Improvement of Health Status of *Litsea monopetala* using Plant Growth Promoting Rhizobacteria

Amrita Acharya, Usha Chakraborty and Bishwanath Chakraborty*

Immuno-Phytopathology Laboratory, Department of Botany, University of North Bengal, Siliguri, West Bengal (734 013), India

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Correspondence to

**E-mail*: bncnbu@gmail.com

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Abstract

Litsea monopetala (Roxb.) Pers. (Lauraceae) is one of the primary host plants of *muga* silk worm in Northeast India, with the local name *Sualu*. In *muga* sericulture, the *muga* silkworm *Antherea assamensis*, is fed on the leaves of Sualu, the primary host plant. A study was made to improve the quality and quantity of the leaves of the plant through the application of different plant growth promoting rhizobacteria (PGPR). Two isolates each of *B. pumilus* (BRHS/C1and BRHS/T82) and *B. altitudinus* (BRHS/P22 and BRHS/S73) and one isolate of *Paenibacillus lentimorbus* (TRS-5) were tested for their growth promoting activity in this plant. These were directly applied as foliar spray and also applied to the soil. Results were computed in terms of increase in plant height, leaf area index and compared with control sets. Among these, *B. pumilus* (BRHS/C1) was found to be more effective on growth and development of plants followed by *B. pumilus* (BRHS/T82), *P. lentimorbus* (TRS-5), *B. altitudinus* (BRHS/P22) whereas *B. altitudinus* (BRHS/S73) showed less effect. A significant increase in phenolics, total protein content as well as defense related enzymes such as peroxidase, phenylalanine ammonia-lyase, chitinase and β-1,3-glucanase were also observed following application of PGPR. In this present study *B. pumilus* (BRHS/C1) was found to be most effective in promoting over-all growth of *Sualu* plants which is a promising trait for utilizing such beneficial PGPR for effective plant growth-promotion that broadens the spectrum of use of beneficial microorganisms for sustainable crop management.

1. Introduction

Sericulture is an agro-based industry playing an important role in rural economy of the country. Among the four types of sericulture, *muga* is the most ancient form practiced in the North-eastern States in India. *Muga* silk is the product of the silkworm *Antheraea assamensis* endemic to Assam. *Litsea monopetala* (*Sualu*) is one of the primary host plant of *muga* silkworms. It is a small tree up to 18 m tall, bole straight to crooked, up to 60 cm in diameter, bark surface longitudinally fissured, dark greyish, inner bark brown mottled. Leaves alternate, 4.5-17 cm×2.5-10 cm, blunt to acute, glabrous above, sparsely hairy below, midrib sunken above, with 6-13 pairs of secondary veins which are sunken above, tertiary venation scalariform, distinct below, petiole 1-2.5 cm long. *Sualu* is a perennial crop which is cultivated mainly as the foliage of the *muga* silkworms. Silkworms feed on the leaves during their entire larval period; therefore, the leaf quality has a direct bearing on the biosynthesis of silk fibre and ultimately the silk production and quality.

Plant growth-promoting rhizobacteria and their utilization for crop improvement as well as for plant disease management have been described by Chakraborty et al. (2012). PGPR enhances plant growth by direct and indirect means. Some of these mechanisms, which can probably be active simultaneously or sequentially at different stages of plant growth, are increased mineral nutrient solubilization and nitrogen fixation, making nutrients available for the plant; suppression of soilborne pathogens (by the production of hydrogen cyanide, siderophores, antibiotics, and/or competition for nutrients); improving plant stress tolerance to drought, salinity, and metal toxicity; and production of phytohormones such as indole-3-acetic acid (Gupta et al. 2000). Moreover, some PGPR have the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyses ACC, the immediate precursor of ethylene in plants (Glick, 1995). The aim of the present study was to investigate the effects of selected PGPR strains to test their efficacy on the growth and improvement of the *Sualu* plant, which in turn will give a good source of food material for the *muga* silkworm larvae for better silk production.

2. Materials and Methods

2.1. Collection of sample

Around 20 saplings of *Sualu* plants were collected from Central Silk Board, Kalimpong, West Bengal and brought to the nursery of Immuno-phytopathology laboratory, Dept of Botany, NBU. These saplings were then transferred to pots and kept in the net-house for 60 days. Five different rhizobacteria with well characterized growth promoting activity were obtained from the Immuno-phytopathology laboratory, Dept of Botany, NBU. They were *Bacillus pumilus* (BRHS/C1), *Bacillus pumilus* (BRHS/T82), *Bacilus altitudinus* (BRHS/P22), *Bacillus altitudinus* (BRHS/S73) and *Paenibacillus lentimorbus* (TRS-5).

