Chapter 11
Enhanced Productivity Associated with Tripartite Symbiosis Between *Phaseolus*, Rhizobia, and *Piriformospora indica*: In Presence of Vermicompost

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11.1 Introduction

Piriformospora indica, a fungus belonging to the member of Basidiomyocotina, is a plant growth promoter discovered by Verma et al. (1998). *P. indica* is structurally and functionally similar to arbuscular mycorrhizal fungi (AMF) in several aspects (Oelmüller et al. 2009; Singh 2004; Varma et al. 1999). However, unlike AMF, *P. indica* can be grown in artificial medium (Singh 2004).

This symbiotic fungus mainly associates with plant roots but does not invade the aerial part of the plant. Within the root cortex, they form inter- and intracellular hyphae, often differentiating into arbuscule-like structure and vesicle-like structures. The fungal hyphae multiply within the host cortical tissues without traversing through the endodermis.

P. indica has the ability to colonize the roots of wide host range. Initially, the effect on the growth was studied on *Zea mays*, *Pisum sativum*, *Nicotiana tabacum*, *Glycine max*, etc. (Rai and Varma 2005). Subsequently, it was discovered that the fungus has the ability to stimulate the growth of wide range of plants ranging from medicinal to timber-yielding plants (Rai et al. 2001; Singh 2004). Growth-promoting ability of *P. indica* has been observed in terrestrial orchids such as *Dactylorhiza purpurella*, *D. incarnate*, *D. majalis*, and *D. fuchsii* (Singh 2004). This endophyte has been proven as an effective growth promoter of hydroponic cultures (Fakhro et al. 2010). Plants associated with vesicular–arbuscular (VA) mycorrhiza are benefited compared to nonmycorrhizal plants. The mycorrhizal

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associations have owed plants with resistance to stress such as toxicity, high salinity, and adverse soil pH (Atlas and Bartha 2000; Bagyaraj and Varma 1995).

More importantly the fungi promote the plant growth by facilitating in the uptake of the frequently limiting macronutrients such as nitrogen and phosphorous (Schachtman et al. 1998). Nitrogen gas though abundant in the atmosphere is not readily available for the plant. It is converted into the plant utilizable form by nitrogen-fixing bacteria. The symbiotic fixation by rhizobia in association with leguminous plants contributes for the largest part of nitrogen in terrestrial habitats (Atlas and Bartha 2000).

Larger part of phosphorous is often present in unavailable form in the soil as a result of adsorption, precipitation, or conversion to the organic form (Holford 1997). The rate of diffusion of phosphate is slow in soil. If the plant uptake rate is high, it creates a zone around the root that is depleted of phosphorous (Schachtman et al. 1998). Increased P absorption in plant has been associated with the presence of fungal symbiont (Harrison et al. 1995). The plant-associated mycorrhiza extends extrametrical hyphae in soil facilitating the adsorption of P followed by its translocation through hyphae and mycelia development within root tissue and eventually from fungus to root cells (Joner and Johansen 2000).

Both the rhizobial and mycorrhizal symbioses can act synergistically on promoting plant growth (Jia et al. 2004). Improving the growth of plant leading to the greater yield in the product has been a strategy in agriculture to meet the ever-increasing demand for food in the world. The use of vermicompost for culturing the bean plant inoculated with both rhizobia and mycorrhizal fungi was aimed to enhance the growth, productivity, and nutritional content of the plant.

11.2 Tripartite Symbiosis Within Mycorrhizal Fungi, Rhizobia, and Host

The synergistic interaction within *Rhizobium*-AMF and legume was found to increase the rate of P uptake and N-fixation resulting in an increased crop biomass (Azcon et al. 1991; Xavier and Germida 2002). In *Rhizobium*-AMF–legume tripartite symbiosis relationships, nodulation of rhizobia and establishment of AMF often occur simultaneously and synergistically. Rhizobia provide fixed nitrogen not only to the plant but also to the fungus. Besides, rhizobia can also assist in mobilizing nutrients from the soil and improving the growth of infected plants. AMF on other hand enhance plant growth by absorbing P from soil and transporting it to the roots (Jakobsen et al. 1992). The increment in the P supply as a result of AMF colonization was consistently associated with increase in N accumulation and N productivity (Jia et al. 2004). Coinoculation of soybean with rhizobia and AMF increased the growth under the condition of low P and N content in soil (Wang et al. 2011).

The interaction between rhizobia, AMF, and legume shares parts of the signaling pathways mediated by specific flavonoids (Antunes et al. 2006; Tajini et al. 2009).

