Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 7 (2): 101-113, 2012
ISSN 1816-4935 / DOI: 10.3923/jm.2012.101.113

Screening of Phosphate Solubilizing Bacterial Isolates for the Growth Improvement of *Tectona grandis* Linn.

V. Mohan and Ayswarya Radhakrishnan
Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore-641 002, India

Corresponding Author: V. Mohan, Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore-641 002, India

ABSTRACT

Microorganisms like Rhizobia, Azotobacters, Azospirillum, Phosphate Solubilizing Bacteria, Mycorrhizal fungi play an important role in plant nutrition through the uptake of critical elements even from nutrient poor and hostile soils. In recent years, use of these beneficial microbes as bio-inoculants for the production of quality planting stock in forest nurseries is well known. Selection of potential isolates of microbial inoculants is essential for application as bio-fertilizer to produce quality seedlings in nurseries. In the present study, twenty three different isolates of Phosphate Solubilizing Bacteria (PSB) were isolated from teak growing areas of Andhra Pradesh, Kerala and Tamil Nadu in India. These isolates were mass cultured and inoculated to teak tissue culture raised plantlets in nursery condition to test their efficacy on the growth and biomass production of teak. It was observed that out of 23 isolates screened, the isolates KED-4 (*Bacillus subtilis*) and TCO-6 (*Pseuodomonas fluorescens*) were the most potential isolates for enhancing growth and biomass production of teak plantlets. An attempt was also made to determine the synergistic effect between these 2 isolates. The present study demonstrated that application of both the isolates together not only enhanced the growth but also increased the biomass and the quality of the plantlets.

Key words: *Bacillus subtilis,* microbial inoculants, phosphate solubilizing bacteria, *Pseuodomonas fluorescens, Tectona grandis*, indole acetic acid

INTRODUCTION

Tectona grandis Linn., commonly known as teak, has gained a worldwide reputation as a high quality timber on account of the attractiveness and the durability of the wood it produces. This tree species belongs to the family Verbenaceae and is a large deciduous tree which in favourable locations develops into a tall, straight and fairly clean cylindrical bole. Teak species occur naturally between latitudes 9-26°N and 73-104°E longitude. It is the principal timber tree of Peninsular India, Burma, Indonesia and Thailand and one of the most important timbers of the world. In India, the main teak forests are found in Kerala, Tamil Nadu, Karnataka, Maharashtra, etc. Teak is commonly found in deciduous forests and it normally grows up to 35 m in height. Due to an enactment banning green felling of timber from the natural forests, the supply is far below the demands resulting in a huge increase in market price. Thus private and corporate entrepreneurs have taken up planting of teak in commercial scale. Several teak improvement programs have been developed. The most widely used methods are generating genetically identical clones of the plus trees. This can be done through cuttings, bud grafting and tissue culturing.

One of global ecological problems of agriculture and forestry is the problem of over phosphatization of soils. Till now process of biological transformation of phosphorus in soil is poorly studied. Several soil bacteria and fungi, notably species of *Bacillus, Pseudomonas, Penicillium, Aspergillus* etc., secrete organic acids and lower the pH in their vicinity to bring about dissolution of bound phosphates in soil (Garretsen, 1948; Rao and Sinha, 1963; Guar and Ostwal, 1972).

PSB such as *Thiobacillus, Bacillus, Pseudomonas* etc., convert non-available inorganic phosphorus present in soil into an available form utilizable by crop plants. These bacteria also produce iron chelating substances, called siderophores which chelate the iron present in the root zone.

Application of PSB as microbial inoculants to agriculture crops is well known (Kucey, 1987; Kundu and Gaur, 1980; Kathiresan *et al*., 1995; Ponmurugan and Gopi, 2006; Trivedi and Pandey, 2007; Vikram and Hamzehzarghani, 2008; Mishra *et al*., 2010; Ardakani *et al*., 2010; Woyessa and Assefa, 2011; Ramteke *et al*., 2012). In forest nurseries, phosphate solubilizing microorganisms are applied individually as well as in combinations with other symbiotic and beneficial microbes. The stimulatory effect of phosphate solubilizing bacterial inoculation on plant growth in phosphorus deficient soil has been reported by Asea *et al*. (1988). Inoculation of *Eucalyptus camaldulensis* in an unsterilized soil with phosphate solubilizing bacteria enhanced root collar diameter, fresh weight and dry weight compared to uninoculated control (Mohammad and Prasad, 1988). Recently, Karthikeyan and Sakthivel (2011) observed that the PGPR (*A. chroococcum*) inoculated cuttings of *Eucalyptus camaldulensis* able to produce significant quantities of IAA for root initiation and higher growth than IBA treated cuttings of this forestry species.

Limited reports are available on the status of PSB population in forest soils especially in Teak. Aditya *et al*. (2009) studied the effect of nitrogen fixing bacterium, *Azotobacter* and phosphate solublising bacterium, *Bacillus megaterium* on the growth of Teak (*Tectona grandis*) and Indian redwood (*Chukrasia tubularis*) under nursery condition. The results of their investigation revealed that the positive effects of these bio-inoculants on the growth improvement of these trees. Hence in the present study, an attempt has been made to investigate the status on the occurrence and distribution of PSB from thirty different plantations and natural forests of Teak in Andhra Pradesh, Kerala and Tamil Nadu states, India and to screen and identify the potential isolates for growth improvement of teak tissue culture plantlets under nursery condition.

