Chapter 4
Piriformospora indica: Perspectives and Retrospectives

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4.1 Introduction

Soil is a dynamic system consisting of soil minerals/clay, soil organic matter, and soil microorganisms which play an important role in improving soil quality, enhancing soil fertility, and maintenance of soil structure stability. This soil microbial diversity is thus a precious resource that indicates the agricultural prosperity of a nation with a single gram of soil that may consist several thousand types of microbes in terms of both species richness and species number (Wardle et al. 2004). Soil microbial diversity encompasses the prokaryotic forms like bacteria, cyanobacteria, actinomycetes, and myxomycetes and eukaryotic forms like fungi (both soil yeasts and molds) and protozoa (amoeba and ciliates) as well as bacteriophages/viruses. Among these diverse life forms, the soil fungi are of great ecological importance pertaining to organic matter decomposition and nutrient recycling. Fungi are multicellular, heterotrophic, uni- to multinucleated microbes having four major classes and several orders. Certain oomycetes and basidiomycetous fungi exhibit symbiosis with higher plants; the "symbiotic root fungi" possess higher potential for application in agriculture as these are known to improve soil quality in terms of soil stabilization by secretion of particle-binding proteins (like glomalin) and function as plant growth promotive as well as plant probiotic

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microbes. The major genera of symbiotic fungi, commonly termed as "mycorrhizal fungi," include the structurally and molecularly diverse ectomycorrhiza, endomycorrhiza, ectendomycorrhiza, and arbuscular mycorrhizal (AM) fungi (*Glomus, Scutellospora, Acaulospora, Gigaspora* genera). The mycorrhizal associations are plant mutualistic interactions that have appeared due to coevolution of certain specific plant genera with these fungi over several eras which resulted in transformation followed by initial colonization of aquatic or semiaquatic plant forms (Ligrone et al. 2007) to true terrestrial vascular land plants (Brundrett 2002). Due to their enormous ecological and agricultural importance, these are one among the elaborately studied symbiotic associations at the molecular genetics, proteome, and interactome levels. These interactions result in formation of certain specific or in general morphologically distinct structures of fungal origin (extramatricular hyphal extensions, mantle, arbuscules, and vesicles) which result in capturing of nutrient elements from the soil solution beyond the depletion zone considering the plant benefit perspectives and in increasing the host–fungal interaction surface volume for better nutrient exchange between both partners. The AM fungal associations are unique and could be exploited for attaining higher crop yields with suboptimal chemical fertilizer and pesticide inputs (Kuo and Huang 1982; Jeffries et al. 2003; Arpana and Bagyaraj 2007; Hu et al. 2009) though the mass production of authentic culture for commercial success suffers a basic hitch due to the obligate symbiont characters of the fungi. Several advancements in the culturing of these fungal forms have paved towards achieving potential culture inoculants but not to match the commercial scale production levels. Any mycorrhizologist would prefer for studies on a cultivable AM fungi-like organism, and the discovery of *Piriformospora indica* puts forth the possible solution for it by offering an AM fungi-like benefits supermounted by the premiere characteristics of easy cultivation on known semisynthetic to synthetic media like potato dextrose agar and Kaefer's agar (Verma et al. 1998).

P. indica belongs to phylum Hymenomycetes, class Basidiomycota, and order Sebacinales having enormous potential as a plant growth-promoting fungal endophytic agent to a variety of plant hosts belonging to diverse families or groups, even the members of families like Cruciferae which are not infected by the AM fungi. It has been named due to formation of typical pyriform chlamydospores which act as perennation bodies similar to the AM spores. Another similarity among the two is the formation of extended hyphal structures that ramify the soil in vicinity of the host roots hunting and accumulating nutrients like phosphorus, iron, manganese, zinc, and many more from a diameter of several square meters of the soil. Within the host plant root tissues, structures similar to arbuscules and vesicles occur in the intercellular spaces as well as may be packed intracellularly which enhances the interaction interface among the microsymbiont and the host plant tissues or may act as storage organ of the microsymbiont. A perusal of current literature has shown that *P. indica* has enormous potential for growth promotion of plants by colonization of roots. So a lot of focus is given on studies related to *P. indica* to fully exploit the enormous potential encapsulated in this fungus. Though it mimics the morphology, it shares physiological and application potentials of arbuscular mycorrhizal

fungi, but it has its unique features, which command its elaborative commercial success over the AM fungal counterparts.

4.2 Discovery and Taxonomic Position

Though the fungal endophytes are known for asymptomatic infection of the root tissues of several aquatic, semiaquatic, and terrestrial plants since ages (Rodriguez and Redman 2008), the first report of plant beneficial root fungal association was given by Kamienski (1881) followed by reports on the mycorrhizal associations in plants. The discovery of plant root-interacting, endophytic, and arbuscular mycorrhiza-like fungus took place in 1998 by isolation of *P. indica* from the sandy rhizospheric soil of woody shrubs *Prosopis juliflora* and *Zizyphus nummularia* in Thar Desert in northwestern Rajasthan, India (Verma et al. 1998). This fungus, though related to *Rhizoctonia,* is known for its ability to infect a variety of host plants and acts as a potent plant growth-promoting microbe through an array of morphological and physiological attributes which are now patented from the European Patent Office, Muenchen, Germany, with patent number 97121440.8-2105 (Nov. 1998) (Varma and Franken 1997). This fungal culture is available from culture repositories in India (National Bureau of Agriculturally Important Microorganisms, Mau Nath, UP, India) and in Europe (Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunsweich, Germany).

On the basis of latest molecular fungal classification including information from molecular analysis of 18S rRNA taxonomically, *P. indica* belongs to order Agaricini of class Hymenomycetes among division Basidiomycota which includes basidiospore-producing, hymenium-bearing fungal forms involved in intimate association with the plant roots particularly related to the ectomycorrhizal genera *Epulorhiza* to *Tulasnella* and *Sebacina*, *Ceratorhiza* to *Ceratobasidium*, or *Moniliopsis* to *Thanatephorus* (Selosse et al. 2002; Hibbett et al. 2007).

