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Vol. 8(32), pp. 2984-2992, 6 August, 2014
DOI: 10.5897/AJMR2014.6928
Article Number: 80F7AAC46526
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Review

From *Piriformospora indica* to Rootonic: A review

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Received 29 May, 2014; Accepted 14 July, 2014

Piriformospora indica **(Hymenomycetes, Basidiomycota) is a cultivable endophyte that colonizes roots and has been extensively studied.** ***P. indica*** **has multifunctional activities like plant growth promoter, biofertilizer, immune-modulator, bioherbicide, phytoremediator, etc. Growth promotional characteristics of** ***P. indica*** **have been studied in enormous number of plants and majority of them have shown highly significant outcomes. Certain secondary metabolites from the fungus are reasons behind such promising outputs. Effect of** ***P. indica*** **has been studied on more than 150 plants. Promising outputs of laboraatory experiments and small field trials indicated the need for its mass cultivation and usage. For field trials, a formulation "Rootonic" was prepared by mixing** ***P. indica*** **biomass in magnesium sulphite. The quantity of formulation (Rootonic) to be used per acre of land for maximum productivity has also been standardized for about 50 plants.** ***P. indica*** **has proved to be highly beneficial endophyte with high efficacy in field. This article is a review on our journey from** ***P. indica*** **to "Rootonic".**

Key words: *Piriformospora indica*, Rootonic, Mycorrhizae, endophyte, biofertilizer.

INTRODUCTION

Piriformospora indica was discovered by Prof Dr. Ajit Varma and his colleagues in Thar Desert of Western India in 1992 from the root system of several xerophytic plants (Varma et al., 1999; Verma et al., 1998). It belongs to *Hymenomycetes, Basidiomycota*. A new family Sebacinaceae and new order Sebacinales Glomeromycota was created for this fungus due to its unique features (Weiß et al., 2004; Qiang et al., 2011). This is a very unique symbiotic fungus which not only promotes plant growth but also has other multifunctional activities such as plant growth promoter, bio-protectant, bio-pesticide, helps in enhanced flowering and fruiting etc. Its properties have been patented in Germany (European Patent Office, Muenchen, Germany, Patent No. 97121440.8-2105, Nov. 1998) dating back to 1997. *P. indica* is deposited at the Deutsche Sammlung fu¨r Mikroorganismen und Zellkulturen, Braunschweig, Germany (DSM 11827). It promotes plant growth, increases the resistance of colonized plants against fungal pathogens and increases their stress tolerance (Harman 2011; Varma et al., 2012a).

Characterization of *P. indica* has shown its multifunctional activities as plant growth promoter, biofertilizer, immune-modulator, etc (Figure 1). It significantly improves plant growth and overall biomass and can be easily cultivated on a variety of synthetic media (Varma et al., 2012a; Oelmüller et al., 2009). Till date about 150 plants have been reported with the fungus to show its effect. Plants

