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Radioisotope labelling (^{32}P) to Study the Functional Linkage Between *Dendrobium* cv. Earsakul and *Piriformospora indica* (PGPRE)

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ABSTRACT
The fungus was radio labelled with ^{32}P and the radio assay was conducted for the presence of ^{32}P in the plant. Autoradiography showed that the orchid roots absorbed ^{32}P from the source i.e. when labelled $N:P_2O_5:K_2O$ (1:2:2) nutrient solution applied to the media inoculated with non-labelled fungus (T_3). The image of the root indicated that, ^{32}P had moved through root from the source of application and translocated into the root tissues. Plants treated with treatment T_1 and T_2 did not give any image. The radioactivity was higher in pseudo bulb portion than in roots and leaves of the Dendrobium cv. Earsakul. Highest radioactivity (240.27 cpm g^{-1}) was recorded in the leaves of the plants where labelled $KH_2{}^{32}PO_4$ applied to the media inoculated with non-labelled fungus (T_1). Lowest radioactivity (209.39 cpm g^{-1}) was recorded in T_2. However, plants which are treated with labelled $N:P_2O_5:K_2O$ (1:2:2) nutrient solution applied to the media inoculated with non-labelled fungus (T_3) showed radioactivity of 235.18 cpm g^{-1}. The radio assay study in case of the plants which are inoculated with labelled fungus indicated that, among plant parts, radioactivity was higher (362.21 cpm g^{-1} and 1638.93 cpm g^{-1}) in pseudobulb portion in treatments T_4 and T_6, respectively. While, highest radioactivity of 530.11 cpm g^{-1} was recorded in roots of the treatment T_5 compared to leaves and pseudo bulb. The treatments T_4 and T_6 recorded lower radioactivity of 253.07 cpm g^{-1} and 245.36 cpm g^{-1}, respectively in roots.
***Key words**: Dendrobium cv. Earsakul, radio isotope ^{32}P, functional linkage, Radio assay*

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INTRODUCTION

Orchids, the most spectacular cut flower among the flowers, are unique with their versatility in colour, form, size, shape and longer life span of the plant and flower. They are famous for their long lasting character and beauty which fetch a very high price in the international market. Taxonomically, it belongs to the most highly evolved monocotyledons family, Orchidaceae with 600-800 genera and 25,000-35,000 species. Orchids have a wide range of growing habit, from terrestrial to epiphytic. In the past couple of decades, they have occupied a coveted position in the international flower market, evolving into a multibillion dollar business. With the recent increase in the world floriculture trade, orchids have become the second most popular plants as cut flowers as well as pot plants with an annual growth rate of 10-20 per cent [5].

Orchids are present in all the countries, except in Antarctica and majority of the cultivated orchids are native to tropical countries, occurring in their greatest diversity in humid tropical forests of South and Central America, Mexico, India, Ceylon, Burma, China, Thailand, Malaysia, Philippines, New Guinea and Australia. Orchids are grown worldwide with almost 8 per cent share in the world's floriculture trade [1]. In many countries, orchid industry plays an important role as a source of foreign exchange.

The root-colonizing fungal mutualist *Piriformospora indica* was discovered in the rhizosphere of the woody shrubs *Prosopsis juliflora* and *Zizyphus nummularia* in the Indian Thar desert and it was named according to its characteristic pear-shaped chlamydospores [13].

Depending on the ultra structure of hyphae (presence of dolipore septa) and 18s-rRNA gene sequence, *Piriformospora indica* was grouped in the class Hymenomycetes (Basidiomycota) [13]. Serological classification showed close antigenic properties with mycorrhizal fungi [12]. In contrast to mycorrhizal

fungi, this fungus can be cultured axenically on various synthetic simple and complex media at 25-35 ^{o}C [11].

Piriformospora indica AM fungi – like fungus, showed prominent positive influence on a wide range of plants of agriculture, forestry and flori-horticultural importance. Fungus has a wide host range of monocots and dicots including legumes, terrestrial orchids (*Dactylorhiza maculata*) and members of the bryophytes (*Aneura pinguis*). The fungus showed potential as an agent for biological control of disease against soil-borne root pathogens. ^{32}P experiments suggest that this fungus is important for phosphorus acquisition by the roots, especially in the arid and semi-arid regions. Mycelium could utilize a wide variety of inorganic and organic phosphate chemicals and produced acid phosphatases at the tip of the hyphae [8, 9].

