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Endophytics fungus *Piriformospora indica* and its mechanism of plant growth promotion

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ABSTRACT

Piriformospora indica has an exceptional ability to promote plant growth, protection and stress tolerance. *P. indica* is endophytics fungus and its mechanism of plant growth promotion which exhibits its versatility for colonizing/hosting a broad range of plant species through directly manipulating plant hormone-signaling pathway during the course of mutualism. And root colonization leads to a better plant performance in all respect, including enhanced root proliferation by indole-3-acetic acid production which in turn results into better nutrient acquisition and subsequently to improved crop growth and productivity. Additionally, *P. indica* can induce both local and systemic resistance to fungal and viral plant diseases through signal transduction. Additionally, this fungus mediated stimulation in antioxidant defense system components and expressing stress-related genes can confer crop/plant stress tolerance. Therefore, the potential of this important fungus and its application promotes plant growth and biomass production. Due to its active growth, provides a model organism for the study of beneficial plant-microbe interactions and a new tool for improving plant production systems.

Keywords: Endophytes, *Piriformospora indica*

1. INTRODUCTION

In natural ecosystems, a variety of microorganisms seek to obtain nutrients for their survival by interacting with plants, where the interaction can be neutral, harmful (parasitism),

or beneficial (mutualism or symbiosis) to the host [1,2]. Endophytes can be defined as, Prokaryotic or eukaryotic organisms with the capability of colonising plants. An endophytic type of different organisms was reported among fungi, bacteria, algae, plants and even insects [3] Plants are potential targets (hosts) for a broad spectrum of microbial organisms like fungal basidiomycete *P. indica*. These associations can be roughly categorised into mutualistic, communalistic or pathogenic relationships. Interactions with certain mutualistic fungal microbes can benefit plants, resulting for example in an improved plant development even under unfavorable environmental conditions [4]. Simultaneously, the microbial partners acquire nutrients from the host and can be protected from environmental stress or competitors [5]. In other cases, it is the microbes that primarily profit from the association, with the host fitness being apparently unaffected (commensalism) [4].

Focusing on fungal microbe's endophytes *P. indica* were defined as organisms that colonize in living plant tissue during their entire life cycle and live in a broad variety of host plant species (e.g. barley, maize, parsley, poplar, tobacco and wheat) without causing disease symptoms. Then, in this review paper attention paid to describe the bio protection performance of *P. indica* against the root parasite and its plant growth promoting activity and its role in enhancement of the tolerance of the host plants against abiotic and biotic stresses as well as strong growth-promoting activities leading to enhancement of host plant yield and give attention on fungal *P. indica* in its evidence for nutrient transfer to the host plants. Furthermore, illustrate the application of *P. indica* in horticulture and agriculture [6].

2. ENDOPHYTIC *P. INDICA* A PLANT GROWTH PROMOTING FUNGUS

P. indica is the most a newly described cultivable endophyte fungus that colonizes roots and ensures the growth of plants. And a wide host root colonizing endophytic fungus which allows the plants to grow under extreme physical and nutrient stress.

The phylogenetic classification of *P. indica* within the newly defined mycorrhizal a basidiomycete of the order *Sebacinales* representing a model for the study of mutualistic symbiosis and, beyond that, the plant immune system. *P. indica* is a root interacting endophytic fungus discovered in the Indian desert in close association with the spores of AM fungi, *Glomus mosseae* [7].

This fungus is interesting for basic research as well as biotechnological applications. It functions as plant promoters and bio fertilizers in nutrient deficient soil as a bio protector against biotic and a biotic stress including root and leaf fungus pathogens and insect invaders, as a bio regulator for plant growth development, early flowering, enhanced seed production, and stimulation of active ingredients in medicinal plants, as well as a bio-agent for the hardening of tissue culture raised plants. Positive interaction is established for many plants of economic importance in arboriculture agro-forestry, flori-horticulture [7,8] described, *P. indica* with plants, especially emphasizing its life strategies in host plants, it has been shown that root colonisation by *P. indica* and its lifestyle in plant may vary depending on environmental factors, the genetic predisposition and the developmental stage of host plants and plant organs, respectively. The fungus is also involved in providing systemic resistance against powdery mildew fungi, *Blumeria graminis hordei*, root rot fungi, *Fusarium culmorum* in barley and *Golovinomyces orontii* in *A. thaliana* [9].