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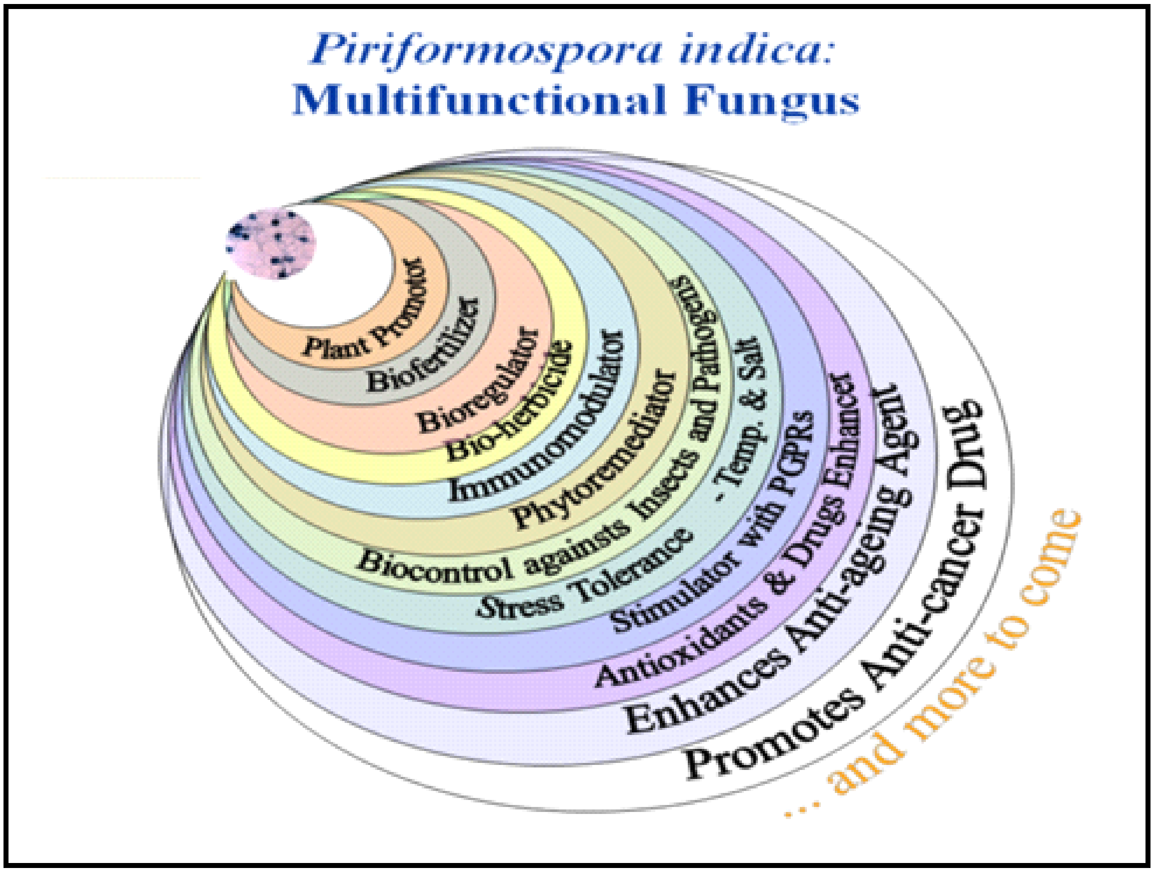

Figure 1. Functional characteristics of fungus (c.f. Varma et al., 2013).

belonging to Bryophytes, Pteridophytes, Gymnosperm and Angiosperm (both mono-dicots), include orchids (Singh and Varma, 2000). *Aneura pinguis L., Cicer arietinum, Adhatoda vasica* L., *Aristolochia elegans, Daucus carota* L., *Arachis hypogaea (*groundnut or peanut*), Petroselinum crispum* L., *Medicago sativa, Centella asiatica, Glycyrrhiza glabra, Cuminum cyminum, Abrus precatorius* L.*, Foeniculum vulgare, Mimosa pudica, Carum capticum, Vigna unguiculata, Coriandrum sativum, Glycyrrhiza glabra, Artemisia annua* L., *Acacia catechu, Spilanthes calva, Stevia rebaudiana, Prosopis chilensis Stuntz sys., Calendula officinalis, Prosopis juliflora, Arnica* spp. are few of the plants showing enhanced flowering, enhanced plant size, increased production of secondary metabolites etc. upon interaction with *P. indica* (Varma et al., 2012a, b, c).

Colonization by *P. indica* increases nutrient uptake, allows plants to survive under water, temperature and salt-stresses, confers (systemic) resistance to toxins, heavy metal ions and pathogenic organisms and stimulates growth and seed production. The valuable secondary metabolites excreted by *P. indica* influence early seed germination, better plant productivity, early flowering, etc. Use of *P. indica* to increase desiccation tolerance in higher plants has been studied by Varma and his colleagues (2012c) and significant increase tolerance was achieved.

Genome wide study revealed that its genome is assembled into 1,884 scaffolds containing 2,359 contigs with an average read coverage of 22 and a genome size of 24.97 Mb. The estimated DNA content of *P. indica* nuclei ranges from 15.3 to 21.3 Mb. To assess the genome completeness of *P. indica* a blast search was performed with highly conserved core genes present in higher eukaryotes (Zuccaro et al., 2009). *P. indica* can be stably transformed by random genomic integration of foreign DNA and that it possesses a relatively small genome as compared to other members of the Basidiomycota (Zuccaro et al., 2011).

