

Growth response of *Dalbergia sissoo* Roxb. to mineral solubilizing bacteria and fungi in nursery conditions

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Abstract: *Dalbergia sissoo* is an important multipurpose tree frequently used in afforestation programmes. Growth analysis of *D. sissoo* seedlings was conducted to evaluate their establishment after inoculation with six phosphate solubilizing fungi, four iron ore solubilizing fungi and five phosphate solubilizing bacteria. The experiment was done in pot culture under polyhouse misting facility. In general, biomass in seedlings was higher under inoculated conditions as compared to control. Seedlings exhibited maximum biomass production when inoculated with *Penicillium chrysogenum* (2) and *Aspergillus* sp. (1). Growth analysis revealed that NAR (net assimilation rate), and LAR (leaf area ratio) accounted for the differences in RGR (relative growth rate) in the treatments. Application of selected microbes can lead to successful establishment of *D. sissoo* in nurseries even in pot soils, and help in producing quality planting material.

Resumen: *Dalbergia sissoo* es un árbol importante de usos múltiples utilizado con frecuencia en programas de forestación. Se llevó a cabo un análisis de crecimiento de plántulas de *D. sissoo* para evaluar su establecimiento después de la inoculación con seis hongos solubilizadores de fosfatos, cuatro hongos solubilizadores de mineral de hierro y cinco bacterias solubilizadoras de fosfatos. El experimento se realizó en cultivo en macetas en una instalación de polietileno (polyhouse) con nebulización. En general, la biomasa de las plántulas fue mayor cuando éstas fueron inoculadas en comparación con el control. Las plántulas tuvieron la máxima producción de biomasa cuando fueron inoculadas con *Penicillium chrysogenum* (2) y *Aspergillus* sp. (1). El análisis de crecimiento reveló que la tasa neta de asimilación (NAR, siglas en inglés) y el cociente de área foliar (LAR, siglas en inglés) explicaron las diferencias en las tasas relativas de crecimiento (RGR, siglas en inglés) entre tratamientos. La aplicación de ciertos microbios seleccionados puede permitir el establecimiento exitoso de *D. sissoo* en los viveros, incluso en suelo de maceta, y ayudar en la producción de material de siembra de calidad.

Resumo: A *Dalbergia sissoo* é uma árvore multiuso importante usada frequentemente em programas de florestação. A análise de crescimento das plântulas de *D. sissoo* foi efectuada para avaliar o seu crescimento após a inoculação com seis fungos solubilizadores de fosfato de ferro, quatro fungos solubilizadores de minério de ferro e cinco bactérias solubilizadoras de fosfato. O ensaio foi feito em cultura em vasos sob estufa de polietileno com de nebulização. Em geral, a biomassa nas plântulas foi maior sob condições de inoculação em comparação com o controlo. As plantas exibiram uma produção máxima de biomassa quando inoculadas com *Penicillium chrysogenum* (2) e *Aspergillus* sp. (1). A análise de crescimento revelou que a NAR (taxa de assimilação líquida) e a LAR (razão de área foliar) contribuíram para as diferenças de RGR (taxa de crescimento relativo) nos tratamentos. A aplicação de micróbios seleccionados pode levar à criação bem sucedida da *D. sissoo* em viveiros, mesmo em solos em vaso, e ajudar na produção de material de plantio de qualidade.

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Introduction

Nitrogen and phosphorus are major plant nutrients which occupy a key place in balanced use of fertilizer. Phosphorus is an important requirement for legumes for their nitrogen fixation process (Huda *et al.* 2007). Microorganisms are useful for biomineralization of bound minerals making nutrients available to their host and /or its surroundings. Most of the tropical soils are phosphate fixing hence free form of phosphate is not readily available to the plants. Application of mineral solubilizers to the seedlings helps in their establishment in such type of problematic soils (Dabas & Kaushik 1998; Gupta *et al.* 2007; Hameeda *et al.* 2006; Sahgal *et al.* 2004; Tilak *et al.* 2005). The process of inoculating microbes to the soil in a forest nursery could be an effective method to achieve higher growth and establishment of tree species on afforestation sites. Since application of fertilizer in forestry operation is minimal or non existent, development of a proper poly-mix (combination of microbial isolates and organic fertilizers) for use in nursery could be a cost effective process. Microbial application at the nursery stage has been reported to enhance the productivity of forest trees like *Albizia*, *Acacia* and *Dalbergia* (Rahangdale & Gupta 1998; Sahgal *et al.* 2004; Thatoi *et al.* 1993; Verma *et al.* 1995).