2.2. Inocula preparation and application

The five selected strains of bacterial cultures (BRHS/C1, BRHS/T82, BRHS/P22, BRHS/S73 and TRS-5) were grown in nutrient broth for 48hrs and then centrifuged at 15000 rpm for 15mins. The pellet obtained was re-suspended in sterile distilled water. The optical density of this suspension was measured using a UV-VIS Spectrophotometer at 600 nm, to obtain a final density of $3x10^6$ $cfuml^{-1}$. Tween20 (few drops) was added to the bacterial aqueous suspension and applied as foliar spray and soil drench @200 ml^{-1} of the two months old potted *Sualu* plants. Each treatment set had a control and three replicates.

2.3. Biochemical analysis

Phenylalanine Ammonia-Lyase (Chakraborty et al. 1993), Peroxidase (Chakraborty et al. 1993), Chitinase (Boller and Mauch, 1988), β-1,3 glucanase (Pan et al. 1991), Total and Orthophenol (Mahadevan and Sridhar 1982) and Total Protein Content (Lowry et al., 1951) were assayed with the young leaves of the plants after 72 hours of soil application of bacterial suspension.

3. Result and discussion

3.1. Effect of PGPR on the growth of the plant

After the application of the five different strains of PGPRs to the leaves and rhizosphere of the two months old potted *Sualu* plants, growth pattern in terms of average height and average leaf surface area of the plant was checked at 15days interval. It was observed that the growth of the plant was enhanced in case of all the treatments. However growth was more prominent with respect to height in case of treatment with *B. pumilus* (BRHS/C1). With respect to leaf surface area growth was more pronounced incase of treatment with *P.lentimorbus* (TRS-5) (Tables 1 and 2, Figure 1). Unni et al. (2008) reported that a combination of five different rhizobacterial strains showed maximum improvement in the quality and quantity of leaves produced by Som (*Persea bombycina*) plants, another host plant of *muga* silk worm. Improvement in growth of different plants by the application of PGPR has earlier been reported by Gholami et al. (2009) in Maize, Chakraborty et al. (2012) in Tea, Meera and Balabhasker (2012) in Rice, Zhang et al. (1997) in Soyabean.

3.2. Effect of PGPR on the biochemical parameters of plant

3.2.1. Estimation of defense enzyme

The various defense enzymes in the plants viz. phenylalanine ammonia lyase (PAL), peroxidase (POX), chitinase and β-1,3 glucanase were assayed using young tender leaves of the plants. It was observed that there was a marked increase in the level of these enzymes in the leaves of the treated plants as compared to the control sets. Among the different types of treatment the plants treated with *B. pumilus* BRHS/C1 showed the highest level of enzyme accumulation within 72 hours of treatment. The results are showed in Figure 2(A-D).

3.2.2. Estimation of phenolics

The total and O-dihydroxy phenol content were estimated with the young and tender leaves of the control and treated plants. There was an increase in the phenolics content following the treatment with the PGPR but the total phenol content was highest in the plants treated with *B. pumilus* (BRHS/T82), whereas the O-dihydroxy phenol content was highest in the case of plants treated with *B. pumilus* (BRHS/C1) (Figure 3a and b)

3.2.3. Estimation of total soluble proteins

The total protein content of the young leaves of the *Sualu*

Table 1: Average height (cm) of the treated and control plants of *Sualu* after 15 and 30 days of application

Treatment	15 Days	30 Days
Control	6.0±1.15	10±1.15
B. pumilus (BRHS/C1)	11.35±1.18	17.3±0.57
B. pumilus (BRHS/T82)	9.5±1.00	16 .0±1.52
B. altitudinus (BRHS/P22)	8.5±0.57	14.0±0.73
B. altitudinus (BRHS/S73)	10.0±1.15	14.6±0.73
P. lentimorbus (TRS-5)	9.5±0.57	15.0±2.00

Table 2: Average leaf surface area (cm^2) of the treated and control plants of *Sualu* after 15 and 30 days of application