P. indica is a model organism used in mycorrhizal research, and its research outputs has been published in highly recognized journals like Nature, PNAS, Plos Pathogen, JBC, Plant Physiology, Molecular Plant Pathology, etc. Extensive research on this organism has brought it to an appreciable state and made its field trials and marketing possible.

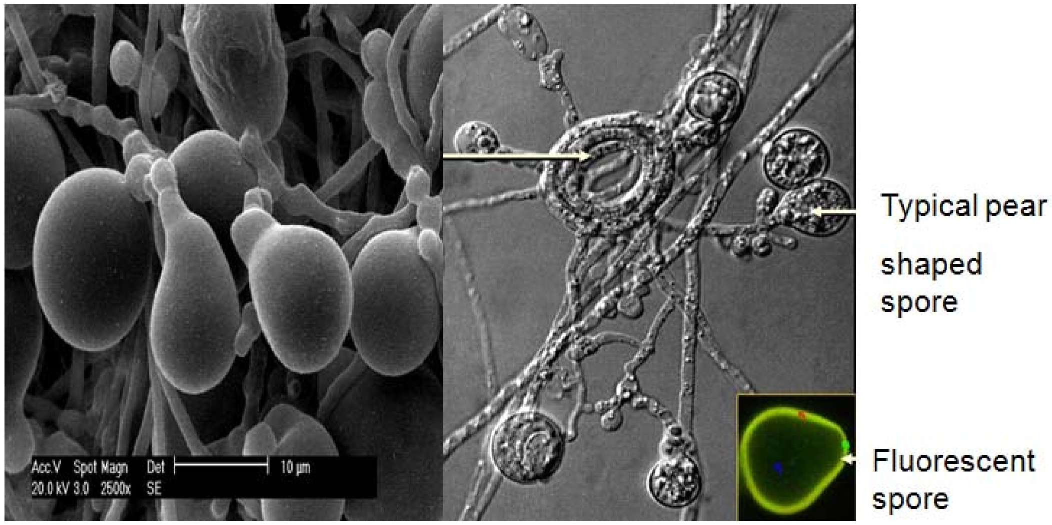

Figure 2. Morphological features of *P. indica* indicating hyphae and pear shaped spores. Scanning electron microscopy view spore (left) (c.f. Varma et al., 2013).

Objective of this review is to give a general view of journey of *P. indica* from laboratory to field and finally towards industrialization.

CULTIVATION AND MORPHOLOGICAL CHARACTERISTICS

Simple morphological features of *P. indica* contain hyphae and pear shaped large spores (Figure 2). It can be cultivated on basic defined medium at an optimum temperature of 25°C ± 2, pH 6.8. Maximum biomass production was obtained after 7 days incubation at 120 rpm (Varma et al., 2013).

FUNCTIONAL CHARACTERISTICS OF *P. INDICA*

Plant growth promotion

P. indica promotes growth of plants of forestry, horticulture and agriculture importance. Numerous plants have been tested for the effect of *P. indica* on their growth and interestingly majority of them have shown beneficial effect. Few results of its effect on sugarcane, Pinus and potato are shown in Figure 3. It is important to note that in addition to enhancement of plant growth, the fungus also helps in enhancement of active ingredients in plants. In the case of Ratoon crop of sugarcane it was seen that plants not associated with *P. indica* turned yellow due to iron deficiency, whereas plants subjected to *P. indica* treatment remained green, indicating that the fungus also helps in iron transport. Almost 39% enhancement in iron content and 16% increase in sugar content were recorded in *P. indica* treated plants (Table 1). Noticeable increase in plant size and tuber size was observed in the case of Pinus and potato, respectively.

