482
N.R. sugar (µg/ml)	130.39	172.57	132.51	171.56	150.87	167.26	4.7563	15.8385

Table 4. The amino acid and non reducing sugar contents in the root exudates of Moong (*Vigna radiata* L.) were estimated by germinating the seeds in deionized water (20 ml) for one week duration followed by lyophilization to one tenth the volume. The amino acid (AA) and non reducing sugar (N.R. Sugar) contents were expressed as µg/ml of the root exudates.

Variety	P-vishal	P-Ratna	P-9531	P-105	P-16	SEm+	CD at 5 %
Amino acid (µg/ml)	25.430	36.087	25.353	41.270	28.403	3.1572	12.1238
N.R. sugar (µg/ml)	29.726	61.362	29.547	54.82	41.783	7.7175	29.6352

as endophytic colonisation ability. Further, the endophytic colonisation ability of the strain is stimulated by co-inoculation with a novel symbiotic fungus *Piriformospora indica*. Therefore, symbiotic fungus behaves in an identical manner to the arbuscular mycorrhizal fungi (AMF) in stimulating the PGPR activity in the rhizosphere of crop, as reported by Werner (2001) and Kohler *et al.* (2007). Roesti *et al.* (2005) reported the stimulation of exophytic PGPR in wheat rhizosphere following inoculation with arbuscular mycorrhizal fungi. The arbuscular mycorrhizal fungi (AMF) was found to play a dominant role in comparison to (wheat) host crop in promoting exophytic colonization. *Piriformospora indica* is an endophyte and resembles AM fungi in many properties. Singh and coworkers (2008) have reported a synergistic interaction between AMF and bacteria on grasses. Our results indicate that *Pseudomonas striata* and *Piriformospora indica* dual inoculation could be an attractive and efficient biological system to augment micro/macro nutrient and water availability to the plants.

The importance of root exudates in mediating root-microbe interactions is well documented (Walker *et al.*, 2003; Weir *et al.*, 2004). Chemical signaling between compatible host and microorganisms like PGPR and AMF triggers a chain of reactions preceding successful root colonization (Buee *et al.*, 2000). PGPR locate plant roots through cues exuded from roots and root exudates such as carbohydrates and amino acids. Chemotaxis is responsible for competitive colonization by extracellular PGPR. For instance, a major outer membrane protein (MOMP) from *Azospirillum brasilense* binds to root exudates from several plant species with differing affinities. The *A. brasilense* MOMP showed stronger adhesion to root exudates of cereals than exudates of legumes and tomatoes (Burdman *et al.*, 2001) indicating the selective influence of host genotype on colonization ability of bacteria. The minor differences in the crop genotypes manifest itself as altered exudation pattern. What is striking is that cultivars of a given crop show different affinity to the added PGPR indicating the significance of minor genetic variations existing among the cultivars in determining the root colonising ability of the added bioinoculant. Our data show variations in two major components of the root exudates namely, sugars and amino acids which serves as a source of carbon and energy for the microbial growth. Root exudates such as carbohydrates and amino acids act as chemo-attractants and thereby stimulate PGPR to root surfaces (Somers *et al.*, 2004; Walker *et al.*, 2003; Weir *et al.*, 2004). In the present study, the metabolites

analysed in root exudates namely, sugars and amino acids were found to vary among the cultivars of both the crops tested. Similar observations were made by Duineveld *et al.* 2001 in Chrysanthemum. The present investigation suggests that composition of root exudates is governed by the crop genotype and associated microorganisms for example symbiotic fungi (Jones *et al.*, 2004).

Root exudates of different tomato genotypes were found to differ chemically and thereby exerted differential effect on seed germination of Broom rape. Haicher and coworkers (2008) using $^{13}\text{CO}_2$ grew wheat, maize, rape and clover and the bacteria assimilating root exudates were characterized by DGGE analysis. The study revealed that the root exudates play a key role in the microbial diversity and abundance in the rhizosphere. Similarly, Bias and coworker (2005) demonstrated that the antimicrobials released from the root exudates impart resistance to plants against pathogenic invasion. By matching the rhizobacterium genotypes with crops for which they have colonization preference, the degree of root colonization could be increased due to the fact that rhizosphere of different plant species differentially support the introduced bio-inoculants. These results have implications for plant breeders for adopting breeding strategies for developing crop genotypes responsive to microbial bioinoculants. This is of particular relevance in organic farming.

ACKNOWLEDGEMENTS

The authors thank Dr. Ajit Kumar Varma, Prof. School of Life Sciences, JNU, New Delhi for providing the culture of *Piriformospora indica*.

REFERENCES

- Bais HP, Weir TL, Perry LG, Gilroy S and Vivanco JM (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Ann. Rev. Plant Biol* 57: 233-266.
- Bais HP, Prithiviraj B, Jha AK, Ausubel FM and Vivanco JM (2005). Mediation of pathogen resistance by exudation of antimicrobials from roots. *Nature* 434: 217-221.
- Benizri E, Baudoin E and Guckert A (2001). Root colonization by inoculated plant growth promoting rhizobacteria. *Biocontrol Sci. Technol* 11: 557-574.
- Bosco M, Baruffa E and Picard C (2006). Organic breeding should select for plantgenotypes able to efficiently exploit indigenous Probiotic Rhizobacteria. *Paper presented at the European Joint Congress 'Organic*