Studies on *Piriformospora indica* have shown fungal-mediated uptake of radio-labelled 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. *P. indica* produces significant amounts of acid phosphatases for the mobilization of broad range of insoluble, condensed or complex forms of phosphates, enabling the host plant the accessibility of adequate phosphorus from immobilized reserves in the soil [10]. Based on the above information, the present investigation for radioisotope labelling (^{32}P) was planned to study the functional linkage between the host plant and *Piriformospora indica* to know the role of fungi (*Piriformospora indica*) in transferring ^{32}P to the host roots.

MATERIAL AND METHODS

The experiment (observational trial- a part of one study) was carried out at the Radio Tracer Laboratory (RTL), College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala to study the functional linkage between the host and *Piriformospora indica* (PGPRE) during 2011-2013.

Table-1. Treatments for the radioisotope study

Sl. No.	Treatments	Notations
1	Labelled $KH_2{}^{32}PO_4$ applied to the roots inoculated with non-labelled fungus (1.7 µCi concentration)	T_1
2	Labelled $KH_2{}^{32}PO_4$ applied to the roots without fungus (1.7 µCi concentration)	T_2
3	Labelled 1:2:2 $N:P_2O_5:K_2O$ nutrient solution applied to the roots inoculated with non-labelled fungus (2.4 µCi concentration)	T_3
4	Labelled fungus applied to the roots (1.29 µCi concentration)	T_4
5	Labelled fungus applied to the roots (1.94 µCi concentration)	T_5
6	Labelled fungus applied to the roots (2.58 µCi concentration)	T_6

Labelled $KH_2{}^{32}PO_4$ applied to the roots inoculated with non-labelled fungus (T_1)

The concentration of 1.7 µCi of ^{32}P was mixed to the 1000 ml of 1000 ppm of KH_2PO_4 and 25 ml was applied to the roots of the plant. For this treatment, the fungus was inoculated to the plant before application of labelled $KH_2{}^{32}PO_4$.

Labelled $KH_2{}^{32}PO_4$ applied to the roots without fungus (T_2)

Roots of the plant was applied with labelled $KH_2{}^{32}PO_4$ (1.7 µCi concentration) without fungus inoculation.

Labelled nutrient solution applied to the roots inoculated with non-labelled fungus (T_3)

For this treatment, 2.4 µCi ^{32}P was taken and mixed with 100 ml 1:2:2 $N:P_2O_5:K_2O$ nutrient solution and applied to the roots of the plants. In this case also, plants were inoculated with *Piriformospora indica* fungus before applying the labelled nutrient solution.

Labelled fungus applied to the roots with three concentrations

Pure culture of *Piriformospora indica* fungus from Potato Dextrose Agar (PDA) media was taken and sub-cultured in Martin's Rose Bengal media. The fungus was labelled with ^{32}P with three concentrations *viz*., 1.29 µCi (T_4), 1.94 µCi (T_5) and 2.58 µCi (T_6) in the broth culture *i.e.* in Martin's Rose Bengal media each treated separately and kept for fungus development at Radio Tracer Laboratory.

After proper growth of the fungus in the Martin's Rose Bengal media, radioactivity present in the growth media was removed by centrifugation before inoculation of labelled fungus into the plant in order to assure whether the radio activity detected in inoculated plant is either from the *P. indica* fungus or from the media *i.e.* ^{32}P should reach the plant from the fungus. For this, the broth culture (Martin's Rose Bengal media) was centrifuged in centrifuge tubes at 5000 rpm for 15 minutes. After each centrifugation, the supernatant was removed and the fungus pelleted at the bottom of the centrifuge tube was re-suspended in sterile distilled water and centrifuged again for removing the radioactivity from the Martin's Rose Bengal media. The process of centrifugation was repeated thrice and the supernatant obtained was tested

for the presence of radioactivity in a scintillation unit. Centrifugation and re-suspension in fresh sterile distilled water was repeated until the supernatant was totally free of radioactivity. Finally, the labelled fungus was re-suspended in 25 ml sterile distilled water and used for root inoculation of the *Dendrobium* cv. Earsakul plants.

