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Colonization of Plant Growth-promoting Microbes in Coconut Seedlings

Shanmugapriya D.[1*], Roy Stephen[1], K.N. Anith[2], Viji M.M.[1] and Prathapan K.[3]
[1]*Department of Plant Physiology, College of Agriculture,*
Vellayani, Kerala Agricultural University (Kerala), India.
[2]*Department of Agricultural Microbiology, College of Agriculture,*
Vellayani, Kerala Agricultural University (Kerala), India.
[3]*DY Patil Agriculture & Technical University, Talsande, Kolhapur (Maharashtra), India.*

(Corresponding author: Shanmugapriya D.)*
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ABSTRACT: Button shedding is one of the main determinants of coconut yield which can be reduced by the application of plant growth regulators such as auxins, and gibberellins. Plant growth promoting microorganisms enhance plant growth by improving nutrient availability, regulation of phytohormones and increasing tolerance to biotic and abiotic stresses. The research was conducted to check the colonization of plant growth-promoting fungal endophyte *Piriformospora indica* and the endophytic bacterium *Rhizobium radiobacter* in the root of coconut seedlings. The roots were evaluated repeatedly to check the colonization of *P. indica* by grid intersect method followed by lactophenol-trypan blue staining. The results revealed that the chlamydospores of *P. indica* were seen colonized in the cortex region of the root hairs of the coconut seedlings which is reported for the first time whereas *Rhizobium radiobacter* was not found colonized in the roots.

Keywords: Plant growth promoting microorganisms, Button shedding, Coconut, Colonization, *Piriformospora indica.*

INTRODUCTION

Coconut (*Cocos nucifera* L.) is a significant perennial crop with a variety of uses. The coconut yield is affected by both biotic and abiotic factors. Immature nut fall and button shedding are the key factors that determine the final yield (Peiris *et al.*, 1995) which is caused by numerous factors including pathogen and insect pest attacks, nutritional deficiencies, variations in soil and climate, problems with pollination and fertilization, structural flaws in the flower, abortion of embryos and the palm's limited ability to bear fruit. Button and nut shedding is observed at various stages of fruit development. Normally, a coconut bunch with an average of 16 female flowers, loses 23.8% of nuts in the first two months, 40.1% in the second two months and 1.9% in the third two months of development. After that, nut losses through the shedding of buttons will be insignificant (Abeywardena and Mathes 1971). Exogenous application of growth regulators lowers pre-harvest fruit drop percentage which leads to an increase in the total number of fruits per plant. Growth regulators such as auxins and gibberellins will prevent early fruit abscission in most cross-pollinated fruits (Bons and Kaur 2019). The research was hypothesized to find out the role of beneficial microbes in auxin production in coconut. The hypothesis proposed was that the application of plant growth-promoting microorganisms in coconut seedlings will produce the phytohormones like auxin which will be transferred to the leaves and help in the control of button shedding.

MATERIALS AND METHODS

Beneficial microbes which are reported to induce phytohormones were selected. The three months old seedlings of west coast tall (WCT) which are in 3rd leaf emerging stage were selected and raised in 45 × 60 cm polybags (500 gauge thickness) with 8–10 perforations at the bottom. The medium used was topsoil mixed with sand in a 3:1 ratio or fertile topsoil, sand or coir dust. The microbial inoculum was applied to the root zone area of the seedlings. The experimental design used is Completely Randomized Design (CRD) which consists of 3 treatments and 10 replications. The treatments were

T1: Control
T2: 1% inoculum of *Piriformospora indica*
T3: *Rhizobium radiobacter* (1×10^8cfu).

Inoculum preparation

(1) *Piriformospora indica.* An inoculum of *P. indica* from the mother culture was inoculated in Potato Dextrose Agar medium plate and incubated for 10 days at 28 °C (Fig. 1). Mass multiplication of the fungus was done in Potato dextrose broth. The freshly grown *P. indica* was inoculated in the broth and kept in a shaker at 110 rpm for uniform growth (Fig. 2). After 10-15 days, the mycelial mat was separated using a muslin cloth. The *P. indica* was confirmed by checking the presence of piriform-shaped chlamydospores by observing a bit of mycelial mat under the microscope (400X) from the broth. To prepare 1% inoculum for 10 seedlings, 15 g of mycelium was mixed with 1.5 kg of

autoclaved vermiculite (repeated thrice at 121ºC; 15Pa). The vermiculite (2 mm diameter) containing *P. indica* was mixed into the soil near the root zone of the coconut seedlings.

