

Influence of *Piriformospora indica* on Growth and Flowering of Tropical Orchid *Dendrobium*

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ABSTRACT: Investigations were carried out to study the influence of nutrients and *Piriformospora indica*, a Plant Growth Promoting Root Endophyte (PGPRE), on growth and flowering of the tropical orchid *Dendrobium cv. earsakul*. The association of *P. indica* in root system of *Dendrobium cv. earsakul* enhanced nutrient absorption and facilitated the floral characters significantly without appreciable change in growth characters. Anatomical studies showed that *P. indica* association was confined to cortical root tissues. Hyphae multiplied within cortical tissues but never traversed to aerial portion of the plant. Radio assay study revealed that radioactivity was highest in pseudobulbs than in roots and leaves.

Key words: Orchid, *Dendrobium*, Nutrients, *Piriformospora indica*, Growth.

INTRODUCTION

Dendrobium is one of the important tropical genera of orchids which have immense commercial significance in the floriculture trade. Orchid hybrids require copious amount of nutrients since their growth and flowering rates are faster. The type of nutrients, their quality and frequency of application play an important role on the quality of flower (Singh, 1992). The members of orchidaceae are characterized by a novel form of mycorrhizal interaction. *Piriformospora indica* is a newly described axenically cultivable phytopromotional endosymbiont, which mimics the capabilities of Arbuscular Mycorrhizal Fungi (AMF). *P. indica* acts as plant growth promoter, biofertilizer, bioregulator, immunomodulator, phytoremediator, imparts resistance against heavy metals, biocontrol against insects and pathogens, stress tolerance - both salt and temperature and works as stimulator with Plant Growth Promoting Rhizobacteria (PGPRs) (Varma *et al.*, 1999; Deshmukh *et al.*, 2006; Sherameti *et al.*, 2008). With the objective of assessing the influence of nutrients and PGPRE on growth and development of *Dendrobium* was taken up in the commercial variety *ersakul*.

MATERIALS AND METHODS

The experiment was conducted at the orchidarium of All India Co-ordinated Floriculture Improvement Project in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, Thrissur, Kerala. The experimental site was located at an altitude of 22.25 m above MSL, latitude 10.30° longitude N 76.15° E and with warm humid tropical climate. Six months old tissue culture plants of commercially cultivated orchid hybrid *Dendrobium cv.*

Table 1
Details of treatments

Treatment	Details
T ₁	Package of practices (POP) recommendations of KAU for orchids (KAU, 2011)
T ₂	POP + Organic mixture
T ₃	POP + Vermiwash
T ₄	POP + Bone meal
T ₅	POP + <i>Piriformospora indica</i> (PGPRE)
T ₆	POP + Organic mixture + <i>P. indica</i> + Bone meal
T ₇	POP + Vermiwash + <i>P. indica</i> + Bone meal
T ₈	POP + Organic mixture + Vermiwash + <i>P. indica</i> + Bone meal
T ₉	Organic mixture + Vermiwash + <i>P. indica</i> + Bone meal

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earsakul was used for the study. The plants were maintained under 50 per cent shade provided by green coloured shade nets. The experiment was laid out in Completely Randomized Block Design with nine treatments and three replications as detailed in Table 1.

POP recommendation (KAU, 2011): Spraying with supernatant liquid of cow dung slurry (1 Kg of fresh cow dung in 5 L of water), foliar feeding with fertilizer mixture of N: P₂O₅:K₂O 3:1:1 during vegetative period and 1:2:2 during flowering period (dose of the mixture is 2-3 g per litre of water, spraying weekly twice). Nutrient combinations of NPK were made using ammonium nitrate, ortho phosphoric acid and potassium nitrate, respectively.

Organic mixture: Bone meal (N 2 to 4%, P 20 to 25%), neem cake (N 5.5%, P 1.1%, K 1.5%) and groundnut cake groundnut cake (N 6.5%, P 1.3%, K 1.5%) 100 g each, soaked in water for 3-4 days and diluted to 10-15 times with water, filtered and sprayed over the plants at 15 days intervals.

Vermiwash: Vermiwash diluted to 3 per cent and sprayed at 15 days intervals.