Root inoculation

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 root was dipped carefully in the fungal suspension for root inoculation.

Autoradiography

Three weeks after application of labelled $KH_2{}^{32}PO_4$ (T_1 and T_2) and labelled 1:2:2 nutrient solution (T_3), the plants were uprooted carefully from the pots. The plants were then arranged in X-ray films in between sheets of an absorbent paper in their original position, labelled and secured with adhesive tape. The specimens sandwiched between absorbent sheets were then pressed in herbarium press and allowed to dry at room temperature in the dark room. After drying, the plant specimens were taken for autoradiography. The plants were removed and the X-ray film was developed and fixed by using a commercial X-ray film developer/ fixer solution.

Counting of ^{32}P in a scintillation unit

After autoradiography, 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 unit for counts per minute (CPM) and data was recorded.

RESULTS AND DISCUSSION

The fungus was radio labelled with ^{32}P and the radio assay was conducted for the presence of ^{32}P in the plant. The brief information on the details and result of autoradiography is furnished in the table 2. Autoradiography showed that the orchid roots absorbed ^{32}P from the source i.e. when labelled $N:P_2O_5:K_2O$ (1:2:2) nutrient solution applied to the media inoculated with non-labelled fungus (T_3). The image of the root (Plate 1 and 2) indicated that, ^{32}P had moved through root from the source of application and translocated into the root tissues. Plants treated with treatment T_1 and T_2 did not give any image (Table 2).

Distribution pattern of ^{32}P assimilate partitioning in various plant parts of *Dendrobium* cv. Earsakul are summarized in Table 2. Results of the radio assay from the table showed variation in radioactive counts of ^{32}P in different parts of the plant. The radioactivity was higher in pseudo bulb portion than in roots and leaves of the *Dendrobium* cv. Earsakul.

Highest radioactivity (240.27 cpm g^{-1}) was recorded in the leaves of the plants where labelled $KH_2{}^{32}PO_4$ applied to the media inoculated with non-labelled fungus (T_1). Lowest radioactivity (209.39 cpm g^{-1}) was recorded in T_2. However, plants which are treated with labelled $N:P_2O_5:K_2O$ (1:2:2) nutrient solution applied to the media inoculated with non-labelled fungus (T_3) showed radioactivity of 235.18 cpm g^{-1}. This would suggest that the fungus acted as the extension of the root system and enhancing the nutrient absorption.

The radio assay study in case of the plants which are inoculated with labelled fungus indicated from the table that, among plant parts, radioactivity was higher (362.21 cpm g^{-1} and 1638.93 cpm g^{-1}) in pseudobulb portion in treatments T_4 and T_6, respectively. While, highest radioactivity of 530.11 cpm g^{-1} was recorded in roots of the treatment T_5 compared to leaves and pseudo bulb. The treatments T_4 and T_6 recorded lower radioactivity of 253.07 cpm g^{-1} and 245.36 cpm g^{-1}, respectively in roots (Table 2). The above findings were in agreement with Finlay and Read [5, 6] who showed that ectomycorrhizal (ECM) hyphae were the principle route for ^{14}C and ^{32}P transfer to the Pinus seedlings. The results obtained in this study are endorsed by Cameron *et al*. (2007) in *Goodyera repens*, Yadav *et al*. [14] in maize and Kumar *et al*. [6] in maize. Yadav *et al*. [14] reported that *P. indica* plays a vital role in phosphate transport to the host plant. The mycorrhiza plays an important role in the life cycle of plants of the family orchidaceae. Mycorrhiza in *Vanilla* roots was first recorded by him and observed the infection of fungi on the roots adhering to their nutrients *via* the fungus. The mechanism underlying phosphate transfer from the fungus to the plant remains unknown, and it is speculated that the process occurs at the plant-fungus interface. However, the physiological pathways responsible for P flow through orchid mycorrhizal networks and to the partner orchid are to be investigated.

Radio assay showed variation in radioactive counts of ^{32}P in different parts of the plant. The radioactivity was higher in pseudobulb portion than in roots and leaves (Table 2). This is in conformity with the findings of Sheehan *et al*. [7] in *Cattleya* 'Trimose'.

