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Growth Promotion and Prevention of Hardening Loss in Banana cv. Grand Naine by *Piriformospora indica* through Enhanced Photosynthetic Pigment Production and Root Formation

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Abstract

Piriformospora indica is a remarkable root endophyte showing growth promotion in diverse plant species. It colonizes the roots of numerous medicinal, ornamental, and agricultural crops and was first isolated from woody shrubs growing in the Thar Desert of Rajasthan, India. Numerous studies conducted since its discovery have highlighted its positive effects, including stimulation of vegetative growth, early flowering, enhanced nutrient acquisition, improved resistance against pathogens, and increased tolerance to abiotic stresses. In the present investigation, roots of tissue-cultured banana (*Musa* spp.) cv. Grand Naine plantlets were colonized with the culture broth of *P. indica* to assess its influence on plant growth and photosynthetic pigments. The treated plantlets showed improved growth performance in terms of vegetative characters and significant increase in photosynthetic pigment content when compared with untreated control plantlets.

Key Message: *Piriformospora indica* promotes the vegetative growth by enhanced production of Photosynthetic pigments in tissue cultured banana cv Grand Naine

Abbreviation: Chl - Chlorophyll; *P. indica* - *Piriformospora indica*

***Key words*:** *Piriformospora indica*, *Musa paradisiaca*, Grand Naine, Plant growth, Photosynthetic enhancement

Banana (*Musa* spp.) is one of the most important fruit crops cultivated worldwide and serves as a staple food and economic commodity in many tropical and subtropical regions. Belonging to the family Musaceae, bananas are herbaceous flowering plants valued for their edible fruits and high nutritional importance. The crop is predominantly cultivated by small-scale farmers and plays a crucial role in ensuring food security and sustaining the livelihoods of millions of rural populations globally. In India, banana cultivation is widespread and extends across nearly all states except regions experiencing extreme cold climatic conditions. The country possesses rich genetic diversity in banana, with more than 600 reported varieties, although several of these are considered synonymous or closely related cultivars [1].

Banana is one of the most extensively consumed fruit crops in India and holds considerable nutritional, cultural, and economic importance. Due to its year-round availability and affordability, the fruit is widely accepted among all sections of society and forms an essential component of daily diets and social functions. Kerala is rich in banana diversity, with cultivars such as Red Banana, Rasakadali, Nendran, Robusta, and Grand Naine being commonly cultivated. Among these, Grand Naine, a commercially important cultivar of the *Musa* AAA group, is highly valued for its superior yield, market demand, and export potential, contributing significantly to the banana industry in India [2]. Banana fruits are utilized in the preparation of several processed products including milkshakes, banana powder, and ice creams. In addition to the fruit, various plant parts possess economic and traditional significance; banana leaves are widely used as serving plates in South Indian cuisine, while the inflorescence is commonly used in culinary preparations. Moreover, previous studies have reported the antibacterial and antioxidant properties of banana peels and flowers, emphasizing their potential pharmaceutical and nutraceutical applications.

Bananas are nutritionally rich fruits and serve as an excellent source of carbohydrates, vitamins, and essential minerals. They contain significant amounts of vitamins A, B-complex, and C, along with minerals such as calcium, sodium, potassium, and magnesium, which contribute to their dietary importance. Due to their rich nutritional profile, bananas are associated with numerous health benefits including improved digestion, maintenance of blood glucose levels, support in weight management, prevention of anaemia, enhancement of skin health, strengthening of bones, regulation of blood pressure, and promotion of cardiovascular health. Despite their

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high nutritional and commercial value, Grand Naine bananas are highly vulnerable to a wide range of pathogenic diseases, which can adversely affect plant growth, yield, and overall economic returns [3-4].

Endophytes are beneficial microorganisms that reside within plant tissues without inducing any apparent disease symptoms in their host plants. These microorganisms are known to establish symbiotic associations that contribute to enhanced nutrient acquisition, improved resistance against pathogenic organisms, and increased tolerance to abiotic stresses such as drought, salinity, and water deficit conditions [5]. In recent years, endophytes have gained considerable attention due to their ability to stimulate vegetative growth and improve yield in several agriculturally important crops. Studies have also indicated that endophytic interactions can influence plant developmental processes, including the reduction of flowering time and enhancement of overall plant vigor. Moreover, endophyte inoculation has been reported to elevate the production of secondary metabolites, phytohormones and other bioactive compounds of medicinal importance in various plant species, thereby increasing their agricultural, nutritional, and pharmaceutical significance.

