Review paper

Piriformospora indica: The versatile and multifunctional root endophytic fungus for enhanced yield and tolerance to biotic and abiotic stress in crop plants

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Abstract

In sustainable agricultural, horticultural and forestry ecosystems, different beneficial microbes are explored to enhance crop production and tolerance of plants to different (a)biotic stress conditions. The members of the order Sebacinales of Basidiomycetes form symbiotic associations with the majority of terrestrial plants. Among Sebacinales, *Piriformospora indica* is a unique and an interesting fungus capable of colonizing roots of many plant species and thus establishing symbiotic relationships, and also with model plants like *Arabidopsis thaliana*, *Nicotiana tabacum* (tobacco) and *Hordeum vulgare* (barley). The fungus lacks host specificity and is cosmopolitan in nature. Moreover, the fungus can be axenically cultivated. Positive interactions of *P. indica* are established for many important agricultural and horticultural plants, which allow them to grow under extreme physical and nutrient stress. The fungus promotes plant growth especially in nutrient-deficient soils, confers tolerance to abiotic (salinity, drought, water, cold, high temperature and heavy metals) and biotic (root and foliar pathogens) stress, regulates plant growth and development, induces early flowering and enhanced seed production, stimulates the production of active ingredients in medicinal plants, and helps in the hardening of micro-propagated or tissue-cultured plants. The interactions of *P. indica* with the model plants *A. thaliana* and *H. vulgare* are used to understand the molecular basis of these beneficial plant-microbe interactions. The current knowledge about the role of *P. indica* in improving the crop productivity and also in enhancing tolerance of the plant to biotic and abiotic stress is described. An attempt is also made to propose the possible mechanisms by which the fungus benefits from the crop plants.

Key words: Abiotic stress; Antioxidants; Biotic stress; Growth promotion; Primary metabolism; Phytohormone; Root endophyte; Signaling cascades; Symbiosis

Introduction

Worldwide crop productivity is being challenged by various biotic and abiotic stress factors (Agrios, 2005). In spite of this, we must increase the yield potential of major food crops to feed the growing population. The major factors affecting the yield potential are failure of the crop to absorb enough nutrients from soil, non-availability of essential nutrient elements in soil, unfavorable environmental conditions, incidence of insect pests and diseases, etc. Many micro-organisms including root endophytes establish symbiotic relationships with plants and play an essential role in maintaining a better soil and plant health (Smith and Smith, 2011). Endophytic beneficial root fungi live in the intercellular space or intracellular of plant tissue resulting in a symbiotic association with the host plants (Smith and Smith, 2011). The endophytic fungi are either constitutive mutualists (type I

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clavicipitaceous endophyte; Epichloe/ Neotyphodium) that are systemic, vertically transmitted through seeds and exclusively infect grass, or inducible mutualists (type II endophyte) which are non-systemic, taxonomically diverse, horizontally transmitted from plant to plant and colonize all plants in ecosystem (Yuan et al., 2010). Non-systemic endophytic fungi identified in a wide range of host plant species are getting increasing attention due to their striking species diversity and multiple functions. Plants use these endophytic fungi to evade different biotic (pathogenic fungi, oomycetes, bacteria and nematodes and also from herbivory) and abiotic (oxidative, salt, drought, water and heavy metal) stress conditions (Rodriguez and Redman, 2008). Among these non-systemic endophytic fungi, *P. indica* is one of the most important fungi which has been extensively studied.

P. indica, originally isolated from the roots of xerophytic woody shrubs e.g. *Prosopis juliflora* and *Zizyphus nummularia* in the Thar desert of North-Western India (Verma et al., 1998; Varma et al., 1999), is easily cultivable, lacks host specificity and is endophytically colonizing roots of many agricultural, horticultural, floricultural, medicinal and agroforestry plants leading to their growth promotion (cf. Oelmüller et al., 2009; Franken, 2012; Qiang et al., 2012; Varma et al., 2012). On root colonization, the endophyte increases nutrient uptake, allows plants to survive under water, temperature, salt, drought and oxidative stress, confers (systemic) resistance to toxins, heavy metal ions, as well as root and foliar pathogens. It also provides other beneficial effects such as plant growth promotion, enhanced nitrate and phosphate assimilation, promotion of adventitious root and root hair formations, early flowering, higher seed yield, alteration in the secondary metabolites, hardening of tissue cultured plants and their preparation for field conditions (Oelmüller et al., 2009; Zuccaro et al., 2011; Varma et al., 2012). Thus, *P. indica* is similar to arbuscular mycorrhizal fungi (AMF) in many physiological effects to host plants, but it can be cultured on artificial medium unlike AMF (Varma et al., 1999). The fungus has vast potential for improving crop productivity and enhancing tolerance especially in the present era of the changing climatic conditions. Hence, *P. indica* is considered as a powerful symbiont candidate for improving plant production and crop yield in sustainable agriculture, flori-horticulture and agroforestry. However, the success of any microbial inoculation in field condition has to be tested for each case, since the effectiveness of symbiosis depends on complex interactions between the plants, symbionts and their environment. The mechanisms leading to the above beneficial effects were elucidated in the model interaction system involving *Arabidopsis thaliana-P. indica* and *Hordeum vulgare-P.indica* (Oelmüller et al., 2009; Achatz et al., 2010; Qiang et al., 2012).

Molecular ecology studies, based on rDNA sequences, reveal that Sebacinales are common and versatile mycorrhizal associates with many plant species all over the world. They are universally present in bryophytes, pteridophytes, angiosperms, gymnosperms including non-mycorrhizal model plant *A. thaliana* (Varma et al., 1999; Peškan-Berghöfer et al., 2004; Barazani et al., 2005; Waller et al., 2005; Serfling et al., 2007; cf. Oelmüller et al., 2009; Franken, 2012; Qiang et al., 2012; Varma et al., 2012). An amazing morphological and physiological diversity is observed among these interactions. Multitude of mycorrhiza-like interactions in Sebacinales might have arosen from an ancestral endophytic habitat by specialization. Considering the endophytic beneficial influence on plant growth and their ubiquity, these Sebacinales have been previously unrecognized in plant ecosystems. Molecular phylogenetic analyses with the nuclear gene for the ribosomal large unit (nrLSU) shed light on the ecology and evolution of Sebacinaceous fungi whose striking biodiversity and ecological importance has now been widely recognized (Selosse et al., 2007; Weiss et al., 2011). Furthermore, molecular phylogenic analyses using 18S rDNA sequences showed that *P. indica* is a member of Sebacinaceae of the order Sebacinales

(Basidiomycota: Agaricomycetes) (Weiss et al., 2011; Waller et al., 2005; Qiang et al., 2012). Genome sequencing of *P. indica* revealed that it possesses at least 6 chromosomes and has a genome size of 15.4 to 24 Mb (Zuccaro et al., 2009; 2011). The *P. indica* genome has 50.68% GC content, 4.68% repeat rate and 11,769 putative protein-encoding genes which also include the genes responsible for mutualistic interaction, early biotrophic and late necrotrophic phases of the fungus. The genome contains 5.16 average exons per gene, a gene density of 471 (number of gene per Mb), sequences for 867 secreted and 386 small secreted proteins, 3,134 unique gene models, 197 unique small secreted proteins and 58 tRNA genes (Zuccaro et al., 2009; 2011). The fungus lacks cystidia and clamp connections, and also forms monilioid hyphae which look like pearls in a chain. This mutualistic endosymbiont has biphasic lifestyle strategies in the colonized barley roots as revealed both by cytological studies and microarray analysis (Deshmukh et al., 2006; Zuccaro et al., 2011). Interestingly, the fungus requires host cell death in the outer epidermal and cortical cell layers through endogenously programmed cell death for its proliferation and symbiotic association in barley (Deshmukh et al., 2006). Within the root cortex, they form inter- and intracellular hyphae without traversing through the endodermis. The symbiotic fungus does not invade aerial parts of the plant (Varma et al., 2012).