This study evaluated the efficacy of different phosphate and mineral solubilizers (bacteria and fungi) on *D. sissoo* under nursery conditions with a view to identify specific microbes useful for the establishment of the tree. *Dalbergia sissoo* Roxb. was chosen as the model plant for this experiment because it is a large deciduous tree and one of the most common versatile multipurpose, drought resistant, frost-hardy and widely distributed indigenous tree species, valued for its timber, fodder and nitrogen fixing quality. It is widely used in agroforestry, afforestation programs and farm forestry in the Indian subcontinent (Chander *et al.* 1998; Gilman & Watson 1993; Huda *et al.* 2007). It is an important plantation species and its nursery and field response were also worked out by several workers (Dabas & Kaushik 1998; Sah *et al.* 1998). Observations have also been made on its usefulness as afforestation species for rehabilitation of

degraded forest land under the inoculated conditions (Sah *et al.* 1998; Shyam Lal 1994; Singh & Bhati 2005). Shah *et al.* (2008) studied the influence of cadmium and chromium on the biomass production of *Dalbergia sissoo* that is also a suitable agroforestry species for phytoremediation of seleniferous soils (Dhillon *et al.* 2008).

Materials and methods

The study was carried out during April-August 2009 in the poly house (equipped with misting system and fitted with iron-pot-holders) placed in experimental fields of the Regional Plant Resource Centre, Bhubaneswar, India. The experiment was done in poly bags (size: 12 x 16 cm containing 2.5 kg sandy loam soil). The soil contained 83.8 % of sand, 8.8 % of silt and 7.4 % of clay. Soil pH was 6.27 and salt content was 0.504 ds m⁻¹. Soil N, P, K was analyzed by wet oxidation method (Greenwood *et al.* 2001) through commercial laboratories. Soil was fumigated with 1 % formalin (25 ml pot⁻¹) for 48 h prior to the experiment. Average nitrogen (N), phosphate (P₂O₅) and potassium (K₂O) contents of the soil were 168.7 kg ha⁻¹, 237.2 kg ha⁻¹ and 645.12 kg ha⁻¹, respectively. Temperature in the poly-house averaged 35 ± 2 °C and relative humidity 80 ± 5 %.

Six phosphate solubilizing fungi (*Penicillium griseofulvum* Diercks, *Penicillium* sp., *Penicillium chrysogenum* Thom. (1), *Aspergillus* sp. (1), *Aspergillus* sp. (2), *Aspergillus wentii* Wehmer), four iron leaching fungi [*Penicillium expansum* Link, *Paecilomyces variotii* Bainier, *Cunninghamella elegans* Lendn and *Penicillium chrysogenum* Thom (2)], five phosphate solubilizing bacteria [*Streptomyces* sp. (1), *Streptomyces* sp. (2), *Micrococcus* sp. (1), *Micrococcus* sp. (2), *Micrococcus* sp. (3)] were used for the inoculation studies (Gupta *et al.* 2007; Gupta & Vastrakar 2009). These organisms were isolated from overburden mine soils of Odisha; the strains were maintained in the microbiology laboratory of the Regional Plant Resource Centre, Bhubaneswar, Orissa (Gupta *et al.* 2007). 25 ml of 7 days old fungal culture prepared in Czapek dox medium (4.5 pH) was added to each pot as fungal inoculum prior to seed sowing. 25 ml of 5 days old bacteria culture pre-

pared in potato dextrose broth (7.0 pH) was added to each pot as bacterial inoculum prior to seed sowing.

The experiment was done in two phases, preliminary screening where single microbial isolates were tested for their effectiveness and secondary screening, where two best result giving isolates from the previous screening were applied individually as well as in combination. In the preliminary screening, 15 microbial inoculants were used along with uninoculated control. Each pot received individual microbial strain (25 ml pot⁻¹) separately, ten days prior to seed sowing. In the second phase of experiment, the strains used were [1] *Aspergillus* sp. (1) 25 ml, [2] *Penicillium chrysogenum* Thom.(2) 25 ml, [3] both the fungal strains in equal amounts (25 ml each), [4] *Aspergillus* sp. (1) + *P. chrysogenum* (2) in 2:1 ratio, [5] *Aspergillus* sp. (1) + *P. chrysogenum* (2) in 1 : 2 ratio. For each treatment, in both experiments, 25 pots were used.

