Journal of Agricultural Technology 2015 Vol. 11(2): 517-523
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

The *Piriformospora indica*, magic fungus and its role on sustainable agriculture

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Danesh, Younes Rezaee (2015). The Piriformospora Indica, magic fungus and its role on sustainable agriculture. Journal of Agricultural Technology Vol. 11(2): 517-523

The conversion of plant production systems from conventional resource exhausting to sustainable systems depends on management of environmental factors. Root-inhabiting fungi are considered as a main factor since their hyphae connect in ideal manner and challenge of the surrounding with the plant. One of these most important root endophytic fungi is *Piriformospora indica*. This fungus has a broad host spectrum and positively affects different aspects of plant performance. *P. indica* has multifunctional activities like plant growth promoter, bio-fertilizer, immune-modulator, bio-herbicide, phytoremediator, etc. Effect of *P. indica* has been studies on more than 150 plants. Promising outputs of laboratory experiments and small field trials indicated the need for its mass cultivation and usage. *P. indica* has proved to be highly beneficial endophytic fungus with high efficacy in filed.

Key words: *Piriformospora indica*, Sebacinales, Root Endophytic Fungi, Plant growth promotion.

Introduction

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 underground root system is under the direct influence of a diverse group of micro-organisms. The mycorrhizal fungi and PGPRs (plant growth promoting rhizobacteria) being mutualistic symbionts, control, in many ways, the plant health. More than 90% of the terrestrial plants (angiosperms, gymnosperms, pteridophytes, bryophytes and some algae) are colonized by mycorrhizal fungi (Singh *et al.,* 2000). The term mycorrhiza refers to the association between fungi and roots of higher plants. This association is usually considered a mutualistic symbiosis because of the highly beneficial

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relationships established between both partners, in which the host plants receive mineral nutrients via the fungal mycelium (mycotrophism), while the heterotrophic fungi obtain carbon compounds from the host plants (Varma *et al.*, 1999). In addition, it has become evident that all plants also harbor nonmycorrhizal root-endophytic fungi, and their colonization often impacts plant growth and development (bio-regulation), plant nutrition (bio-fertilization) and plant tolerance and resistance to abiotic and biotic stresses (bio-protection). Therefore, root-endophytic fungi have to be taken into account in order to understand the interaction of the root with its environment, and moreover, they could be used as biological agents to improve plant production systems (Franken, 2012). Despite the numerous important role and ecological function of AM fungus, mass pure inoculum production and axenic cultivation of this group of symbiotic fungi have not grown even till date. These fungi cannot grow like any other fungi apart from their host (obligate photosymbionts). Because of the absence of an authentic pure culture, the commercial production is the greatest bottleneck in use and application of mycorrhizal biotechnology at large.

Piriformospora indica- Model Symbiotic Fungus

Scientists have discovered an endophyte named *Piriformospora indica*, a member of the Sebacinales. *P. indica* has received worldwide attention as it promotes the growth of several plant species as well as it can be axenically cultivable easily on synthetic media in contrast to obligate biotrophic AM fungi. Originally, this fungus was isolated during the screening for AM fungi in the the soil samples collected from the rhizosphere of woody shrubs *Prosopsis juliflora* and *Zizyphus nummularia* growing in the Thar Desert of Rajasthan, India (Verma *et al*., 1998; Singh *et al*., 2000). The fungus has been named as *Piriformospora indica* based on its characteristic pear shaped chlamydospores (Figure 1). *P. indica* is related to the Hymenomycetes of the Basidiomycota and belongs to order Sebacinales. The fungus is able to associate with the roots of various plant species in a manner similar to arbuscular mycorrhizal fungi and promotes plant growth (Singh *et al*., 2003). Hence, it provides a promising model organism for the investigations of beneficial plant–microbe interactions. The properties of *P. indica* have been patented (European Patent Office, Muenchen, Germany, Patent number 97121440.8-2104, Nov. 1998). The culture has been deposited at Braunsweich, Germany (DMS number 11827) and 18S rDNA fragment deposited with GenBank, Bethesda, USA (AF 014929).