The role of *P. indica* in conferring drought and salt tolerance is also reported [10]. These mutualists improve the growth of crops on poor soils with lower inputs of chemical fertilizers and pesticides [11].

2. 1. *P. indica*: A new root symbiotic fungus

The axenically cultivable plant growth-promoting root endophyte, *P. indica*, has been characterized in collaboration with several European scientists [12]. *P. indica* tremendously improves the growth and overall biomass production of different plants (Figure 1), like herbaceous mono and dicots, trees, including medicinal plants (*Bacopa monniera*, *Artemesia annua*), and several economically important crops [13]. Pronounced growth promotional effects were also seen with terrestrial orchids (Figure 2). The fungus promises to be a potential source for colonizing the orchids, their better growth and higher rate of survival of seeds [12]. A recent report indicated the ability of *P. indica* to colonize the rhizoids of a liverwort (bryophyte) and the thalli failed to grow under *in situ* conditions in the absence of this fungus.



Figure 1. Maize plants (*Zea mays* L.) were grown in surface sterilized plastic pots containing washed expanded clay.

Pots on the left were supplied with dead fungal biomass and those on the right were treated with freshly grown fungus [13]. The interactions of *P. indica* with plant provide and increase the development of roots and leaves (Figure 2).

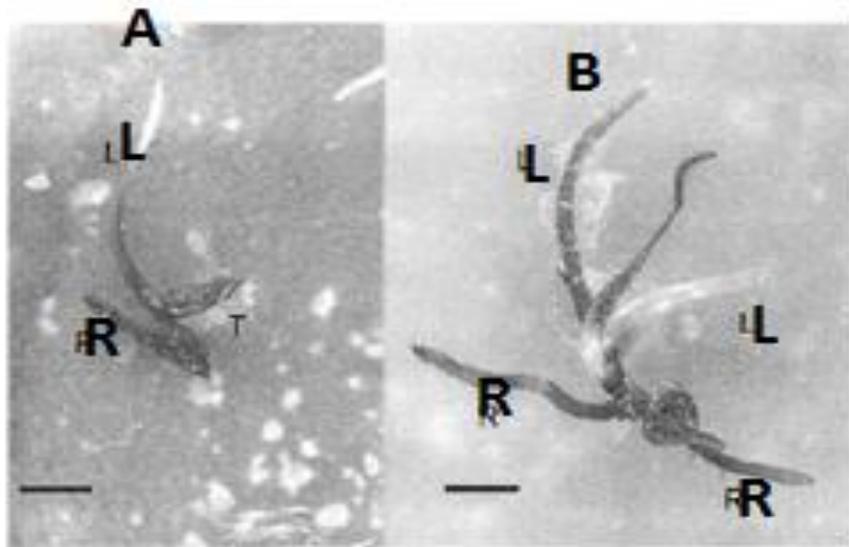


Figure 2. A, B. *Dactylorhiza maculata* was asymbiotically pre-grown for 2 years and then inoculated with *P. indica*. A, Control plants without fungus, differentiating only short roots (R) and leaf (L); B, symbiotic plant interacting with the fungus for 3 months with well-developed roots (R), leaves (L) [13].

2. 2. Characteristics of *P. indica*

The high potential of multifaceted fungus, *P. indica* has tremendous applications in future as biofertilizer, protector and immune regulator which will be helpful in improving quality of not only plants but also ultimately of food, nutrition, medicine and overall quality of human life (Table 1) [14]. *P. indica* is analogous to AM fungi with regard to plant growth promotional effects and used as bio fertilizer, stimulates nutrient uptake in the roots and solubilizes insoluble phosphates and sulphur components in the soil [7,8,15]

Table 1. General Characteristics of *P. indica* [14]

General characteristics
<ul style="list-style-type: none"> • Increase the potential of plants and their essential ingredients. • Acts as a plant stimulator and pathogenic inhibitor. • Applicable in agriculture, floriculture, viticulture and reclamation of degraded and heavily mined soils. • Used in form of culture filtrate for further development and formulation into a bio fertilizer. • <i>P. indica</i> emphasizes importance of individual component of the culture filtrate involved in

- ✓ Stimulation of growth of the plants.
- ✓ bio fertilizer,
- ✓ bio protector,
- ✓ immune regulator and agent for biological hardening of tissue cultured plants.

