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Novel Cultivable Mycobiont *Piriformospora Indica* as Plant Growth Promoting Endophyte

Krishnaveni. N[1], Geetha Ramani[2], Ranjitha. L[1] and Cibichakravarthy. B[3]
[1]Department of Microbiology, PSG College of Arts and Science, Coimbatore, Tamil Nadu, INDIA
[2]Department of Microbiology, Dr. NGP College of Arts and Science, Coimbatore, Tamil Nadu, INDIA
[3]Department of Biotechnology, Bharathiar University, Coimbatore, Tamil Nadu, INDIA

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Abstract

The novel endophytic fungi Piriformospora indica was found to have vast applications as a plant growth promoter. The fungus has been observed comprehensively in the present study for its indigenous applications in some of the crop plants cultivated in this locale. The morphological and cultural characteristics of the endophyte were verified. Further to enhance the applications of fungi in plant growth promotion, the–vitro growth parameters of fungi were optimized. The endophyte has been experimented for germination studies with maize plants. Pot culture of the maize plants that were colonized with Piriformospora indica has been observed to show considerable difference in growth parameters when compared with the control plants. The plants were also compared for physical parameters such as percentage colonization, root height, shoot height, fresh weight, dry weight etc., with the control plants. The present study has added light to the understanding that Piriformospora indica is an effective plant growth promoting endophytic fungi.

Keywords: *Piriformospora indica*, endophytic fungi, maize growth, plant growth promoter.

Introduction

Arbuscular mycorrhizhal fungi are obligate endosymbionts that inhabit several plant roots yet cannot be cultured at in-vitro conditions in the laboratory media. Production of some of the earlier known cultivable mycorrhizae also has been time consuming and expensive. This difficulty has been solved by a novel endophytic fungi *Piriformospora indica*, an arbucular mycorrhizae (AM)-like fungus, being culturable in axenic cultures, where it asexually forms chlamydospores[1]. The fungus *Piriformospora indica* was discovered in the Thar Desert from the rhizospheric soil of two xerophytic plants, namely *Prosopis juliflora* and *Ziziphus nummularia*[2]. *Piriformospora indica* has the capacity to colonize a wide variety of plants. Some important plants that were studied earlier and had formed definite hosts are *Zea mays (maize), Nicotiana taba-cum, Oryza sativa, Pisum sativum*, *Hordeum vulgare* L. etc.[3,4]. Being a potential plant growth booster, the fungus has engrossed researchers across the world to exploit its fullest potential in the field of sustainable agriculture. A review of literature advocates the fact that a significant contribution has been made on various aspects of *P.indica* with particular reference to its plant growth promoting capability and its ability to induce resistance to abiotic and biotic stresses[1-3].

Piriformospora indica as a mycofertilizer has produced a greater passion among mycorrhizologists, due to being easily culturable, plant-growth enhancement and induction. In the present work, the recently discovered root endophytic-fungi *Piriformospora indica* has been optimized and tested for its growth enhancement in test plants.

Methodology

Culture of *P.indica:* *P.indica* (DMS 11827)[1], was kindly provided by Professor Varma and his co-scientists, from the School of Microbial Technology, Amity University, Noida. The culture was maintained on Hill and Kaefer medium[5]. The cultures were incubated at 30±2°C in incubator for a week.

To optimize the various growth parameters of the fungus in laboratory conditions, several physical parameters such as temperature, pH, incubation time required for optimum growth, oxygen requirement and growth in various media were optimized.

Germination and Pot culture studies: Seeds of maize were surface-sterilized with sterile distilled water and rinsed in 0.1% $HgCl_2$ for 1 min and washed thrice with sterile distilled water[1] to eliminate naturally occurring microorganisms. Seeds were germinated on MS medium[6]. After germination the plantlets of maize were placed in pots containing sand and soil in 3:1 ratio. Initially, the plants were inoculated with *P.indica* by mixing the culture directly in sterile soil and control plants being grown without fungus. A little sterile tap water was gently sprinkled to moisten the upper soil layer.