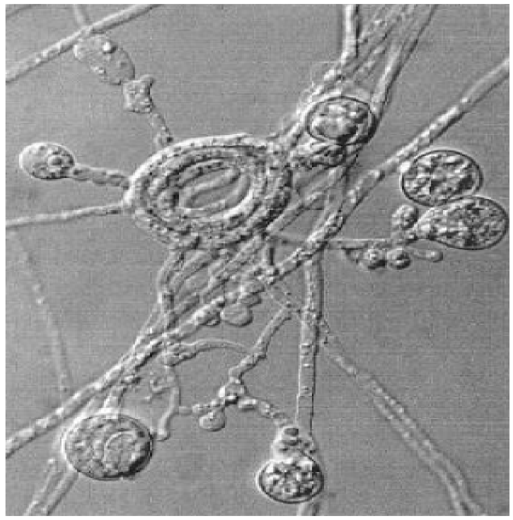

Fig. 1 *Piriformospora indica* hyphae and chlamydospores

Applications and Diverse Functions of *P. indica*

The endophyte *P. indica* has an encouraging influence on growth and development on host plants. This fungus mimics the capabilities of typical AM fungus. It tremendously improves the growth and overall biomass production of a wide host spectrum, like herbaceous mono- and dicotyledons, and trees, including medicinal plants and several economically important crops (Shrivastava and Varma, 2014). *P. indica* enhanced nutrient uptake, helped plants to survive in extreme drought, temperature and salt conditions, exhibited systemic resistance to toxins, acted as bio-fertilizer, bio-protector, stimulator of growth, increased seed production, and played a key role in increasing the tolerance to insects (Bagde *et al.,* 2014). It helps in biological hardening to tissue culture raised plants, provides protection against shock of transplantation' and pathogens of roots (Prasad *et al.,* 2008a, 2008b). It is observed that among the compounds released in root exudates infected with *P. indica,* flavonoids are found to be present. Flavonoids have been suggested to be involved in stimulation of pre-contact hyphal growth and branching, which is consistent with their role as signaling molecules in other plant-microbe interactions (Bagde *et al.,* 2010). Cell wall degrading enzymes like cellulase, polygalactouronase and xylanase were found in significant quantities both in the culture filtrate and in the roots exudates colonized by *P. indica* fungus. Also *P. indica* showed profound effect on disease control when challenged with a virulent root and seed pathogen of *Gaeumannomyces graminis* by completely inhibiting the growth of this pathogen. It indicates that *P. indica* acted as a potential agent for biological control of root diseases, however, chemical nature of the inhibitory factor is still unknown (Varma *et al.*, 2001). Like AM fungi, *P. indica* functions as bio-regulator, bio-fertilizer and bio-protector as well as delays wilting and withering of the leaves. In addition, it also prolongs life-span of callus tissues. Several studies have demonstrated that *P. indica* may be used for phytoremediation, because it accumulates heavy metals and prevents their uptake into the plants (Oelmüller *et al*., 2009).

Mechanisms behind the Functions of P. indica

The fungal interactions are mainly due to increasing in efficiency of nutrient uptake from soil based on better hyphal penetration as compared to thicker root hairs. Plants deliver phosphorous assimilates to fungus and during mycorrhizal associations plants acquire phosphates from extensive network of extra radical hyphae. Interaction of *P. indica* with plant alters pathway for nitrogen metabolism, thereby helping plants to absorb more nitrogen. This phenomenon gives higher resistance to water deficiency and makes plants drought tolerant. Enhanced growth of plants under mycorrhizal condition amplifies its starch requirement. This starch is obtained from deposition in root amyloplasts. Thus, it is interpreted that one of the major starch degrading enzymes, the Glucan-water dikinase is activated by *P. indica* (Shrivastava and Varma, 2014). Uptake and transportation of important macronutrients like iron, zinc, copper, etc. are also regulated by the fungus. Along with this, beneficial phytohormones are synthesized by plants associated with fungus. The cumulative effect of macro-micro-nutrients and phytohormones regulates plant metabolism leading to value addition, early flowering, plant growth promotion, etc. Massive proliferation of useful rhizospheric microorganisms sustains soil fertility (Shrivastava and Varma, 2014).

Commercial Application

In contrast to the obligate biotrophic arbuscular mycorrhizal fungi, *P. indica* can also be propagated in axenic cultures as a saprophyte and grows on numerous different natural and artificial cultivation media (Verma *et al.*, 1998). However, the choice of substrate for inoculum production influences the impact on the plant (Andrade-Linares *et al.*, 2012). The choice of N source, for example, is critical because propagation on a substrate containing only ammonium results in a strong negative effect on the plant after inoculation (Kaldorf *et al.*, 2005). Also, to produce inocula which can be commercially applied, it is necessary to obtain a larger quantity of spores. This can be achieved by optimization of substrate composition and environmental conditions (Kumar *et al.*, 2011) and by the application of certain nanomaterials (Suman *et al.*, 2010). Additionally, for distribution, the inoculum must be combined with a carrier, and two such carriers have already been tested (Sarma *et al.*, 2011). Other important parameters include the amount of inoculum being applied, the time point of inoculation (Fakhro *et al.*, 2010) and the choice of soil or substrate for plant cultivation (Fakhro *et al.*, 2010). Further, to place *P. indica* on the market, it must be registered as an inoculum. Regulations for registration vary between countries, but one bottleneck concerning safety might be that sometimes negative effects on plant growth can be observed. This is probably based on the mode of colonization, which includes a dependency on programmed cell death (Deshmukh *et al.*, 2006). Another concern is that the fungus was isolated in India, and at present, only this one isolate of *P. indica* exists. Because *S. vermifera* is distributed worldwide and shows similar characteristics to *P. indica*, it will be useful to obtain more *S. vermifera* isolates from different regions and to analyze their impact on the plant and for their mode of colonization. The fact that the hyphae of *P. indica* and related Sebacinales contain bacteria, which promote plant growth and disease resistance and which can at least partially be cultivated (Sharma *et al.*, 2008), opens up the possibility of using such bacteria as inoculum. Another alternative would be the application of culture filtrate, since such filtrate can also promote plant growth and development (Bagde *et al.*, 2011; Kumar *et al.*, 2012) and influences the synthesis of particular valuable compounds (Bagde *et al.*, 2011; Kumar *et al.*, 2012).

Future Prospects of P. indica

The interaction of *P. indica* with plant roots has been intensively studied, and genome sequence and transformation systems are available. However, in order to use the root endophyte in agricultural practice, a product for commercial use must be established and registered. The high potential of multifaceted fungus, *P. indica* has tremendous applications in future as bio-fertilizer, 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. Also the fungus has been reported to possess good quantity of antioxidants. Mechanism by which *P. indica* promotes the growth of plants is unclear but some studies have implicated various factors induced by it in plants that were responsible for its positive effects. For this purpose, future research concerning applications should concentrate on the following points:

– Evaluation of alternatives to *P. indica*, including related fungal isolates, endophytic bacteria and culture filtrate

– Inoculum production conditions

– Inoculum formulation and stability

– Persistence of the fungus in the environment

After which, it will be possible to specify scopes for application and to define the conditions which support the beneficial effects. Finally, it will be necessary to calculate ecological and economic costs and benefits to guide *P. indica* and related products to successful agricultural application.

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