The demand for P is found to be greater especially when the N supply in legumes depends upon the rhizobial symbiosis as up to 20 % of total plant P is allocated to nodules (Tajini et al. 2009). The nodule biomass is correlated with the availability of P in plant (Hellsten and Huss-Danell 2001) as the deficiency of P resulted in reduced nodule size (Gunawardena et al. 1992).

11.3 Tripartite Symbiosis Between *P. indica*, *Rhizobium leguminosarum*, and *Phaseolus vulgaris* in Presence of Vermicompost

Vermicompost is a product of biodegradation of organic materials through interactions between earthworms and microorganisms (Sallaku et al. 2009). It is produced by earthworms in the form of worm cast upon feeding on biodegradable materials. Being rich in nitrogen, phosphorus, and potassium (NPK) and important plant growth hormones, vermicompost has been popular organic compost known to enhance the biomass production of numbers of crops (Hidalgo 1999; Pashanasi et al. 1996). Vermicompost has been known to improve the texture and properties of soil (Edwards and Burrows 1988). Besides, it has been found to increase the soil microflora. There has been a significant increase in the colonization of the mycorrhiza in the plant in presence of vermicompost (Kale et al. 1992).

Dual inoculation of legume with mycorrhizal fungi and *Rhizobium* improved nodulation, mycorrhizal colonization, dry weight, and nitrogen and phosphorus content (Manjunath et al. 1984). The *Phaseolus* bean inoculated with *P. indica* and *R. leguminosarum* in presence of vermicompost showed increased length and dry weight of both root and shoot during harvesting stage of plant compared to treatment lacking vermicompost or single inoculation (Fig. 11.1).

The experiment was carried out in soil-filled earthen pot, and vermicompost was supplemented where it is required. Inoculation of *P. indica* and *R. leguminosarum* were done where necessary following the germination of seed. The length and weight of the root and shoot were measured 30 days after the sowing of the seeds for flowering stage. Similarly harvesting was done after 50 days.

The growth of plant in terms of shoot and root parameters was relatively highest in dual inoculation during the flowering stage (Figs. 11.1 and 11.2), however, not significantly different from the dual treatment supplemented with vermicompost. The growth of plant was found to be highest in the dual treatment supplemented with vermicompost during the harvesting stage (Figs. 11.1 and 11.3).

Similarly the yield was highest in the plant treated with *P. indica* and *R. leguminosarum* in presence of vermicompost (Fig. 11.4) followed by *P. indica*- and *R. leguminosarum*-treated plant without vermicompost. The yield was estimated from the number of bean pods in each plant.

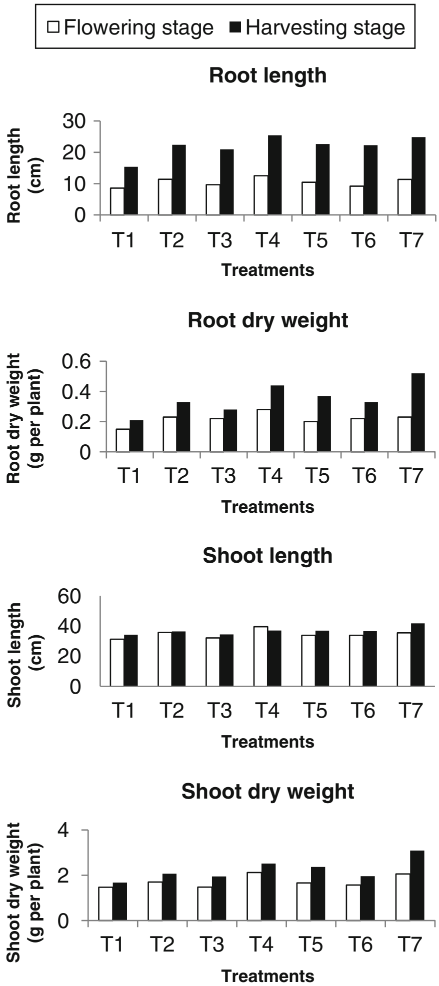

Fig. 11.1 Root and shoot length and dry weight production during flowering stage and harvesting stage in different plant treatments T1 (control), T2 (*P. indica*), T3 (*Rhizobium*), T4 (*P. indica* and *Rhizobium*), T5 (*P. indica* and vermicompost), T6 (*Rhizobium* and vermicompost), and T7 (*P. indica*, *Rhizobium*, and vermicompost)

11.4 Mycorrhizal Colonization Associated with Vermicompost

Measurements of the extent to which roots are mycorrhizal have been used to indicate the abundance of mycorrhizal fungi in soil (Hayman and Stovold 1979; Sparling and Tinker 1978). The active symbiotic phase is reflected from the mycorrhizal root colonization (Singh 2004). The mycorrhizal root colonization was highest in the plant inoculated with *P. indica* and Rhizobium with vermicompost supplement incorporated (Fig. 11.5). There was an increased colonization in the harvesting stage compared to the flowering stage (Fig. 11.5). The root colonization of *P. indica* in *Adhatoda vasica* was estimated to 95 % after 6 months (Rai and Varma 2005).