Piriformospora indica (PGPRE): *P. indica* mixed with vermiculite @ 1g per 100g of vermiculite and applied near the root zone at the time of planting.

Bone meal: Applied near the root zone at the time of planting @ 15 g per plant.

Observations were recorded on plant height (cm), number of leaves per plant, number of shoots per plant, number of roots per plant, root length (cm), root volume (ml), root colonisation of the *P. indica* (per cent), days to flowering, length of the spike (cm), number of flowers per spike and size of the flower (cm). The method proposed by Giovannetti and Mosse (1980) was followed for assessment of root colonization of *P. indica*.

To investigate the structural linkage, anatomical studies were undertaken as per the procedure proposed by Phillips and Hayman (1970). Root cells were examined microscopically (40X and 100X) and photographed.

For investigations on functional linkage between PGPRE and orchid, radio trace studies were undertaken. The fungus was labelled with ³²P with three radioactive concentrations viz., 1.29 µCi, 1.94 µCi and 2.58 µCi in the broth culture (Martin's Rose Bengal media). The plants were treated separately and kept seven days for fungal growth. After completion of centrifugation and final testing of radioactivity in scintillation unit, the fungal suspension (25 ml) was taken in small plastic tubes and the tip of the orchid root was dipped carefully in the fungal suspension

for root inoculation for 24h. The dried plant parts were ground into fine powder, digested with diacid mixture made up to 100 ml and filtered solution was taken in vials and kept in a scintillation counter for counts per minute (CPM) was recorded.

RESULTS AND DISCUSSION

Different treatments did not show appreciable difference in plant height, number of leaves and number of shoots per plant during different growth stages (Table 2). The results indicated that the action of *P. indica* had significant influence in plant height at later stage of plant growth. The increase in plant height may be due to root endophyte inoculation which has the potential to added nitrogen to crop growth through associative symbiosis and increased production of growth hormones like NAA, GA and cytokinins (Zou and Tan, 1999, Varma *et al.*, 1999 and Singh *et al.*, 2000). These phytohormones might have caused morphological changes in roots, thereby causing an increase in uptake of nutrients resulting in better growth. The possible reason for such an effect could be that *P. indica* increases the cell division and cell elongation in the region of auxiliary buds.

Flower characters in orchid *Dendrobium* cv. earsakul are found to be influenced by nutrients and *P. indica* (Table 4). Positive influence of nutrients along with *P. indica* resulted in increased spike length. In general, flowering could be enhanced by two months. The combination of POP + *P. indica* recorded maximum spike length and took minimum days for flowering. This may be attributed to the role of *P. indica* which would have influenced the root system and absorption of nutrients which further enhances the spike length and early flowering.

The treatment POP + organic mixture + *P. indica* + bone meal recorded higher number of roots per plant (33.00) in the early stages of growth (Table 3). At bud formation stage, the treatment organic mixture + vermiwash + *P. indica* + bone meal recorded significantly higher root length (23.33 cm). The treatment POP + *P. indica* recorded maximum root volume (13.330 ml) and root colonization of *P. indica* (79.89 per cent) at the time of flower bud formation. Root colonization study revealed that *P. indica* enters through the roots and colonize within the cortical cells of the root tissue (Fig. 1). It is evident that POP + *P. indica* was the best treatment combination for root colonization of *P. indica*. The positive influence of *P. indica* for the above root parameters was clearly evident from the study. *P. indica* having a broad host spectrum shows pronounced growth promotional effects. It mobilizes

Table 2
Influence of nutrients and *Piriformospora indica* on growth characters of *Dendrobium cv. earsakul*