MATERIALS AND METHODS

Collection of soil samples: During soil sample collection, the base of 10 healthy trees was cleaned by removing the top soil and other forest litter. The rhizosphere soil was collected from all the 10 trees of one site and pooled together to form a sampling. This was placed in polythene bags and brought to the laboratory in ice boxes. All the samples were analyzed for the presence of PSB in laboratory by adopting standard procedure.

Isolation, identification and propagation of the phosphate solubilizing bacteria: A known quantity (1 g) of rhizosphere soil sample was suspended in a known volume of sterile water (100 mL) and serial dilutions of the suspension made in sterile water blanks. Appropriate dilutions were plated on phosphate-containing solid media (Pikovskaya's medium-modified by Rao and Sinha (1963) for obtaining microorganisms capable of dissolving phosphates. The plates were incubated

for 4-5 days. Transparent zones of clearing around microbial colonies indicated the extent of phosphate solubilization (Rao and Sinha, 1963). Such cultures were isolated, identified and the extent of solubilization determined quantitatively. Number of colonies on the respective dilution was calculated and expressed as colony forming units (CFU g^{-1}) of soil. The colony of the largest halo zone from each of the soil samples was isolated and further tests were performed to confirm the presence of PSB. Purified PSB isolates were subjected to various staining techniques such as Gram Staining (Gram, 1884) and Endospore Staining technique (Schaeffer and Fulton, 1933) as an initial screening. After confirmation by performing biochemical tests (Cappucino and Sherman, 1983), all the isolates of PSB were mass cultured in King's medium and then mixed with carrier material (lignite) for inoculation studies in nursery.

Indole acetic acid production by PSB: The potential for the production of IAA was determined in culture filtrates of the isolates grown in King's B broth supplemented with 100 ppm tryptophan as per the procedure of Tien *et al.* (1979). Based on the IAA production potential, the isolates were selected for further study.

Plant source: *Tectona grandis* plantlets were raised at the Tissue culture lab of the Biotechnology Research Center, Tirupati andhra Pradesh (India) by culturing the Teak auxiliary buds under *in vitro* conditions followed by rooting and hardening under *ex vitro* condition and the same were used for the nursery experiment (Bhojwani and Razdan, 1996).

Transplantation of teak plantlets and inoculation of PSB isolates: One month old teak plantlets after hardening were transplanted to polythene bags (10×20 cm in size) which were filled with the potting mixture of sand, soil and farmyard manure (FYM) (1: 2: 1). Ten grams of inoculum of 23 isolates of PSB were inoculated individually to tissue culture plantlets in polythene bags. Totally 24 treatments including uninoculated control were made and each treatment was replicated 6 times with 10 plantlets per replication. There were a total of 1440 (24x60) plantlets set up in the nursery in a randomized block design. The plants were maintained under nursery conditions and observation was taken at three months after inoculation. The plantlets in various treatments were harvested and the growth parameters such as shoot height, root length, collar diameter, shoot and root dry weights, quality index, volume index and shoot: root ratios were recorded. All harvested plants after measurement were then oven dried at 70 to 80°C for 48 h for the determination of dry weight. The seedling quality index, volume index and shoot root ratio were calculated by using the following formula:

$$\text{Shoot root ratio} = \text{Shoot dry weight}/\text{Root dry weight}$$

$$\text{Volume Index} = \text{Diameter}^2 \times \text{height (cm)}$$

where,

$$\text{Height} = \text{Root length} + \text{Shoot length})$$

$$\text{Quality Index} = \frac{\text{Seedlings dry weight (g)}}{\text{Height (cm)}/\text{Diameter (cm)} + \text{Shoot dry weight (g)}/\text{Root dry weight (g)}}$$

Analysis of data: Analysis of Variance (ANOVA) was performed on all data and the means were separated by using Duncan's Multiple Range Test (DMRT) (SPSS, version 10).

RESULTS

Isolation and characterization of phosphate solubilizing bacteria (PSB): A total of 23 isolates of phosphate solubilizing bacteria were isolated based on the halo zone formation by the organisms due to phosphate solubilization (Table 1). Based on the above observation, 23 isolates were characterized and among these, 13 isolates (AAN-1, AAN-2, AAN-5, KED-1, KED-2, KED-4, KED-5, TCO-2, TCO-4, TKE-1, TKE-2, TKE-4, TKE-6) were gram positive, showed the presence of endospores, produced acid and gas in lactose fermentation, hydrolyzed starch and gelatin, utilized citrate and were catalase positive. These were identified tentatively as *Bacillus* sp. The remaining 10 isolates (AAN-3, AAN-4, KED-3, TCO-1, TCO-3, TCO-5, TCO-6, TCO-7, TKE-3, TKE-5) were gram negative and not able to produce acid and gas in lactose broth. Starch hydrolysis was negative in all the isolates and exhibited gelatinase by liquefying gelatin. These were identified tentatively as *Pseudomonas* sp. Fluorescence was noticed under UV light and the isolate TCO-6 gave positive result. (Table 1). Among the 23 isolates, 5 were isolated from Allapalli natural teak forest (AAN-1, AAN-2, AAN-3, AAN-4, AAN-5) of Andhra Pradesh and 5 were isolated from Edavanna teak plantation (KED-1, KED-2, KED-3, KED-4, KED-5) of Kerala. In Tamil Nadu, 7 strains from Coutralam (TCO-1, TCO-2, TCO-3, TCO-4, TCO-5, TCO-6, TCO-7) and 6 strains from Keeriparai (TKE-1, TKE-2, TKE-3, TKE-4, TKE-5, TKE-6) were isolated.