Piriformospora indica is a root endophytic fungus, that comes under the order Sebacinales. It can colonize roots of a wide range of plants and tremendously improves the growth and overall biomass production [6-7]. It can also stimulate nutrient uptake and confers resistance to various biotic and abiotic stresses [8]. Recently the modulation of biotic stress in pants by *Piriformospora indica* has received a lot of attention. Atia *et al*. [9] reported that *Piriformospora indica* significantly reduced the root knot nematode infection in cucumber plants. Further it also increased the vegetative growth and chlorophyll content resulting in high yield. Another report indicated that co-inoculation of *Piriformospora indica* and the root pathogen *Plasmodiophora brassicae* in *Brassica campestris* increased the plant biomass and resulted in 60% decrease in gall production [10]. *Piriformospora indica* serves as a promising root endophytic fungus that significantly enhances vegetative growth, photosynthetic pigments, nutrient uptake, stress tolerance, and disease resistance in plants, highlighting its potential application in improving the growth and productivity of tissue-cultured Grand Naine banana plantlets.

Fakhro *et al*. [11] reported an increase in biomass of tomato plants inoculated with *Piriformospora indica*. It also reduced the disease severity caused by *Verticillium* sp. in tomato. Moreover, the biomass of tomato fruit was also increased by *Piriformospora indica*. Deshmukh *et al.* [12] reported that inoculation with *Piriformospora indica* in barley decreased the root rot symptoms caused by *Fusarium* and ultimately resulted in greater yield. In another study Baltruschat *et al*. [13] found that under conditions of salt stress, *Piriformospora indica* markedly increased the concentration of ascorbic acid and increased the activities of antioxidant enzymes in barley roots. The present study was undertaken to evaluate the effect of *Piriformospora indica* on vegetative growth and photosynthetic pigment enhancement in tissue-cultured Grand Naine banana plantlets.

MATERIALS AND METHODS

Tissue culture of Grand Naine banana

Murashige and Skoog (MS) medium was used for the in vitro culture of Grand Naine banana. The media was prepared by adding 34.06 g MS media powder into 1l distilled water. Then 3 ml BAP was added and the medium's pH has been adjusted to 5.8. Then it was solidified with 7.5g agar and was autoclaved at 121 °C and 20 psi pressure for 3o min. The cultures were maintained by regular subculturing every four weeks.

Piriformospora indica culture

The pure culture of *Piriformospora indica* was obtained from College of Agriculture Vellayani, Thiruvananthapuram. It was cultured on potato dextrose agar media and sub-cultured every two weeks. *Piriformospora indica* plugs from the PDA plates were placed in 200 ml potato dextrose broth medium and maintained under normal room temperature in a shaker incubator at 30 rpm to prepare the fungal inoculum.

Endophyte treatment of tissue cultured plants

Plants of 4-5 leaf stage was selected for endophyte inoculation. The roots of these plantlets were immersed in *Piriformospora indica* culture broth for 24 hours so that the roots remain in close contact with the fungal mycelium. Then the plants were transplanted into paper cups containing autoclaved cocopeat and sand mixture (1:1). The plantlets treated with PDB without fungal inoculum were used as control. Thirty days post inoculation the endophyte colonization was detected by histochemical analysis. For histochemical analysis, roots were taken from *Piriformospora indica* treated and control plants. They were washed thoroughly and cut into small pieces. Then they were kept in 10% KOH solution overnight. After washing the roots, they were acidified with 1M HCl for 2-3 min and stained with trypan blue. Then the roots were observed under light microscope at 10- 40X magnifications [14].

Analysis of hardening loss and vegetative characters

Three months after *Piriformospora indica* inoculation, the number of plants survived and lost was analyzed. Vegetative characters such as height of plant, number of leaves, leaf length, leaf width and number of roots were taken after 3 months. Plant height was measured from the soil base to the base of youngest leaf and was expressed in centimeters. Pseudo stem girth was measured at the base above soil surface.

Photosynthetic pigments measurements

The photosynthetic pigments were determined according to Arnon [15] method (1996). 0.5 g of leaves was homogenized in 20ml of 80% acetone. The mixture was centrifuged in 6000 rpm for 10 min. Saved supernatant and the pellet were re-extracted twice. All the supernatants were pooled and made upto 50 ml with adding 80% acetone. Absorbance was recorded at 663nm, 645nm, 480nm and 510nm. The conc. of chl a, chl b, total chlorophyll and carotenoids was calculated using Arnon's equation.