- Farming and European Rural Development*. May 30-31: 376-377.
- Bucio JL, Campos-Cuevas JC, Hernandez-Calderon E, Valasquez-Bacerra C, Farias-Rodriguez R, Macias-Rodriguez LI and Valencia-Cantero E (2007). *Bacillus magisterium* rhizobacteria promote growth and alter root system architecture through an auxin and ethylene-independent signaling mechanism in *Arabidopsis thaliana*. *Mol. Plant Microbe Interactions* 20: 207-217.
- Bucee M, Rossignol M, Jauneau A, Ranjeva R and B'ecard G (2000). The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. *Mol. Plant-Microbe Interact* 13: 693-98.
- Burdman S, Dulguerova G, Okon Y and Jurkevitch E (2001). Purification of the major outer membrane protein of *Azospirillum brasilense*, its affinity to plant roots, and its involvement in cell aggregation. *Mol. Plant-Microbe Interact* 14: 555-58.
- Chabot R, Antoun H. and Cesias MP (1996). Growth promotion of maize and lettuce by phosphate solubilizing *Rhizobium leguminosarum* biovar. Phaseoli. *Plant Soil* 184: 311-321.
- Duineveld BM, Kowalchuk GA, Keijzer A and Van Elas JD (2001). Analysis of bacterial communities in the rhizosphere of chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. *Appl. Environ. Microbiol.*, 67: 172-178.
- Germida JJ, Sicillano SD, Defreitas JR and Seib AM (1998). Diversity of root-associated bacteria associated with field grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) *FEMS Microb. Ecol* 26: 43-50.
- Haichar FZ, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J, Heulin T and Achouak W (2008). Plant host habitat and root exudates shape soil bacterial community structure. *Eur. J. Plant Pathol* 122: 395-401.
- Jones DL, Kuzyakov Y and Hodge A (2004). Plant and mycorrhizal regulation of rhizodeposition. *New Phytol* 163: 459-480.
- Kaefer E (1977). Meiotic and Mitotic recombination in *Aspergillus* and its chromosomal aberrations. *Advances Genet* 19: 33-131.
- Kohler J, Caravaca F, Carrasco L and Roldan A (2007). Interaction between a PGPR, an AM fungus and a phosphate solubilizing fungus in the rhizosphere of *Lactuca sativa*. *Appl. Soil Ecol* 35: 480-487.
- Lemanceau P, Corberand T, Gardan L, Labour X, Laguerre G, Boeufgras J and Alabouvette L (1995). Effect of two plant species, flax (*Linum usitatissimum* L.) and tomato (*Lycopersicon esculentum* Mill.) on the diversity of soil-borne populations of fluorescent Pseudomonads. *Appl. Environ. Microbiol* 61: 1004-1012.
- Marschner H, Treeby M, Romheld V (1989). Role of root-induced changes in the rhizosphere for iron acquisition in higher plants. *Z Pflanzenernahr Bodenk* 152: 197-204.
- Miller JH (1972). Experiments in molecular genetics. Cold spring Harbour Lab., Cold Spring Harbour, New York.
- McInroy JA and Kloepper JW (1995). Population dynamics of endophytic bacteria in field grown sweet corn and cotton. *Can. J. Microbiol* 41: 895-901.
- Moore S and Stein WH (1948). In: *Methods Enzymol.* (Eds. Colowick SP and Kaplan ND), Academic press, New York, 3: 468.
- Nehl DB, Allen S and Brown JF (1997). Deleterious rhizosphere bacteria: an integrating perspective. *Appl. Soil. Ecol* 5: 1-20.
- Peters NK and Long SR (1988). Alfalfa root exudates and compounds which promote or inhibit induction of *Rhizobium meliloti* nodulation genes. *Plt. Physiol* 88: 396-400.
- Pikovskaya RE (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiologia* 17: 362.
- Roesti D, Ineichen k, Braissant O, Redecker D, Wiemken A and Aragno M (2005). Bacteria Associated with Spores of the Arbuscular Mycorrhizal Fungi *Glomus geosporum* and *Glomus constrictum*. *Appl. Environ. Microbiol* 71: 6673-6679.
- Simon R, Prierer U and Pahter A (1983). A broad host range mobilization system for *in vivo* genetic engineering : Transposon mutagenesis in gram negative bacteria. *Biotechnol* 1: 784-791.
- Smith SE and Read DJ (1997). Mycorrhizal symbiosis. Academic Press, San Diego. Calif.
- Singh BK, Nunan N, Ridgway KP, McNicol J, Young JPW, Daniell TJ, Prosser JI and Millard P (2008). Relationship between assemblages of mycorrhizal fungi and bacteria on grass roots. *Env. Microbiol* 10: 534-541.
- Singh G and Mukerji KG (2006). Root exudates as determinant of rhizospheric microbial biodiversity. *Soil Biol* 7: 39-53.
- Somers E, Vanderleyden J, Srinivasan M (2004). Rhizosphere bacterial signalling: a love parade beneath our feet. *Crit. Rev. Microbiol* 30: 205-235.
- Steinkellner S, Mammerler R, Vierheilig H (2008). Germination of *Fusarium oxysporum* in root exudates from tomato plants challenged with different *Fusarium oxysporum* strains. *Eur. J. Plant Pathol* 122: 395-401.
- Varma A, Verma S, Sudha N, Buetehorn B and Franken P (1999). *Piriformospora indica*, a cultivable plant growth promoting root endophyte. *Appl. Environ. Microbiol* 65: 2741-2744.
- Walker TS, Bais HP, Grotewold E and Vivanco JM (2003). Root exudation and rhizosphere biology. *Plant Physiol* 132: 44-51.
- Weir TL, Park SW and Vivanco JM (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Curr. Opin. Plant Biol* 7: 472-479.
- Werner D (2001). Organic signals between plants and microorganisms. In: *Rhizosphere, biochemistry and organic substances at the soil-plant interface*, (Eds. Pinton R, Varanini Z, Nannipieri P) Marcel Dekker, New York, 197-222.