Ultrastructurally, the hyphae of *P. indica* bear characteristic dolipore septa with continuous parenthosomes under transmission electron microscope, while serologically this endophyte shares many antigenic properties with that of the mycorrhizal fungi (Varma et al. 2001; Weiss et al. 2004). The 5′ terminal large subunit rRNA sequence analysis and the ultrastructural analysis have led to reclassification of *P. indica* in a separate newly defined order monotypic Sebacinales housing globally distributed great variety of ericoid, orchid, cavendishioid, jungermannioid, and ectomycorrhizae (Setaro et al. 2006; Kirk et al. 2008). On basis of the phylogenetic analysis, order Sebacinales contains two distinct groups or clades, viz*.,* clade/group A comprising of basidiomes and sebacinoid mycobionts harboring ectomycorrhizas and orchid mycorrhizas and clade/group B forming a heterogeneous group including ericoid, cavendishioid, and jungermannioid mycorrhizas (Weiß et al. 2004; Setaro et al. 2006). Order Sebacinales has a single family Sebacinaceae comprised of eight genera and 29 species (Cannon and Kirk 2007; Kirk et al. 2008). *P. indica* has been classified as a member of genus *Sebacina* spp. in the group B of order

Sebacinales. Similar to the other members of its order, *P. indica* bears tremendous plant growth-promoting potentials which result in enormous improvement in the overall plant biomass and thus yield (Singh et al. 2003). Moreover, due to its wider host range, it can effectively infect the orchids and may act as a specific orchidaceous mycorrhizal fungus particularly for genus *Dactylorhiza* (Deshmukh et al. 2006).

4.3 Structure, Life Cycle, and Host Range

P. indica shows morphological, physiological, functional, and serological similarities with AM fungal genera *Glomus* and *Gigaspora* though due to the difference in the large subunit rRNA sequence and presence of dolipore septa, it is not classified among zygomycetous AM fungi. The fungus can easily be axenically cultivated on a number of defined, complex, synthetic media (Hill and Kaefer 2001) which facilitate mass cultivation of this fungus without the requirement of the living organs/tissues of the host plant as are required for cultivation of obligatory symbionts AM fungi.

Morphologically, *P. indica* produces thin-walled, irregularly septate, white, and almost hyaline hyphae which exhibit multinucleate character though there exists variability in number of nuclei in each hyphal segment. These hyphae typically range from 0.7 to 3.5 μm in diameter and show frequent anastomosis, i.e., fusion between branches of the same or different hyphae. The ramifying hyphae bear multinucleate (8–15 nuclei), large pyriform chlamydospores (16–25 μm × 10–17 μm) at their tips and/or at the intercalary positions, individually or in clusters that are formed in the root cortical cells and later are present in soil; however, the sexual structures, hyphal knots, and clamp connections have yet to be reported in this fungus (Varma et al. 2001; Varma 2008). It infects the host roots due to germination of the chlamydospores in soil lying in the vicinity of the host roots and forms inter- and intracellular hyphae in the root cortex, often differentiating into dense hyphal coils and chlamydospores in higher plants (Singh and Varma 2000).

P. indica being a versatile root endophyte possesses a wide spectrum of host plants encompassing angiospermous dicots and monocotyledonous plants including herbaceous, shrubs, as well as tree species like poplar (Pham et al. 2004; Prasad et al. 2005). The host plants being benefited by *P. indica* may involve economically important cash crops like sugarcane, maize, and wheat (Rai et al. 2001; Waller et al. 2005; Baltruschat et al. 2008) to medicinal plants like *Aloe vera, Adhatoda, Artemisia, Abrus, Bacopa, Chlorophytum, Coleus, Spilanthes, Tridax, and Withania* (Kumari et al. 2004; Oelmuller et al. 2009); legume crops like *Glycine max, Pisum sativum,* and *Nicotiana tobaccum* (Barazani et al. 2005) to members of family Chenopodiaceae and Brassicaceae like spinach, mustard, cabbage, and *Arabidopsis thaliana* which are non-mycorrhizal owing to secretion of large amounts of glucosinolates (Kumari et al. 2003; Peskan-Berghofer et al. 2004); and terrestrial orchids (Bougoure et al. 2005; Kaldorf et al. 2005) to bryophytes

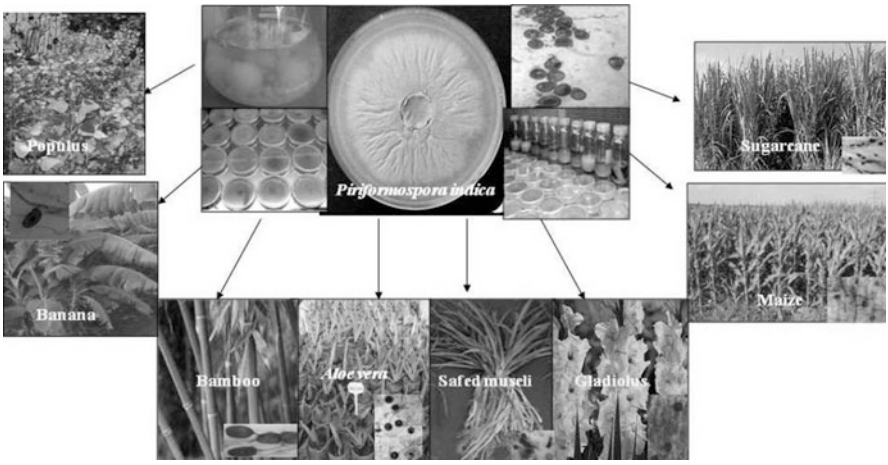

Plate 4.1 Different host plants studied for *P. indica* infection and colonization under green house and field conditions

like liverwort (*Aneura pinguis*) as well as pteridophytes (*Pteris ensiformis*) and gymnosperms (*Pinus halepensis*) (Kottke et al. 2003; Urban et al. 2003; Bidartondo and Duckett 2010). A variety of plant hosts have been studied to exhibit infection and colonization by *P. indica* inoculation in greenhouse as well as field experiments at Punjab Agricultural University, Ludhiana, Punjab, India (Plate 4.1).