Statistical analysis

All the measurements represent mean value of three replicates ± standard deviation. Statistical significance was analyzed by ANOVA in Micro Soft Excel 2019 software. Differences were considered significant only when the *P*-value was less than 0.05.

RESULTS AND DISCUSSION

Piriformospora indica colonization

Endophyte colonization of root was analyzed by histological analysis. After 1 month, pear shaped chlamydospores and hyphae was detected in *Piriformospora indica* treated plants (Fig 1 a-b). There was no fungal colonization in control plants.

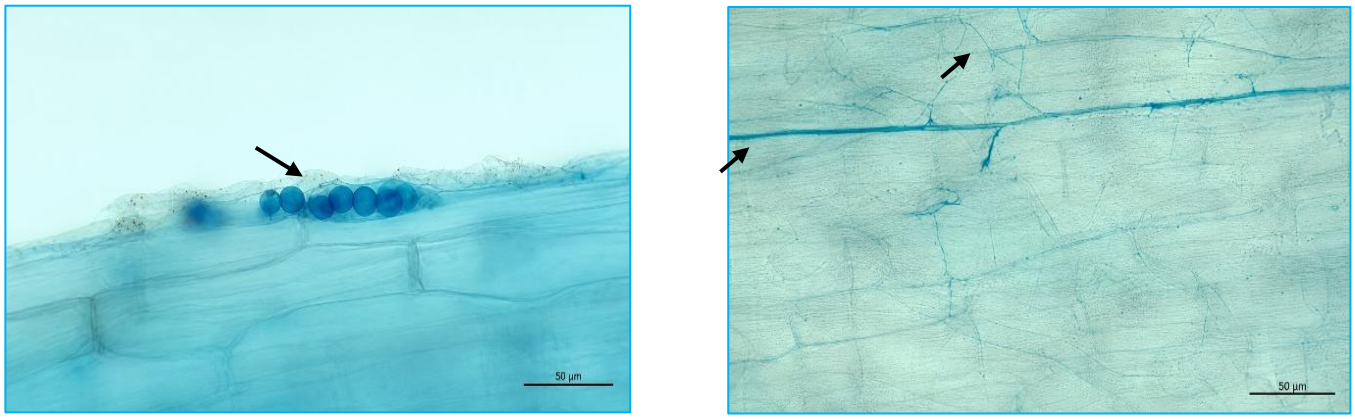

Fig 1 (a) Chlamydospores of *P. indica* in root cortex, (b) hyphae of *P. indica*. Scale bar indicates 50µm

Table 1 Number of plants survived in control and *P. indica* treated group

Treatment	No. of plants at the time of transplantation	No. of plants survived
Control	8	2
Piriformospora indica	8	6

Analysis of hardening loss and vegetative characters

The number of plants survived in control and *Piriformospora indica* treated group were noted after 3 months. There was 75% loss in control plants and 25% loss in *Piriformospora indica* treated plants (Table 1). The effect of *Piriformospora indica* in terms of vegetative growth promotion was examined 3 months after inoculation. *Piriformospora indica* inoculated plants exhibited significantly increased number of leaves, leaf length and width, pseudo-stem girth and more number of roots when compared to the uninoculated control (Table 2). Also, the plant height of *P. indica* treated group were higher when compared to control plants (Fig 2-3).

Table 2 Growth parameters of control and *Piriformospora indica* treated plants

Growth parameters	Control	*Piriformospora indica*
Plant height	8.4 ± 1.5	10.42 ± 0.5**
Number of leaves	7.6 ± 1.3	8.75 ± 0.4*
Leaf length	14.35 ± 2.1	16.35 ± 0.7*
Leaf width	5.13 ± 1.3	6.41 ± 0.5*
Pseudo stem girth	3.28 ± 0.5	3.82 ± 0.1*
Number of roots	7.87 ± 0.7	11.25 ± 0.6**

Results expressed as mean ± standard deviation. Asterisk indicates significant difference (*p< 0.05, **p < 0.005)

Fig 2 Control (left) and *P. indica* (right) treated plants 3 months after inoculation