Fig. 1. Culturing of *P. indica* in Petri plate

Fig. 2. *P. indica* broth

(2) *Rhizobium radiobacter*. Pure culture of *R. radiobacter* was cross streaked on the nutrient agar plates and incubated (Fig. 3). After 2 days, 10 mL of autoclaved distilled water was added to the plates and kept for 1 hour. The suspension was collected from the plates and tested for population density and the cell density was adjusted to 10^8 cfu/mL by measuring optical density (0.8) using a UV-1900i spectrophotometer at 660 nm. 100 mL of the solution was poured into the soil around the root zone.

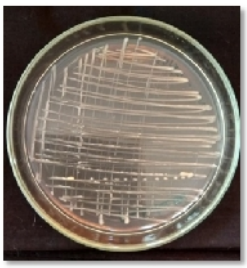

Fig. 3. *Rhizobium radiobacter*.

Evaluation of Colony Morphology

(3) Evaluation of *P. indica* Colonization. Root colonization was examined by the grid intersect method followed by staining root segments with lactophenol-trypan blue. The roots were cut (3-5 cm from the root tip) 15 days after the inoculation of plant growth promoting micro-organisms in the seedlings. The roots were washed thoroughly in water to remove the adhered soil particles and cut into small bits of 1cm. Then, the root bits were soaked in freshly prepared 10% KOH solution overnight and boiled in 10% KOH for 1-2 hours. After boiling, it was washed in water 3 times and acidified with 1N HCl for 3 minutes. The acid is drained and root bits are kept in lactophenol-trypan blue (LTB) for 10 min for staining. After 10 min, the root bits were transferred to lactophenol solution for destaining (Paul *et al.*, 2021). The above-mentioned procedure is repeated on the 30th day and 45th day and repeated until the results are obtained.

(4) Evaluation of *Rhizobium radiobacter* colonization. The roots were cut 15 days after the inoculation of *R. radiobacter* in the seedlings. The Yeast Extract Mannitol Agar (YEMA) media was prepared and poured into the plates. The roots were washed thoroughly in water to remove the soil particles and cut into small bits in sterile conditions. The roots were kept in 1% sodium hypochlorite for surface sterilization and washed in sterile water seven times. The edges of the root were removed and crushed to get the root extract. The root extract was then streaked in the YEMA plates and kept for incubation.

Fig. 4. Field view of the experiment.

RESULTS

The young and mature roots were collected from the control and treated seedlings from the field CRS (Coconut Research station, Balaramapuram) (Fig. 4) after 15 days of inoculation with beneficial microbes and the colonization was evaluated. The characteristic chlamydospores of *P. indica* were observed in the root segments of the coconut. Piriform-shaped chlamydospores of *P. indica* were found single in the cortex region of the treated roots when compared to the non-colonized plants (Fig. 5.) which is viewed under the fluorescence microscope (400X) whereas *Rhizobacterium radiobacter* were not found colonized in the root region. Result demonstrates that this *P. indica* successfully colonizes the root segments of coconut. It is capable of interacting with and overall development of coconut.

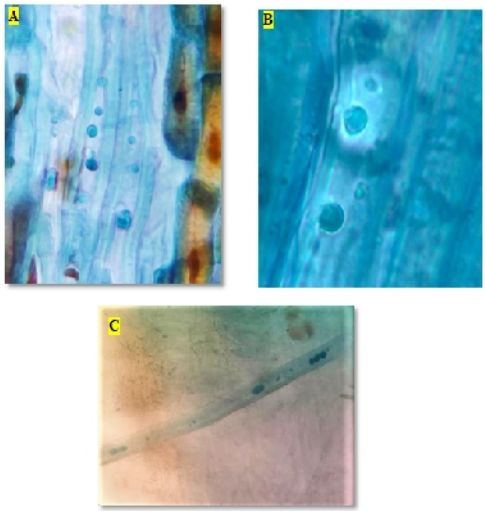

Fig. 5. Root colonization and sporulation of *P. indica* in coconut seedlings. A. Young developing chlamydospores present in the cortex region similar to *P. indica* spores; B. Mature piriform shaped chlamydospores in root cortex; C. Sporulation in root especially in elongation, meristematic regi on.