Seeds of *Dalbergia sissoo* were soaked in water for 48 h and used for pot experiments. Each pot received 5 seeds but ultimately a single seedling was maintained in each pot. Plants were watered twice a day through sprinkler mist system for 2 h. Hogland nutrient solution was applied to each pot at a rate of 25 ml pot⁻¹ month⁻¹. Observations on 20 seedlings from each treatment after 4 months from DAS (date of sowing) were recorded for shoot height, and numbers of leaves and branches, dry biomass of leaves and stem, and collar diameter. In addition, sampling was done on the 21st day (2 plants per treatment were uprooted) to take the initial reading for leaf and seedling dry biomass and leaf area for the calculation of RGR (relative growth rate), NAR (net assimilation rate) and LAR (leaf area ratio). Standard procedures for growth analysis (Basak *et al.* 2004; Leopold & Kriedemann 1975; Noggle & Fritz 1986) were followed. A quality index was calculated according to Tewari *et al.* (2006) and Dickson *et al.* (1960). The following formulae were used to calculate the above parameters:

$RGR (mg\ g^{-1}\ d^{-1}) = (W_2 - W_1) / (T_2 - T_1)$, where, W_2 is final biomass, W_1 is initial biomass, T_1 is initial day (21st day), T_2 is final day (121st day).

$LAR (cm^2\ g^{-1}) = (L_1 + L_2) / (W_1 + W_2)$, where, L_1 is initial leaf area in cm², L_2 is final leaf area in cm², W_1 is leaf dry weight initial in gram and W_2 is leaf dry weight final in gram.

$NAR (mg\ cm^{-2}\ d^{-1}) = (W_2 - W_1) / t \times (L_2 - L_1)$, where, L_1 is initial leaf area in cm², L_2 is final leaf

area in cm², W_1 is initial leaf dry weight in gram and W_2 is final leaf dry weight in gram, t is interval between initial and final reading i.e. 100 days.

$QI = TW / [H/D + SW/RW]$, where, TW is total seedling dry weight in gram, H is seedling height in cm, D is collar diameter in mm, SW is shoot dry weight in gram and RW is root dry weight in gram.

Average and standard deviation for all parameters were calculated; the data were subjected to one-way ANOVA to see the significance of the effect of treatments on various seedling attributes. Correlation coefficients among RGR, NAR and QI were also calculated across treatments.

Results

Inoculation of different fungal and bacterial strains resulted in enhancement of plant height as compared to uninoculated control (Table 1). ANOVA indicated significant effect of the treatments at $P < 0.001$. The application of *Aspergillus* sp. (1) showed maximum plant height (145.66 ± 11.76 cm) as compared to other treatments whereas inoculation of *Penicillium expansum* exhibited good root growth (21.41 ± 3.16 cm). Differential response due to inoculations was observed in numbers of leaves and branches. However, no significant difference was observed in the number of leaves between control plants and those in other treatments except for plants inoculated with *Aspergillus* sp. (1) which showed highest number of leaves (115.0 ± 27.87). Mean dry biomass (33.44 ± 3.31 g) and collar diameter (42.66 ± 4.93 mm) measured after four months indicated maximum increment in growth of plants inoculated with *Penicillium chrysogenum*. It is evident that seedlings inoculated with *Aspergillus* sp. (1) and *P. chrysogenum* showed better growth and biomass compared to other treatments. Due to enhancement in dry biomass, stem height and leaf area, the relative growth rate (RGR) also changed in all the treatments. With the inoculation of *P. chrysogenum* where growth of seedlings was maximum, net assimilation rate (NAR) measured $1477.3\ mg\ cm^{-2}\ d^{-1}$ (Table 1). Across the treatments, RGR correlated positively with NAR ($r = 0.94$, $P < 0.001$) and negatively with LAR ($r = -0.75$, $P < 0.01$). NAR and LAR were also negatively related ($r = -0.71$, $P < 0.01$). It appears that differences in growth rates were due to differences in NAR and LAR. QI was positively related to RGR ($r = 0.90$, $P < 0.001$) and NAR ($r = 0.91$, $P < 0.001$)