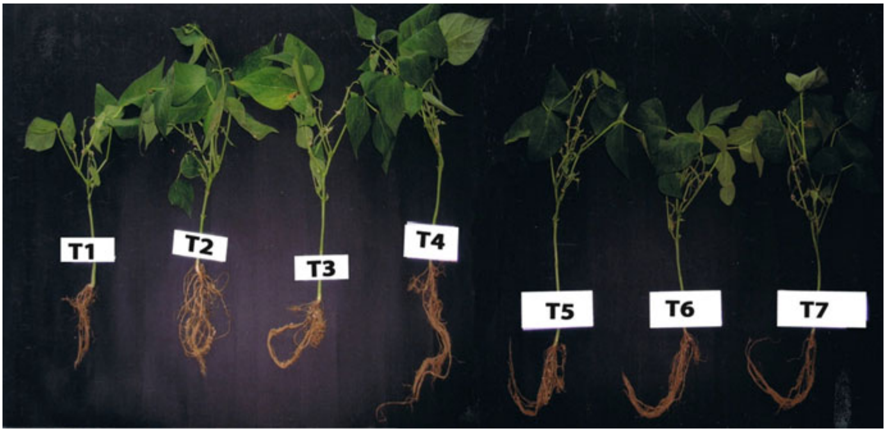

Fig. 11.2 *Phaseolus* plants of different treatment at flowering stage. T1 (control), T2 (*P*. *indica*), T3 (*Rhizobium*), T4 (*P*. *indica* and *Rhizobium*), T5 (*P*. *indica* and vermicompost), T6 (*Rhizobium* and vermicompost), and T7 (*P*. *indica*, *Rhizobium*, and vermicompost)

Fig. 11.3 *Phaseolus* plants of different treatment at harvesting stage. T1 (control), T2 (P. *indica*), T3 (*Rhizobium*), T4 (*P*. *indica* and *Rhizobium*), T5 (*P*. *indica* and vermicompost), T6 (*Rhizobium* and vermicompost), and T7 (*P*. *indica*, *Rhizobium*, and vermicompost)

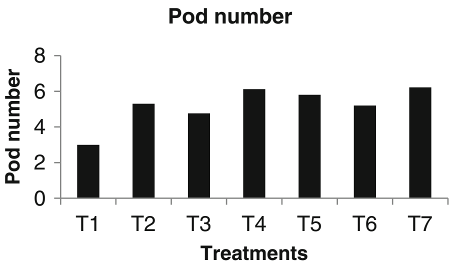

Fig. 11.4 Yield in *Phaseolus* pod followed by different treatments during the harvesting stage. The seven different treatments as mentioned in Fig. 11.1

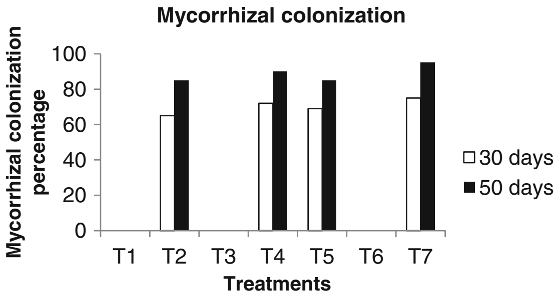

Fig. 11.5 Percentage of mycorrhizal colonization of root in *Phaseolus* in different treatments during flowering and harvesting stage. The seven different treatments as mentioned in Fig. 11.1

11.5 Nitrogen, Phosphorus, and Potassium Content in the Plant

Nitrogen, phosphorus, and potassium are most essential nutrients required for the growth of the plant. Nitrogen being the key building block of the protein and present in the nucleic acid is indispensable component of the cell. Phosphorous is present in biomolecules such as nucleic acid, phospholipids, and ATP making it important for plant. Potassium promotes the root growth in plants and assists the absorption of minerals.