Value addition in spices and plants of pharmaceutical importance

Effect of *P. indica* has been studied on large number of spices and plants of medicinal importance. To name few are *Curcuma longa, Spilanthus calva*, *Artemisia annua, Tridax procumbens, Abrus precatoriu, Bacopa monnieri, Coleus forskohlii, Adhatoda vasica, Withania somnifera, Chlorophytum tuberosum*, *Foeniculum vulgare*, *Linum album*, *Podophyllum* sp., etc (Das et al., 2012). The organism has shown significant increase in concentration of active ingredients like curcumin, artemisnin, podophyllotoxin and bacoside leading to value addition to the plant.

Interaction of *P. indica* with *C. longa* resulted in approximately 21, 19 and 13% increase in essential oil, Curcumin and rhizome yield. Field trials showed that increase in rhizome yield after treatment with *P. indica* would benefit a farmer with Rs. 16,000/ (US $ 280.00) per hectare of land. It is also probable that healthy and shiny rhizomes would fetch better price. Increase in plant size, secondary metabolite release and increase ability to fight against infections was observed upon interaction of *P. indica* with *A. annua*, *B. monnieri*, etc (Figure 4).

The tissue culture results obtained were evaluated for field trial and similar promising outputs were obtained. Field trial of *A. annua* was done in Central India. Improved plant growth and 1.6 fold increased concentration of active ingredient artemisinin was observed. In the case of *B. monnieri* 3.5 fold increase in bacoside concentration was observed.

Interaction of *Ephedra ciliata* (used for treatment of hay fever, asthma etc.) with *P. indica* also led to enhanced growth of treated plants (Varma et al., 2013).