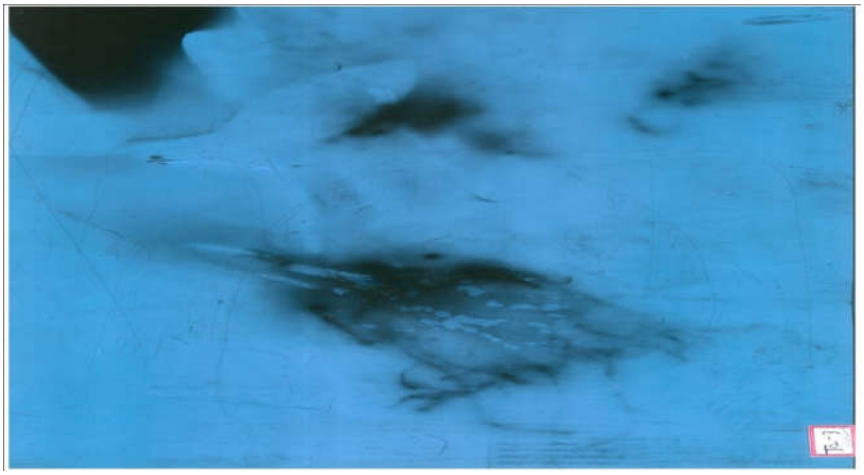

Plate 1. Autoradiography showing ^{32}P labelled fungus in orchid roots (T_2).

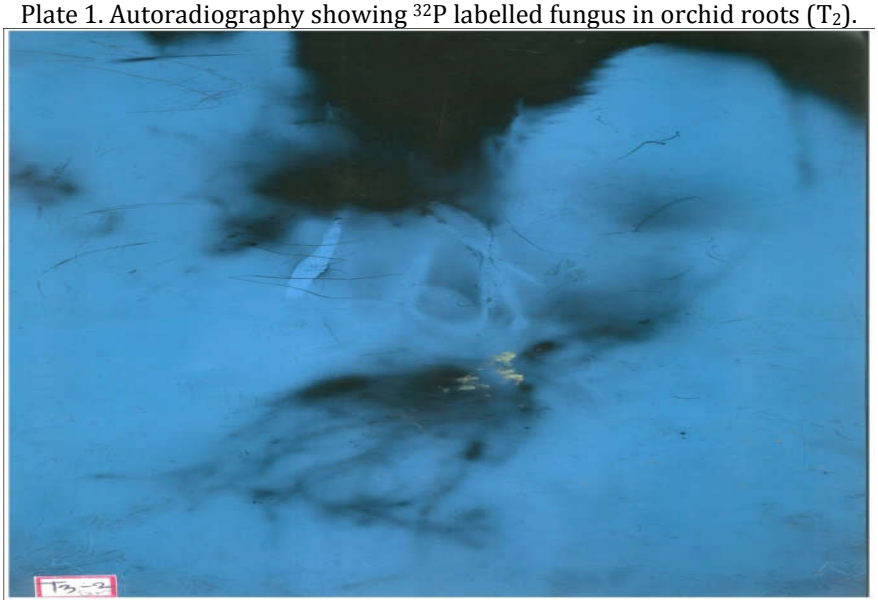

Plate 2. Autoradiography showing ^{32}P labelled fungus in orchid roots (T_3)

Table- 2. Distribution pattern of ^{32}P assimilate partitioning in *Dendrobium* cv. Earsakul

Treatments	Details of the treatment	Autoradiography	Radioassay	
			Plant parts digested	Radioactive counts (cpm g^{-1})
T_1	Labelled $KH_2{}^{32}PO_4$ applied to the roots inoculated with non labeled fungus (1.7 µ Ci)	No image in X ray film	Leaves	240.27
T_2	Labelled $KH_2{}^{32}PO_4$ applied to the roots without fungus (1.7 µ Ci)	No image in X ray film	Leaves	209.39
T_3	Labelled $N:P_2O_5:K_2O$ (1:2:2) nutrient solution applied to the roots inoculated with non labelled fungus (2.4 µ Ci)	Image in X ray film was observed	Leaves	235.18
T_4	Inoculated with labelled fungus (1.29 µ Ci)	-	Roots	253.07
			Pseudobulb	362.21
			Leaves	287.21
T_5	Inoculated with labelled fungus (1.94 µ Ci)	-	Roots	530.11
			Pseudobulb	325.95
			Leaves	364.35
T_6	Inoculated with labelled fungus (2.58 µ Ci)	-	Roots	245.36
			Pseudobulb	1638.93
			Leaves	575.91