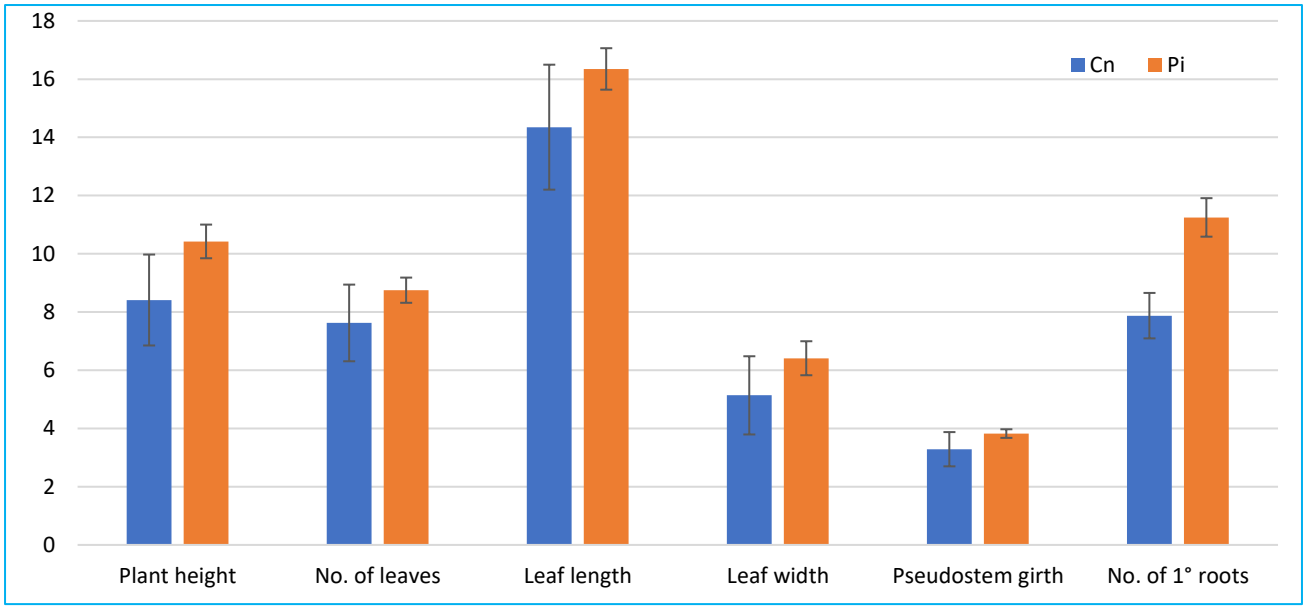

Fig 3 Graph showing growth parameters of *P. indica* treated and control plants. Error bars indicate standard deviation

Photosynthetic pigments

Chlorophyll 'a', Chlorophyll 'b', total chlorophyll and carotenoid were measured in both *Piriformospora indica* treated and control leaves. Photosynthetic pigments were significantly increased in *Piriformospora indica* colonized plants. Furthermore, the carotenoid content was also enhanced in plants colonized with *Piriformospora indica* compared to the control (Table 3, Fig 4).

Table 3 Effect of *Piriformospora indica* on photosynthetic pigments

Photosynthetic pigments	Control	*Piriformospora indica*
Chl a	0.72 ± 0.17	1.5 ± 0.13**
Chl b	0.33 ± 0.06	0.69 ± 0.06*
Total chl	1.05 ± 0.23	2.19 ± 0.2**
Carotenoid	0.68 ± 0.32	1.29 ± 0.13*

Data represents mean ± SD. Asterisk indicates significant difference (*p<0.05, **p<0.01)