Plant growth promotion

Similar to AM fungi, the beneficial interaction of *P. indica* with a large number of crops ultimately leads to growth promotion by enhanced root and shoot biomass, increased root hair, secondary and tertiary root formation, enhanced vegetative growth and early and enhanced flower and seed production (Oelmüller et al., 2009; Franken, 2012; Qiang et al., 2012; Varma et al., 2012; 2013). Once endosymbioses is established inside the roots, the fungus gets access to photo-assimilates and other plant nutrients, which further promotes colonization and proliferation of the fungus in roots, and thus significantly enhances plant growth (Oelmüller et al., 2009). Plants benefit from this relationship because the fungus reprograms their transcriptomes, proteomes and metabolomes that includes phytohormone synthesis and signaling which affect growth, nutrient uptake, flowering, seed production and protection against drought, salinity and phytopathogens (Oelmüller et al., 2009; Franken, 2012; Qiang et al., 2012; Varma et al., 2012). The modulation of gene expression, proteins and metabolites of the endosymbionts and host plants helps both partners to keep the interaction mutually beneficial (Johnson and Oelmüller, 2009).

P. indica interaction resulted in growth promotion of major crop plants like maize (*Zea mays*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), sugarcane (*Saccharum officinarum*), potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), brinjal (*Solanum melongena*), rape seed (*Brassica napus*), onion (*Allium sepa*), chickpea (*Cicer arietinum*), ground nut (*Arachis hypogaea*), red gram (*Cajanus cajan*), mung bean (*Vigna mungo*), coriander (*Coriandrum sativum*), black pepper (*Piper nigrum*) and chinese cabbage (*Brassica campestris* ssp. *chinensis*). Positive growth enhancement was observed also in the model plants like *Arabidopsis thaliana*, *Nicotiana tabaccum* and *N. attenuata*, as well as medicinal plants like *Abrus precatorius, Artemesia annua*, *Bacopa monniera*, *Centella asiatica*, *Chlorophytum tuberosum*, *Coleus forskohlii*, *Curcuma longa*, *Mimosa pudica*, *Spilanthes calva*, *Stevia rebaudiana*, *Withania somniferra*, etc. (Table 1; Franken, 2012; Varma et al., 2012; 2013). Plant growth promotion is observed in all the crop plants inoculated with *P. indica* under *in vitro*, greenhouse and field conditions. Co-inoculation with *P. indica* resulted in the enhanced growth promotion in rice plants (Prajapati et al., 2008). *P. indica*-inoculated rice plants had higher number of adventitious roots in addition to the enhanced root and shoot lengths as well as fresh and dry weights (Table 1). Further the colonized plants also had 70-100% more amount

of chlorophyll (Chl) and carotenoids (Jogawat et al., 2013). The roots of winter wheat varieties were also colonized efficiently resulting in the increased shoot and root biomass especially when the crop was grown on poor substrates or under nutrient limiting conditions (Serfling et al., 2007). Similarly *P. indica*-colonized maize plants showed an increased biomass production, root length and root number compared to the non-colonized plants (Varma et al., 2001; Kumar et al., 2009). Extensive studies on the interaction of *P. indica* with barley have shown an enhanced barley grain yield (Waller et al., 2005). This mutualistic symbiotic interaction causes host cell death for the proliferation of the fungus in the differentiated roots for its better colonization in barley (Deshmukh et al., 2006). The fungus in *Brassica napus*, an economically important oil seed crop, resulted in significant increase in the size and number of leaves, biomass of roots and shoots, early flowering, increased seed yield and oil content (Table 1; Chen et al., 2014 unpublished). Similar results were also shown in other oil seed crops like sunflower (Bagde et al., 2011). Colonization of the fungus in sugar yielding crop like sugarcane resulted in the increased number of tillers and canes per clump in addition to the enhanced biomass of canes and their sugar content (Varma et al., 2012; Gosal et al., 2013). In hydroponic cultivation of tomato, 100% increase in fresh weight and 20% increase in dry matter content of tomato fruits were observed in *P. indica*-inoculated plants as compared to uninoculated control (Fakhro et al., 2010). The fungus also promotes the growth of tropical legumes like chickpea (*Cicer arietinum*), *Phaseolus aureus*, mung bean (*Vigna mungo*), peas (*Pisum sativum*) and soybean (*Glycine max*) (Table 1; Varma et al., 2012). The above studies and the available literature clearly indicate that this root endophytic fungus could be commercially used for enhanced crop production.

P. indica promoted growth and enhanced active ingredients of the medicinal plants by forming symbiotic associations in *Artemesia annua, Bacopa monnieri, Coleus forskohlii*, *Spilanthes calva* and *Withania somnifera,* both under greenhouse and field conditions (Table 1; Das et al., 2013). The fungus also enhanced flowering and fruit development in *C. forskohlii*, *S. calva* and *W. somnifera* (Das et al., 2013; 2014). It promotes growth of *A. annua*, which produces the antimalarial drug Artemisinin, under green house and field conditions. The beneficial effects were also seen in tissue culture-raised plantlets under *in vitro* conditions by enhancing the shoot and root biomass, higher rate of shoot and root development, bio-hardening of tissue cultured-plantlets, higher survival of tissue cultured-plantlets when transferred to the natural field conditions and by a 3-fold stimulation of the Artemisinin content (Das et al., 2013). Similar results were also obtained with other medicinal plants like *Tridax procumbens* which is extensively used in Indian traditional medicines as anticoagulant, antifungal and insect repellent, in jaundice, bronchial catarrh, diarrhea and dysentery (Kumari, 2005; Das et al., 2013). *Abrus precatorius* (Syn. *Glycyrrhiza glabra*) containing abrin, abraline, choline, precatorine, abricin and abridin used against inflammation, vitiligo, skin disease, wounds, ulcer, alopecia, asthma, stomatitis and fever also gave comparable results with the fungus (Kumari, 2005, Das et al., 2013). *B. monnieri* (Brahmi) is another medicinal plant used as a common nerve tonic, containing bacosides which is used to treat asthma, insanity, epilepsy, hoarseness, enlargement of spleen, snake bites, rheumatism, leprosy, eczema, and ringworm as well as diuretic, appetitive and cardiotonic diseases that gave similar results with the fungi (Prasad et al., 2008). *C. forskohlii* (Syn. *C. barbatus*) (Das et al., 2013; 2014) which contains forskolin (coleneol), an activator of adenylate cyclase involved in the production of cyclic adenosine mono-phosphate (cAMP), barbastusin used against lung carcinoma and lymphatic leukemia (Das et al., 2013), *Adhatoda vasica* (Malabar nut) (Table 1) which contains glycodin used against bronchitis and other pulmonary disorders, *W. somnifera* (Das et al., 2013) producing several anti-cancerous drugs, *S. calva*

having anti-ageing property (Das et al., 2013), *Curcuma longa* (turmeric) (Das et al., 2013), *Chlorophytum* sp. (safed musli) (Gosal et al., 2010) having aphrodisiac, anti-stress and immuno-modulatory properties used for the cure of physical weakness, diabetes and arthritis, *Foeniculum vulgare* (Fennel) (Dolatabadi et al., 2011) used against digestive disorders, and *Linum album* (Baldi et al., 2008) which contains podophyllotoxin having anticancer and antiviral properties (Table 1) are plants in which beneficial growth results were observed.