REFERENCES

1. Asghar, S.; Ahmad, T, Hafiz, I. A. and Yaseen, M. (2011). *In vitro* propagation of orchid (*Dendrobium nobile*) var. Emma white. *Afr. J. Biotechnol*. 10 (16): 3097-3103.
2. Cameron, D.D.; Jhonson, I., Leake, J.R., and Read, D.J. (2007). Mycorrhizal acquisition of inorganic phosphorus by the green-leaved terrestrial orchid *Goodyera repens*. *Ann. Bot*. 99: 831-834.
3. Finlay, R. D. and Read, D. J. (1986a). The structure and function of the vegetative mycelium of ectomycorrhizal plants. II. The uptake and distribution of phosphorus by mycelium interconnecting host plants. *New Phytol*. 103: 157-165.
4. Finlay, R.D. and Read, D.J. (1986b). The structure and function of the vegetative mycelium of ectomycorrhizal plants. I. Translocation of ^{14}C labelled carbon between plants interconnected by a common mycelium. *New Phytol*. 103: 143-156.
5. Hossain. (2010). Therapeutic orchids: traditional uses and recent advances – an overview. *Fitoterapia*. 82(2): 102-140.
6. Kumar, M.; Yadav, V., Kumar, H., Sharma, R., Singh, A., Tuteza, N., and Johri, A.K. (2011). *Piriformospora indica* enhances plant growth by transferring phosphate. *Plant Signaling and Behav.* 6(5): 723-725.
7. Sheehan, T.J.; Joiner, J.N. and Cowart, J.K. (1967). Absorption of ^{32}P by *Cattleya* 'Trimos' from foliar and root applications. *Florida Agric. Exp. Stn. J. Ser.* 2812: 400-404.
8. Singh, A.N.; Singh, A.R., Kumari, M., Kumar, S., Rai, M.K., Sharma, A.P., and Varma, A. (2003a). Biotechnological importance of *Piriformospora indica* Verma *et al*.- a novel symbiotic mycorrhiza- like fungus: an overview. *Indian J. Biotechnol*. 2: 65-75.
9. Singh, A.N.; Singh, A.R., Kumari, M., Kumar, S., Rai, M.K., Sharma, A.P., and Varma, A. (2003b). Unmassing the accessible treases of the hidden unexplored microbial world. In: Prasad, B.N. (ed.), *Biotechnol. in Sus. Biodiversity and Food Security.* Science Publishers, Enfield, NH, pp.101-124.
10. Singh, A.; Sharma, J., Rexer, K. H., and Varma, A. (2000). Plant productivity determinants beyond minerals, water and light: *Piriformospora indica* - A revolutionary plant growth promoting fungus. *Curr. Sci.* 79: 1548–1554.
11. Varma, A.; Singh, A., Sahay, N.S., Sharma, J., Roy, A., Kumari, M., Rana, D., Thakran, S., Deka, D., Bharti, K., Franken, P., Hurek, T., Blechert, O., Rexer, K.H., Kost, G., Hahn, A., Hock, B., Maier, W., Walter, M., Strack, D., and Kranner, I. (2001). Mycota. In: Hock, B. (ed.), *Piriformospora indica*: *an axinically cultivable Mycorrhiza- like endosymbiotic fungus*. Springer series, Heidelberg, Germany. pp.125–150.
12. Varma, A.; Verma, S., Sudha, S., Sahay, N.S., and Franken, P. (1999). *Piriformospora indica*: a cultivable plant growth promoting root endophyte with similarities to arbuscular mycorrhizal fungi. *Appl. Environ. Microbiol.* 65: 2741-2744.
13. Verma, S.; Varma, A., Rexer, K.H., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Bütehorn, B., and Franken, P. (1998). *Piriformospora indica*, a new root-colonizing fungus. *Mycologia.* 90: 896-903.
14. Yadav,V.; Manoj, K., Deepak Kumar, D., Kumar, H., Sharma, R., Tripathi, T., Narendra, T., Saxena, A.K., and Atul Kumar, J. (2010). A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. *J. Biol. Chem*. 285(34): 26532-26544.

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