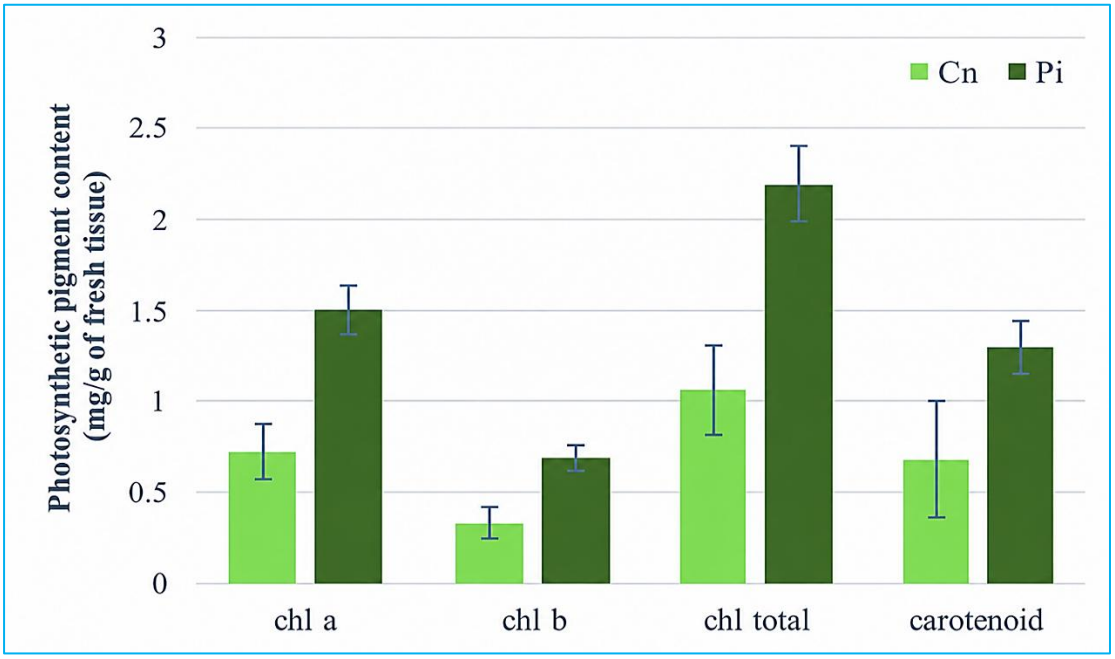

Fig 4 Photosynthetic pigments of *P. indica* treated and control plants. Error bars indicate standard deviation

Piriformospora indica, one of the most beneficial root endophytes, is well known for its beneficial effects including better nutrient acquisition, providing tolerance to various stresses such as salt, water, temperature and drought stress, and also resistance to different pathogenic organisms. In this study we examined the growth promoting effect of the root endophyte, *P. indica* on tissue cultured banana variety Grand Naine. We also analyzed the difference in the photosynthetic pigment content in *P. indica* treated and un treated control plants. For this the tissue cultured plantlets were treated with *P. indica* culture broth. Plants treated with sterile broth served as control. In order to confirm *P. indica* colonization the root

samples were taken and was subjected to histochemical analysis. Pear shaped chlamydospores was observed in the inoculated plant roots. We also observed the fungal mycelium as shown in (Fig 1). Three months after inoculation vegetative parameters were analyzed. It was shown that *Piriformospora indica* treated plants exhibited significantly increased growth parameters. Plant growth promotion by *P. indica* was shown by other researchers also. Similar results were reported by Shah *et al*. [16] in *Cymbidium aloifolium* the plantlets colonized by *P. indica* exhibited higher number of root and shoot and also shoot and root length. Zhen *et al*. [17] colonized the roots of *Brassica napus* with *P. indica* and observed enhanced root growth along with increased root length and lateral roots which was also observed in our study. Ye *et al*. [18] reported a 2-fold increase in root number in *P. indica* colonized *Oncidium* roots compared to the control roots. The stem diameter and leaf number were also increased in *P. indica* colonized plants.

A combined effect of *Piriformospora indica* and two *Pseudomonads* strain were reported in *Vigna mungo* by Kumar *et al*. [19]. They observed a significant increase in dry root weight and shoot weight in *P. indica* treated plants when compared to the control plants. Increase in the number of nodules and pods were also observed in the *P. indica* colonized plants. *P. indica* mediated improvement in vegetative growth was also reported in *Dimocarpus longan* seedlings [20] *P. indica* promoted the root growth and chlorophyll content in longan seedlings. Johnson *et al*. [21] reported that the colonization of Chinese cabbage roots by *P. indica* elevated shoot and root biomass by increasing the auxin concentration. They identified an upregulation in genes related to auxin metabolism and transport in *P. indica* treated plants.