Treatment	15 Days	30 Days
Control	6.6±0.63	8.03±1.47
B. pumilus (BRHS/C1)	8.8±0.611	11.5±0.57
B. pumilus (BRHS/T82)	8.5±0.57	11.1±1.21
B. altitudinus (BRHS/P22)	7.3±0.63	10.2±0.63
B. altitudinus (BRHS/S73)	7.2±0.63	10.2±0.57
P. lentimorbus (TRS-5)	8.2±0.63	12.2±0.60

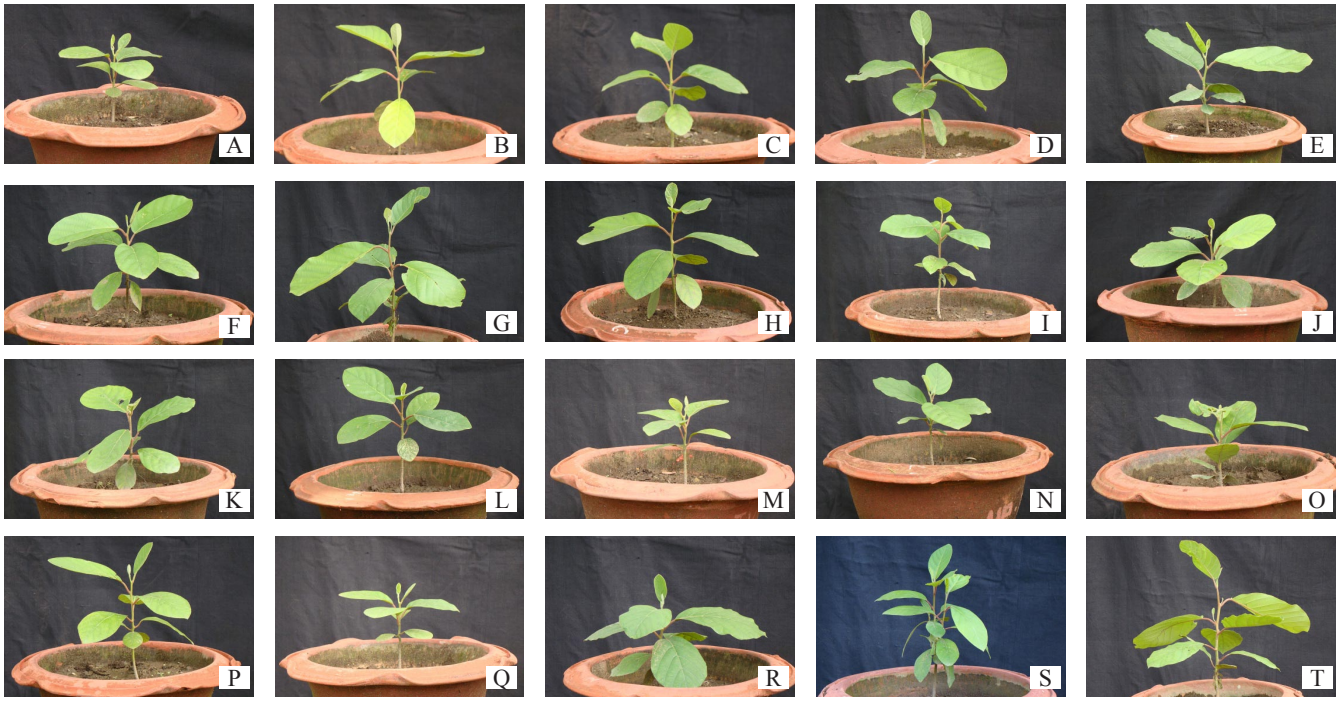

Figure 1: A-Control; B,C,D-*Bacillus pumillus* (BRHS/C1); E-Control; F,G,H-*Bacilus pumillus* (BRHS/T82); I-Control; J,K,L-*Penibacillus lentimorbus* (TRS-5); M-Control; N,O,P-*Bacillus altitudinus* (BRHS/S73); Q-Control; R,S,T-*Bacillus altitudinus* (BRHS/P22)