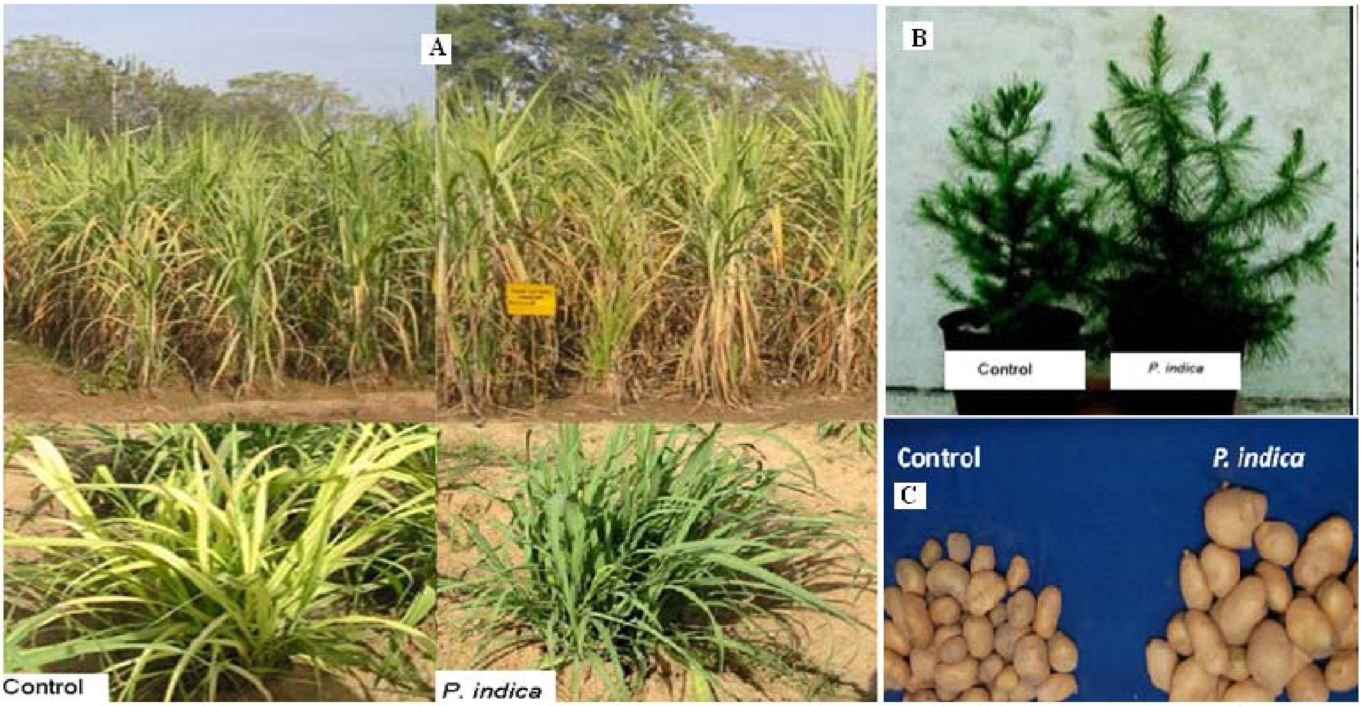

Figure 3. (A) Effect of *P. indica* on sugarcane (*Saccharum officinarum*) cultivated in field at Punjab. (B) Growth promotion of Cyprus plants (pinus) upon interaction with *P. indica* grown on rocky sand. (C) Increases size and improves texture of potato plants upon interaction with *P. indica*, cultivated in field at Punjab. (c.f. Varma et al., 2013).

Table 1. Enhanced iron and sugar content of the sugarcane upon interaction with *P. indica*. (c.f. Varma et al., 2013).

Parameter	Iron (ppm)	Sugar (°Bx*)
Control	202.2	18.35
P. indica	281.4	21.4

* °Bx (degree Brix) is the sugar content of an aqueous solution. One degree Brix is 1 g of sucrose in 100 g of solution.

P. indica as bioprotectant, rejuvenate fruiting, promoting early flowering

Experimental data suggests that *P. indica* suppresses the growth of a large number of pathogens like *Geaumannomyces graminis, Alternaria* sp., *Colletotrichum falcatum, Fusarium oxysporum, Fusarium udum, Rhizoctonia bataticola, R. solani, Sclerotinum rolfsii, Verticillium* sp. and many more (Dolatabadi et al., 2011; Ghahfarokhy et al., 2011). Field trials on *Lagenaria siceraria* and *Tagetes* sp. showed that interaction with *P. indica* suppressed the infestation by plant pathogens including viruses (Figure 5). *P. indica* protecting a *Tagetes* sp. from mite infection has also been recorded.

Rigorous fruiting was observed in kinnow (*Citrus reticulata*) plant after interaction with *P. indica* with a limitation that this fruiting was time dependant and seen only at the early stage. *P. indica* also possess unique characteristics of inducing early flowering. This property is seen in case of plants viz., tobacco, *Coleus*, *Brassica,* etc.. Orchids are well known ornamental plants and their cultivation is expensive. Another limiting factor to its growth is that among millions of tiny seeds, unfortunately 99% do not germinate and transform into fully grown and mature plants unless they establish contact with mycorrhiza. Its interaction with *P. indica* has shown increased seed germination. *P. indica* has also shown early seed generation and enhanced plant growth in highly valuable plants like *Jatropha curcas* and *Populus deltoides* (Kaldorf et al., 2005).

Unique features of *P. indica*

P. indica shows striking unique features upon interaction with various plants. Field trials to investigate activity of the fungus have been conducted in North, Central and in extreme cold deserts (Ladakh-Leh) of India. *P. indica* mixed with frozen Leh soil (temperature -30°C) showed significant results where all the fifteen seeds of *Cichorium endivia* germinated within 12-25 days (Singh and Varma,