Treat- ments	Plant height (cm)															Number of leaves per plant															Number of shoots per plant																																																																																																																																																															
	2			4			6			8			10			12			14			2			4			6			8			10			12			14																																																																																																																																																						
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T ₁	8.69 ^a	12.63 ^a	14.09 ^{ab}	15.82 ^a	17.33 ^a	18.75 ^a	20.79 ^{ab}	6.11 ^a	5.67 ^a	6.56 ^a	4.11 ^a	5.11 ^a	7.00 ^a	9.89 ^a	6.11 ^a	4.00 ^a	3.89 ^a	6.11 ^a	6.67 ^a	8.22 ^a	10.00 ^{ab}	9.72 ^a	14.23 ^a	14.73 ^{ab}	14.82 ^a	15.09 ^a	17.17 ^a	20.37 ^{ab}	7.67 ^a	6.22 ^a	6.56 ^a	5.67 ^a	6.11 ^a	7.00 ^a	9.89 ^a	6.11 ^a	4.00 ^a	3.89 ^a	6.11 ^a	6.67 ^a	8.22 ^a	10.00 ^{ab}	9.60 ^a	11.53 ^a	12.00 ^b	12.29 ^a	13.64 ^a	15.77 ^a	18.12 ^b	5.56 ^a	5.44 ^a	4.21 ^a	5.45 ^a	6.78 ^a	8.11 ^a	5.11 ^a	4.11 ^a	3.67 ^a	5.22 ^a	5.78 ^a	7.22 ^a	9.67 ^a	11.22 ^a	9.47 ^a	12.83 ^a	12.91 ^b	12.97 ^a	13.18 ^a	14.98 ^a	20.89 ^{ab}	7.56 ^a	5.33 ^a	7.00 ^a	4.22 ^a	5.56 ^a	6.10 ^a	10.11 ^a	5.78 ^a	4.22 ^a	4.00 ^a	5.44 ^a	7.44 ^a	8.78 ^a	10.44 ^{ab}	10.61 ^a	14.83 ^a	17.66 ^a	16.02 ^a	17.43 ^a	18.78 ^a	25.33 ^a	8.34 ^a	6.00 ^a	7.00 ^a	5.00 ^a	6.22 ^a	8.67 ^a	12.33 ^a	5.33 ^a	4.76 ^a	3.89 ^a	7.00 ^a	7.56 ^a	10.11 ^a	12.11 ^a	10.11 ^a	14.42 ^a	15.28 ^{ab}	16.87 ^a	17.11 ^a	18.20 ^a	24.27 ^{ab}	6.67 ^a	5.22 ^a	6.54 ^a	3.89 ^a	4.66 ^a	6.44 ^a	10.11 ^a	5.11 ^a	3.56 ^a	4.00 ^a	4.89 ^a	5.44 ^a	6.89 ^a	8.55 ^{ab}	11.14 ^a	13.38 ^a	14.00 ^{ab}	15.01 ^a	15.05 ^a	17.32 ^a	24.29 ^{ab}	6.89 ^a	5.33 ^a	6.67 ^a	3.44 ^a	4.53 ^a	6.56 ^a	9.11 ^a	4.78 ^a	3.89 ^a	4.11 ^a	5.11 ^a	5.89 ^a	6.44 ^a	8.22 ^a	10.89 ^{ab}	9.00 ^a	12.87 ^a	14.58 ^{ab}	16.13 ^a	16.73 ^a	17.74 ^a	25.98 ^{ab}	6.44 ^a	5.00 ^a	6.78 ^a	5.00 ^a	5.78 ^a	8.22 ^a	11.78 ^a	5.44 ^a	4.22 ^a	3.88 ^a	5.44 ^a	6.22 ^a	9.22 ^a	10.89 ^{ab}	9.33 ^a	11.81 ^a	13.39 ^b	15.12 ^a	15.54 ^a	16.00 ^a	20.82 ^b	6.11 ^a	5.11 ^a	5.78 ^a	4.00 ^a	5.22 ^a	5.78 ^a	9.77 ^a	5.00 ^a	4.56 ^a	3.77 ^a	5.67 ^a	5.77 ^a	8.56 ^a	9.78 ^{ab}

MAP - Months after planting; Comparison along the column. Figures with same superscript do not differ.

Table 3
Influence of nutrients and *P. indica* on root parameters of *Dendrobium cv. earsakul*