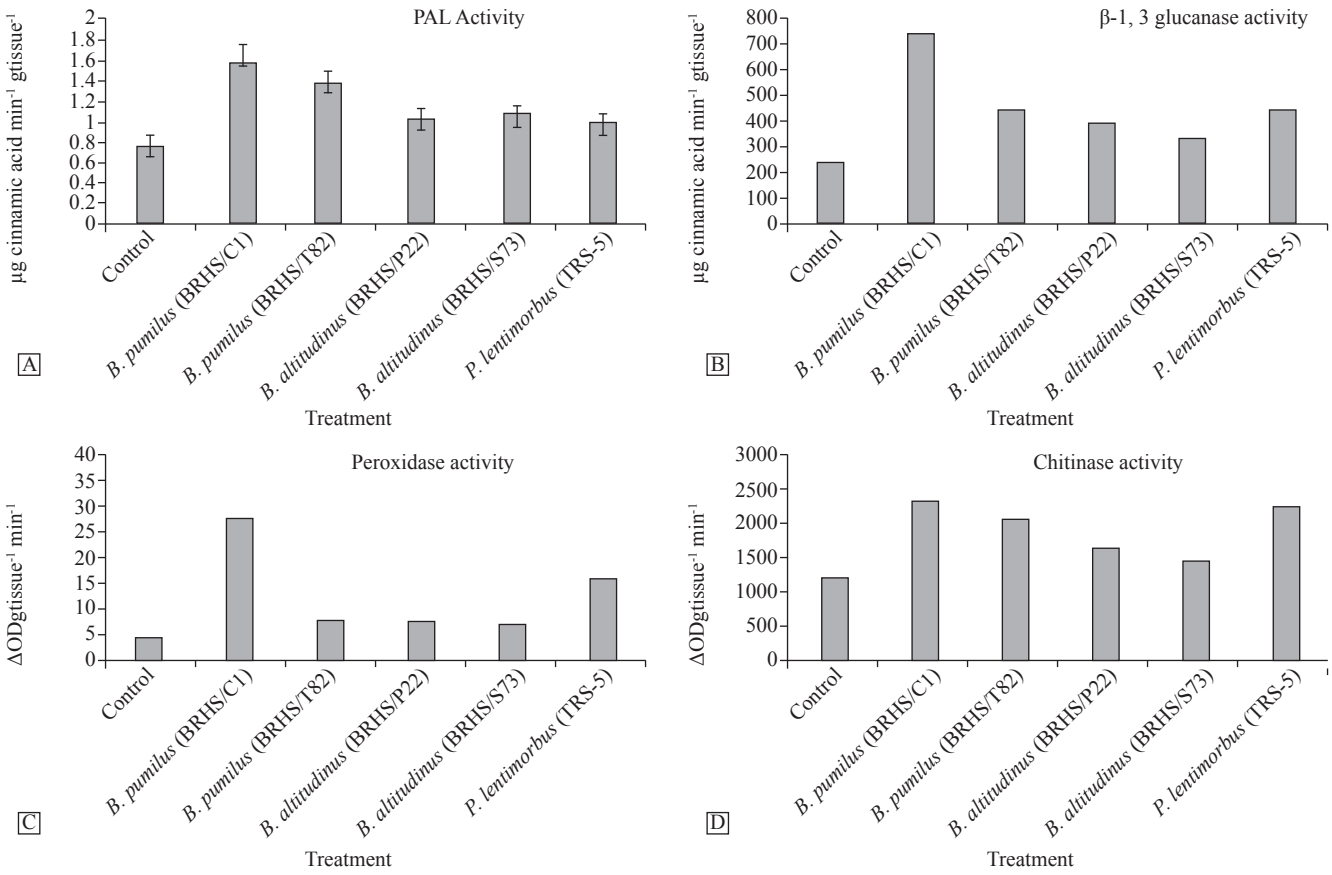

Figure 2: (A-D): Activities of phenylalanine ammonia lyase (A), β,1-3 glucanase (B), peroxidase (C), and chitinase (D) in leaves of *Sualu* following treatment with different PGPR

plants were assayed according to the mentioned method and was found that the protein content was high in the treated plants than in the control *Sualu* plants (Figure 4).

These result are in accordance with various previously stated reports on the enhacement of defense related enzymes in different plants with the application of PGPR. Ganeshamoorthi et al. (2008) reported that the activity of defense enzymes peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), β-1,3-glucanase, chitinase and defense chemical total phenols were found to be significantly higher in combined application of biocontrol agents *Pseudomonas fluorescens* isolate Pf1 and Py15 and *Bacillus subtilis* isolate Bs16 treated Mulberry plants when compared to individual treatments and control. Attia et al. (2011) also reported that soil application with PGPR significantly increased the activity of peroxidase, chitinase, β-1,3-glucanase and PAL and the accumulation of phenolics in soybean plants when compared to untreated control. Chakraborty et al. (2010) reported the increase in growth and induction of disease resistance in Tea with the use of various PGPRs. Induction of defense enzymes and phenolics by treatment of plant growth promoting rhizobacteria *Serratia marcescans* was evaluated in betelvine by Lavania et al. (2006). *Bacillus* sp. is one of the most important and abundant PGPR that alone or in combination