IAA production of PSB isolates under *in vitro*: These isolates were screened to test the ability to produce Indole Acetic Acid (IAA) under *in vitro* conditions. It was found that the PSB isolate

Table 1: List of PSB isolates obtained from the rhizosphere of *Tectona grandis* plantations and natural forests in Andhra Pradesh, Kerala and Tamil Nadu states (India)

Treatment No.	Isolate code	Location	IAA (μg mL^{-1})
1	Control	-	-
2	AAN-1	Andhra Pradesh	20.5
3	AAN-2	Andhra Pradesh	18.6
4	AAN-3	Andhra Pradesh	21.2
5	AAN-4	Andhra Pradesh	33.5
6	AAN-5	Andhra Pradesh	26.4
7	KED-1	Kerala	21.0
8	KED-2	Kerala	19.5
9	KED-3	Kerala	26.4
10	KED-4	Kerala	45.2
11	KED-5	Kerala	27.1
12	TCO-1	Tamil Nadu	23.6
13	TCO-2	Tamil Nadu	22.8
14	TCO-3	Tamil Nadu	23.1
15	TCO-4	Tamil Nadu	24.8
16	TCO-5	Tamil Nadu	26.4
17	TCO-6	Tamil Nadu	38.6
18	TCO-7	Tamil Nadu	24.1
19	TKE-1	Tamil Nadu	23.2
20	TKE-2	Tamil Nadu	22.1
21	TKE-3	Tamil Nadu	18.4
22	TKE-4	Tamil Nadu	16.2
23	TKE-5	Tamil Nadu	20.8
24	TKE-6	Tamil Nadu	28.7

(KED-4, Kerala) has produced maximum amount of IAA (45.2 μg mL^{-1}), followed by the PSB isolate (TCO-6, Tamil Nadu) (38.6 μg mL^{-1}), the PSB isolate (AAN-4 andhra Pradesh) (33.5 μg mL^{-1}), the PSB isolate (TKE-6, Tamil Nadu) (28.7 μg mL^{-1}) and the PSB isolate (KED-5, Kerala) (27.1 μg mL^{-1}) (Table 1). Since IAA production was maximum in KED- 4 and TCO- 6, these two isolates were selected for further studies to evaluate their potential in growth enhancement of teak.

Screening of different PSB isolates on growth performance of teak plantlets: All the thirty three PSB isolates were mass cultured and inoculated as individual application to Teak tissue culture plantlets in order to select the potential isolate for enhancing the growth and biomass. Data on growth parameters such as plant height, root length, collar diameter, shoot and root biomass, total biomass, shoot root ratio, volume index and quality index of tissue culture raised plantlets of Teak (90 days after inoculation) in the nursery are given in Table 2-4. The results indicated that all the inoculated plantlets had better growth performance as compared to uninoculated (control) plantlets. Among the 23 different isolates of PSB screened, the isolates KED-4 (*Bacillus subtilis*) and TCO-6 (*Pseudomonas fluorescens*) were found to be the most efficient and showed statistically significant values of growth, biomass, shoot-root ratio, volume and quality indices. It was interesting to note that the same PSB isolates produced high concentration of phytohormone (IAA) under *in vitro* assay (KED-4-45.2 and TCO-6-38.6 μg mL^{-1}) when compared to the other isolates of PSB (Table 1).

Table 2: Effect of the different PSB isolates on the growth improvement of tissue culture plantlets of *Tectona grandis*

Treatment No.	Isolate code	Shoot length (cm)	Root length (cm)	Root collar diameter (mm)
1	Control	2.2^{a}	4.2^{b}	1.35^{a}
2	AAN-1	3.2^{g}	4.8^{ef}	1.72^{b}
3	AAN-2	3.9^{j}	4.5^{d}	1.99^{c}
4	AAN-3	3.6^{h}	5.2^{hi}	2.31^{fg}
5	AAN-4	2.3^{b}	4.4^{c}	2.09^{d}
6	AAN-5	3.1^{f}	4.1^{a}	2.22^{e}
7	KED-1	4.5^{m}	5.4^{k}	2.47^{ij}
8	KED-2	3.2^{g}	4.7^{e}	2.54^{jk}
9	KED-3	4.3^{l}	5.2^{gh}	2.38^{gh}
10	KED-4	6.1^{n}	6.2^{n}	3.15^{n}
11	KED-5	4.2^{k}	5.3^{jk}	1.94^{c}
12	TCO-1	3.5^{h}	5.4^{jk}	2.41^{hi}
13	TCO-2	3.3^{g}	5.4^{k}	2.14^{d}
14	TCO-3	3.1^{f}	4.6^{e}	1.34^{a}
15	TCO-4	2.9^{d}	4.7^{e}	2.76^{l}
16	TCO-5	3.6^{h}	4.8^{f}	2.77^{l}
17	TCO-6	6.1^{n}	6.7^{o}	3.46^{o}
18	TCO-7	3.5^{h}	5.8^{m}	2.86^{m}
19	TKE-1	4.2^{k}	5.5^{l}	2.61^{k}
20	TKE-2	2.9^{e}	5.7^{m}	2.29^{ef}
21	TKE-3	2.5^{c}	5.3^{jk}	2.11^{d}
22	TKE-4	3.2^{g}	5.4^{jk}	2.46^{ij}
23	TKE-5	4.4^{l}	5.3^{ij}	2.48^{ij}
24	TKE-6	3.7^{i}	5.1^{g}	2.41^{hi}