Treatment	Number of roots/plant			Root length (cm)			Root volume (ml)			Root colonization of <i>P. indica</i> (per cent)		
	At the time of flower bud formation			At the time of flower bud formation			At the time of flower bud formation			At the time of flower bud formation		
	6	MAP	6	6	MAP	6	6	MAP	6	6	MAP	6
T ₁	32.67 ^a	52.67	11.33	18.50 ^{ab}	3.333	11.670 ^{ab}	—	—	—	—	—	—
T ₂	25.33 ^{ab}	58.00	11.43	15.97 ^b	2.667	11.000 ^{abc}	—	—	—	—	—	—
T ₃	17.67 ^b	46.33	9.77	14.33 ^b	2.000	6.333 ^c	—	—	—	—	—	—
T ₄	26.00 ^{ab}	55.67	8.43	18.00 ^{ab}	2.667	6.333 ^c	—	—	—	—	—	—
T ₅	31.33 ^a	64.33	10.30	19.50 ^{ab}	3.667	13.330 ^a	60.00	79.89 ^a	—	—	—	—
T ₆	33.00 ^a	48.33	8.80	16.30 ^b	3.333	8.000 ^{bc}	48.89	68.89 ^{ab}	—	—	—	—
T ₇	24.67 ^{ab}	58.33	10.10	15.67 ^b	3.000	7.000 ^{bc}	53.33	73.33 ^{ab}	—	—	—	—
T ₈	29.33 ^{ab}	59.67	10.43	15.20 ^b	3.000	9.000 ^{abc}	55.55	64.44 ^b	—	—	—	—
T ₉	19.00 ^b	53.33	10.73	23.33 ^a	2.667	8.000 ^{bc}	—	—	—	—	—	—

Comparison along columns. Figures with same superscript do not differ.

Table 4
Influence of nutrients and *P. indica* on flowering of *Dendrobium cv. earsakul*

Treatment	Spike length (cm)	No of flowers	Size of the flower (cm)		Days for flowering
			6	MAP	
T ₁	34.066 ^a	6.830	8.356	462.00 ^{ab}	—
T ₂	30.486 ^{ab}	5.596	8.003	465.00 ^{ab}	—
T ₃	30.553 ^{ab}	6.996	6.750	466.00 ^{ab}	—
T ₄	28.606 ^b	5.773	8.000	469.67 ^a	—
T ₅	34.083 ^a	7.093	8.376	393.00 ^c	—
T ₆	33.220 ^a	6.486	7.400	450.00 ^b	—
T ₇	34.820 ^a	7.843	8.320	397.67 ^c	—
T ₈	31.276 ^{ab}	7.996	8.273	400.00 ^c	—
T ₉	28.663 ^b	5.720	7.520	480.00 ^a	—

Comparison along columns. Figures with same superscript do not differ.

the insoluble phosphates and translocates the phosphorus to the host in an energy-dependent process. *P. indica* made the P available to the plants which in turn increase the root characters in orchid (Singh *et al.*, 2000).

Anatomical studies were undertaken to study the structural linkage of *P. indica* and orchid. After inoculation of *P. indica* into the roots of *Dendrobium* cv. earsakul, hyphae of the fungus entered into the tissue of the root through the root tip (Fig. 2). Hyphae, first touching the root surface and entered through velamen tissue. The hyphal growth of the fungus was detected on the root surface between the outer cell layers of the cortex and within the cortical cells. The fungus produced chlamydospores at the apex of undifferentiated hyphae and within the cortical cells of the root tissue. The fungal hyphae invade the cortical cells and form tightly interwoven coils called 'pelotons' characteristic of orchid mycorrhizae. In the cortical cells of the roots, development of intracellular hyphal coils and round bodies could be observed. Hyphae multiplied within the cortical tissues and never traversed through endodermis. These are in agreement with findings of, Varma *et al.*, (2001) in maize and Stein *et al.*, (2008) in *Arabidopsis*.

The radio assay study in the plants which are inoculated with labelled fungus indicated that, among

plant parts, radioactivity was higher in pseudobulb portion in the treatments with labelled fungus applied to the roots (1.29 μ Ci concentration) and labelled fungus applied to the roots (2.58 μ Ci concentration). While, highest radioactivity of 530.11 cpm g^{-1} was recorded in roots compared to leaves and pseudobulb (Table 5).