3. BIOTECHNOLOGICAL APPLICATIONS OF *P. INDICA*

The endophyte promotes nutrient uptake, allows plants to survive under water, temperature and salt stress, confers (systemic) resistance to toxins, heavy metal ions and pathogenic organisms and stimulates growth and seed production [7]. *P. indica* is an interesting endophytic fungus capable of colonising roots and forming symbiotic relationship with every possible plant on earth.

P. indica has also been shown to increase both crop yield and plant defense of a variety of crops (barley, tomato, maize) against root pathogens.

3. 1. *P. indica* confers drought stress tolerance

Plant growth is greatly affected by drought stress and plants must adapt to this stress to survive. Drought resistance mechanisms have been divided into several types. At the first level, the phenomenon may be distinguished into desiccation postponement (ability to maintain tissue hydration), desiccation tolerance (ability to function when dehydrated) which are sometimes referred to as drought tolerance at high and low water potentials respectively and drought escape which comprises plants that complete their lifecycles during the wet season, before the onset of drought and the only true drought avoiders. The water savers use water conservatively saving some in the soil for later use in the life cycle, whereas the water spenders aggressively absorb water, often using prodigious quantity. Drought stress induces a range of physiological and biochemical responses in plants such as stomatal closure [16].

Repression of growth and photosynthesis [17] and activation of respiration. Many drought inducible genes have been identified which can be classified into two major groups: proteins that function directly in abiotic stress tolerance and regulatory proteins, which are involved in signal transduction or expression of stress-responsive genes [8]. Many genes for drought stress signaling components themselves are up regulated under drought stress. *P. indica* was isolated from an desert; it is likely that the fungus may confer drought tolerance to plants. When *Arabidopsis* is exposed to mild drought stress, seedlings co-cultivated with the fungus *P. indica* continue to grow, while the uncolonized controls do not and show symptoms of withering [8].

P. indica confers drought stress tolerance to *Arabidopsis*, and this is associated with the priming of the expression of a quite diverse set of stress-related genes in the leaves. When seedlings are first exposed to drought stress and then transferred to soil, many colonized seedlings reach the flowering stage and produce seeds, while the percentage for uncolonized seedlings is much lower.

3. 2. Performance of *P. indica* in plant against pathogens

The endophyte *P. indica* colonizes roots of a range of host plants and increases biomass production and resistance to fungal pathogens and, thus has been considered a biocontrol fungus. The performance of this fungus in different substrata under greenhouse and practical field conditions. Roots of winter wheat were colonized efficiently, and biomass was particularly increased on poor substrata. In greenhouse experiments, symptom severity of a typical leaf (*Blumeria graminis*), stem base (*Pseudocercospora herpotrichoides*), and root (*Fusarium culmorum*) pathogen was reduced significantly [11]. However, in field experiment symptoms caused by the leaf pathogen did not differ in *P. indica* colonized compared with control plants. In the field, *Pseudocercospora herpotrichoides* disease severity was significantly reduced in plants colonized by this endophyte. Earlier work established that *P. indica* increased the biomass of several host plants belonging to a wide range of taxa [7,12,18.]

The inoculated wheat with *P. indica* may subsequently reduce the symptoms caused by several fungal pathogens on leaves, stems, and roots were addressed under greenhouse and field conditions and also indicated by increased hydrogen peroxide formation in leaves challenged with powdery mildew. There are varieties of fungal species including fungal endophytes, which demonstrate plant disease control properties in different rhizospheres [19]. As demonstrated by the present work, *P. indica* provides protection against *F. graminearum* root rot in barley.

Table 2. Mechanisms of biological plant disease protection by *P. indica*

Major mechanisms of biological plant disease protection by <i>P. indica</i>	<ul style="list-style-type: none"> • Competition for occupying niches or nutrients • Production of antibiotics. • Induced resistance and mycoparasitism [14]
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P. indica or *F. graminearum* do not exhibit direct antagonistic effects on each other, when confronted under axenic culture conditions. These results demonstrated that production of antimicrobials or myco parasitism mechanisms are not involved in the bio protective action provided by *P. indica*. The main sites of plant penetration were the lateral roots and root hairs, where the fungus directly penetrates epidermal cells. [16] found that fungal growth at this stage was mainly intercellular. These findings demonstrate the common life history strategy of *Fusarium* in plant roots, in which the pathogens first attach to the lateral. Root hairs penetrate directly through the epidermis of these tissues.