Means, in a column, followed by common letter(s) are not significantly different at p = 0.05 level according to DMRT

Table 3: Effect of the different PSB isolates on the biomass production of tissue culture plantlets of *Tectona grandis*

Treatment No.	Isolate code	Shoot biomass (g)	Root biomass (g)	Total biomass (g)
1	Control	0.8317^{a}	0.7083^{a}	1.5400^{a}
2	AAN-1	1.0383^{c}	0.8333^{b}	1.8717^{b}
3	AAN-2	1.1350^{ef}	0.9200^{c}	2.0550^{d}
4	AAN-3	1.3300^{k}	1.1317^{e}	2.4617^{h}
5	AAN-4	0.8200^{a}	1.1367^{e}	1.9567^{c}
6	AAN-5	1.4017^{l}	1.2050^{f}	2.6067^{i}
7	KED-1	1.2200^{gh}	1.5867^{i}	2.8067^{k}
8	KED-2	1.2483^{hi}	1.3383^{g}	2.5867^{i}
9	KED-3	1.2100^{gh}	1.4183^{h}	2.6283^{i}
10	KED-4	2.7817^{m}	1.4100^{h}	4.1917^{m}
11	KED-5	0.9217^{b}	1.1117^{de}	2.0333^{d}
12	TCO-1	1.2750^{ij}	1.1067^{de}	2.3817^{g}
13	TCO-2	1.0967^{de}	1.2433^{f}	2.3400^{fg}
14	TCO-3	1.2983^{jk}	1.5750^{i}	2.8733^{k}
15	TCO-4	1.0850^{d}	1.2150^{f}	2.3000^{f}
16	TCO-5	1.2417^{ghi}	1.1433^{e}	2.3850^{g}
17	TCO-6	2.7567^{m}	1.3900^{h}	4.1467^{m}
18	TCO-7	1.2350^{ghi}	1.1250^{de}	2.3600^{fg}
19	TKE-1	1.4183^{l}	1.3000^{g}	2.7183^{j}
20	TKE-2	1.4117^{l}	1.6850^{j}	3.0967^{l}
21	TKE-3	1.2433^{ghi}	0.8333^{b}	2.0767^{d}
22	TKE-4	1.1500^{f}	1.0750^{d}	2.2250^{e}
23	TKE-5	1.1983^{g}	1.4333^{h}	2.6317^{i}
24	TKE-6	1.0317^{c}	1.1400^{e}	2.1717^{e}

Means, in a column, followed by common letter(s) are not significantly different at p = 0.05 level according to DMRT

Table 4: Effect of the different PSB isolates on the shoot root ratio, volume and quality index of tissue culture plantlets of *Tectona grandis*

Treatment No.	Isolate code	Shoot root ratio	Volume index	Quality index
1	control	1.1767^{h}	11.7283^{a}	0.2583^{a}
2	AAN-1	1.2483^{i}	23.6900^{b}	0.3167^{b}
3	AAN-2	1.2367^{i}	33.7583^{d}	0.3733^{c}
4	AAN-3	1.1750^{h}	46.9333^{f}	0.4950^{g}
5	AAN-4	0.7217^{a}	29.5417^{c}	0.4983^{g}
6	AAN-5	1.1633^{h}	35.6300^{d}	0.5883^{k}
7	KED-1	0.7717^{b}	60.5850^{h}	0.5867^{k}
8	KED-2	0.9317^{f}	51.2417^{g}	0.6350^{l}
9	KED-3	0.8517^{cde}	54.0417^{g}	0.5383^{h}
10	KED-4	1.9733^{l}	123.1117^{k}	0.7083^{n}
11	KED-5	0.8283^{c}	35.8617^{d}	0.3550^{c}
12	TcO-1	1.1567^{h}	51.9950^{g}	0.4883^{fg}
13	TcO-2	0.8850^{def}	40.2200^{e}	0.4700^{f}
14	TcO-3	0.8250^{c}	14.0783^{a}	0.4317^{e}
15	TcO-4	0.8950^{ef}	58.0683^{h}	0.6317^{l}
16	TcO-5	1.0883^{g}	64.6217^{i}	0.5783^{jk}
17	TcO-6	1.9817^{l}	152.9533^{l}	0.7300^{o}

Table 4: Countinued

Treatment No.	Isolate code	Shoot root ratio	Volume index	Quality index
18	T^{c}O-7	1.1000^{g}	76.7633^{j}	0.5417^{hi}
19	TKE-1	1.0917^{g}	66.4717^{i}	0.5633^{ij}
20	TKE-2	0.8383^{cd}	45.8317^{f}	0.6667^{m}
21	TKE-3	1.4933^{j}	35.1433^{d}	0.3983^{d}
22	TKE-4	1.0717^{g}	52.5933^{g}	0.4850^{fg}
23	TKE-5	0.8350^{cd}	59.6033^{h}	0.5567^{hij}
24	TKE-6	0.9067^{f}	51.2100^{g}	0.4783^{fg}

Means, in a column, followed by common letter(s) are not significantly different at p = 0.05 level according to DMRT