Table 5
Distribution pattern of ^{32}P assimilate partitioning in *Dendrobium* cv. earsakul

Details of the treatment	Radioassay	
	Plant parts digested	Radioactive counts (cpm g^{-1})
Inoculated with labelled fungus (1.29 μ Ci)	Roots	253.07
	Pseudobulb	362.21
	Leaves	287.21
Inoculated with labelled fungus (1.94 μ Ci)	Roots	530.11
	Pseudobulb	325.95
	Leaves	364.35
Inoculated with labelled fungus (2.58 μ Ci)	Roots	245.36
	Pseudobulb	1638.93
	Leaves	575.91

Thus, on the basis of the findings of the present investigation it may be concluded that the treatment combination of spraying the supernatant liquid of cowdung slurry (1 kg of fresh cow dung in 5 litres of

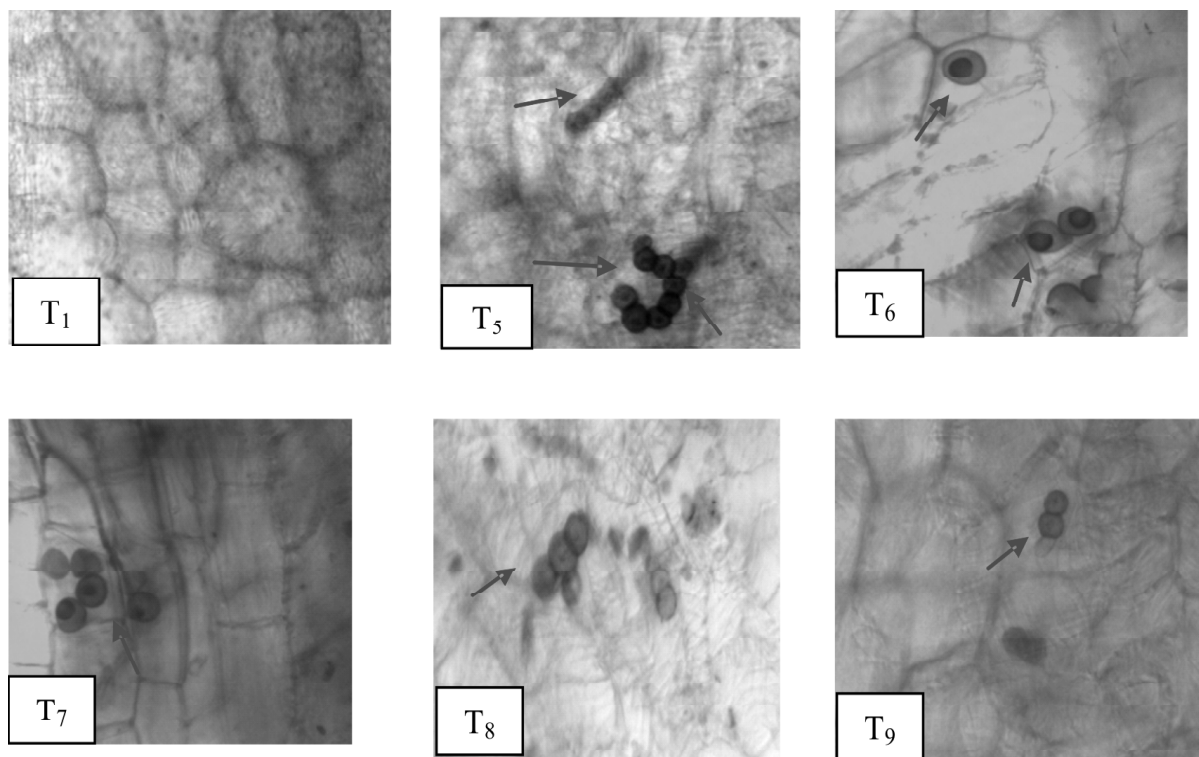


Figure 1: Colonisation of *P. indica* on root system of *Dendrobium* cv. earsakul at different combinations of nutrients and PGPRE