Unlike AM fungi, *P. indica* also interacts with the non-mycorrhizal host *A. thaliana* and promotes its growth (Peškan-Berghöfer et al., 2004). The growth-promoting effect was also demonstrated during the entire life time of the plant: when Arabidopsis seedlings were co-cultivated with the fungus under *in vitro* conditions, there was an enhanced shoot and root biomass production of the seedlings. When transferred to soil, the rosette leaves were slightly larger and bolding occurred earlier. Consequently, the colonized plants grew faster, contained more leaves, started to flower earlier and had a higher seed yield per plant (Peškan-Berghöfer et al., 2004; Johnson et al., 2011). Priming the seeds of another model plant, *Nicotiana attenuata* with axenic culture of *P. indica* resulted in enhanced seed germination and increased stalk elongation and plant growth (Barazani et al., 2005). Furthermore, the colonized seedlings showed more than 75% increase in root and shoot biomass in soil experiments (Sherameti et al., 2005; Johnson et al., 2011; Schuck et al., 2012). The ability of the fungus to form endosymbioses with the well characterized model plants like *A. thaliana*, *N. tabaccum*, *N. attenuata* and *H. vulgare* makes them, powerful model systems to study beneficial plant-microbe interaction at the molecular level.

The mechanisms involved in *P. indica*-mediated growth promotion

P. indica-interaction studies with different model plants and its mutants clearly demonstrated that the fungus-induced growth promotion is achieved by the enhanced nutrient uptake and its translocation, increased efficiency of photosynthesis and modulation of phytohormones involved in growth and development (Oelmüller et al., 2009; Franken, 2012; Qiang et al., 2012; Varma et al., 2012).

a. Nutrient uptake and translocation

The major feature of any symbiotic plant-microbe interactions is the ability of the microbial endosymbionts to assimilate nitrogen (N), phosphorus (P) and micronutrients that limit plant growth and to trade these nutrients with the plant in exchange for a carbon source derived from photosynthesis (Varma et al., 2013). *P. indica*-mediated growth promotion is accomplished by the massive transport, absorption and mobilization of nutrients from soil and further its efficient translocation to aerial parts (Shahollari et al., 2007). *P. indica* is able to extract, mobilize and transport N, P, potassium (K), sulphur (S) and magnesium (Mg) and minor nutrients like iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu). P, one of the most essential mineral nutrient for plant growth and development, is required in significant quantities to perform diverse regulatory, structural and energy transfer roles. Plants take up phosphate either directly through its own transporters or indirectly through endophytic / mycorrhizal associations. *P. indica* is an efficient phosphate mobilizer and produces phosphatase enzymes which cleave phosphate ester bonds of insoluble polyphosphates and organic phosphates (Malla et al., 2004) and different organic acids which in turn solubilize the insoluble polyphosphates. Both acid and alkaline phosphatases are involved in P-solubilization in soil. Acid phosphatases are present in both symbionts, localized in plasma and cell membranes of mycorrhizal roots, and involved in P uptake whereas, alkaline phosphatases are present only in the hyphal membrane of the fungal symbiont and are involved in assimilation of P (Fries et al., 1998). The ability of the fungus to grow on a variety of P

sources like inorganic, organic and polyphosphates also emanates its role as an active P-solubilizer apart from being P-mobilizer. Shahollari et al. (2007) demonstrated *P. indica*-mediated growth promotion in Arabidopsis is associated with a massive uptake of radio-labeled P from the growth medium. Uptake and transport of P is also stimulated by the fungus in the colonized roots of maize (Yadav et al., 2010). Furthermore, *P. indica*-phosphate transporter (PiPT) gene, which helps to transport phosphates from the soil to the plant as a result of *P. indica* colonization, has been identified (Yadav et al., 2010; Pedersen et al., 2013). PiPT exhibits 12 transmembrane helices connected by a large hydrophilic loop in the middle and belongs to the high affinity phosphate transporter family. *PiPT* has 1815 bps and the activity of the enzyme is localized to the external hyphae of *P. indica*-colonized host roots (Yadav et al., 2010; Pedersen et al., 2013). *P. indica* knockdown mutant of *PiPT* transported significantly lower amount of phosphate to host plant than the wild type *P. indica* which clearly establishes the role of PiPT in phosphate transportation in *P. indica*-colonized plants (Yadav et al., 2010). Recently the PiPT protein was purified, crystallized and characterized (Pedersen et al., 2013). The fungus also induces phosphate transporters of *A. thaliana* eg: PhT1-1 to PhT1-5 upon colonization to enhance P mobilization from soil by the host plant (Fig. 1; Johnson, 2014). The fungus-colonized mung bean plants had significantly higher amount of N, P and K compared to the non-colonized plants (Table 1; Kumar et al., 2012).

The beneficial fungus-root interaction leads to an early protein alterations in the plasma membrane and the endoplasmic reticulum which involves the synthesis of a leucine-rich repeat (LRR) protein (Peškan-Berghöfer et al., 2004, Shahollari et al. 2007); the increased expression of nitrate reductase, the β-glucosidase - PYK10 and starch-degrading enzymes SEX1 (Fig. 1; Sherameti et al., 2005; 2008b). Uptake and assimilation of N are enhanced by *P. indica* upon root colonization in different host plants. Interaction of the fungus with Arabidopsis roots is accompanied by a considerable requisition of nitrogen from the environment (Peškan-Berghöfer et al., 2004). The fungus enhances NADH-dependent nitrate reductase (Nia2) activity in roots of Arabidopsis and tobacco which helps in the increased uptake and assimilation of nitrate from the soil (Fig. 1; Sherameti et al., 2005). In contrast to AMF where N is preferentially absorbed as ammonium, *P. indica* mediates nitrate uptake from the soil. Further Sherameti et al. (2005) identified a *cis*-regulatory element in the *Nia2* promoter that is crucial for the expression of *Nia2* gene and is targeted by the transcription factor BHL1. Similarly, the fungus also upregulated the expression of a major starch degrading enzyme, *SEX1*, which encodes a glucan water dikinase through *cis*-regulatory element, a target of BHL1 (Fig. 1; Sherameti et al., 2005). Similar results were also demonstrated in the fungus-colonized tobacco plants (Sherameti et al., 2005). *P. indica*-colonized tomato plants showed improved N acquisition and was not affected by the increase in P level in the soil and the degree of colonization compared to AMF-colonized plants (Cruz et al., 2012; 2013). Similar studies also showed that the fungus stimulated several plastid-localized genes involved in sulphur metabolism in *A. thaliana* and the studies on loss of function of these genes further confirmed its requirement for the beneficial effect on plants (Oelmüller et al., 2009). The major micronutrients essential for plant growth and development includes Fe, B, Cu, Zn, Mo, Mn and Cl which act as cofactor for several enzymes involved in the electron transport systems in plastids and mitochondria (Fig.1). *P. indica* is known to mobilize micronutrients from soil and makes them available to plants through its colonization (Gosal et al., 2013; Achatz et al., 2010). It is not clear whether the transport occurs directly into the plant cell or via the fungal hyphae. The positive agronomic and yield characters in the fungus-colonized *Brassica napus* is correlated to the increased accumulation of N, P, K, S and Zn (Chen et al., unpublished). *P. indica*-mediated growth promotion in mung bean (3-4 fold)