Microscopic Observation of Root Colonization by fungus:

After a considerable plant growth in pots, the roots of randomly chosen plants were subject to microscopic observation to evidence colonization of the endophyte in the roots. The roots were washed thoroughly in running tap water, cut into about 1-cm pieces, transferred to 10% KOH, boiled for 10 min, then about 1 M HCl was added and kept for 10 min, and at last stained with 0.02% of Trypan blue in lactophenol overnight[7]. Samples were destained with lactic acid: glycerol: water solution in the ratio of 14:1:1(Volumetric ratio). The stained root samples were observed under microscope. Scanning Electron Microscopic examination of root was done to observe the fungal hyphae in the roots of maize plants[8].

Estimation of physical parameters for plant growth: For assessment of plant growth promotion of endosymbiotic maize plants; shoot and root length, leaf length, fresh weight and dry weight of the plants were measured. The parameters were compared with their respective values in control plants.

Results and Discussion

The *Piriformospora indica* culture obtained from Dr. Varma's laboratory was maintained on Hill and Kaefer medium. The cultures were revived and optimized to grow in laboratory conditions. *P. indica* showed optimum growth at a temperature of 28^{0}C, pH of 6 under aerobic conditions and it took about 25 days for superior growth of the fungus. The fungus was characterized and identified by standard staining techniques and was confirmed of its morphological features as described in earlier studies by Varma et al., 1999, 2000, 2009. Hill and Kefer medium have shown to demonstrate better growth among all the other nutrient media that were used earlier[9,10].

Germination and Pot culture studies: Germination studies of *P. indica* in MS medium[6,11] have shown considerable advancement in growth compared to control plant. Pot culture studies have also revealed better plant growth promotion which is evidenced by comparing the physical parameters of endosymbiotic plant with the control plant (figure-1a. and 1b).

Figure-1a
Germination studies in *P. indica* colonized seeds, A. Control plant showing less germination, B. *P. indica* colonized seed showing increased length of root and shoot during germination

Figure-1b
Pot culture studies in *P. indica* colonized plantlets, A. Control plants showing lesser growth, B. Endophytic plants colonized by *P. indica* showing increased growth

***P. indica* development in maize roots:** To observe the endophytic development in maize, root colonization were observed by light microscopic inspection through trypan blue staining (figure-2a). In plants from pot culture, hyphal growth of *P.indica* was detected on the root surface by SEM analysis (figure-2b).

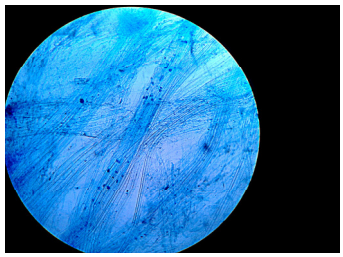

Figure-2a
Root of maize plant colonized with *P.indica* stained by trypan blue

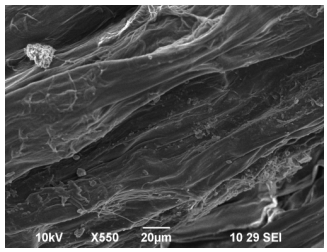

Figure-2b
SEM analysis of *P.indica* colonized root showing hypae of fungus)

The percentage of colonization inside the roots was calculated with a suitable formula.

$$\text{Root colonization \%} = \frac{\text{No of root segments colonized}}{\text{Total No of segments observed}} \times 100$$

Root colonization % = 11 / 20 x 100 = 55%

Totally of all 20 root samples that were grown in pot culture were selected, stained and visualized. Out of the 20 samples; the colonization by the fungus *P. indica* was observed in 11

samples. The colonization of root has been thus calculated as 55% by using the formula.The inhabitation of maize plant roots by *P.indica* has led to its augmented growth. Due to the tremendous growth promotional activities in the maize plants, when compared to non-colonized plants, plants colonized with *P.indica* showed greater increase in leaf length, root and the shoot length (figure-3a, 3b).