4.4 Plant–Fungal Interactions and Mycorrhizosphere Associations

Rhizosphere is the most dynamic zone in soil exhibiting interactions which could be positive, negative, or neutral among a variety of microbial forms with plant roots. Mycorrhizosphere is an extended version of the soil region encompassing plant roots, soil, and extramatricular hyphal extensions of the symbiotic mycorrhizal/endophytic fungi along with the interacting bacteria, fungi, protozoa, and viruses. The dynamicity of the rhizo- or mycorrhizospheric interactions lies in the intricate microbe–microbe or plant–microbe cross talks using secretion of (may be minute quantities of) signaling compounds which may be phenolics or the modified glucosides (Harrison 2005; Paszkowski 2006; Bonfante and Anca 2009). Mycorrhizosphere is characterized by involvement of the bacterial component among the subsets of beneficial rhizomicroflora existing in the vicinity of the growing plant roots due to presence of abundant nutrients. These mycorrhiza-associated

rhizobacteria or helper bacteria may either exist in the vicinity (loosely) or cling tightly on the surface of the fungal extramatricular hyphae to provide the additional benefits to plants for being mycorrhizal host by supporting establishment of the mycorrhiza.

The extent of interaction state and interface between the plant–microbial symbionts is speculated to exist in equilibrium with both partners being equally benefitted in a mutualistic interaction. Over the evolutionary scale, microsymbionts (bacteria, fungi, actinomycetes, cyanobacteria) have modified their external structures and signals to cope up with the plant defense mechanisms as well as both have maneuvered each other using finely orchestrated signal molecules to develop an inter/intracellular coexistence. The microbes particularly alter (initiate or subdue) the defense-related molecules or phytohormones, i.e., salicylic acid, jasmonic acid, and ethylene along with minor players like abscisic acid, gibberellins, and auxins, which are intricately dependent on each other to form a network (Wang et al. 2007; Asselbergh et al. 2008; Koornneef and Pieterse 2008; Navarro et al. 2008). The most popular bacterial symbionts rhizobia secrete chitooligosaccharides, i.e., nod factors which result in changes that lead to suppression of salicylic acid accumulation and reactive oxygen species production in the host plant, and these suppressions help in intrusion of rhizobia through root-hair cells (Shaw and Long 2003).

Arbuscular mycorrhizal associations have been most elaborately studied in symbiotic plant–fungal associations that involve production of diffusible signaling molecules (Myc factors) followed by their release by fungal hyphae and perception by the plant without even actual physical contact between the two members (Kosuta et al. 2003). Similarly, the plant roots excrete few chemicals that result in induction of hyphal branching of the germinating mycorrhizal spores, e.g., presence of strigolactone 5-deoxystrigol compound in root exudates enhances hyphal branching (Akiyama et al. 2005). At the tissue level, mycorrhizal infection of the epidermis followed by the outer and inner cortex of the plant roots occurs largely intercellular and then intracellular by formation of branched arbuscules to increase the fungal–plant interaction interface.

Being an endophytic mycorrhiza, *P. indica* exhibits many common signal and receptor molecules to that of AM fungi though this symbiosis differs from plant–AMF associations in terms of extent of intrusion of the host root tissue which is extensive for the former with both cellular and extracellular fungal growth in epidermal and cortical tissue. The infection process starts with the germination of the pyriform chlamydospores occurring in the vicinity or on the surface of the root followed by invasion of the host roots within one to 2 days by the fungal hyphae that penetrate the epidermal cells *via* anticlinal cell walls to reach intercellular space between cells, while the intracellular invasion is particularly through the plasmodesmata of the plant cell (Deshmukh et al. 2006). The sites of *P. indica* infection include the root tip, root hairs, and the root differentiation zone from where the fungus reaches the cortical tissue and starts producing spores in epidermis and cortex within a week. The intracellular existence of this fungus indicates its

potential to exist inside root cells as hyphae and spores, without provoking tissue necrotization (Deshmukh et al. 2006).

The spectrum of benefits imparted by interaction of this fungus with its host plant includes the growth promotion, production of higher yields, as well as stress resistance to several biotic and abiotic factors. The former two benefits may be attributed to the production of phytohormone by the fungus itself as well as modulation of the host phytohormone profile (amount and type of phytohormone). This endophytic fungus is also known to produce phytohormones like auxins and cytokinins, though amounts of fungal auxins produced are minuscule, but it is known to modulate the plant auxin production and also enhances auxin production by the plant *in vivo* and produces substantial amount of cytokinins itself as well as increases plant cytokinin levels (Vadassery et al. 2008). However, the stress resistance is *via* the suppression/elevation of the molecules/products involved in the host defense-related circuits. A study by Camehl et al. (2010) exhibits the role of the ethylene signaling components and ethylene-targeted transcription factors in balancing or maintaining the beneficial interaction between *P. indica* and the infected host.

