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Plant Growth Promotional Effect of *Azotobacter chroococcum*, *Piriformospora indica* and Vermicompost on Rice Plant

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Abstract

The activities of rhizospheric organisms have been well recognized in non-leguminous plants such as tropical grasses, rice and maize. Such activities include nitrogen fixation, phosphate solubilization and mineralization that are beneficial for the overall growth and development of the plant. An experiment was carried out to study the growth promotion of rice (*Oryza sativa* L.) due to dual inoculation of *Azotobacter chroococcum* and *Piriformospora indica* along with vermicompost. The effects on shoot length, root length, fresh shoot and root weight, dry shoot and root weight, and panicle number on 45th day and 90th day were investigated. In both the stages these parameters of all the treated plants were significantly ($P<0.05$) greater than the uninoculated control. Dual inoculated plants in presence of vermicompost gave better positive effects on both 45th day and 90th day, in comparison to single inoculation of *A. chroococcum*, *P. indica* and vermicompost. However, *A. chroococcum* treated plants showed significant decrease in dry root weight as compared to control plants on 90th day observation. This suggested that dual inoculation of *A. chroococcum* and *P. indica* had beneficiary response on growth of rice plant.

Key words: rice, *Azotobacter chroococcum, Piriformospora indica*, vermicompost, mycorrhizae nitrogen fixation

Introduction

The agriculturally important rhizospheric microorganisms play a remarkable role in nutrient acquisition for plants that ultimately lead improved growth parameters. In pursuit of that goal, various workers have used arbuscular mucorrhizal fungi and nitrogen fixing bacteria, as single inoculants and in combination with each other in various plants (Lauchli & Bieleski 1983).

Azotobacter represents the main group of heterotrophic free living nitrogen-fixing bacteria. They are Gram negative, large ovoid pleomorphic cells of 1.5-2.0 µm or more in diameter ranging from rods to coccoid cells. They occur singly, in paired or irregular clumps and sometime in chains of varying length. They do not produce endospores but form cysts. They are motile by peritrichous flagella or non motile. They are motile by peritrichous flagella or non-motile. *Azotobacter* spp. are most specifically noted for their nitrogen fixing ability but they have also been noted for their ability to produce different growth hormones (IAA and other auxins, such as gibberllins and cytokinins), vitamins and siderophores (Narula *et al.* 1981, Neito & Frankenberger 1989, Tindale *et al.* 2000). *Azotobacter* is capable of converting nitrogen to ammonia (Bishop *et al.* 1980), which in turn is taken up by the plants.

Vesicular arbuscular mycorrhizae (VAM) are beneficial fungi that penetrate and colonize the roots of the plant, then send out filaments (hyphae) into the surrounding soil. The term mycorrhizae literally means fungus-root and VA mycorrhizae (VAM) are considered endomycorrhizal since they colonize the interior parts of the roots. They are associated with the plant in a mutually beneficial relationship. The VAM fungi, nestled inside the roots, send out long filaments or hyphae to explore up to the soil area available to the root alone. The hyphae literally form a bridge that connect the plant root with large areas of soil and serve as a pipeline to funnel nutrients back to the plant. In return, the plant must supply the VAM fungi with carbon for the fungal growth and energy needs.

An axenically cultivable mycorrhiza-like fungus was discovered by Varma and his co-workers (Varma *et al.* 1998). The fungus was named *Piriformospora indica*, based on its characteristic pear-shaped spores. Based on the 18S and 28S rRNA analysis and the ultra-structural details of the septal pores, the phylogenetic relationship of this fungus was established within the Hymenomycetes (Basidiomycotina) and closest to Rhizoctonia group and *Sebacina vermifera* (Varma *et al.*1998, Singh 2004). *P. indica* is the first symbiotic fungus, known in the literature which can be grown on root of a living plant and under axenic culture (Singh 2004). The properties *P. indica* have been patented at the European patent office, Muenchen, Germany (Patent No. 97121440.8-2105, Nov. 1998). It enters the root cortex to form inter- and intra-cellular hyphae. Within cortical cells the fungus often forms dense hyphal coils or branched structures intracellularly. *P. indica* also forms spore or vesicles like structures within or between the cortical cells. Like in AM fungi, hyphae multiply within the host cortical tissues and never traverse through the endodermis. Likewise, they also do not invade the aerial portion of the plant (stem and leaves). Interestingly, the host spectrum of *P. indica* is very much like AMF. The hyphae are highly interwoven, often adhered together and give the appearance of a simple intertwined cord. Hyphae are thin- walled and of different diameters ranging from 0.7 to 3.5 ì m. The septate hyphae often show anastomosis and are irregularly septate. They often intertwine and overlap each other. Chlamydospores are formed from thin-walled vesicles at the tips of the hyphae. The chlamydospores appear singly or in clusters and are distinctive due to their pear shaped appearance. The chlamydospores are 16-26 ìm in length and 10-17 ìm in width. The cytoplasm of the chlamydospores is densely packed with granular material and usually contains 8-25 nuclei. Very young spores have thin, hyaline walls. At maturity, these spores have walls up to 1.5 ìm thick, which appear two-layered, smooth and pale yellow. Neither clamp connections nor sexual structures are observed (Varma *et al.* 2002, Singh 2004).