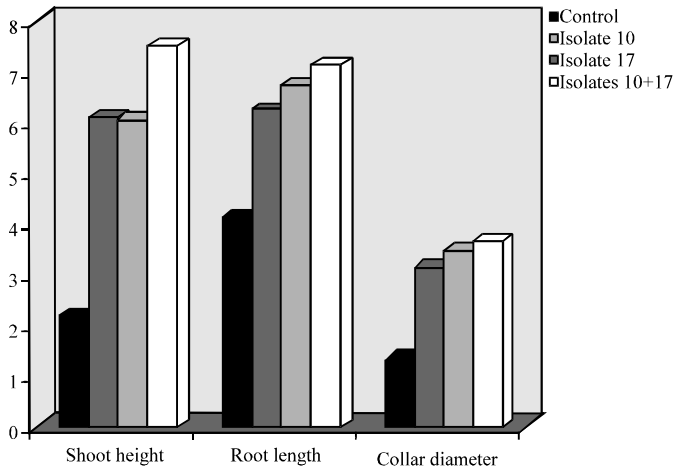

Fig. 1: Effect of the dual inoculation of PSB isolates (Isolate 10-KED-4+Isolate 17-TCO-6) on the shoot and root lengths (cm) and collar diameter (mm) of tissue culture plantlets of *Tectona grandis*

Determine the efficacy of selected isolates of PSB on growth enhancement of teak plantlets: Based on the above results, the two isolates of PSB (KED-4, TCO-6) were selected and tested again on Teak tissue cultured plantlets in nursery in order to find out the best one and also the efficacy of combined application of these two isolates together. The results revealed that the combined application of both the isolates of *B. subtilis* and *P. fluorescens* showed significantly better growth, biomass, shoot-root ratio, volume and quality indices of the plants over uninoculated control (Fig. 1-4) and it clearly indicates that the synergistic association of both the isolates.

DISCUSSION

Beneficial microbes play an important role in production of quality planting stock for afforestation programmes in disturbed and marginal lands. Revegetation efforts are likely to remain incomplete and unsuccessful if the beneficial microbial component is ignored. The beneficial microorganisms can solubilize bound phosphate in soil and bring into solution making it available for plant uptake. Pikovskaya (1948) was the pioneer in isolating from soils and phosphorite, an organism capable of actively solubilizing tricalcium phosphate which is termed as 'Bacterium P'. Menkina (1951) isolated *Bacillus megaterium* var. *phosphaticum* and *B. megaterium* var. *serratia* which liberated inorganic forms of P from organic compounds. Such bacteria, known as

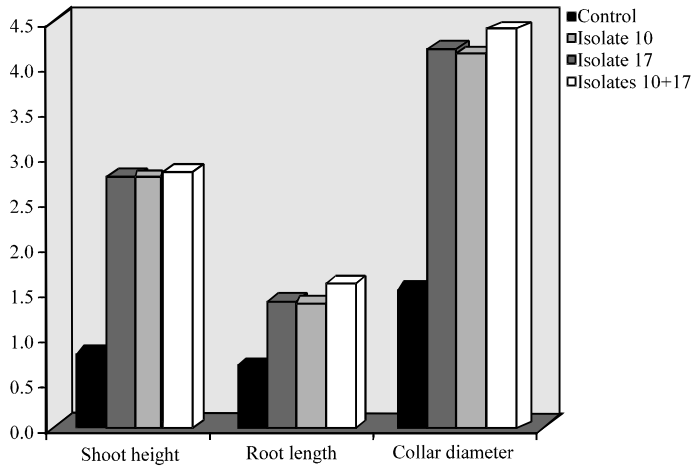

Fig. 2: Effect of the dual inoculation of PSB isolates (Isolate 10-KED-4+Isolate 17-TCO-6) on the biomass (g) production of tissue culture plantlets of *Tectona grandis*

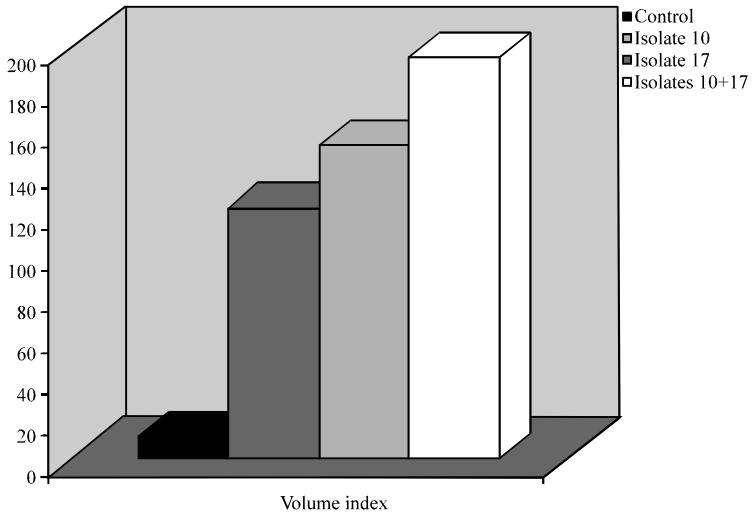

Fig. 3: Effect of the dual inoculation of PSB isolates (Isolate 10-KED-4+Isolate 17-TCO-6) on the volume index of tissue culture plantlets of *Tectona grandis*

PSB which are often present in the rhizosphere of plants (Alexander, 1977). Phosphate solubilizing bacteria include members of *Pseudomonas, Micrococcus, Bacillus* and *Flavobacterium*.