4.5 Molecular Dissection of Interaction at Genome and Proteome Level

As most of the information on the molecular sequences of majority of mycorrhizal plants and mycorrhiza are still fragmentary, there is plenty of room for the development of innovative tools and techniques for deciphering the mycorrhizal–host plant interactions at the molecular scales (Schafer et al. 2007). With the popularization of the novel tools and techniques of the -omic sciences, it is now possible to dissect the level of interactions and explore the diversity of interaction signals during development of symbiosis and signal pathways at the molecular levels, be it genetic/phylogenomic, proteomic, to metabolomic to reveal the share of contribution of each partner in the association as the genomic sequences of both mycorrhiza and host plant are now readily available (Hata et al. 2010). Both rhizobia and mycorrhizal fungi share a common signaling pathway with many signaling proteins of same origin or type (Table 4.1). This suggests a common ancestry of the mode of infection by the two endosymbionts which later segregated or rather became specialized for both the counterparts in their respective hosts. The rhizobia specialized to interact with the signaling compounds of the legume hosts, while the mycorrhiza retained the broad host spectrum range and only curtailed few plants from getting benefits from the association. Molecularly, the plant–mycorrhizal associations result in elicitation of responses among which the rapid and transient shifts in the intracellular calcium (most common intracellular messenger in signal transduction by plants) happen most commonly as the mycorrhizal signals are usually perceived by the receptor-like kinases which elicit phosphorylation

Table 4.1 The genes involved in the common symbiosis pathway (CSP) shared by rhizobia and mycorrhiza

Plant genes	Role or function	References
Nodulation receptor kinase (NORK)	Contains three leucine-rich repeats (LRRs) involved in ligand binding and cell signaling Plays an intermediary part and connects the activity of Myc factor receptors to subsequent steps	De Mita et al. (2007), Gianinazzi-Pearson et al. (2007), Markmann et al. (2008)
Symbiosis leucine-rich repeat receptor kinase (SYMRK)	Epistatic to other common symbiosis pathway genes	Stracke et al. (2002), Parniske (2004), Reinhardt (2007)
CASTOR and POLLUX (cation channels)	Predicted to be membrane-integrated channel proteins showing structural homology with Ca-gated potassium channels Involved in upstream of intracellular calcium spiking Localized in the plastids of root cells, indicating plastid role in controlling intracellular symbioses	Imaizumi-Anraku et al. (2005), Banba et al. (2008)
NUP85 and NUP133 (nucleoporins)	Components of the nucleopore complex involved in trafficking and/or localization of factors essential for induction of Ca signals	Kanamori et al. (2006), Saito et al. (2007), Banba et al. (2008)
Ca/calmodulin (CaM)-dependent protein kinase (CCaMK)	Structural similarity to CaMKII in mammals Act as a key regulator protein for the downstream genes leading to activation of the downstream signaling pathways	Tirichine et al. (2006), Banba et al. (2008)
CYCLOPS/IPD3	Known to interact with CCaMK Activates the downstream gene cascade(s) leading to successful infection of mycorrhiza	Messinese et al. (2007), Yano et al. (2008)
Does not Make Infection genes (DMI1, DMI2, and DMI3)	Involved in formation of the transient prepenetration apparatus as revealed by the *dmi* mutants that exhibit total or partial block of epidermal penetration by *Gigaspora* hyphae	Genre et al. (2005)
MtMSBP1 gene	Encodes a membrane-bound steroid-binding protein Involved in mycorrhizal development as evidenced by occurrence of aberrant mycorrhizal phenotype with thick and septated appressoria, decreased number of arbuscules, and distorted arbuscule morphology by RNAi downregulation of gene Speculated to play role in sterol homeostasis in root	Kuhn et al. (2009)

followed by cation channel-based induction of calcium changes in nucleus and finally activation of calcium calmodulin-dependent kinase (Navazio et al. 2007). Vadassery et al. (2009a) have reported induction of intracellular calcium in roots of *Arabidopsis* in response to cell wall extract of *P. indica*. The inevitability of the presence of certain genes responsible for production of signaling components could be ascertained by pinpointing the absence of gene homologs of several signaling components in certain non-mycorrhizal plants which suggests the exact genetic basis of the non-mycorrhizal behavior of members of Chenopodiaceae and Brassicaceae.

P. indica possesses 6 chromosomes comprising a total genome size of 15–20 Mb, and this would be helpful in identification of the loci involved in production of signaling proteins/receptor or enzymes for initiation and establishment of infection in host root cells. Moreover, the fungus is transformable which provides an added advantage of scrutinizing the events of infection during interaction using conventional and advanced microscopy techniques like confocal laser scanning microscopy.

P. indica exhibits similarities as well as variations in the expression or repression of certain common genes involved in initiation and establishment of mycorrhizal–plant interaction (Peskan-Berghofer et al. 2004). The majority of reports on molecular basis of *P. indica* host interactions including the elucidation of the molecular mechanisms responsible for host recognition, root colonization, and subsequent beneficial activities have been obtained by studies on plants whose genome is known, particularly the model plant *Arabidopsis thaliana* and *Nicotiana tobaccum*. The structural proteomic tools, i.e., 2D gel electrophoresis and mass spectrometry, are most useful techniques to identify the amount and type of the expressed proteins (Kalia and Gupta 2005). The physiological changes brought about on infection of this microsymbiont in the plant can be traced down even at the molecular level particularly using the structural proteomic tools. The physiological alterations involve the changes in the protein profile of the membrane (MATH, i.e., meprin and tumor necrosis factor receptor-associated factor (TRAF) homology domain containing protein) and endoplasmic reticulum proteins (a leucine-rich repeat protein LRR2 and PYK10 beta-glucosidase) (Shahollari et al. 2007; Sherameti et al. 2008). Similarly, other genes like nitrate reductase and glucan water dikinase are upregulated by *P. indica* infection (Sherameti et al. 2005). Shahollari et al. (2005) have also documented transient upregulation of a plasma membrane receptor kinase during the recognition period of both organisms. Vadassery et al. (2009b) have reported the crucial role of monodehydroascorbate reductase 2 and dehydroascorbate reductase 5 for development of a mutualistic interaction between *P. indica* and *Arabidopsis*.

There are certain genes which are prerequisite for mycorrhizal colonization but are not required by *P. indica* to cause infection like deactivation of *dmi-1* gene encoding an ion carrier which affects the mycorrhizal infection but not infection by *P. indica*. Gutjahr et al. (2009) have reported induction of starch accumulation in *Lotus japonicus* roots due to release of certain presymbiotic factors by *Gigaspora margarita*.