DISCUSSION

The wide host root-colonizing endophytic fungus *Piriformospora indica* belongs to the order Sebacinales (Basidiomycota). It is a phytopromotional, biotrophic mutualistic root endosymbiont that mimics the functions of typical Arbuscular Mycorrhizal (AM) fungi, (Unnikumar *et al.,* 2013). The host range of fungus includes bryophytes, pteridophytes, gymnosperms, and all tested monocot and dicot plants. It can enter and colonize a variety of plants including wheat, barley, maize, tobacco, parsley, and poplar but the plants themselves show no symptoms of any fungus (Sherameti *et al.,* 2005). In this work, we demonstrate that *P. indica* successfully colonized with the roots of coconut (WCT).

P. indica increases the host plant's ability to absorb nutrients, induces early blooming and enhances the accumulation of secondary metabolites (Liu *et al.,* 2019). The endophyte also improves the plant's tolerance to biotic and abiotic challenges like insects, nematode infections, high-temperature stress, salinity, drought and radiation. The colonization results in the production of biomolecules including betaine, glycine and proline and this relationship help plants survive under stressful conditions by causing the release of biomolecules (Gill *et al.,* 2016).

Numerous crucial processes in plant development and adaptive growth are coordinated by auxins (IAA), the most prevalent auxin in nature (Naser and Shani 2016). *P. indica* disrupts plant auxin metabolism or signaling to stimulate growth and developmental processes in its hosts. Auxin plays a role in organ formation, cell growth, and organ division in addition to defense and stress responses (Egamberdieva *et al.,* 2017). When *P. indica* interacts with Arabidopsis, it is thought to secrete IAA, which could be the cause of the observed enhanced growth (Sirrenberg *et al.,* 2007). Jisha and Sabu (2019) also reported that the endogenous IAA levels were higher in *P. indica* colonized roots in *Cucumis sativus* L. compared to the control. Kundu et al. (2022) reported that *P. indica* stimulates putrescine to increase growth phytohormone levels, which ultimately promotes plant growth. Lee *et al.* (2011) explained the genes upregulated to increase the auxin content in *P. indica* colonized Chinese cabbage. *P. indica* colonized roots had twice as much auxin as the control. Three classes of auxin-related genes showed upregulation such as genes that produce auxin signal proteins, intercellular auxin transport carrier proteins, and proteins involved in the acidification of cell walls (AUX1). *P. indica* significantly improved the Chinese cabbage seedlings' shoot and root development as well as their fresh weight twofold.

The colonization of *P. indica* was evaluated after 15 days of inoculation and recurred until the result was obtained. The chlamydospores of *P. indica* were colonized in the cortex region of the root hairs of the coconut seedlings. A gram-negative, soil-dwelling bacterium, *Rhizobium radiobacter* has possibly the broadest host range. Singh *et al.* (2020) reported that the *R. radiobacter* has the ability to synthesize IAA which made the inoculated plant roots proliferate more than the control plants and vigorous growth was observed in those plants. This was the first report where *R. radiobacter* was a bacterial endophyte in the cereal (maize) crop, that could enhance the host plant growth. In our research, colonization of *Rhizobium radiobacter* was not observed in the roots of coconut seedlings.

CONCLUSION

In conclusion, this research delivers evidence of the symbiotic relationship between *P. indica* and coconut. *Piriformospora indica* has a wide variety of uses in many crops under different environmental conditions. Thus *P. indica* exhibits its versatility for colonizing various plant species with direct alteration of auxin signaling. The role of beneficial microbes on auxin production in coconut and the effect of *P. indica* in the coconut crop has to be studied in the future. These beneficial effects recommend the significant potential for the effective use of *P. indica* for future microbial bio-fertilizer application in Coconut to enhance the growth promotion and stronger resistance to button shedding.

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Conflict of Interest. None.

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