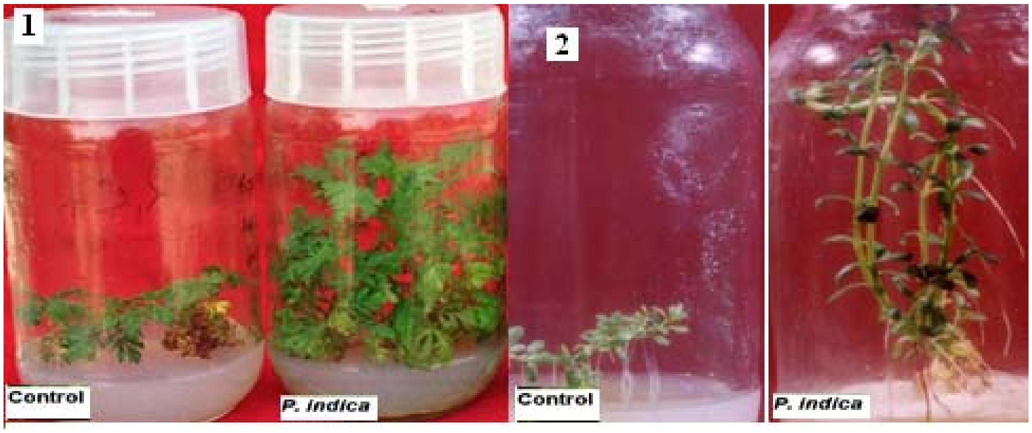

Figure 4. Co-cultivation of *Artemisia annua* and *Bacopa monnieri* in plant tissue culture with *P. indica* showing increased plant size (1 and 2 respectively) (c.f. Varma et al., 2013).

Figure 5. (1) Field trial on interaction of *P. indica* with *Lagenaria siceraria* showing how it suppresses the infestation by plant pathogens including viruses (upper); a magnified view of the same (lower). (2) Interaction of *P. indica* with plant of *Tagetes* sp. Note: Control plant with folded leaf due to mite infestation; no such symptoms were observed on treated plants (c.f. Varma et al., 2013).

2000). The germinated plantlets when transferred to macro-plots attained full growth and imparted better productivity than the control. In contrast, not a single seed broke dormancy in absence of the fungus was noticed.

Liquid fertilizer from *P. indica*

Culture filtrate (liquid fertilizer) of *P. indica* also acts as an excellent plant growth promoter. In order to prepare culture filtrate, *P. indica* was grown in broth and for 10 days followed by removal of biomass. Culture filtrate helped in early seed generation and early flowering as well (Figure 6). It was then interpreted that the fungus secretes some secondary metabolites that works as fertilizers (Bagde et al., 2010a, b, 2011).

Mechanisms behind the unique action of *P. indica*

The fungal interactions are characterized by increase in

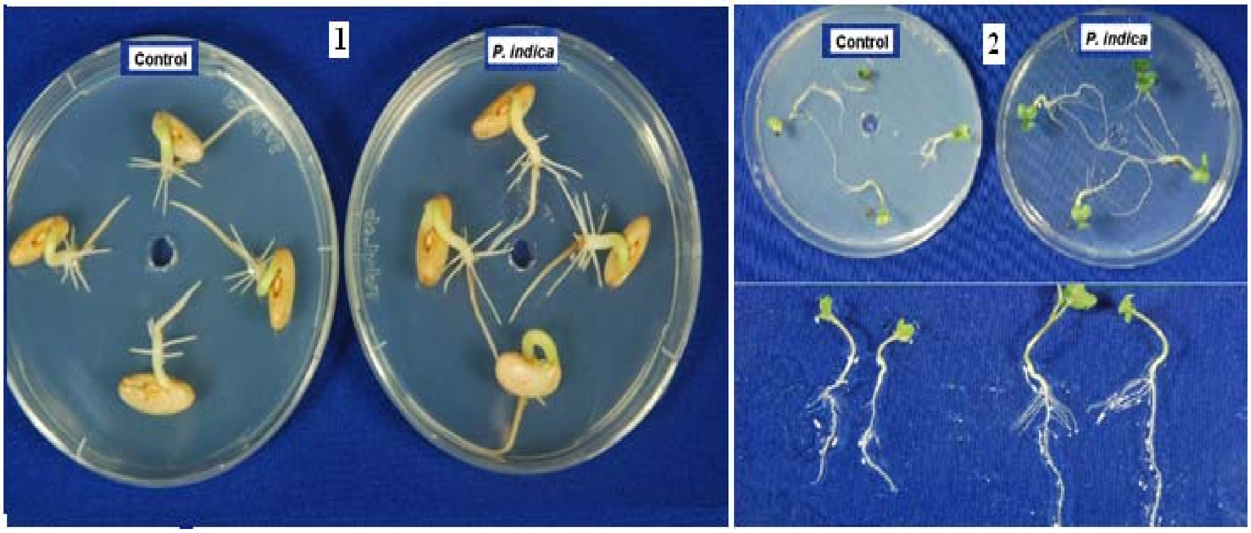

Figure 6. (1) Interaction of *Phaseolus vulgaris* (rajma) with *P. indica*. (2) Co-cultivation of *Brassica oleracea* (Broccoli) with *P. indica*. (c.f. Varma et al., 2013).

efficiency of nutrient uptake from soil due to better hyphal penetration as compared to thicker root hairs. Plants deliver phosphor 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 phenol-menon 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* (Iris et al., 2010).