Table 1: P. indica-induced growth promoting effect in different crops/plants

Plant/crop species		Growth promotion (biomass-g/plant) -*Pi*	+*Pi*	References
Maize	Root (FW)	48.6±2.5	75.6±4.8	Varma et al. (1999)
(*Zea mays*)	Shoot (FW)	45.0±3.1	90.6±6.7	
	Biomass-seedlings	0.4	0.9*	Kumar et al. (2009);
	P content-µM	2.5	6.0*	Yadav et al. (2010)
Rice	Shoot length	13.1±0.5	15.3±0.3	Jogawat et al. (2013)
(*Oryza sativa*)	Root length	11.9±1.5	18.1±1.3	
	Fresh weight	81.1±3.1	105.0±1.5	
	Dry weight	22.0±1.3	31.6±0.7	
Wheat	Root (FW)	2.0	3.5*	Serfling et al. (2007)
(*Triticum aestivum*)	Shoot (FW)	1.1	2.1*	
Sugarcane	Cane no./clump	8.1	15.9*	Varma et al. (2012)
(*Saccharum officinarum*)	Cane height-cm	179.0	191.0	
	Sugar content-Brix	18.2	21.4	
Black Pepper	Root (FW)	9.0	11.0*	Anith et al. (2011)
(*Piper nigrum*)	Shoot (FW)	27.0	29.0*	
Chinese cabbage	Root (FW-mg)	8.5±1.2	20.4±1.1	Sun et al. (2010);
(*Brassica campestris*	Shoot (FW-mg)	20.5±2.1	45.5±2.6	Lee et al. (2011)
ssp. *Chinensis*)				
Mung bean	Dry root wt.	1.2±0.1	2.9±0.1	Kumar et al. (2012)
(*Vigna mungo*)	Dry shoot wt.	13.9±1.8	34.9± 2.1	
	No. of pods/plant	17.6±1.8	65.7±5.5	
	N-mg/g plant	5.6±0.4	8.8±0.3	
	P-mg/g plant	3.1±0.4	4.2±0.2	
	K-mg/g plant	7.1±0.5	10.2±0.1	
Arabidopsis thaliana	Seed wt-mg/plant	154.9±3.3	188.3±5.1	Sherameti et al. (2005);
	Biomass-mg/seedling	38.5±5.2	59.5±2.6	Shahollari et al. (2007)
Tobacco	Root (FW)	3.4±6.2	6.2±0.4	Varma et al. (1999);
(*Nicotiana tabaccum*)	Shoot (FW)	4.3±0.3	7.8±0.5	Sherameti et al. (2005)
Tobacco	Stalk length-cm	42.0	50.0*	Barazani et al. (2005)
(*Nicotiana attenuata*)	No. of flower/plant	67.0	95.0*	
	Seed wt-mg/capsule	20.0	33.0*	
	Root (FW-mg)	4.4	8.2*	Schuck et al. (2012)
	Shoot (FW-mg)	10.1	21.3*	
Brahmi	Root (FW)	2.0±0.2	6.9±0.5	Varma et al. (1999)
(*Bacopa monnieri*)	Shoot (FW)	2.7±0.1	7.8±0.4	
	Shoot length-cm	11.1	23.1*	Das et al. (2013)
	Root length-cm	2.1	6.5*	
Sweet wormwood	Root (FW)	4.0±0.3	9.9±0.8	Varma et al. (1999)
(*Artemisia annua*)	Shoot (FW)	8.6±0.1	15.0±1.2	
	Height (cm)	158.5±6.5	246.0±36.0	Das et al. (2013)
	Dry weight	20.5±1.5	73.0±9.0	
Parsley	Root (FW)	2.1±0.1	5.1±0.3	Varma et al. (1999)
(*Petroselinum crispum*)	Shoot (FW)	2.6±0.2	5.9±0.3	
Poplar	Root (FW)	2.4±0.2	4.6±0.3	Varma et al. (1999)
(*Populus tremula*)	Shoot (FW)	4.6±0.2	8.7±0.5	
Coat buttons	Shoot length-cm	57.3±1.0	73.0±0.9	Das et al. (2013)
(*Tridax procumbens*)	Root length-cm	30.5±0.9	35.4±0.8	
	Root (FW)	6.9±0.1	11.2±0.5	
	Shoot (FW)	19.0±0.4	15.0±1.2	
	Dry root weight	2.4±0.3	4.7±0.3	
	Dry shoot weight	6.8±0.3	9.2±0.3	

Indian liquorice	Shoot length-cm	66.9±1.5	88.0±1.6	Das et al. (2013)
(*Abrus precatorius*)	Root length-cm	35.3±2.7	43.4±0.8	
	Root (FW)	6.3±0.5	8.3±0.6	
	Shoot (FW)	31.6±1.4	35.7±0.7	
	Dry root weight	2.1±0.1	3.1±0.2	
	Dry shoot weight	14.0±1.3	16.6±0.5	
Makandi/Mayani	Shoot length-cm	43.9±2.4	55.1±1.7	Das et al. (2013)
(*Coleus forskohlii*)	Root length-cm	16.4±0.8	24.1±0.9	
	Dry root weight	8.5±3.6	4.3±3.0	
	Dry shoot weight	16.6±1.3	30.8±3.3	
Malabar nut	Height-cm	21.2	26.1*	Das et al. (2013)
(*Adhathoda vasica*)	Root (FW)	160.5	390.4*	
	Shoot (FW)	215.5	455.5*	
	Dry root weight	45.5	135.5*	
	Dry shoot weight	75.5	155.5*	
Toothache plant	Shoot length-cm	26.5	30.5*	Das et al. (2013)
(*Spilanthes calva*)	Root length-cm	11.2	15.1*	
	Flower/ inflorescence	11.5±3.6	48.6±0.4	
	Seeds/fruit	716.0±0.4	1006.0±7.6	
Indian gensens	Shoot length-cm	42.5	68.5*	Das et al. (2013)
(*Withania somnifera*)	Root length-cm	11.4	16.5*	
	Flower/inflorescence	81.8±3.6	307.4±0.5	
	Seeds/fruit	35.3±4.9	46.3±5.8	
Indian Pennywort	Plant wt.-FW	21.4	29.4*	Satheesan et al. (2011)
(*Centella asiatica*)	Leaf number	8.0	11.8*	
	Root number	14.6	19.8*	
Fennel	Plant height-cm	92.0	108.0*	Dolatabadi et al. (2011)
(*Foeniculum vulgare*)	Shoot dry wt.	3.0	3.7*	
	Root dry wt.	1.0	1.3*	
	No. of Inflo./pl.	18.5	27.5*	