For establishment of successful infection by *P. indica* in host, certain specific genes need to be up/downregulated, e.g., transient upregulation of the membrane receptor leucine-rich repeat protein while partial deactivation of the sphingosine kinase gene. Schafer et al. (2009) have reported the role of gibberellin (GA) synthesis and perception for mutualisitic interaction with *P. indica*. The various benefits imparted by *P. indica* to its inoculated host can also be traced at the molecular level to ascertain the role of particular gene or set of genes in a specific function and would be more elaborately discussed in the applications section.

4.6 Applications

There are several benefits of *P. indica* inoculation in a variety of plants which may range from phenotypically identifiable enhanced growth and development to better biomass and grain yields. Moreover, the benefits may also be traced down at the biochemical or molecular level with increased production of certain phytochemicals (biochemical), endurance to sustain/withstand abiotic stresses like cold, drought, and salt-stress tolerance (physiological), as well as development of resistance to several potential phytopathogens. The fungus possesses positive phytopromotional effects due to production/modulation of phytohormone levels (plant bioregulation ability), apart from its role in mobilization and transportation of the plant unavailable phosphorous reserves in soil beyond the depletion zone (P-mobilizer ability). The role of this endophytic fungus in relieving the abiotic and biotic stresses could be elaborately utilized for biological hardening of micropropagated plantlets and as biological control agent. It is also well established that *P. indica* exhibits synergistic interactions with other plant growth-promoting rhizobacteria (PGPRs) similar to mycorrhiza (Bonfante and Genre 2008; Sharma et al. 2008).

4.6.1 Nutrient Uptake

Similar to AM fungi, *P. indica* is well known to possess ability to extract, mobilize, and transport two major macronutrients (phosphorus and nitrogen) as well as several micronutrients from soil and is actively involved in transferring these nutrients to the infected host plant *via* plant–fungal interfaces.

4.6.1.1 Macronutrient Uptake

P. indica acts as a potential phosphate mobilizer as it produces high amounts of phosphatase enzymes (cleave phosphate ester bonds to hydrolyze insoluble polyphosphates and organic phosphates) (Yadav et al. 2004) as well as can indirectly mobilize soil P-reserves by interacting/communicating with diverse

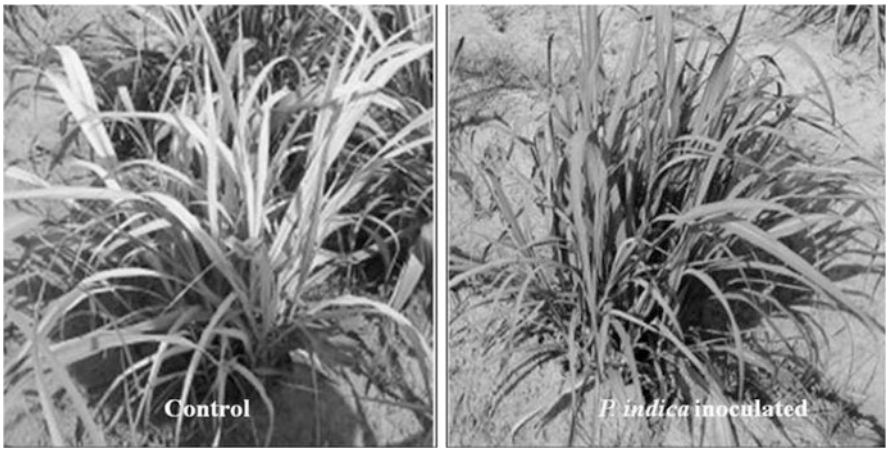

Plate 4.2 Effect of *P. indica* inoculation on iron acquisition in ratoon sugarcane var CoJ 88

rhizobacteria having inorganic P-solubilizing capabilities by virtue of production of a variety of organic acids (Singh et al. 2009). The recent studies using molecular tools have revealed that coinoculation of *P. indica* with P-solubilizing rhizobacteria (PSRB) results in enhanced P-uptake and P-content in host plant due to better establishment of PSRB in the mycorrhizosphere (Meena et al. 2010). Moreover, the *in vitro* laboratory studies have also revealed the ability of *P. indica* to grow on a variety of P-sources, i.e., inorganic, organic, and polyphosphates, which emanates its role as an active P-solubilizer apart from being P-mobilizer. *P. indica* also mediates nitrate uptake from the soil, which is in contrast to AMF, where nitrogen is preferentially absorbed as ammonium (Sherameti et al. 2005).

4.6.1.2 Micronutrient Uptake

Micronutrients are the elements essentially required in very small quantities for maintaining plant growth and health, among which include iron, boron, copper, zinc, molybdenum, manganese, and chlorine. The majority of micronutrients act as cofactors for several enzyme/enzyme complexes and have a greater role as essential members of the electron transport system proteins. Similar to mycorrhiza, *P. indica* is known to extract, mobilize, and translocate micronutrients particularly zinc, iron (Plate 4.2), manganese, and copper from soil and make it available to the plant (Gosal et al. 2010b; Achatz et al. 2010; Gosal et al. 2008a; Gosal et al. 2007).