Inhibition of systemic spread of *Fusarium* during early stage of *Fusarium* infection under conditions that favor symptomless infection, fungal growth may be restricted to specific tissues, where *Fusarium* penetrates only specific cells and in which it reproduces without damaging the surrounding cells [20].

Under conditions that favor pathogenic development, more mycelium develops and the fungus switches to a more aggressive phase that probably involves secretion of hydrolytic enzymes and toxins. In experiments with *P. indica* and *Fusarium*, the biomass (fresh weight) of *Fusarium* inoculated and control plants were measured. Interestingly, no reduction in

growth promotion was found in plants, which were colonized by both *P. indica* and *F. graminearium* [19].

3. 3. Bacterial endosymbiotic associations within *P. indica*

Recent molecular analyses have shown that both *P. indica* and *S. vermifera* are intimately associated with bacteria. Based on PCR analyses and sequencing of the 16S ribosomal RNA, an association of *P. indica* with *Rhizobium radiobacter*, a gram-negative α -proteobacterium, was traced back to the original *P. indica* isolate deposited in the culture collection of the German Resource Centre for Biological Material, Braun-schweig bacterial cells are not present in culture filtrates of *P. indica* [19]. They are released after crushing the fungal mycelium, suggesting that *R. radiobacter* is closely associated with the hyphal walls or even lives endosymbiotically. Isolated bacteria show biological activities on barley similar to those mediated by *P. indica*, including systemic resistance induction against powdery mildew and growth promotion.

Since *R. radiobacter* has not been successfully eliminated from *P. indica*, it remains an open question to what extent fungus and bacterium contribute to the biological effects on their host plants. There are several reports showing a mutualistic association of mycorrhizal fungi with bacteria in which, for instance, bacteria improve spore germination and the formation of mycorrhizal interactions. In addition, plant growth-promoting bacteria (PGPR) have been shown to interact physically with fungal hyphae.

True endosymbiotic bacteria have been reported in only a few fungi, including members of the *Glomeromycota* (e.g. *Gigaspora*, *Geosiphon pyriforme*) and the ectomycorrhizal basidiomycete *Laccaria bicolor*. Endosymbiotic bacteria were first identified in the AM fungus *Glomus margarita*, and this association is the best studied interaction of AM fungi and endobacteria [21]. In the plant pathogen *Rhizopus microspores* endosymbiotic bacteria play a crucial role in fungal infection strategies. Recently, *R. microspore* was thought to produce a toxin that kills plant root cells. However, [15] demonstrated that, the toxin was not produced by the fungus but by endogenous bacteria. On the basis of the 16S ribosomal RNA gene sequence, they found that the bacteria belong to the genus *Burkholderia*, a member of the beta subdivision of proteobacteria. The bacteria and bacteria-free fungus were each isolated in pure culture. There was a strong correlation between the presence of bacteria and the toxin-producing capability of *Rhizopus*. In the absence of endobacteria, *Rhizopus macrosporus* was not capable of vegetative reproduction [15]. Formation of sporangia and spores was restored only upon reintroduction of endobacteria. The motile rod-shaped bacteria appeared to be prone to chemotaxis, since they migrated toward the tips of the hyphae, the region best supplied with nutrients and where sporangia were formed. Particularly, *P. indica* seems to mediate and transport phosphorous to plants [8, 12].

3. 4. *P. indica* determine plant productivity beyond abiotic factors

P. indica is a newly described axenically cultivable Phyto promotional endosymbiotic. This fungus having a broad host spectrum shows pronounced growth-promotional effects. It mobilizes the insoluble phosphates and translocates the phosphorus to the host in an energy dependent process. The axenic cultivability of *P. indica* on economically viable synthetic media makes it suitable for mass scale inoculum production for application in agro-forestry and horticulture. According to [13] in sum up, solar energy, water and soil nutrients are

undoubtedly essential for plant productivity but the interaction with useful and friendly microbe called *P. indica* also exert a tremendous impact. It is a general belief that plants, because they are autotrophs, can carry out all the functions of life with the availability of the so-called abiotic factors such as solar energy, moisture and mineral nutrients. However, what is not generally realized is that the plants, as all living organisms, also interact with the biotic factors, and their plant growth promoting fungus like *P. Indica* being mutualistic symbionts control, in many ways, the plant health and productivity [22].