In the present study, 23 different isolates of PSB were isolated from the rhizosphere of Teak in Andhra Pradesh, Kerala and Tamil Nadu states and screened their efficacy on IAA production under *in vitro* conditions. This study is in accordance with the findings made by many earlier researchers (Burr *et al*., 1978; Gardner *et al*., 1984; Van Peer and Schipper, 1988; Ponmurugan and Gopi, 2006; Trivedi and Pandey, 2007) and they found that plant growth hormone stimulation by fluorescent *Pseudomonas*. Further, the release of plant available P is not only the beneficial action of PSBs but also the production of biologically active substances like IAA,

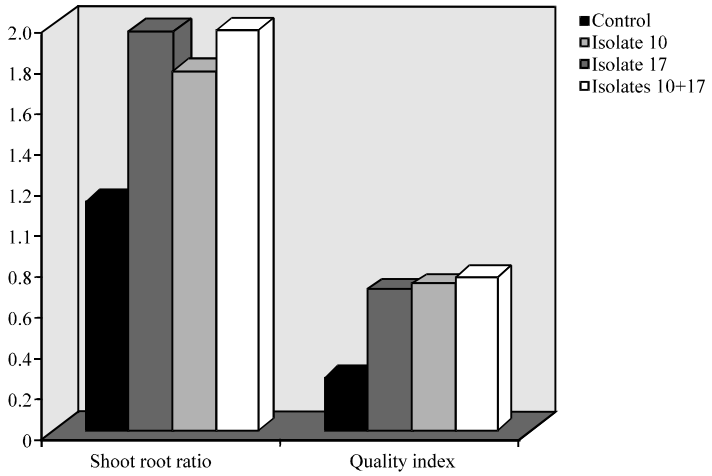

Fig. 4: Effect of the dual inoculation of PSB isolates (Isolate 10-KED-4+Isolate 17-TCO-6) on the shoot root ratio and quality index of tissue culture plantlets of *Tectona grandis*

gibberellins and cytokinins (Kucey, 1988). Katznelson and Cole (1965) reported that *Pseudomonas fluorescens* produced 1-14 μg gibberellic acid per litre of broth. They also reported that the plant could derive maximum benefit from microbial synthesis of gibberellins and related substances only at the root surface. Dowling and O' Gara (1994) reported that many strains of *Pseudomonas* produced IAA which stimulated root elongation by stimulating the growth of root hairs. Trivedi and Pandey (2007) have screened four hundred and fifty bacterial isolates obtained from different regions of Indian Himalayas for their low temperature phosphate solubilization activity and plant growth promotion abilities such as production of siderophores and IAA. They found that the bacteria isolated from the Himalayan soils have been able to evolve with the ability to solubilize phosphate and also produce plant growth hormones at lower temperature.

Inoculation of PSB as bio-fertilizers to agriculture crops was studied by many researchers (Kundu and Gaur, 1980; Kucey, 1987; Kathiresan *et al*., 1995; Ponmurugan and Gopi, 2006; Trivedi and Pandey, 2007; Vikram and Hamzehzarghani, 2008; Mishra *et al*., 2010; Ardakani *et al*., 2010; Woyessa and Assefa, 2011; Ramteke *et al*., 2012). In forestry, such studies are very scanty. However, studies were carried out by inoculating phosphate solubilizing microorganisms individually as well as in combination with other symbiotic and non-symbiotic beneficial microbes to some of the forestry crops in nursery (Mohammad and Prasad, 1988; Poi *et al*., 1989; Young, 1990; Aditya *et al*., 2009; Karthikeyan and Sakthivel, 2011).

In the present study, attempt has been made to determine the efficacy of 23 different PSB isolates for selecting the best isolate for growth and biomass production of tissue culture plantlets of Teak in nursery. The PSB isolates of *Bacillus subtilis* (Isolate KED-4 of Kerala) and *Pseudomonas fluorescens* (Isolate TCO-6 of Tamil Nadu) were found superior in terms of better growth performance and also production higher concentration of IAA as compared to other isolates and control. This is in accordance with the earlier reports where inoculation of PSB improved the plant height, collar diameter and shoot weight in *Acacia mellifera* and *A. farnesia*; root length in *A. farnesiana*, number of leaves and leaf area in *A. seyal*; total biomass in *A. seyal* and *A. farnesiana* and shoo-root ratio in *A. planifrons* and *A. farnesiana* (Saravanan, 1991). Enebak *et al*. (1998) studied the effect of 12 strains of PGPR on Loblolly (*Pinus taeda*) and Slash