4.6.2 Mechanism of Nutrient Uptake

Phosphorus solubilization from soil is performed by action of two types of phosphatase enzymes, viz., acid and alkaline phosphatases with the former shared by both the symbionts and involved in uptake of phosphorus, while the latter is mainly of fungal origin and involved in assimilation of phosphorus (Tisserant et al. 1993; Tarafdar and Rao 1996; Fries et al. 1998). Cytochemically, acid phosphatases could be localized on the membrane surfaces or plasmalemma in mycorrhizal roots, while alkaline phosphatases are localized on plant membranes surrounding the arbuscular or periarbuscular membrane of intercellular fungal hyphae. The *P. indica*-specific phosphate transporter has recently been deciphered to be a high-affinity P-transporter belonging to major facilitator superfamily and structurally contains 12 transmembrane helices divided into two halves connected by a large hydrophilic loop in the middle. The expression profiling of this 1,815 bp P-transporter has showed activity to be localized to the external hyphae of *P. indica* colonized with host plant root and shares significant sequence similarity with the already known GvPT (*Glomus versiforme*) and PHO84 (*S. cerevisiae*) phosphate transporters (Yadav et al. 2010). Similar to arbuscular mycorrhiza, *P. indica* also exhibits uptake and assimilation of the nitrogen species from the soil. *P. indica* stimulates NADH-dependent nitrate reductase activity in roots of *Arabidopsis* and tobacco plants with the enhanced enzymatic activity that could be correlated with an increased transcription of the corresponding plant gene (Sherameti et al. 2005). *P. indica* mediates nitrate uptake from the soil, which is in contrast to AMF, where nitrogen is preferentially absorbed as ammonium.

4.6.3 Biotization or Biological Hardening

Biotization is a common term used to signify inoculation of a plant beneficial microbe on surface of seed, roots, leaves, or other aerial parts of the plant that results in enhancement of growth, vigor, and finally yield (in biomass or grains) of inoculated plant. The germinated seed or even vegetatively propagated plants exhibit elaborate cross talks with the soil rhizomicrobes and thus have attained knowledge regarding the inevitability of the microbial counterparts as well as show dependence on beneficial microbes for better growth and development. However, the micropropagated plants are reared in sterile or aseptic conditions creating a microbiological vacuum, and thus these plants have to be primed with beneficial microbes for redemption of the microbiological void which equips these plants to acclimatize well to counteract the problems concerning survival and development of plantlets (Gosal et al. 2010a, b, 2008b). The micropropagated plants exhibit higher mortality rate due to the "transient transplant shock," on transfer of plantlets from lab to land. Above all, several survived plants may exhibit stunted growth or are often attacked by pathogenic soil microbes which further decrease the economic benefits

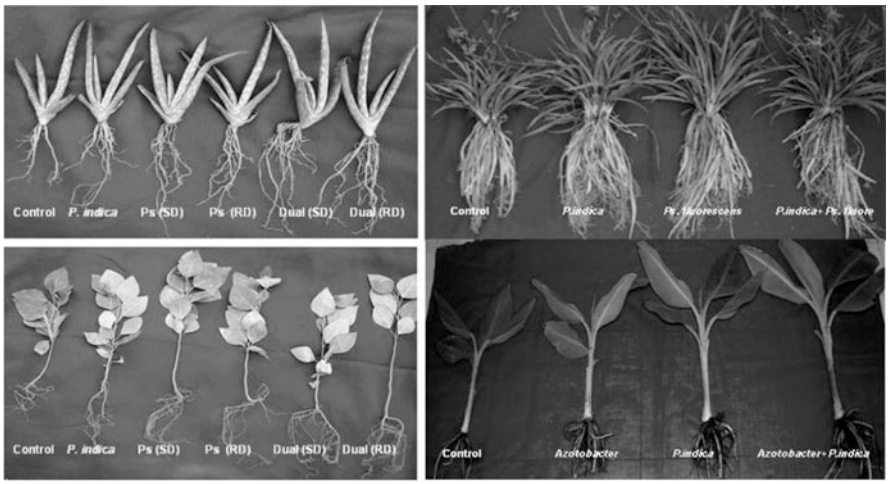

Plate 4.3 Effect of inoculation of *P. indica* and plant growth promoting bacteria on growth and development of different micropropagated plants under green house conditions

particularly in case of tissue-cultured floricultural or horticultural crop plants. However, at the weaning stage, about 10–40 % of plantlets either die or do not attain market standard, thereby causing significant losses at the commercial level.

The application of arbuscular mycorrhizal fungi (AMF) as a tool for biological hardening has solved a part of the problem. The lack of an authentic AMF axenic culture is an inherent problem for commercial application. Because of its ease of culture and plant growth promotional effect, Sahay and Varma (1999) evaluated the potential of *P. indica* to improve the survival and establishment of tissue-culture-raised plants. They recorded 88–94 % survival rate among the *P. indica*-inoculated regenerated plantlets of tobacco over uninoculated control plantlets and also observed maximum revival capacity of the inoculated plantlets to overcome the stress as compared to the control. Thus, the fungus has the potential to render protection to the micropropagated plantlets and help them escape the "transient transplant shock" (Shende et al. 2006). *P. indica* is known to exhibit similar brilliant positive effects on several plants belonging to diverse families like micropropagated *Chlorophytum borivilianum*, *Aloe vera*, *Populus*, *Dendrocalamus strictus*, sugarcane, and maize plants (Gosal et al. 2010a, b, 2009, 2008a, b, 2007) (Plate 4.3). Above all the inoculation of *P. indica* in medicinal plants also increases the production of amount of the medicinally active compound in the inoculated plants (Gosal et al. 2010b; Baldi et al. 2008). The saponin content of safed musli has been reported to increase on inoculation (Gosal et al. 2010b). Similarly, the amount of podophyllotoxin and 6-methoxypodophyllotoxin production (possess pharmacological anticancer properties) (Farkya et al. 2004) was increased by about four- and eightfold by co-cultivation of cell suspensions of *Linum album* with *P. indica* and *S. vermifera* (Baldi et al. 2008).