3. 5. *P. indica* plays in Phosphate transport to the host plant

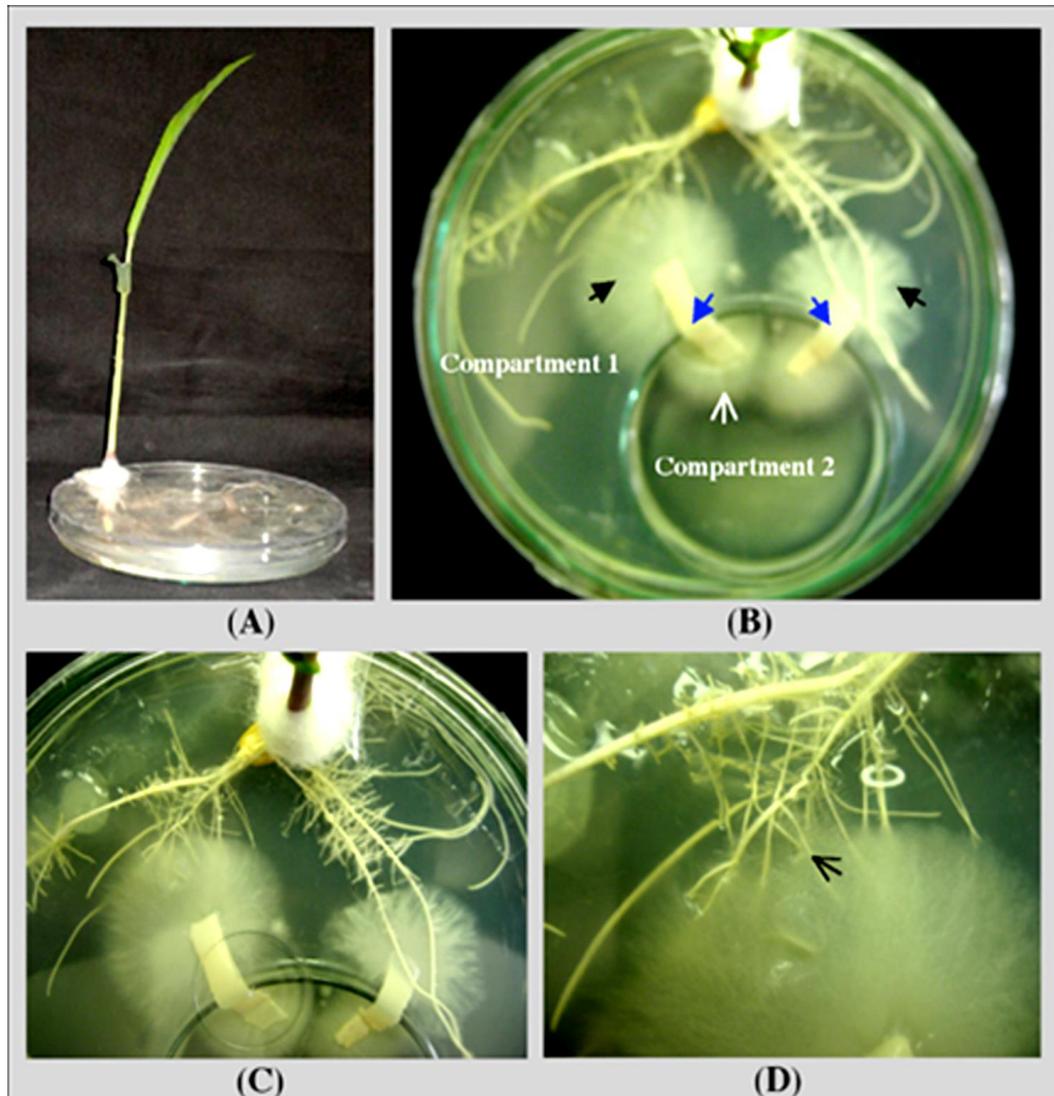


Fig. 3. Bi-compartment Petri dish culture system to study the transport of radio labeled (P) orthophosphoric acid to maize plants via *P. indica*. A, lateral view. B and C, top view showing both compartments separated by a small glass Petri plate (used as compartment 2). The black arrow indicates the *P. indica* growth in compartment 1, and the white arrow indicates the growth of *P. indica* in compartment 2 (D).