Pine (*Pinus elliottii*) seedlings by inoculating the seed at sowing under greenhouse conditions and they found that treatment with rhizobacteria had a significant positive and negative effect on seedling growth and biomass which depended on tree species. Garcia *et al.* (2004) studied the effect of four bacterial strains showing *in vitro* metabolic capacities of plant growth-promoting action on the growth of holm-oak (*Quercus ilex*) and pine (*Pinus pinea*) plants at a forest nursery and found that all strains significantly increased some of the parameters studied (stem length, neck diameter and shoot dry weight). Solano *et al.* (2007) carried out screening of 270 PGPR isolates to improve growth of*Cistus ladanifer*seedlings. They observed that fifty-eight percent of the isolates showed phosphate solubilisation and siderophore production. Seven of the 11 assayed strains were phosphate solubilisers and able to produce siderophores, only one was really effective in increasing all biometric parameters in*Cistus ladanifer*seedlings. Teixeira *et al.* (2007) tested PGPR mainly *Psuedomonas* sp. and *Bacillus subtlis* obtained from the rhizosphere of *Eucalyptus* clones for rooting of cuttings and mini-cuttings. They found that ten isolates were capable of providing gains of up to 110% in root formation and up to 250% in root biomass over non-inoculated control cuttings. Mafia *et al.* (2009) evaluated the root colonization and interaction among isolates of PGPR and *Eucalyptus* species. They found that there was interaction among isolates of rhizobacteria and *Eucalyptus* species for seed germination and seedling growth. It was found that *Pseudomonas* sp. was the best rhizobacteria for growth promotion of *E. cloezi*ana and *E. grandis*. *Bacillus subtilis* was the most effective inoculant for *E. globulus*. *Pseudomonas* sp. *P. fulva* and *Stenotrophomonas maltophilia* were the most promising isolates for *E. urophylla*. Aditya *et al.* (2009) tested the effect of nitrogen fixing bacterium, *Azotobacter* and phosphate solublising bacterium, *Bacillus megaterium* on the growth of Teak (*Tectona grandis*) and Indian redwood (*Chukrasia tubularis*) trees under nursery condition. The results of their study revealed that the co-inoculation of N-fixing and P-solublizing organisms improved the plant growth (seedling height and collar diameter) for *C. tubularis* and *T. grandis*. The present study is the first attempt to determine the status of PSB in Teak growing areas and subsequent screening and selection of the potential isolates of the same for application to tissue culture raised Teak plantlets in nursery.

CONCLUSION

Inoculation of forest tree seedlings with potential PSB in nurseries will increase the growth and quality of seedlings and in turn improve their performance in the field. Also, use of these microbial inoculants as bio-fertilizer in nurseries may considerably reduce the requirement for chemical fertilizer as well as save cost, time and labour. Present study was an effort to identify superior isolates and their synergistic interactions under a given set of environmental conditions for enhancing the growth and biomass production of tissue culture raised plantlets of *Tectona grandis*. The study clearly demonstrates that the application of selected native PSB isolates of *Bacillus subtilis* (KED-4) and *Pseudomonas fluorescens* (TCO-6) are very effective in improving the quality of clonal plantlets of Teak.

ACKNOWLEDGMENTS

The authors are also thankful to the Director, Institute of Forest Genetics and Tree Breeding, Coimbatore for providing necessary facilities and encouragement. One of the authors (Ayswarya Radhakrishnan) is highly grateful to the Director, Biotechnology Research Center, State Forest Department, Tirupati andhra Pradesh for providing JRA fellowship, all necessary facilities and kind cooperation during the Ph.D. study.

REFERENCES

Aditya, B., A. Ghosh and D. Chattopadhy, 2009. Co-inoculation effects of nitrogen fixing and phosphate solubilising microorganisms on Teak (*Tectona grandis*) and Indian redwood (*Chukasia tubularis*). J. Biol. Sci., 1: 23-32.

Alexander, M., 1977. Introduction to Soil Microbiology. 2nd Edn., John Wiley and Sons Inc., New York, USA., ISBN: 9780471021797, Pages: 467.

Ardakani, S.S., A. Heydari, L. Tayebi and M. Mohammadi, 2010. Promotion of cotton seedlings growth characteristics by development and use of new bioformulations. Int. J. Botany, 6: 95-100.

Asea, P.E.A., R.M.N. Kucey and J.W.B. Stewart, 1988. Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. Biol. Biochem., 20: 459-464.

Bhojwani, S.S. and M.K. Razdan, 1996. Plant Tissue Culture: Theory and Practice. Revised Edn., Elsevier, Netherlands, Pages: 767.

Burr, T.J., M.N. Schroth and T.V. Suslow, 1978. Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology, 68: 1377-1383.

Cappucino, J.G. and N. Sherman, 1983. Microbiology: A Laboratory Manual. Addison-Wesky Publishing Co., California, Sidney, Pages: 461.

Dowling, D.N. and F. O'Gara, 1994. Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. Trends Biotechnol., 12: 133-141.

Enebak, S.A., G. Wei and J.W. Kloepper, 1998. Effects of plant growth-promoting rhizobacteria on loblolly and slash pine seedlings. For. Sci., 44: 139-144.

Garcia, J.A.L., J. Domenech, C. Santamaria, A. Daza and F.J.G. Manero, 2004. Growth of forest plants (pine and holm-oak) inoculated with rhizobacteria: Relationship with microbial community structure and biological activity of its rhizosphere. Environ. Exp. Bot., 52: 239-251.

Gardner, I.C., D.M. Clelland and A. Scott, 1984. Mycorrhizal improvement in non leguminous nitrogen fixing associations with particular reference to *Hippophae rhamnoides* L. Plant Soil., 78: 189-200.

Garretsen, F.C., 1948. The influence of microorganisms on the phosphorus uptake by the plant. Plant Soil, 1: 51-51.

Gram, C., 1884. The differential staining of schizomycetes in tissue sections and in dried preparations. Fortschitte Der Med., 2: 185-189.

Guar, A.D. and K.P. Ostwal, 1972. Influence of phosphate dissolving bacilli on the yield and phosphate uptake of wheat crop. Indian J. Exp. Biol., 19: 393-393.