Plate 4.4 Effect of *P. indica* inoculation on growth and development of ratoon sugarcane var CoJ 88 under field conditions

4.6.4 Plant Growth-Promoting Activity

As this endophytic fungus exhibits AM fungi-like mechanisms regarding colonization of the roots of a wide variety of plant species and positive benefits imparted to even non-mycorrhizal hosts, it could be well speculated that on inoculation it will act by several known/unknown mechanisms to impart benefits. In general, as discussed above, *P. indica* exhibits root growth promotion even before noticeable root colonization which may be attributed to the enhanced nutrient (P particularly) availability and massive transfer to the aerial parts of the inoculated plant/seedlings (Shahollari et al. 2005). However, experimental input by Barazani et al. (2005) elaborates the plant growth promotional effects of inoculation of *P. indica* and also comprehends that the improvement of the nutritional status (P and N status) of the plant is not always responsible for visible enhanced vegetative or reproductive performance. They recorded that seed inoculation of axenic cultures of *P. indica* and *Sebacina vermifera* in *Nicotiana attenuata* resulted in stimulated seed germination and increased growth/stalk elongation. Plant growth promotion was observed in all the crop plants inoculated with *P. indica* under greenhouse as well as field conditions. Gosal et al. (2008a) have reported the increase in the shoot and root length of the maize plants under field conditions. Similar increase in the plant height was recorded by *P. indica* inoculation in *Dendrocalamus strictus* grown under greenhouse conditions (Gosal et al. 2007). A significant increase in tiller number, cane number, cane height, and cane yield were reported in *P. indica*-inoculated ratoon sugarcane plants as compared to uninoculated conventionally propagated sugarcane var CoJ 88 (Gosal et al. 2010c) (Plate 4.4). The plant growth promotional (PGP) activities of *P. indica* encompass both production of the phytohormones by itself and ordered alteration in the secretion pattern or amount of phytohormone production by the inoculated plant. Fakhro et al. (2010) reports

increase in *P. indica*-inoculated tomato fruit biomass in hydroponic culture with an approximately 100 % increase in fresh weight and approximately 20 % increase in dry matter content. Even with the application of fungicides like Bavistin, *P. indica* exhibited infection and colonization and finally increased yield in maize crop under field conditions (Gosal et al. 2009). Prajapati et al. (2008) have demonstrated the enhanced plant growth promotional effect on rice plant by coinoculation of *P. indica* with a known PGPR *Azotobacter chroococcum* and application of vermicompost. However, a report by Ray and Valsalakumar (2010) exhibits the nongrowth promotional behavior of *P. indica* in green gram, and the report concludes that *P. indica* is not a good synergist on green gram.

4.6.5 Tolerance to Abiotic and Biotic Stresses

Apart from these effects on vegetative and generative plant development, *P. indica* mediates stress tolerance to infested plants. The stress tolerance could be imparted in the inoculated host towards a variety of factors which could be largely classified or categorized into abiotic factors (including temperature extremes, saline/alkaline soil conditions, low moisture/soil–water conditions) and biotic factors (plant disease-causing microbial agents particularly including phytopathogenic bacteria and fungi) (Yuan et al. 2010).

4.6.5.1 Abiotic Stress Tolerance

Plant exhibits complex responses to abiotic stresses like salinity, heat, and drought stresses and involves elicitation of complex signal transduction pathways that are manifested as elaborate responses at the genetic as well as physiological scales. Plants being living organisms do respond, in positive or negative manner, towards various types of abiotic stresses; however, relatively very few plant species are known to withstand elevated abiotic stress levels (Alpert 2000). During their initial years of coevolution with the beneficial soil microbes, plants have marked out few mechanisms to overcome the abiotic stresses which either may be controlled by the plant genome or may involve the active role of the colonizing microbe, i.e., endophyte-mediated plant tolerance (Baltruschat et al. 2008). The abiotic stress tolerance attribute of the mycorrhizal fungi has been well deciphered. The mechanisms of plant growth promotion by *P. indica* are quite similar to the mycorrhizal fungi as a lengthened endophytic period in the inoculated host suggests its coevolution of common signaling and response pathways similar to the elaborately explored mycorrhizal fungi (Kogel et al. 2006); however, the pathways involved in imparting the abiotic as well as biotic stress tolerance are quite dissimilar to the mycorrhizal infections.

P. indica is known to impart tolerance towards many abiotic stress factors like extreme cold temperature, drought, and saline soil conditions. The saline and

drought-stress tolerance are most deciphered responses though meager research regarding the cold temperature stress tolerance due to *P. indica* inoculation has been reported. Many researchers have designed experimental setups to study the various mechanisms of *P. indica*-induced salt-stress tolerance. A report by Baltruschat et al. (2008) provides insight on several biochemical mechanisms responsible for induction of salt-stress tolerance in *P. indica*-inoculated barley plants by monitoring the key physiological markers affected by salt stress. They observed that on colonization of salt-stressed plant roots, *P. indica* attenuated the salt-induced lipid peroxidation, metabolic heat efflux, and fatty acid desaturation in plant leaves while elevating the amount of antioxidants such as ascorbic acid and antioxidant enzymes. These findings suggest that antioxidants might play a role in both inherited and endophyte-mediated plant tolerance to salinity.

Similar to induction of salt-stress tolerance, *P. indica* also confers drought-stress tolerance to inoculated plants which could be traced down at the molecular scale by figuring out alteration in expression of quite diverse set of stress-related genes (Seki et al. 2002). A report by Sheramati et al. (2008) suggests the induction of drought tolerance in *Arabidopsis* by co-cultivating or mock treating the plants with *P. indica* before exposing to mild drought stress. The further transfer of plants to soil resulted in better survival in comparison to the uninoculated controls. At the molecular level, the *P. indica*-inoculated plants exhibited faster and stronger upregulation of the message levels for several key drought-stress inducible enzymes, viz., phospholipase Dδ, CBL1, and HAT. The drought-stress inducible genes may either produce proteins that have a direct role in conferring tolerance to low water availability stress (like chaperones, late embryogenesis-abundant proteins, osmotin, antifreeze proteins, mRNA-binding proteins, key enzymes for osmolyte biosynthesis, water channel proteins, sugar and proline transporters, detoxification enzymes, and various proteases) or indirectly regulate the stress response genes (like transcription factors, protein kinases/phosphatases, phospholipid metabolism enzymes, signaling molecules such as calmodulin-binding proteins) (Shinozaki and Yamaguchi-Shiozaki 2007) by playing a vital role in the signal transduction cascades involved in induction/suppression of direct drought-related genes (Shinozaki et al. 2003).