Table 1. Effect of bioinoculants (bacterial and fungal strains) on the growth of *Daibergia sissoo* seedlings in pot culture. (Mean \pm SD)

Tr	NL	NB	RL	SH	DBL	TSDB	CD	QI	NAR	LAR	RGR
1	68.00 \pm 9.08	3.60 \pm 3.28	12.46 \pm 2.36	99.9 \pm 11.7	6.71 \pm 0.66	13.90 \pm 3.68	27.66 \pm 3.78	1.46 \pm 0.36	800.49	258.43	0.20
2	88.20 \pm 44.95	9.00 \pm 3.16	15.73 \pm 4.50	130.34 \pm 17.09	6.64 \pm 3.68	18.98 \pm 6.44	34.66 \pm 1.15	2.83 \pm 1.12	931.96	339.42	0.19
3	44.40 \pm 9.52	3.40 \pm 1.81	17.20 \pm 1.34	118.02 \pm 17.92	3.76 \pm 0.79	10.84 \pm 2.26	27.66 \pm 10.96	1.46 \pm 0.81	473.13	531.98	0.11
4	24.80 \pm 9.41	0.00 \pm 0.00	6.70 \pm 1.50	88.90 \pm 10.02	2.03 \pm 0.54	5.29 \pm 1.95	15.00 \pm 2.64	0.58 \pm 0.22	247.71	1301.74	0.05
5	115.00 \pm 26.87	3.80 \pm 1.09	18.46 \pm 2.00	145.66 \pm 11.76	8.64 \pm 1.87	24.27 \pm 10.10	36.33 \pm 2.08	3.40 \pm 1.25	1190.82	261.11	0.24
6	51.80 \pm 14.65	3.80 \pm 1.92	19.10 \pm 3.85	126.86 \pm 14.86	3.08 \pm 0.66	12.53 \pm 2.74	29.66 \pm 8.73	1.57 \pm 1.04	632.60	481.31	0.12
7	27.80 \pm 7.04	0.20 \pm 0.44	10.46 \pm 2.28	96.82 \pm 12.16	1.78 \pm 0.20	5.37 \pm 0.66	16.33 \pm 1.52	0.50 \pm 0.21	240.39	926.28	0.07
8	65.80 \pm 19.86	4.20 \pm 2.16	21.41 \pm 3.16	130.12 \pm 11.56	5.95 \pm 1.65	19.74 \pm 2.14	28.00 \pm 3.46	2.77 \pm 0.35	1157.63	383.16	0.19
9	55.20 \pm 7.79	4.00 \pm 1.87	17.16 \pm 6.10	142.80 \pm 4.66	7.02 \pm 0.73	18.77 \pm 3.72	23.66 \pm 3.78	2.16 \pm 0.37	910.76	341.55	0.18
10	79.40 \pm 16.75	4.80 \pm 2.68	14.33 \pm 5.85	142.56 \pm 8.20	8.58 \pm 0.58	26.58 \pm 1.37	39.66 \pm 1.16	4.22 \pm 1.41	1477.33	276.25	0.26
11	86.20 \pm 22.47	5.40 \pm 3.20	18.23 \pm 3.57	142.60 \pm 22.71	11.66 \pm 2.41	33.44 \pm 3.31	42.66 \pm 4.93	5.09 \pm 1.35	1330.21	181.48	0.33
12	68.40 \pm 16.97	3.40 \pm 2.07	11.40 \pm 1.22	125.30 \pm 23.61	6.61 \pm 1.87	18.68 \pm 8.09	37.00 \pm 5.56	2.16 \pm 1.03	806.26	305.28	0.18
13	64.00 \pm 24.82	3.20 \pm 1.48	12.66 \pm 2.85	134.48 \pm 9.84	6.54 \pm 1.27	17.96 \pm 1.20	38.66 \pm 3.21	2.15 \pm 0.40	788.36	576.80	0.18
14	45.40 \pm 8.93	1.60 \pm 1.51	11.66 \pm 3.93	110.68 \pm 30.34	4.30 \pm 1.03	10.47 \pm 5.59	36.66 \pm 8.50	1.54 \pm 0.79	478.58	322.92	0.10
15	27.20 \pm 5.21	0.00 \pm 0.00	11.96 \pm 1.02	125.30 \pm 23.61	1.86 \pm 1.35	5.42 \pm 4.85	34.33 \pm 5.03	1.13 \pm 0.46	145.36	653.97	0.05
16	38.20 \pm 22.28	0.00 \pm 0.00	17.73 \pm 1.30	128.86 \pm 14.27	3.23 \pm 1.82	14.72 \pm 2.91	40.33 \pm 1.52	2.97 \pm 0.34	805.84	658.67	0.13