Figure-3a
***P.indica* colonized root showing increased root length. A. *P. indica* inoculated seed showing increased length of root during germination. B. Control plant showing less root length**

Figure-3b
***P.indica* colonized shoot of maize plantlet in pot culture showing increased shoot length and leaf length. A. *P. indica* inoculated seed showing increased shoot and leaf length during germination. B. Control plant showing lesser shoot and leaf length**

Estimation of physical parameters in *P.indica* colonized plants: To validate the ability of *Piriformospora indica* to enhance better growth and development in the study plants when compared to control plants, the physical parameters such as the dry weight and fresh weight were measured and the difference in weight between the colonized and control plants were compared to understand the efficiency of the fungus in increasing plant growth[12-14].

To calculate the fresh weight and dry weight of the randomly chosen harvested plants from pot culture, externally cleaned plants and the dried plants were weighed using a standard electronic weighing balance. In the study maize plants in case of pot studies; the fresh weight was found to be as 25g and dry weight as 5g in fungus colonized plants whereas in control plants the fresh weight was 17g and the dry weight was 4.5g. The endosymbiont colonized plants have shown excellent growth, and gain in weight when compared to that of control plants in pot studies and their differences have been shown in table-1.

The height of shoot and root of plants has beencalculated in study plants, the randomly chosen and harvested plants were straightened to avoid curves to ensure getting the correct measurement. In Maize plant in pot studies; the shoot height was measured as 16cm and root height was 6cm in *P. indica* colonized plants whereas in control plants the shoot height was 12cm and the root height was 4.5cm. The plants that were colonized by the fungus showed excellent growth in height when compared to that of control plants and their difference in shoot and root height in pot studies has shown that the fungus could effectively induce increase in growth of colonized plants. The results have been shown in Table 2. Similar results in increase of plant growth and root colonization has been observed in earlier studies[13,15-17].

Table-1

Fresh and Dry weight of plants studied with *Piriformospora indica* – In Pot study

S. No	Plant Groups	Maize Plant			
		Fresh Weight in gms	Dry Weight in gms	Shoot Height in cms	Root Height in cms
1	*P. indica* colonized plants	25± 0.2	5±0.3	16±0.3	6±0.1
2	Control plants	17±0.1	4.5±0.1	12±0.2	4.5±0.1

Estimation of chlorophyll content: In order to estimate the chlorophyll content of plants the estimation was done by using Arnon and Witham's method in Maize plant. The *P.indica* colonized plants have shown comparatively higher chlorophyll content when compared to that of control plants which indicates that *P.indica* helps to enhance the chlorophyll production in the plants[18]. This actively induces an enhanced role of light reaction and hence making them metabolically more active and thus results in superior growth. The chlorophyll values of Pi colonized and control study plants have been represented in table-3.

In *P.indica* inoculated maize plants, it has been showed that there is 1.39g/tissue of chlorophyll content, whereas in non-colonized control plants it showed only 0.72g/tissue of chlorophyll content and values have proven that *P. indica* helps to improve the biochemical activity of the colonized plants. Similar studies have been found in plant studies as refered in Hearath et al.[19].

Thus it is advocated that *Piriformospora indica* is a superior

fungus among the plant endosymbionts that effectively mediates plant growth promotion.

Table-3

Chlorophyll content of plants studied with *P. indica*

Determination of Chlorophyll concentration	
Maize Plant	Chlorophyll content in g/tissue
P.indica colonized plants	1.3
Control plants	0.72

Conclusion

P.indica unlike AM fungi, can easily be proliferated on a large scale in artificial media in the absence of host plant[1] unlike its mycorrhiza counterpart which are noncultivable in laboratory conditions, Development of *P.indica* may not only enhance crop-growth promotion strategies but will also serve as a model for root endophytes to study its physiological functions, molecular behaviors that result in disease resistance, increased yields and production of many additional growth mediating components, as reported earlier in reviews. The present investigation has focussed on plant growth promotion effect of the fungus, which may be due to production of growth promoting metabolites produced or induced by the fungus. The fungi may contribute for many functional components in plants including hormones and enzymes etc that mediate growth promotion.

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