Organic fertilizers are known to contain NPK essential for plant. Vermicompost has been superior organic manure enhancing biomass production of number of crops (Hidalgo 1999). It has high percentage of NPK and water retention ability which help increase the soil fertility (Acharya 1997; Edwards and Burrows 1988). The efficient uptake of these elements by plant is equally important for the growth of the plant, and this ensures the maximum utilization of the vermicompost.

The presence of rhizobia has been reported to significantly increase the uptake of N, P, and K by rice plants (Biswas et al. 2000). Nitrogen uptake has been increased in dual inoculation of *Bradyrhizobium japonicum* with endomycorrhizal fungi compared to the single (Shalaby and Hanna 1998). The importance of phosphorus in the rhizobia-AMF–legume tripartite symbiosis is reflected from the fact that for one molecule of nitrogen to be fixed to ammonia, 16 ATP is required (Theodorou and Plaxton 1993). Presence of vermicompost will enhance in the content of the essential nutrient in the soil as it led to significant increase in soil enzyme activities such as urease, phosphomonoesterase, and phosphodiesterase (Albiach et al. 2000). Besides plant growth-promoting bacteria stimulate solubilization of nutrients (Rodriguez and Fraga 1999) and production of growth hormones (Correa et al. 2004).

This will directly affect the nutrient content in the plant. In this experiment, the dual inoculation with *P. indica* and *Rhizobium* in presence of vermicompost led to highest percentage of NPK in the shoot compared to the rest of the treatment (Fig. 11.6). Similar result was observed in the percentage of NPK in the root (Fig. 11.7). The control plant without any treatment showed the lowest percentage of NPK content.

The significant increase in the shoot and root potassium and nitrogen content in the dual inoculation as compared with the single inoculation is consistent with the fact that the symbiosis, rather than compete with each other for the nutrient, complement each other by enhancing the plant's nutrient acquisition strategies.

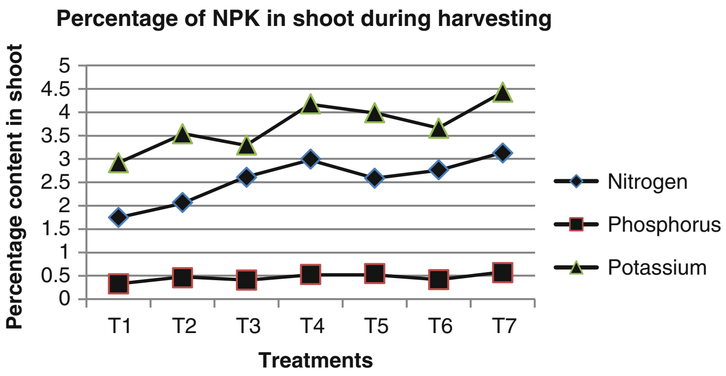

Fig. 11.6 Percentage of nitrogen (*diamond marked*), phosphorus (*square marked*), and potassium (*triangular marked*) present in the shoots of plants under different treatments during the harvesting stage

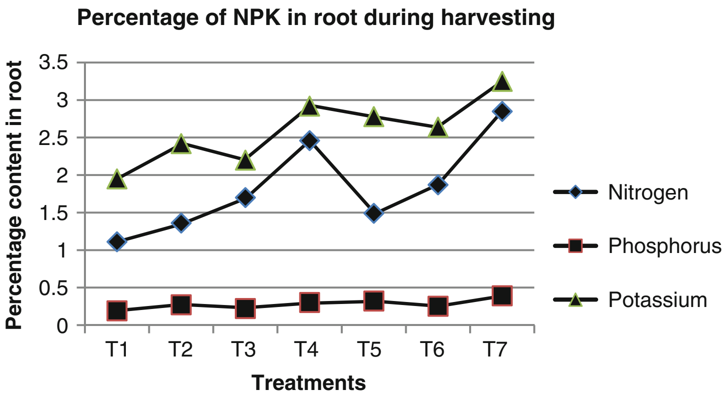

Fig. 11.7 Percentage of nitrogen (*diamond marked*), phosphorus (*square marked*), and potassium (*triangular marked*) present in the roots of plants under different treatments during the harvesting stage

11.6 Conclusion

The soil rich in NPK and other nutrients increases the rhizobial and mycorrhizal symbiotic relationship with plant which in turn have benefit on the growth and nutritional content of the plant. Vermicompost serves as the superior grade of compost for enriching the soil. This study opens possibility for further assessing the tripartite symbiotic relationship in presence of nutritional rich soil for the resistance of plant against potential plant diseases.

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