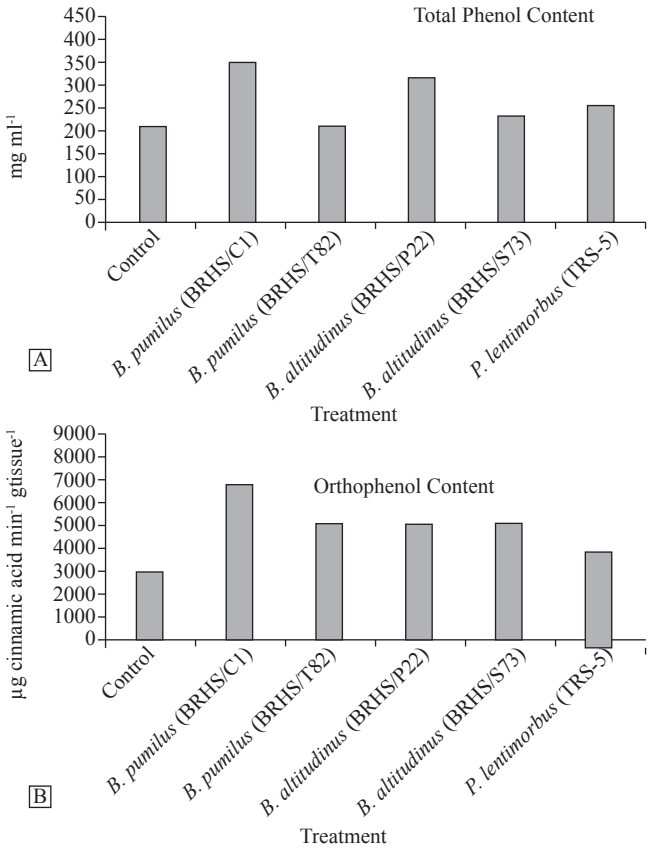

Figure 3: Activities of total (a) and orthophenol (b) in the leaves of *Sualu* plants following treatment with PGPR

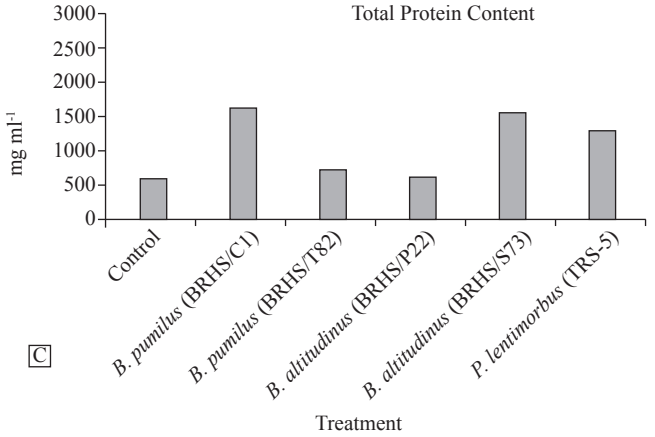

Figure 3: Activities of total (a) and orthophenol (b) in the leaves of *Sualu* plants following treatment with PGPR

has improved the growth as well as increased the levels of defense related enzymes as reported by Liang et al. (2011) in Cucumber and Li et al. (2008) in Tomato. Chakraborty et al. (2009) also quoted increase in defense enzymes PO, CHT, β 1,3-GLU and PAL during plant growth promotion of tea and induction of resistance by *Ochrobactrum anthropi*.

4. Conclusion

Increase in growth and vigour of plants with the treatment of PGPR has already been reported in various plants as well as in different plants involved in sericulture viz Mulberry (*Morus alba*) and Som (*Persea bombycina*). In this present work we have seen that a variety of PGPR strains showed significant increase in the growth parameters of *Sualu* plant as well as in the level of different defense related enzymes, phenols and proteins in the leaves of the treated plants. However among the five PGPRs used, *B. pumilus* (BRHS/C1) showed to have the maximum effect in the plants. This study hence proved that the PGPRs that showed enhanced growth in other plants also showed positive effect on the *Sualu* plants and this trait therefore could be further exploited to increase the quality and the quantity of the leaves of this plant which would in turn increase the quality of the silk produced.

5. Acknowledgement

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