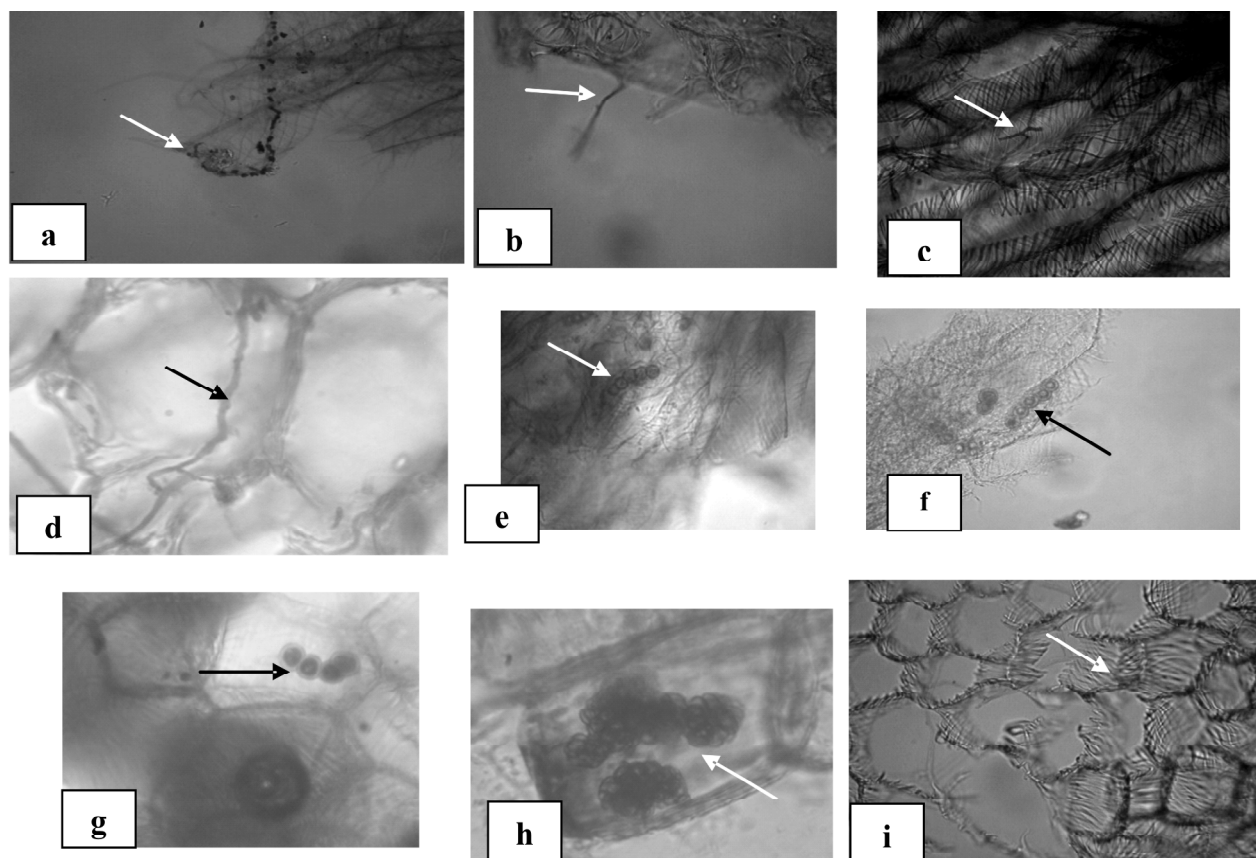


Figure 2: Anatomical studies in roots for structural linkage between *Dendrobium* cv. earsakul and *P. indica*

- a. Fungus entry through the root tip (60x) b. Hyphae touches the root surface (60x) c. Fungus entry through velamen of the root tissue (60x) d. Hyphae of the fungus was detected in cortical cells (100x) e. The fungus produced chlamydospores at the apex of undifferentiated hyphae (60x) f. Pear-shaped chlamydospores in root tissue (60x) g. Cortical cells showing round bodies (60x) h. Cortical cells with highly coiled intracellular structures (60x) i. Fungus hyphae in cortical cells (60x)

water) and foliar spray with $N:P_2O_5:K_2O$ @ 30:10:10 during vegetative period and 10:20:20 at the flowering period at 0.2 per cent applied weekly twice and basal application of *P. indica* fungal culture (20 g per plant) near root zone, resulted in positive influence on floral characters of *Dendrobium* cv. earsakul without much change in its biometric characters. In *P. indica* inoculated plants, the hyphal growth of the fungus was detected on the root surface between the outer cell layers of the cortex and within the cortical cells. The highest radioactivity of $530.11 \text{ cpm g}^{-1}$ was recorded in roots of the treatment labelled fungus applied to the roots ($1.94 \mu\text{Ci}$ concentration) compared to leaves and pseudobulb.

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