* significant <P 0.05%

is positively correlated to the significant uptake of N (1.6 fold), P (1.4 fold) and K (1.4 fold) from the soil under glass house and field conditions (Kumar et al., 2012). Taken together, all studies support the fact that *P. indica* influences the primary metabolism in the roots by delivering more nutrients for growth and development (Figure 1).

b. Increased efficiency of photosynthesis

Chl *a*, Chl *b* and total Chl contents together with different fluorescent and biophysical parameters with respect to the photosynthetic activity are considered as various indices of plant fitness (Maxwell and Johnson, 2000). It is determined by measuring various Chl fluorescence parameters e.g. maximum quantum yield of photosystem II (PSII) (F_v/F_m), quantum yield of PSII ($Ö_{PSII}$), photochemical quenching or proportion of closed PSII (1-qP) and non-photochemical quenching (NPQ) which indicates either the efficiency of the photosynthetic electron transport or the ability of heat dissipation of photo and non-photochemical energy during photosynthesis (Maxwell and Johnson, 2000). Total Chl, Chl *a*, Chl *b*, and carotenoid contents were measured in inoculated and non-inoculated plants of rice (Figure 1; Jogawat et al., 2013) and Arabidopsis (Johnson, 2014). The photosynthetic pigments especially Chl *a* increased in *P. indica*-colonized plants. Furthermore, *P. indica* colonization significantly enhanced the carotenoid content (Jogawat et al., 2013). In Arabidopsis, *P. indica*-colonization resulted in an efficient transfer to electron flow in PSII and enhanced level of photochemical and non-photochemical quenching (Johnson, 2014).

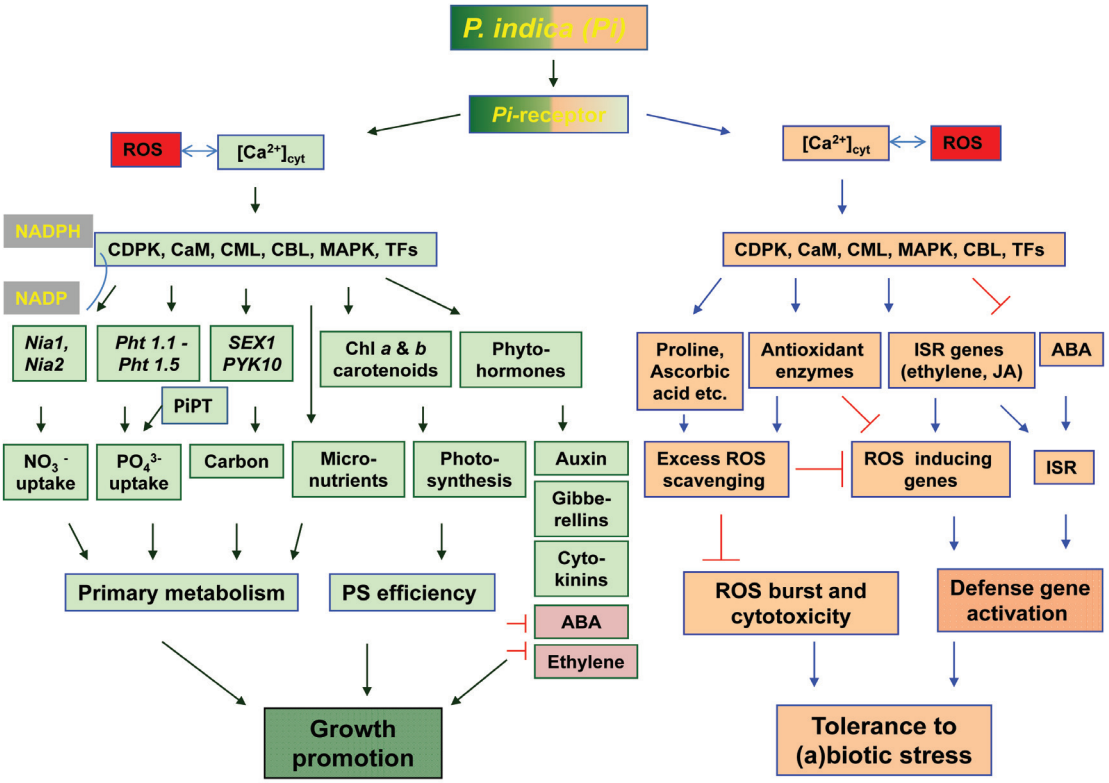

Figure 1. Proposed model showing *Piriformospora indica-mediated* beneficial effects in crop plants**.** The signaling cascades, transcriptome, proteome and metabolome regulations by *P. indica* leading to growth promotion are shown in green lines and boxes whereas blue lines and orange boxes represent events leading to (a)biotic tolerance. Perception of *P. indica* by its receptor(s) invokes cytosolic calcium and ROS signaling which are translated by CDPKs, CaMs, CBL, MAPKs, transcription factors, etc. to activate downstream responses involved in nutrient uptake and metabolism, photosynthesis, and phytohormone signaling and synthesis. This ultimately lead to enhanced primary metabolism, photosynthetic efficiency, growth and development. Similarly, transcriptome and proteomic changes involved in antioxidation processes and defense activation are induced to confer tolerance to biotic and abiotic stress. (ABA, abscisic acid; $[Ca^{2+}]_{cyt}$, cytosolic calcium; CaM, calcium calmodulin; CML, calmodulin-like protein; CBL, calcineurin B-like calcium sensor; CDPK, calcium dependent protein kinase; Chl, chloroplast; JA, jasmonic acid; ISR, induced systemic resistance; MAPK, mitogen activated protein kinase; NADP, nicotineamide diphosphate; NADPH, nicotineamide diphosphate, reduced form; Nia, nitrate reductase; Pht, phosphate transporter; Pi, *Piriformospora indica*; PiPT, *P. indica* phosphate transporter; PS, photosystem; PYK10, β-glucosidase; ROS, reactive oxygen species; SEX1, starch degrading enzyme 1; TFs, transcription factors).

c. Modulation of phytohormone levels

Auxin, cytokinin, ethylene, abscisic acid and gibberellins are the main phytohormones involved in growth and development of new organs in plants. It has been well established that these phytohormones play important roles in *P. indica*-induced growth promotion in Arabidopsis, barley and Chinese cabbage (Sirrenberg et al., 2007; Vadassery et al., 2008; Schäfer et al., 2009a, b; Lee et al., 2011; Johnson et al., 2013a). Growth promotion of Chinese cabbage and barley seedlings is attributed to the increased levels of auxin and gibberellins in the colonized roots, even though the auxin level in the leaves is not affected by the fungus (Schäfer et al., 2009b; Sun et al., 2010; Lee et al.,