4.6.5.2 Biotic Stress

The plant has to bear several biotic stresses like the infection by a variety of phytopathogens belonging to diverse groups of viruses, bacteria, fungi, nematodes, insects, and higher animals particular concern for the herbivores. The beneficial rhizomicrobes exhibit a tendency to prime the plant from the attack by the phytopathogens, thereby imparting inducible systemic stress resistance to the pathogen attack. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism, and production of inhibitory compounds.

P. indica colonizes the cortex of roots of a wide variety of plant species and promotes their growth and induces resistance against soilborne fungal pathogens in a manner similar to arbuscular mycorrhizal fungi (Ghahfarokhi and Goltapeh 2010). The inoculation of *P. indica* followed by colonization of the internal tissues of the plant may impart systemic resistance to various fungal and bacterial pathogens though the molecular mechanisms for these benefits have yet to be deciphered. Waller et al. (2008) have reported induction of systemic resistance against the leaf pathogenic fungus *Blumeria graminis* f. sp. *hordei* by colonization of *P. indica* and *Sebacina vermifera* strains in barley roots. Similar report of induction of resistance by *P. indica* in *Arabidopsis* for powdery mildew pathogen *Golovinomyces orontii* has been provided by Stein et al. (2008) which not only shows the reduction in conidia formation by the pathogen but also provides insight on the mechanism of *P. indica*-conferred ISR.

The inoculation of *P. indica* may also result in increase in the content of plants' own antimicrobial component(s). A report by Raj et al. (2004) advocates the enhancement of the antifungal activity of the *Spilanthes calva* plant extract of *P. indica*-inoculated plant against *Fusarium oxysporum* and *Trichophyton mentagrophytes*. On the other hand, Knecht et al. (2010) have reported the expression of *Beta vulgaris* plant germin-like proteins BvGLP-1 gene in transgenic *Arabidopsis* that elevated the H_2O_2 content and conferred significant resistance to two fungal pathogens *Verticillium longisporum* and *Rhizoctonia solani*. They have also reported that BvGLP-1 expression in *Arabidopsis* constitutively activates the expression of a subset of plant defense-related proteins such as PR-1 to PR-4 and PDF1.2 but not PDF2.1 and PDF2.3. An elaborate report of Felle et al. (2009) provides the molecular alteration exhibited by the *P. indica*-inoculated barley (*Hordeum vulgare* L.) plant. They suggested alteration in the surface pH characteristics by a constant flow of *P. indica* chlamydospores along primary roots. The root zones exhibit enhanced H^+ extrusion resulting in occurrence of transiently alkalized root-hair zone, while the elongation zone remains acidified within short period (8–10 min) of *P. indica* inoculation. The initial response is also observed in the aerial portion like leaves where the leaf apoplast began to acidify, thus providing potentiated systemic response to *Blumeria graminis* f. sp. *hordei* induced by *P. indica* in barley. Contrasting reports of involvement of the phytohormones in imparting systemic resistance in *P. indica*-induced resistance have also been proposed.

P. indica is also known to possess the ability to recruit the plant hormone signaling in order to manipulate plant defense. Schafer et al.'s (2009) experimental data would help identify gibberellin signaling as potential target for successful fungi and also reveals the complexity of compatibility mechanisms in host–microbe interactions. The traditional stress-related molecule like salicylic acid is largely not much affected by the *P. indica* inoculation. However a report by Stein et al. (2008) correlates the role of jasmonic acid and priming of jasmonic acid-responsive vegetative storage protein expression and its subsequent elevation to powdery mildew fungus (*Golovinomyces orontii*) infection. This report further suggests the

involvement of the reminiscent of induced systemic resistance for the resistance conferred by *P. indica*.

On inoculation, *P. indica* vigorously infects host root tissue and blocks the receptors for the attachment and adsorption of the phytopathogens which could be considered as the nonspecific physical way of this beneficial fungus to safeguard the inoculated plant. The fungus exhibits a scorable bioprotectant activity against a known fungal phytopathogen *Fusarium verticillioides*. The *P. indica*-primed or *P. indica*-inoculated maize plants showed a decrease in the antioxidant enzyme activity which curtails the *F. verticillioides* infection of new tissues as well as further controls the colonization of already infected tissues. This phytopathogen proliferates in the host tissue by playing and befooling the antioxidant enzyme machinery of the host. It causes increased antioxidant enzyme activity which minimizes the chances of oxidative burst (excessive production of reactive oxygen species), and therefore *F. verticillioides* might be protected from the oxidative defense system during colonization. Thus, this report explains the bioprotection ability of *P. indica* against the phytopathogens dissected at the molecular scale (Kumar et al. 2009). Similar report of biocontrol action of *P. indica* in tomato against *Verticillium dahliae* has been provided by Fakhro et al. (2010) which states a more than 30 % decrease in the disease severity caused by *V. dahliae* on tomato plants colonized by the endophyte.

4.7 Conclusions

P. indica possesses the phytopromotional to biocontrol properties, imparts stress tolerance to abiotic as well as biotic factors, and also exhibits enhanced nutritional availability to the inoculated plant. It has enormous application potentials for the biotization of the micropropagated plants and thus has a pivotal role to play in the new age phenomena of sustainable agriculture for enhanced productivity. The multifunctional abilities of this novel endophyte are well known though the underlying molecular mechanisms are yet to be deciphered in several hosts. The dissection of the molecular mechanisms to these activities would not only be useful to understand the basics of its action spectrum but would also provide the required knowledge for manipulation of the genomics of endophyte which could be harnessed for formation of multiple spectrum biofertilizer for a varied host plants and for the traditional grain and forage crops.

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