Analysis of fungus to plant transfer of phosphate transportation of phosphate was observed using the bi-compartment system. The phosphorous was transferred to maize plants through the fungal mycelium and across the hyphal bridge between both compartments. Very little radioactive counts were observed in the agar media of the second compartment confirming that the amount of phosphorous present in the maize plants was exclusively transferred by *P. indica* and not because of leaching by the fungus in the second compartment. In set B, very less radioactivity was detected in maize plants colonized with KD-*PiPT* transformants of *P. indica*, confirming the direct role of *PiPT* in phosphate transport to maize plants (Figure. 3A, B). In the case of set 'c' no radioactivity was observed (Figure. 3A, C), and hence, the movement of 'phosphorous' from one chamber to another was not due to diffusion but through fungus only. *Role of PiPT* in Phosphate Nutritional Improvements of Host Plant. We found that inoculation of *P. indica* significantly increased the average above ground biomass of maize plants than that of the non-colonized plants as well as from the KD-*PiPT P. indica* colonized plants, respectively (Figure. 3A).

Colonization of the maize roots was by *P. indica* [23]. *P. indica* colonize the root cortex to obtain carbon from their host plant, while assisting the plant with the improved and rapid uptake of phosphorus and other mineral nutrients of low mobility from the soil, and their translocation to the host root [23]. This is beneficial to the plant as phosphorus is essential for plant growth and development. Studies on *P. indica* have shown fungal-mediated uptake of radio labeled phosphorus from the medium and its translocation to the host in an energy-dependent process, evident by a sharp increase in its content in the shoot.

3.6. Effects imposed by *P. indica* on plants grown in the field

P. indica, a new root colonizing endophytic fungus was discovered by [7]. *P. indica* colonizes a wide range of monocot and dicots plants. Also convey several benefits to host plants like wheat, better tolerance to various biotic and abiotic stresses, as well as improved plant fitness by increasing growth performance under normal and stress conditions [14]. The contribution of *P. indica* symbiosis to improve plant drought and salinity tolerance might point towards the natural habitat of its desert origin [10]. The ability of *P. indica* to improve growth rate of various host plants and also has stimulatory effects on adventitious root formation in ornamental stem cuttings and to activate nitrate reductase that plays a major role in nitrate acquisition and also a starch-degrading enzyme, glucan-water dikinase, involved in early events of starch degradation in the plants such as tobacco and Arabidopsis [9]. In addition, improvement of plants tolerance to biotic and abiotic stresses following colonisation by *P. indica* have also been widely described and considered as a promising means to achieve sustainable agricultural production.

4. MECHANISM OF INTERACTION OF *P. INDICA* WITH PLANTS

The interaction of *P. indica* were responsible for its positive effects [24]. [25] also contended that colonization pattern of *P. indica* in barley roots fungal hyphae enter roots via root hairs to old plants. The fungus forms pear-shaped chlamydo spores within root hairs and proceeds into rhizodermis cells. The fungus grows into the root cortex tissue. The fungus was not detected in the central part of the roots beyond the endodermis and fungal structures were

visualized by acid fuchsin-lactic acid red in, or they were stained for mitochondrial respiratory activity by the succinate dehydrogenase assay.

As per as [24] reported, mechanism by which *P. indica* promotes the growth of plants is not yet very clear, at least in Arabidopsis, it is observed that the effect was due to diffusible factor that could be IAA, as *P. indica* produces IAA in culture filtrate. And some studies have implicated various ingredients from *P. indica* or induced by it in plants for its positive effects, such as Indole-3-acetic acid (IAA) production in culture filtrate by *P. indica* or induction of IAA in plants, proteins in *P. indica* showing similarity to myrosinase binding and myrosinase associated proteins raising IAA that triggered growth promotion in plants.

Leucin rich repeated protein production in plants, mediated by the endophyte, phytohormones, endophyte acting as modulator elevating nitrate assimilation and or starch degradation in plants could be responsible for its positive impact on plants. Also, improvement in the growth of the plant may be due to induction of systemic resistance by *P. indica* against diseases in the plants as has been reported by many researchers [24]. In *P. indica*, colonized plants number of sheath layers and hydrogen peroxide concentration increased after *B. graminis* attack, suggesting that root colonization caused induction of systemic resistance or priming of the host plant. *Pseudocercospora herpotrichoides*), and root (*Fusarium culmorum*) *P. herpotrichoides* disease severity was significantly reduced in plants colonized by the endophyte *P. indica* colonized plants as compared to untreated plants control.