2011; Johnson et al., 2013a), whereas cytokinins especially *trans*-zeatin plays a crucial role in *P. indica*-mediated growth promotion in Arabidopsis (Fig. 1; Vadassery et al., 2008). Large scale microarray analysis of *P. indica*-colonized Arabidopsis roots did not reveal many auxin-related genes as a target of the fungus. Mutants with impaired auxin levels (*ilr1-1*, *nit1-3*, *tfl2*, *cyp79 b2b3*) responded to *P. indica*, revealing that severe alterations in auxin homeostasis in Arabidopsis do not prevent *P. indica*-mediated growth response (Vadassery et al., 2008). However, different cytokinin receptor genes e.g. *CRE1, AHK2, AHK3,* the cytokinin-responsive gene *ARR5*, and *trans*-zeatin cytokinin biosynthesis genes are significantly upregulated in the colonized roots demonstrating the positive role of cytokinin in *P. indica*-mediated growth promotion in Arabidopsis (Vadassery et al., 2008). This is different from the response of Chinese cabbage to *P. indica*. A double-subtractive expressed sequence tag (EST) library from Chinese cabbage roots grown in the presence or absence of the fungus revealed that many genes involved in auxin signaling, metabolism and function are upregulated by *P. indica* in the colonized roots demonstrating the positive role of auxin in *P. indica*-mediated growth promotion in Chinese cabbage (Fig. 1; Lee et al., 2011; Johnson et al., 2013a). Furthermore, small molecule(s) secreted from the fungus promoted the growth of Arabidopsis, Chinese cabbage and tobacco which clearly indicate that in addition to phytohormones, other fungus-derived factors are involved in the *P. indica*-mediated growth promotion in these crops (Vadassery et al., 2009; Lee et al., 2011; Johnson, 2014). Interestingly, Schäfer et al., (2009a) reported a positive role of gibberellins in the mutualistic interaction of *P. indica* with barley. However, *P. indica* inhibits ethylene biosynthesis and its signaling, which contribute to plant growth promotion in tobacco, barley and Arabidopsis (Fig. 1; Barazani et al., 2005; Schäfer et al., 2009a; b; Camehl et al., 2010; Khatabi et al., 2012). This is evident from the fact that growth of *etr1*, *ein2* and *ein3D eil1* (impaired in ethylene biosynthesis) and activation of ethylene responsive genes, for example, *ETR1, EIN2 and EIN3 D EIL1* are not promoted or even inhibited by the fungus (Camehl et al., 2010). Other phytohormones synthesized or manipulated by the root endophyte include abscisic acid and brassinosteroids (Figure 1; Schäfer et al., 2009a; Vadassery et al., 2008; Camehl et al., 2011). It seems that the whole orchestra of phytohormones and its signaling networks are involved in generating compatible endosymbioses between the fungus and plants (cf. Oelmüller et al., 2009; Franken, 2012; Qiang et al., 2012; Johnson et al., 2013a).

P. indica-colonized plants have enhanced tolerance to root and foliar pathogens

Being sessile in nature, plants are permanently confronted with environmental cues throughout their lifetime which evolutionarily resulted in their adaptation to different biotic and abiotic environmental constraints. Even with evolution-driven and genetically determined adaptation, successful pathogens can invade and affect plant health or may cause plant lethality. Similar to AM fungi, *P. indica* not only induces resistance against many soil-borne and root pathogens but also impart systemic resistance to different foliar pathogens after colonizing on/in roots of wide variety of plant species (Table 2; Qiang et al., 2012). In wheat, *P. indica*-colonized plants control root pathogenic fungi like *Cochliobolus sativus* and *Fusarium culmorum* causing root rot diseases besides growth promoting effects (Waller et al., 2005). *P. indica*-colonized wheat and barley plants diminished the infection of the root and seed pathogen, *Gaeumanomycetes graminis* (Varma et al., 2001; Serfling et al., 2007). The endophyte also reduced the negative impact of the root pathogens *F. culmorum* and *Pseudocercosporella herpotrichoides* on winter wheat (Serfling et al., 2007). In the field, significantly fewer symptoms of the stem-base pathogen *P. herpotrichoides* was observed. In barley, *P. indica*-colonized plants could successfully diminish the infection of the root pathogenic fungi *C. sativus* and *F. culmorum* causing root rot diseases besides growth promotion effects (Waller et al.,

Table 2: Plant diseases controlled due to *P. indica*-root colonization in different crops/plants (Modified after Qiang et al., 2012)

Disease/symptom	Pathogen	Host	References
Powdery mildew	*Blumeria graminis* f.sp. *hordei*	Barley	Waller et al. (2005); Felle et al. (2009)
Fusarium root rot	*Fusarium culmorum*	Barley, wheat	Waller et al. (2005); Serfling et al. (2007)
Root rot	*Cochliobolus sativus*	Barley	Waller et al. (2005)
Fusarium root rot	*Fusarium graminearum*	Barley	Deshmukh and Kogel (2007)
Rhizoctonia root rot	*Rhizoctonia solani*	Barley	Qiang et al. (2012)
Powdery mildew	*Blumeria graminis* f.sp. *tritici*	Wheat	Serfling et al. (2007)
Eye spot (stem base)	*Pseudocercosporella herpotrichoides*	Wheat	Serfling et al. (2007)
Root disease	*Fusarium verticillioides*	Maize	Kumar et al. (2009)
Fusarium wilt	*Fusarium oxysporum*	Tomato	Qiang et al. (2012)
Black root rot	*Thielaviopsis basicola*	Tomato	Qiang et al. (2012)
Yellow leaf mosaic	Pepino mosaic virus	Tomato	Fakhro et al. (2010)
Verticillium wilt	*Verticillium dahliae*	Tomato	Fakhro et al. (2010)
Powdery mildew	*Golovinomyces orontii*	Arabidopsis	Stein et al. (2008)
Verticillium wilt	*Verticillium longisporum*	Arabidopsis	Knecht et al. (2010)
Root rot	*Rhizoctonia solani*	Arabidopsis	Knecht et al. (2010)
Cyst nematode	*Heterodera schachtii*	Arabidopsis	Daneshkhah et al. (2013)
Leaf blight	*Alternaria brassicae*	Arabidopsis	Johnson et al. (2013b)
Verticillium wilt	*Verticillium dahliae*	Arabidopsis	Sun et al. (2014)

2005). Studies on the bioprotection performance of *P. indica* against another root pathogen *F. verticillioides* in maize plants demonstrated that *P. indica*-primed roots suppress further colonization of the pathogen (Kumar et al., 2009). Furthermore, Fakhro et al. (2010) demonstrated bioprotection ability of *P. indica* by significantly reducing the root infection of *Verticillium dahliae* in *P. indica*-colonized tomato plants. In Arabidopsis, *P. indica*-colonized plants could completely diminish the infection of different root pathogens like *V. longisporum*, *V. dahliae*, *Rhizoctonia solani* and *Heterodera schachtii*, a sedentary endoparasitic beet cyst nematode (Knecht et al., 2010; Daneshkhah et al., 2013; Sun et al., 2014). This supports its potential for the management of root diseases in different crops species. The ability of *P. indica*-colonized plants to control different root pathogen may be due to its ability to produce antimicrobial compounds (antibiosis) and/or a direct antagonism to pathogens. The direct antagonistic effect of *P. indica* has been demonstrated on a number of phytopathogenic root fungi like *R. solani*, *R. bataticola*, *F. oxysporum*, *F. udum*, *F. solani* and *Phytophthora nicotianae* var. *parasitica* due to antibiosis and production of lytic enzymes such as chitinases and β-1,3-glucanases (Johnson et al., 2013b). Kumar et al. (2009) demonstrated the bioprotection performance of *P. indica* against the root pathogen *F. verticilloides* in maize and is related to the increased activity of the antioxidant enzymes catalase, glutathione reductase, glutathione S-transferase and superoxide dismutase in the *P. indica*-colonized plants (Fig.1).