Uptake and transportation of important macronutrients like iron, zinc, manganese, copper, etc. are also regulated by the fungus. Along with this, beneficial phytohormones are synthesized by plants associated with *P. indica.* The cumulative effect of macro-micro-nutrients and phyto-hormones regulates plant metabolism leading to value addition, early flowering, plant growth promotion, etc. Massive proliferation of useful rhizospheric micro-organisms sustains soil fertility (Varma et al., 2013).

STEP TOWARDS COMMERCIALIZATION

Laboratory scale data and field trials have evidently concluded that *P. indica* can be extensively used to increase plant growth quantitatively and qualitatively (Sahay and Varma, 1999, 2000; Rai et al., 2001). Main motive behind such huge previous experimental subject was further applying it in normal fields so that it is available to all. For making this target come true, a step towards commercialization was taken. Fungal biomass is mass cultivated in fermentor (Bagde et al., 2010b). These fungal biomass formulated with magnesium sulphite (carrier) was prepared (Figure 7). Most effective formu-lation was standardized to 2% (w/w). Moisture content and colony forming unit was maintained at 20% and 10^9, respectively. This formulation is named "Rootonic". Seed treatment was done by mixing Rootonic to seed and incubating it under shade overnight. Protocol for seed treatment has been given as Figure 8.

Quantity of formulation required for seed treatment has been standardized for large number of plants. Details are included in Table 2.

Preliminary studies with nanoparticles

Nanotechnology has significant benefits on food and agri-culture system. Preliminary work on interaction of nano-particle embedded *P. indica* biomass with Broccoli has shown better growth promotional property as compared to the control (*P. indica* without nano material); as tested in our laboratory (Suman et al., 2010).

CONCLUSION

P. indica is a rewarding organism with its huge and distinguished properties. Colonization by *P. indica* increases nutrient uptake, allows plants to survive in drought, salt-stress and temperature stress. Excellent plant growth promotion, growth at extremes of climate and bio-protecting capability of the organisms has paved way for its varied field applications. Large field trials at

Figure 7. Steps for the preparation of the formulation (c.f. Varma et al., 2013).

various locations in India showed beneficial effects of *P. indica* on plant growth and development. Promising outputs of field trials showed that it should be used at large scale so that common farmers are benefited and finally countries economy is at profit. Increase in productivity of certain crop upon interaction with *P. indica*

Figure 8. Protocol for seed treatment (c.f. Varma et al., 2013).

will increase total land usage. Enhanced field usage of the microorganisms requires its mass production. Field trials of the same are done by formulating biomass with powder and inoculating the mixture into root of plants. The formulation is termed “Rootonic”. The journey from *P. indica* to Rootonic is exciting and very fulfilling. Large scale production and application of the product is still under process and we are looking forward to its commercialization soon.

ACKNOWLEDGEMENTS

The authors are thankful to DBT, ICAR and DRDO, India

Table 2. Quantity of formulation required for the seed treatment. (c.f. Varma et al., 2013).

Crops	Seed treatment (g/acre)
Tomato, chillies, brinjal, capsicum, cabbage, cauliflower	25.00
Muskmelon, watermelon, long melon, cucumber, bottle gourd, bitter gourd, sponge gourd, round melon	50.00
Sunflower, ladies finger, onion, spinach, fenugreek, mustard, cotton	100.00
Maize, paddy and millets (barley, pearlmillet, sorghum)	300.00
Pulses (gram, pea, lentil, mungbean, Uradbean, pigeonpea, cowpea, soyabean)	500.00
Potato, wheat, sugarcane	1,000.00

for partial financial funding. The authors are also thankful to various research organizations involved in studying *P. indica*.

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