In the field *P. indica* is a wide-host root colonizing endophytic fungus which allows the plants to grow under extreme physical and nutrient stress. It functions as a plant promoter, and biofertilizer in nutrient deficient soils, as a bioprotection against biotic and a biotic stress including root, leaf pathogens and insect invaders, inducing early flowering, enhanced seed production and stimulation of active ingredients in medicinal plants. Positive increments are established for many plants of economic importance [18].

As [5] explained that, *P. indica* colonizes root cortical cells and enhances yield in different plant species (eg. Barley plants). Microscopic inspection of barley plants grown in *P. indica* inoculated substrate showed that the fungus enters roots primarily via root hairs and, later, grows intracellularly in the root cortex, hyphae were detected neither in the central part of the roots beyond endodermis, nor in stems or leaves.

4. 1. Factors involved by Plants Colonized with *P. indica*

Failed colonization may be accompanied by the development of disease symptoms [5]. *P. indica* has not been shown to possess distinct host specificity, nor have non-host plants been detected. The fungus colonizes monocotyledonous and dicotyledonous plants equally well. Hosts include orchids (*Dactylorhiza* species) and members of the *Poaceae* (e.g. barley, maize, rice, wheat) and Brassicaceae (e.g. *A. thaliana*; [7,14,26] and colonisation is asymptomatic, although these plants are both inter- and intracellularly colonized.

Some common defense reactions were found in plant endophyte interactions, e.g. papillae formation, cell wall lignification, H₂O₂ accumulation, enhanced peroxidase activity, or accumulation of phenolic compounds [5]. Studies with tobacco and *Nicotiana sylvestris* constitutively expressing different plant chitinases demonstrated that defence related proteins do not per se exhibit antimicrobial activity against the AM fungus *Glomus mosseae* [5]. Certain chitinases are even reported to support mycorrhizal root colonization by hydrolysing

chitin which would be recognized by the plant innate immunity system and induce pathogen associated molecular pattern (PAMP)-triggered immunity [27].

4. 2. Antioxidant enzyme activities plants colonized with *P. indica*

Antioxidants are molecules that function to reduce oxidative stress by scavenging or quenching ROS and these molecules are crucial for beneficial plant/microbe interactions [28]. Plant and fungal antioxidants might contribute to the protection of the invaded cell against defense-associated ROS production. Generally, biotic and abiotic stresses result in the production of H₂O₂ or another ROS, which may or may not result in an oxidative burst.

In order to study how *P. indica* protects itself from the oxidative defense systems of the plant during colonization, and to determine the impact of colonization with *P. indica* on the antioxidative systems of the plant, antioxidative enzyme activities were checked in the presence and absence of *F. verticillioides*, as well as after delayed inoculation. With *P. indica* showed improvements in biomass, and root length and number as compared with plants grown with *F. verticillioides* alone. That as the colonization by *P. indica* increases, the presence of colonization by *F. verticillioides* decreases [29]. Decreased antioxidant enzyme activities due to the presence of *P. indica* help the plant to overcome the disease load of *F. verticillioides*.

We propose that *P. indica* can be used as a bioprotection agent against the root parasite *F. verticillioides*. It is a common belief that an antioxidant enzyme plays an important role in fungal symbiosis conferring abiotic stress tolerance [29]. The antioxidants include the low molecular- weight compounds glutathione, ascorbate and tocopherol and the enzymes superoxide dismutases, catalases, ascorbate- or thiol-dependent peroxidases, glutathione reductases, dehydroascorbate reductases and monodehydroascorbate reductases. These enzymes are involved in the removal of ROS either directly (superoxide dismutases, catalases and ascorbate- or thioldependent peroxidases) or indirectly through the regeneration of the two major redox molecules in the cell, ascorbate and glutathione (glutathione reductases, dehydroascorbate reductases and monodehydroascorbate reductases). An interesting feature of the interplay between oxidants and antioxidants is that it occurs in all subcellular compartments including plastids and mitochondria.

Under salt stress conditions *P. indica* increases the tolerance of salt-sensitive barley (*Hordeum vulgare*) cultivar to severe salt stress. *P. indica*-colonized plants contained higher ascorbate concentrations in roots compared with non-colonized plants, while the ratio of ascorbate dehydroascorbate was not significantly altered and catalase, ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase and monodehydroascorbate reductase activities were increased. These modifications are consistent with the decrease of leaf lipid peroxidation [28].