In addition to diminishing the infection of root pathogens, *P. indica* can also reduce foliar diseases caused by multitude of pathogens by activating induced systemic resistance (ISR) (Table 2). In barley, *P. indica*-colonized plants could successfully control the foliar pathogens *Blumaria graminis* f. sp. *hordei* causing powdery mildew disease and *P. herpotrichoides* also causing necrotic leaf spot disease besides growth promotional effects (Waller et al., 2005). Similar results were also demonstrated in winter wheat both in pot culture and field experiments against *B. graminis.* f. sp. *tritici* and *P. herpotrichoides* (Serfling et al., 2007). Further it was demonstrated that root colonization of *P. indica* induces ISR against the foliar pathogenic powdery mildew fungi *B. graminis.* f. sp. *hordei* in barley (Waller et al., 2005; Felle et al., 2009) and

Golovinomyces orontii in Arabidopsis (Stein et al., 2008). Additionally, the *P. indica*-priming in Arabidopsis could effectively diminish the foliar infection by the necrotrophic fungi *Alternaria brassicae* and *V. dahliae* (Table 2; Johnson et al., 2013b; Sun et al., 2014). How the root colonized *P. indica* could protect the plants from foliar pathogens is still not clearly understood as the fungus did not colonize in the shoot. The ability of *P. indica* to diminish the foliar diseases clearly demonstrate that there should be efficient translocation of antimicrobial compounds produced in roots to shoot or there is efficient flow of communication from root to shoot to prime the aerial parts against the foliar pathogens.

P. indica reduced *V. dahliae*-mediated disease development in Arabidopsis, by inhibiting the growth of the pathogen after pretreatment of Arabidopsis roots with *P. indica*. The *P.indica*-pretreated plants grew better after *V. dahliae* infection and the production of Verticillium microsclerotia was dramatically reduced, all without activating stress hormones and defense genes in the host. Sun et al. (2014) concluded that *P. indica* is an efficient biocontrol agent that protects Arabidopsis from *V. dahliae* infection. Since growth of the pathogen is restricted in the presence of *P. indica*, the authors postulate that besides ISR, direct growth inhibition of pathogens by *P. indica* participates in repressing disease development in plants.

It has been well demonstrated that the root colonization of the endophyte could induce reactive oxygen species (ROS)-scavenging genes and enzymes in leaves (Fig.1; Johnson, 2014). *P. indica* activated plant antioxidant system which further enhanced the biotic stress tolerance (Waller et al., 2005, Druege et al., 2007; Kumar et al., 2009). The systemic resistance response against *B. graminis* f. sp. *hordei* is positively correlated to the transient changes in H^+ concentrations in the *P. indica*-colonized root hair and elongation regions, which could also be measured in the apoplast of leaves leading to its acidification thus providing ISR in barley plants (Felle et al., 2009). *P. indica* also enhances the synthesis of different antimicrobial compounds in the colonized plants which help the plants to counter the pathogen attack (Johnson, 2014). *P. indica*-colonized *S. calva* plant extract showed an enhanced antifungal activity against *F. oxysporum* and *Trychophyton mentagrophytes* (Raj et al., 2004). The fungus also recruits phytohormones to manipulate plant defense thus imparting ISR in *P. indica*-colonized plants against a number of foliar pathogens (Table 2). Schäfer et al. (2009a) proved the involvement of gibberellins signaling in conferring ISR against powdery mildew fungus in barley. The role of jasmonic acid (JA), JA-responsive genes, ethylene (ET) and ET-responsive genes were demonstrated in the enhanced resistance of *P. indica*-colonized plants against biotrophic powdery mildew fungi like *G. orontii*, *B. graminis* f. sp. *tritici* and *B. graminis* f. sp. *hordei* in Arabidopsis, wheat and barley (Waller et al., 2005, Serfling et al., 2007; Stein et al., 2008; Khatabi et al., 2012). Beneficial fungal and bacterial colonization in plants transiently activate the innate immune system in order to counter different pathogens through multi-layered innate immune system (Jones and Dangl, 2006) which relies on Ca^{2+} and ROS signaling, activation of Ca^{2+}-dependent protein and MAP kinases (CDPK, MAPK) and transcription factors, and rapid production of stress hormones such as jasmonic acid, salicylic acid, ethylene and abscisic acid (Fig. 1; Boller and Felix, 2009). After recognition of *P. indica*, a very early event of cytosolic Ca^{2+} influx, transient ROS burst, rapid apoplastic alkalization, CDPK and MAPK activations and defense gene induction were reported independently in Arabidopsis and barley (Fig.1; Deshmukh and Kogel, 2007; Felle et al., 2009; Vadassery et al., 2009; Johnson, 2014). Different defense genes were activated only in the early phase of *P. indica* colonization but at later stages, most of the defense genes were strongly down regulated which was further confirmed by microarray studies (Schäfer et al. 2009a, b; Johnson, 2014; Sun et al., 2014). Further studies in

Arabidopsis demonstrated that *P. indica*-induced early immune responses are important for the priming of the shoot parts against foliar pathogens to impart ISR (Schäfer et al., 2009a; Jacobs et al., 2011).

P. indica-colonization confers tolerance to different abiotic stress conditions

Soil drought, water stress and salinity are the most important abiotic stress factors that obstacle growth, survival, production and productivity of different crop plants. Salinity due to Na^+ and Cl^- reduces the availability, mobility and transport of Ca^{2+} and K^+ to the growing plant parts thus affecting the quality of vegetative and reproductive organs (Kohler et al., 2009), increases the ratio of Na^+/Ca^{2+} and Na^+/K^+ making the plants more susceptible to osmotic and specific ion injury and also to nutritional disorders including N and P (Kaya et al., 2002). *P. indica*-colonized plants show increased tolerance to many abiotic stress like extreme cold, temperature, drought, water stress and salt conditions compared to other beneficial microbes (Table 3; Zarea et al., 2012; 2013). Salt and drought stress tolerance are the most often deciphered responses due to *P. indica*-colonization in many plants. Zarea et al. (2012) reported that the fungus could survive salt concentration of 200-400 mM under *in vitro* condition. *P. indica*-colonized barley, wheat, rice and tobacco plants were able to survive in moderate salt (NaCl) concentration of 100 mM to 300 mM with higher biomass production whereas this concentration was detrimental to the uncolonized control plants (Table 3; Waller et al., 2005; Baltruschat et al., 2008; Zarea et al., 2012; 2013; Alikhani et al., 2013; Jogawat et al., 2013; Trivedi et al., 2013; 2014). Under this condition, *P. indica* induced higher levels of ascorbic acid and proline, increased activities of antioxidant enzymes like catalase, ascorbate peroxidase, dehydro-ascorbate reductase, mono dehydroascorbate reductase and glutathione reductase to scavenge the ROS, and reduced degradation of poly-unsaturated lipids (Fig.1; Waller et al., 2005; Baltruschat et al., 2008; Kumar et al., 2009; Sun et al., 2010; Kumar et al., 2012; Zarea et al., 2012). Salt tolerant property of *P. indica* can be explored for the establishment of (salt sensitive) crops in salt affected area.