P. indica also increased the photosynthetic pigments compared to the uninoculated control plants. There was significant difference in chlorophyll a, chlorophyll b, total chlorophyll and carotenoid in plants treated with *Piriformospora indica*. Similar results were observed in *Trigonella foenum graceum* where *P. indica* inoculated plants showed an increase in biomass and chlorophyll contents [22]. The effect of *P. indica* on photosynthetic efficiency was also observed in ornamental plants. *P. indica* colonized *Anthurium* plants exhibited higher concentration of chlorophyll content and photosynthesis rate compared to the uninoculated control plants [23]. Wu *et al*. [24] reported an increase in chlorophyll content *P. indica* treated gerbera plants. They also exhibited better plant growth than the untreated controls. Jogawat *et al*. [25] reported a better performance of rice seedlings under salt stress condition when colonized with *P. indica*. There was a significant increase in chl a, chl b and carotenoids in *P. indica* treated rice seedlings. Suthar and Purohit [26] observed significant increase in chlorophyll content and plant biomass in *P. indica* treated *Boswellia serrata*. When *Cucumis sativus* was colonized with *P. indica* and was given water stress, an increase in chl a, chl b, total chlorophyll were reported by Abdelaziz *et al*. [27]. Genes involved in chlorophyll biosynthesis was also found to be increased in *P. indica* treated plants. In *P. indica* colonized date palm Sabeem *et al*. [28] identified that there was an increase in plant growth, lateral roots and chlorophyll content under salt stress and normal state.

Ghorbani *et al*. [29] reported that the colonization of tomato plant roots by *Piriformospora indica* significantly increased the chlorophyll content and photosynthetic efficiency under salt stress when compared to non-colonized control plants. In the soil contaminated with cadmium and polycyclic aromatic hydrocarbon, phenanthrene, *P. indica* treated *Medicago sativa* plants exhibited better performance and significant increase in chl a and chl b [30]. *P. indica* colonized barley plants was able to tolerate the stress induced by lead treatment. The plant biomass was increased in *P. indica* colonized plants compared to the control plants. Also, *P. indica* treated plants exhibited significantly higher photosynthetic pigments than the control plants [31]. Achatz *et al*. [32] also studied the effect of *P. indica* on barley plants. They reported an increased yield along with plant growth and photosynthetic efficiency. In black pepper *P. indica* inoculation resulted in an increased chl a and total chlorophyll content compared to the non-inoculated control. It also increased the yield of black pepper plants [33]. Thus, it is clear that *P. indica* can colonize a wide range of hosts and can improve the performance of different crop plants by enhancing the growth and photosynthesis efficiency.

Hardening loss is a major impediment facing tissue culture industry and results in loss of revenue and hinders the universal acceptance of tissue cultured plants. *Piriformospora indica* significantly reduced hardening loss of tissue cultured banana cv Grand Naine compared to the uninoculated plants. *Piriformospora indica* has been reported to reduce hardening loss and improve survival of micro propagated plantlets by enhancing acclimatization efficiency and minimizing transplant shock. Our findings are in agreement with earlier reports demonstrating the growth-promoting effects of *Piriformospora indica* in tissue-cultured plants during acclimatization and hardening stages. Improved survival and reduced hardening loss observed in the present study may be attributed to the beneficial root colonization by *P. indica*, which has previously been reported to enhance acclimatization efficiency in micro propagated plants [34]. Improved survival and reduced hardening loss observed in the present study may be attributed to the beneficial root colonization by *P. indica*, which has previously been reported to enhance acclimatization efficiency in micro propagated plants [35]. The increased vegetative growth and photosynthetic pigment content observed in the treated plantlets corroborate previous findings on the plant growth–promoting potential of *P. indica* [35]. Enhanced acclimatization and establishment of in vitro-raised plantlets colonized by *P. indica* have also been documented in orchids and other economically important crops [36].

CONCLUSION

Our study proves that *Piriformospora indica* can successfully colonize the roots of banana cv Grand Naine, which was confirmed by histological analysis. It improved the host plant's performance through increased number of leaves, leaf length and width, pseudo-stem girth and a greater number of roots when compared to the uninoculated control plants. The plants colonized by *P. indica* showed increased photosynthetic pigments in tissue culture grand Naine plants compared to the uninoculated control plants. They also showed increased root length. The increased vegetative performance of *P. indica* colonized plants can be attributed to increased photosynthetic pigment synthesis leading to enhanced photosynthetic efficiency. The improvement of root length of *P. indica* colonized plants results in augmentation of water and mineral absorption from soil boosting vegetative characteristics. Thus, our results show *P. indica* can be considered an efficient bio-stimulant for the growth of tissue cultured grand Naine plantlets and preventing hardening loss.

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Author contributions

Data availability: *All the data of this study are available within the paper.*

Statements and declarations

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Conflict of interest

There are no financial or nonfinancial competing interest.

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