5. TAXONOMIC POSITION OF *P. INDICA*

P. indica a plant root-interacting fungus can be easily grown on various complex and minimal substrates, where it asexually forms chlamydospores containing 8 to 25 nuclei. Stages of a sexual life cycle have not been observed. Analysis for taxonomic position by molecular methods based on 18S rRNA sequences and by electron microscopy suggests that this fungus is related to the Hymenomycetes of the Basidiomycota [9]. The genetically

tractable endophytic fungus *P. indica* is able to colonize the root cortex of a great variety of different plant species with beneficial effects to its hosts and it represents a suitable model system to study symbiotic interactions. Recent cytological studies in barley and *Arabidopsis* showed that upon penetration of the root, *P. indica* establishes a biotrophic interaction during which fungal cells are encased by the host plasma membrane. Large-scale transcriptional analyses of fungal and plant responses have shown that perturbation of plant hormone homeostasis and secretion of fungal lectins and other small proteins (effectors) may be involved in the evasion and suppression of host defenses at these early colonization steps. At later stages *P. indica* is found more often in moribund host cells where it secretes a large variety of hydrolytic enzymes that degrade proteins [5]. This review describes current advances in understanding the components of *P. indica* endophytic lifestyle from molecular and genomic analyses. Electron microscopy and genomic studies employing the analysis of a part of 18s and 28s rRNA placed it in Hymenomycetes (Basidiomycota). The mutualistic *A. thaliana* of *P. indica* association is a new model system for the elucidation of the molecular mechanisms responsible for host recognition, root colonization and subsequent beneficial activities accompanied by microbial plant symbiosis. Based on the 18S rDNA analysis and the ultra-structure of the septal pore, its phylogenetic relationship is within the Hymenomycetes (Basidiomycota). The introduction of proteomic approaches combined with ethyl-methane sulfonate (EMS) mutagenesis has led to the identification of several *P. indica* responsive *Arabidopsis* proteins like a (meprin and tumor necrosis factor receptor-associated factor (TRAF) homology) domain containing protein, a leucine-rich repeat protein LRR2 [11,22]. and PYK10, a -glucosidase located in the endoplasmic reticulum [10]. These proteins are expressed during early interaction stages and are crucial for growth promotion response. The current thesis seeks to identify factors that are released by the fungus, signaling pathways they activate and the role they play in the interaction [30].

6. MASS SCALE PRODUCTION OF *P. INDICA*

Producing large quantities of pure inoculums of the mycorrhizal fungi, free from pathogens, with high infectivity potential, easy to be transported across the country, and assessing their use in field and green-house conditions are substantial in view of the wide range of benefits which they accrue. Presently, these fungi can be grown with host plants in pot culture containing soil, sand or expanded clay. They have also been grown by using hydroponics, aeroponics and root organ culture, all of which are not a cost-effective proposition. *P. indica* can be propagated on several economically viable synthetic media, potato dextrose media. Fermentation technique should be optimized to devise a simple, cheap and commercially viable technique for the mass scale inoculums production for application in agro forestry and horticulture. Especially for the better establishment of tissue culture-raised plants, fungal inoculums will be much needed in the plant industry [30].

7. CONCLUSIONS

Perimospora indica is a wide-host root-colonizing endophytic fungus which allows the plants to grow under extreme physical and nutrient stress. The fungus can be cultivated on

complex and minimal substrates. It belongs to the Sebaciniales in Basidiomycota. The fungus is interesting for basic research as well as biotechnological applications because: it functions as a plant promoter and biofertilizer in nutrient-deficient soils, as a bio protector against biotic and abiotic stresses including root and leaf fungus pathogens and insect invaders, it also used as bioregulator for plant growth development, early flowering, enhanced seed Production and stimulation of active ingredients in medicinal plants as well as a bio-agent for the hardening of tissue culture raised plants.

Positive interaction is established for many plants of economic importance in arboriculture, agro-forestry, flori-horticulture including Orchids, and those utilized for energy production and paper industry. Similar to Arbuscular mycorrhizal fungi, *P. indica* stimulates nutrient uptake in the roots and solubilizes insoluble phosphates and sulphur components in the soil. The interaction of *P. indica* with the model plants *Arabidopsis thaliana* and barley is used to understand the molecular basis of this beneficial plant/microbe interaction. An attempt is made to compare it with pathogenic and mycorrhizal plant/microbe interactions and also propose possible biotechnological applications.

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