Vermicomposting is largely a biological process in which of aerobic and anaerobic microorganisms decompose organic matter and lower the carbon-nitrogen ratio of the substrate. Vermicompost contains major and minor nutrients in plant-available forms, enzymes, vitamins and plant growth hormones (Kerala Agricultural University 2002). It is an excellent soil additive made up of digested and undigested compost. Earthworm castings are much higher in nutrient contents than microbial life and have a high value product.

Materials and Methods

The rice variety used in this study was Khumal-4, which was collected from Annapurna Seed Bhandar of Kathmandu valley. A pure culture of *P. indica* was obtained from the Central Department of Microbiology, Kirtipur while *A. chroococcum* was isolated from vermicompost at the Biotechnology Laboratory of Nepal Academy of Science and Technology (NAST), Khumaltar.

A. chroococcum inoculum was prepared in Jensen's broth on a rotary shaker for 5-7 days at 26 °C- 28 °C at 20 rpm. Literature suggests that for plant inoculation, the cultures should reach mid-log phase, which is equivalent to 10^7-10^8 cells per ml (Zhang *et al.* 2002). *P. indica* inoculum was prepared on PDA by incubating at 25 °C- 27 °C for about one week. *P. indica* culture was cut into pieces of 4 mm diameter. Vermicompost was obtained from NAST, Khumaltar.

There were five treatments which were control plants without any inoculation (T1), plants inoculated with vermicompost only (T2), plants inoculated with *A. chroococcum* alone (T3), plants inoculated with *P. indica* alone (T4) and plants inoculated with *P. indica*, *A. chroococcum* and vermicompost (T5). The earthen pots used for the pot culture experiment were filled with sterilized soil. Vermicompost was initially added to the soil. Ten healthy seeds were sown in each pot at a depth of 2 cm of soil and left for germination. After 7 days of seed germination, the *P. indica* inoculum was carefully placed near the root surface. After 5 days of the application of *P. indica*, 1ml *Azotobacter* broth with inoculum size 1.6×10^7 cfu/ml was applied to each plant per pot. After 25 days of seed sowing the seedlings were thinned manually leaving eight healthy seedlings per each pot. Growth parameters (shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and panicle number) were measured on 45^{th} (vegetative stage) and 90^{th} (reproductive stage) days of cultivation.

Results

Effect of dual inoculation of *A. chroococcum* and *P. indica* on various growth parameters of rice plant at vegetative (45th day) and reproductive (90th day) stages grown in the soil treated with vermicompost have been presented in Table 1 and 2 respectively. Significant differences were observed in all of the treatments for all the parameters at $p<0.05$. All the growth parameters showed positive response against the treatment over control except dry root weight in plants treated with *A. chroococcum* in reproductive stage where negative response was observed against control.

Table 1. Effects of treatment on growth parameters of rice plant at vegetative stage

Treatment	ShootLength (cm)	Root length (cm)	Fresh shoot wt. (gm)	Dry shoot wt. (gm)	Fresh root wt. (gm)	Dry root wt. (gm)
Control	31.33^{a}	10.68^{a}	0.68^{a}	0.22^{a}	0.24^{a}	0.05^{a}
T1	35.63^{b}	14.75^{b}	1.22^{b}	0.27^{a}	0.42^{a}	0.07^{a}
T2	35.0^{b}	13.71^{b}	0.84^{a}	0.24^{a}	0.36^{a}	0.12^{b}
T3	36.7^{b}	15.38^{b}	1.03^{a}	0.4^{b}	0.76^{b}	0.18^{b}
T4	37.38^{b}	17.08^{a}	1.74^{c}	0.66^{c}	0.93^{b}	0.25^{b}
Mean	35.21	14.32	*1.10*	*0.36*	*0.54*	*0.14*
LSD at 0.05	2.23	0.95	0.10	0.08	0.21	0.09