Waller et al., (2005) demonstrated that *P. indica* could diminish the adverse effect of salt stress in barley seedlings by elevating ascorbic acid and the increased activities of antioxidant enzymes. Further, Baltruschat et al. (2008) have shown that *P. indica* attenuated the salt-induced lipid peroxidation, metabolic heat influx and fatty acid desaturation (indicators of physiological stress), in barley leaves of the colonized plants. *P. indica* modulates the defense system and alters the metabolism to compensate the loss in photosynthesis and prevents oxidative damage caused by stress. Under salt stress condition, *P. indica* maintains a high antioxidative environment to detoxify ROS (Fig. 1). In this process, the activities of different superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, peroxidase, mono-dehydroascorbate reductase and dehydroascorbate reductase were induced in *P. indica*-colonized plants (Baltruschat et al., 2008; Zarea et al., 2012). *P. indica* also modulated ion accumulation in colonized barley plants by increasing the foliar K^+/Na^+ ratio, an indicator of salt stress tolerance (Alikhani et al., 2013). Proteomic analysis showed that proteins of different functional categories including photosynthesis, cell antioxidant defense, protein translation and degradation, energy production, signal transduction and cell wall arrangement are induced in fungus-colonized roots (Alikhani et al., 2013). The increased salt tolerance in *P. indica*-colonized rice plants were due to higher content of the photosynthetic pigments Chl *a*, Chl *b* and carotenoids in addition to the enhanced levels of osmolytes such as proline, polyamines, etc. (Jogawat et al., 2013). Therefore, high ROS scavenging enzyme levels and compounds together with elevated concentrations of osmolytes leads to maintenance of plastid integrity and enhanced photosynthetic efficiency in *P. indica*-colonized plants under salt stress condition (Fig.1). A major breakthrough could be studies of Trivedi et al.

(2013; 2014) who identified a cyclophilin A-like protein from *P. indica* (PiCypA), which shows higher expression levels during salinity stress. The transgenic tobacco plants overexpressing PiCypA develop osmotic and salt tolerance, and also exhibit normal growth under salt and osmotic stress conditions.

Similar to induction of salt-stress tolerance, *P. indica* also confers tolerance to drought stress to the fungus-colonized Arabidopsis plants (Sherameti et al., 2008a), Chinese cabbage (Sun et al., 2010) and barley (Ghabooli et al., 2013) under *in vitro* and greenhouse conditions (Table 3). Exposure of *P. indica*-colonized and mock-treated plants to drought did not affect the performance of the fungus-colonized Arabidopsis seedling whereas the mock-treated plants could not survive or perform better under greenhouse conditions (Sherameti et al., 2008a). *P. indica*-colonized seedlings exhibited faster and stronger upregulation of drought-tolerance related phospholipase Dδ, CBL1 and HAT in addition to the enhanced activities of ROS scavenging enzymes localized in cell membranes, chloroplasts, peroxisomes and mitochondria (Sherameti et al., 2008a). Similarly, when colonized Chinese cabbage plants were exposed to 20% polyethylene glycol (PEG) to mimic drought stress, the colonized-plants could very well survive due to the enhanced activities of peroxidases, catalases and superoxide dismutases in the leaves, whereas the non-colonized plants could not survive (Fig.1; Sun et al., 2010). Further, the fungus retarded the drought-induced decline in the photosynthetic efficiency and the degradation of Chls and thylakoid proteins; and also the stress marker malondialdehyde. The expression levels of the drought-related genes DREB2A, CBL1, ANAC072 and RD29A were also upregulated in the drought-stressed leaves of colonized plants (Sun et al., 2010). Similarly, proteome analysis of *P. indica*-colonized barley leaves under well-treated and water-deficit conditions demonstrate that *P. indica* increased the level of proteins involved in photosynthesis, antioxidative defense system and energy transport (Fig.1; Ghabooli et al., 2013). Therefore, *P. indica* root-colonization enhances water stress tolerance based on general and non-specific plant-species mechanism.

P. indica-colonized Arabidopsis and wheat plants could perform better under heavy metal stress conditions (Table 3; Peškan-Berghöfer et al., 2004; Shahabivand et al., 2012). The fungus also showed an enhanced seed germination of vegetables like cabbage, beet root, endive, radish, onion and swisschord even up to 100 per cent in a period of 25 days at extreme low temperature (-30 to 4ºC) in an altitude of 3500 m, whereas not a single seed germinated in untreated control (Table 3; Murugan, 2011; Varma et al., 2012). The micropropagated plants are highly susceptible to biotic and abiotic

Table 3. Abiotic stress tolerance due to *P. indica*-root colonization in different crops/plants

Crop plants	Abiotic stress	References
Barley	NaCl (300 mM)	Waller et al. (2005); Balstruschat et al. (2008); Alikhani et al. (2013)
Wheat	NaCl (300 mM)	Zarea et al. (2012)
Rice	NaCl (300 mM)	Jogawat et al. (2013)
Arabidopsis	Drought (withholding water) (opening the lid of the petri-dishes after co-cultivation for 84h)	Sherameti et al. (2008a)
Chinese cabbage	Drought (PEG-20%)	Sun et al. (2010)
Barley	Drought (withholding water)	Ghabooli et al. (2013)
Vegetables	Low temperature (<4ºC)	Murugan (2011); Varma et al. (2012)
Arabidopsis	Cd (0.2 mM)	Peškan-Berghöfer et al. (2004)
Wheat	Cd (0.9 mM)	Shahabivand et al. (2012)

stress due to the transient transplantation shock. Extensive studies on hardening of micropropagated plants demonstrated that *P. indica*-colonized tissue-cultured plants like sugarcane, maize, *Dendrocalamus strictus*, *Populus* spp., *Aloe vera* and *Chlorophytum borivilianum* survived more and established well in pot culture and field experiments (Gosal et al., 2010; 2013). The increased survival and establishment of micropropagated plants were positively correlated to *P. indica*-root colonization, thus the fungus helps the tissue-cultured plantlets to overcome the transplantation shock (Sahay and Varma, 1999; Gosal et al., 2010). Further, *P. indica*-colonization also enhanced the production of active compounds in tissue-cultured medicinal plants (Baldi et al., 2008; Gosal et al., 2010).

Conclusion

P. indica is one of the most important and promising root endophyte for the practical application under field condition because it is simple to propagate *in vitro* and accessible to basic physiological and genetic researches. Furthermore, it has a wide host range, and is highly beneficial for the host plant to tolerate moderate to extreme biotic and abiotic stress conditions. The fungus confers resistance to root and soil pathogens and tolerance to foliar pathogens in addition to different abiotic factors like salinity, drought, water, oxidative and heavy metal stress. Another remarkable feature of *P. indica* is its ability to colonize a variety of unrelated host plants, which led to the promotion of this endophyte as a putative biofertilizer, biomodulator and biocontrol agent.

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