Karthikeyan, A. and K. M. Sakthivel, 2011. Efficacy of Aztobacter chroococcum in rooting and growth of *Eucalyptus camaldulensis* stem cuttings. Res. J. Microbiol., 6: 618-624.

Kathiresan, G., G. Manickan and P. Parameswaran, 1995. Efficiency of phosphobacteria addition on cane yield and quality. Cooperative Sugar, 26: 629-631.

Katznelson, H. and S.E. Cole, 1965. Production of gibberellin like substances by bacteria and actinomycetes. Can. J. Microbiol., 11: 733-741.

Kucey, R.M.N., 1987. Increased phosphorus uptake by wheat and beans inoculated with a phosphorus solubilizing *Penicillium bilaji* strain and with vesicular arbuscular mycorrhizal fungi. Applied Environ. Microbiol., 52: 2699-2703.

Kucey, R.M.N., 1988. Effect of *Penicillium bilajii* on the solubility and uptake of micronutrients from soil by wheat. Can. J. Soil Sci., 682: 261-270.

Kundu, B.S. and A.C. Gour, 1980. Establishment of nitrogen fixing and phosphate solubilizing bacteria in rhiozsphere and their effect on yield and nutrient uptake of wheat crop. Plants Soil, 57: 223-230.

Mafia, R.G., A.C. Alfenas, E.M. Ferreira, D.H.B. Binoti, G.M.V. Mafia and A.H. Mounteer, 2009. Root colonization and interaction among growth promoting rhizobacteria isolates and *Eucalyptus* species. R. Arvore Vicosa-MG., 33: 1-9.

Menkina, L.N., 1951. Release of phosphorus from insoluble phosphate material in acidic low land rice compounds. J. Applied Bact., 22: 227-230.

Mishra, A., K. Prasad and G. Rai, 2010. Effect of bio-fertilizer inoculations on growth and yield of dwarf field pea (*Pisum sativum* L.) in conjunction with different doses of chemical fertilizers. J. Agron., 9: 163-168.

Mohammad, G. and R. Prasad, 1988. Influence of microbial fertilizer on biomass accumulation in polypotted *E. camaldulensis* seedlings. J. Trop. For., 4: 74-77.

Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil in connection with vital activities by some microbial species. Microbiologia, 17: 362-370.

Poi, S.C., G. Ghosh and M.C. Kabi, 1989. Response of chickpea (*Cicer arietinum* L.) to combined inoculation with *Rhizobium*, phosphobacteria and mycorrhizal organisms. Fur. Microbiol., 114: 249-253.

Ponmurugan, P. and C. Gopi, 2006. Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. J. Agron., 5: 600-604.

Ramteke, P.W., B. Joseph, A. Mani and S. Chacko, 2012. *Pisum sativum* and associated plant growth promoting rhizobacteria: Effect of normal and sewage irrigation. Int. J. Soil Sci., 7: 15-27.

Rao, W.V.B.S. and M.K. Sinha, 1963. Phosphate dissolving organisms in the soil and rhizosphere. Indian J. Agric. Sci., 33: 272-278.

Saravanan, P.P., 1991. Studies on nutrient amendments on *Acacia* species. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore-3, Tamil Nadu, India.

Schaeffer, A.B. and M.D. Fulton, 1933. A simplified method of staining endospores. Science, 77: 194-194.

Solano, B.R., M.T.P. de la Iglesia, A. Probanza, J.A.L. Garcia, M. Megias and F.J.G. Manero, 2007. Screening for PGPR to improve growth of *Cistus ladanifer* seedlings for reforestation of degraded Mediterranean ecosystems. Dev. Plant Soil, 102: 59-68.

Teixeira, D.A., A.C. Alfenas, R.G. Mafia, E.M. Ferreira, L. de Siqueira, L.A. Maffia and A.H. Mounteer, 2007. Rhizobacterial promotion of *Eucalyptus* rooting and growth. Braz. J. Microbiol., 38: 118-123.

Tien, T.M., M.H. Gaskins and D.H. Hubbell, 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on growth of pearl millet (*Pennisetum americanum* L.). Applied Environ. Microbiol., 37: 1016-1024.

Trivedi, P. and A. Pandey, 2007. Low temperature phosphate solubilization and plant growth promotion by psychrotrophic bacteria, isolated from Indian Himalayan region. Res. J. Microbiol., 2: 454-461.

Van Peer, R. and B. Schipper, 1988. Plant growth responses to bacterization with selected *Pseudomonas* sp. strains and rhizosphere microbial development in hydroponic cultures. Can. J. Microbiol., 35: 456-463.

Vikram, A. and H. Hamzehzarghani, 2008. Effect of phosphate solubilizing bacteria on nodulation and growth parameters of greengram (*Vigna radiata* L. Wilczek). Res. J. Microbiol., 3: 62-72.

Woyessa, D. and F. Assefa, 2011. Effect of plant growth promoting rhizobacteria on growth and yield of Tef (*Eragrostis tef* Zucc. Trotter) under greenhouse condition. Res. J. Microbiol., 6: 343-355.

Young, C.C., 1990. Effects of phosphorus solubilizing bacteria and vesicular arbuscular mycorrhizal fungi on the growth of tree species in subtropical-tropical soils. Soil Sci. Plant Nutr., 36: 225-231.