Note : In a column followed by a common letter are not significantly different at the 5% level by MRT

Table 2: Effects of treatment on growth parameters of rice plant at reproductive stage

Treatment	ShootLength (cm)	Root length (c)	Fresh shoot wt. (gm)	Dry shoot wt.(gm)	Fresh root wt.(gm)	Dry root wt.gm)	Panicle Number
Control	100.67^{a}	35.21^{a}	23.31^{a}	16.39^{a}	19.81^{a}	9.7^{a}	0.79^{a}
T1	101.21^{a}	35.26^{b}	24.21^{a}	17.96^{b}	26.19^{b}	10.34^{a}	2.64^{b}
T2	100.88^{a}	35.22^{b}	24.06^{a}	16.56^{a}	20.14^{a}	8.95^{a}	2.43^{b}
T3	101.19^{a}	37.86^{b}	29.05^{b}	18.56^{b}	23.33^{b}	10.33^{a}	2.7^{b}
T4	107.18^{b}	39.63^{b}	32.29^{c}	23.38^{c}	38.54^{c}	16.93^{b}	3.6^{c}
Mean	102.23	36.64	26.59	18.57	25.60	11.25	2.41
LSD at 0.05	2.02	2.23	1.56	0.73	2.38	0.97	0.31

Note: In a column followed by a common letter are not significantly different at the 5% level by MRT

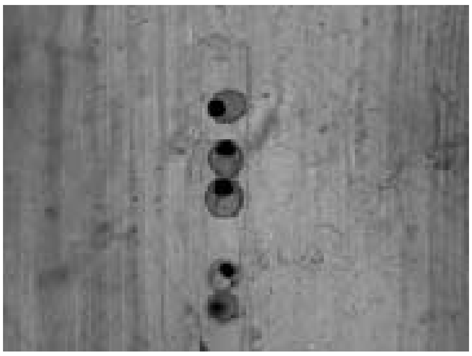

Fig. 1. Chlamydospores of P. indica within the cortical cells

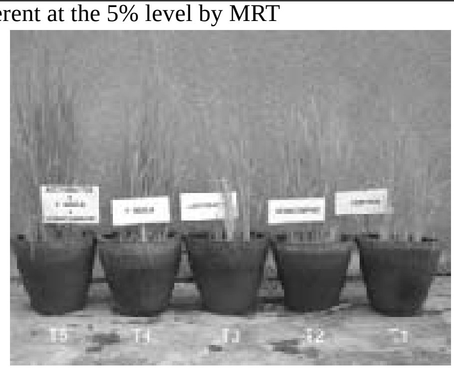

Fig. 2. Effect of treatments on growth of rice plant at vegetative stage

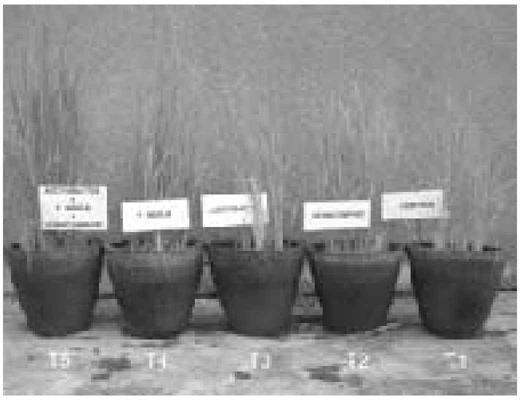

Fig. 2. Effect of treatments on growth of rice plant at reproductive stage

Discussion

The experiment was conducted in sterilized soil so that effect of the inoculant could be studied without the interference of normal flora and fauna of the soil. The phytopromotional effect of treatments at vegetative and reproductive stages are shown in Fig. 2 and 3.

A significant positive response was observed with single inoculation of *A. chroococcum* in all growth parameters (viz. shoot length, root length, shoot fresh and dry weight, root fresh and dry weight and panicle number) of both vegetative and reproductive stages of rice plants can be attributed to the ability to fix atmospheric nitrogen. Nitrogen is usually the nutrient that limits plant production in wetlands (Buresh *et al.*1990) and, under N-limited conditions, plant roots excrete compounds with high C/N ratios, favoring rhizospheric N_2 fixation (Klein *et al.* 1990). Till now, the explanations offered to account for the beneficial action of non-symbiotic microorganisms on plants have been two fold, first is the nitrogen fixing ability of the microorganisms and second is the ability of microorganisms to elaborate growth promoting substances such as vitamins, hormones and amino acids (Azcón *et al.* 1978). Shende *et al*. (1977) attributed the observed beneficial response of crop plants to inoculation with *A. chroococcum* to growth substances produced by the organisms in addition to the fixed nitrogen made available to the plants. In a similar study done by Barik and Goswami (2003), seed inoculation with *A. chroococcum* strains significantly influenced the growth attributes, yield attributes and yield of wheat. So, plant growth promotion in terms of shoot length, root length, shoot fresh and dry weight, fresh root weight and panicle number of both vegetative and reproductive stage over the control by *Azotobacter* inoculation may be due to synergistic effects of several factors. But the decrease in dry root weight in reproductive stage in *Azotobacter* inoculated plants though the length and dry weight of root is superior to the controls, may be due to variable water content that is dependent in plants water status.

The rice plants inoculated with *P. indica* showed significant beneficial effect in all the growth parameters in both vegetative and reproductive stages over the uninoculated control plants. *P. indica* inoculated plants were superior to *A. chroococcum* inoculation and vermicompost treatment in shoot length, root length and dry shoot weight in vegetative stage, and root length, dry shoot weight and panicle number in reproductive stage. In case of fresh root weight in vegetative stage and dry root weight in reproductive stage, the effect of *P. indica* was the same as that of vermicompost. *P. indica*, an endophytic fungus of the Sebacinaceae family, resembles AM fungi in several functional and physiological characteristics. It improves the growth and biomass of wide host range including rice plant and is an efficient phosphate solubilizer and transporter. *P. indica* is able to enhance the absorption of nutrients from the soil which could have moved to the roots principally by mass flow, in addition to those, which could have diffused through the soil slowly. It is envisaged that AM fungal mycelium acts as a key component in a close 'cause and effect interchange' of mineral nutrients, carbon compounds, and signal between the plant and rhizosphere population and soil aggregation. Maize, another important cereal crop was reported to produce more root and shoot biomass than control plants when inoculated with *P. indica* (Singh 2004). Waller *et al*. (2005) studied the interaction of *P. indica* in monocotyledonous plants by establishing *P. indica*-barley system in the laboratory. Infestation of barley roots with *P. indica* led to growth promotion and a modulation of resistance not only in the roots, but also in the leaf.

Beneficial effect on host plant as a result of mycorrhizal infection is usually associated with improved plant nutrition, especially phosphorus by virtue of extensive root system that extend the functional mycelium into surrounding soil, making a greater pool of nutrients available to the plant. This leads to increased plant growth, often as high as several hundred-fold increases in biomass (Menge 1983). *P. indica*, an AM-like fungi, has been found to mediate phosphorus uptake from the medium and translocate it to the host in an energy dependent process by producing significant amount of acid phosphates for the mobilization of broad range of insoluble forms of phosphates, enabling the host plant the accessibility of adequate phosphorus from immobilized reserves in the soil (Varma *et al.* 2000).

Plants treated with vermicompost also showed significant improved growth over the untreated control plants in both vegetative and reproductive stages. Vermicompost treated plants were even promoted than *A. chroococcum* in all the above measured parameters except in case of shoot length in reproductive stage whereas its treatment was better than *P. indica* in fresh shoot weight and dry root weight in vegetative stage and shoot length and fresh root weight in reproductive stage.

Vermicompost is an excellent soil additive that contains water soluble colloidal worm cast, nitrogen, phosphorus, and potassium contents along with a large amount of enzymes and growth hormones. When such compost is applied to the plants, due to the water soluble nature of vermicompost, the nutrients they contain become easily available to the plants roots. Basnet (2006) found that vermicompost sample of cow dung contain organic matter (50.25%), nitrogen (1.96%), phosphorus (2.33%) and potassium (4.99%). The vermicompost sample contained several times more nitrogen, phosphorus and potassium than the soil sample. These added mineral nutrients may be the factors responsible for the enhanced growth of the vermicompost treated plants.

In the pot culture trial, plants treated with dual inoculation of *A. chroococcum* and *P. indica* in vermicompost treated soil showed significant beneficial effect on all the measured parameters.

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