NL - No. of leaves, NB - No. of branches, RL - Root length (cm), SH - Shoot height (cm), DBL - Dry biomass of leaves (g), TSDB - Total shoot dry biomass (g), CD - Collar diameter (mm), QI - Quality index, NAR - Net assimilation rate ($\text{mg cm}^{-2} \text{d}^{-1}$), LAR - Leaf area ratio ($\text{cm}^2 \text{g}^{-1}$) and RGR - Relative growth rate ($\text{mg g}^{-1} \text{d}^{-1}$).

Treatments: 1-control, 2-*Penicillium griseofulvum*, 3-*Penicillium restrictum*, 4-*Penicillium chrysogenum* (1), 5-*Aspergillus* sp. (1), 6-*Aspergillus oryzae*, 7-*Aspergillus wentii*, 8-*Penicillium expansum*, 9-*Paecilomyces variotii*, 10-*Cunninghamella elegans*, 11-*Penicillium chrysogenum* (2), 12-*Streptomyces* sp. (1), 13-*Micrococcus* sp. (1), 14-*Micrococcus* sp. (2), 15-*Micrococcus* sp. (3), 16-*Streptomyces* sp. (2).

Table 2. Effect of selected bioinoculant fungal strains on the growth of *Dalbergia sissoo* grown in pot culture. (Values are means; the SD values are in parentheses).

Attributes	Treatments					
	1	2	3	4	5	6
NL	90.8 (4.09)	137.4 (8.79)	131.4 (15.71)	132.2 (20.84)	134.0 (8.69)	140.2 (13.33)
NB	1.0 (0.71)	2.4 (1.14)	2.20 (1.30)	2.20 (1.30)	2.40 (0.55)	2.20 (0.84)
RL	31.84 (8.40)	23.3 (1.84)	31.62 (4.72)	33.48 (6.64)	32.26 (6.64)	32.76 (3.77)
SH	97.56 (11.21)	141.86 (15.86)	140.56 (13.43)	145.36 (05.14)	143.82 (8.89)	144.62 (5.74)
DBL	9.43 (2.95)	16.99 (3.74)	16.16 (3.77)	17.75 (2.41)	17.06 (2.09)	15.25 (2.90)
TSDB	24.79 (7.65)	44.05 (4.40)	44.47 (1.92)	46.30 (04.01)	44.85 (6.65)	45.47 (04.01)
CD	25.6 (3.36)	30.0 (1.73)	31.20 (3.49)	29.60 (3.05)	28.40 (1.52)	31.40 (3.85)
QI	1.48 (0.44)	1.81 (0.41)	2.38 (0.38)	2.63 (0.42)	2.02 (0.10)	2.46 (1.02)
NAR	800.52	1960.40	1832.52	2288.15	1957.16	1918.92
LAR	179.94	141.64	135.78	147.15	164.61	170.49
RGR	0.25	0.44	0.44	0.46	0.45	0.45

NL - No. of leaves, NB - No. of branches, RL - Root length (cm), SH- Shoot height (cm), DBL - Dry biomass of leaves (g), TSDB - Total shoot dry biomass (g), CD - Collar diameter (mm), Qi - Quality index, NAR - Net assimilation rate ($\text{mg cm}^{-2} \text{d}^{-1}$), LAR - Leaf area ratio ($\text{cm}^2 \text{g}^{-1}$) and RGR - Relative growth rate ($\text{mg g}^{-1} \text{d}^{-1}$).

Treatments: 1- Control, 2- *Aspergillus* sp. (1), 3- *Penicillium chrysogenum* (2), 4 - Both fungal strains equal amount , 5- *Aspergillus* sp.(1) + *P. chrysogenum* (2) in 2:1 ratio, 6 - *Aspergillus* sp.(1) + *P. chrysogenum* (2) in 1:2 ratio.

and negatively to LAR ($r = -0.65$, $P < 0.01$). The quality index, therefore, was a function of RGR and NAR.

Inoculants using selected fungal strains (*P. chrysogenum* and *Aspergillus* sp.) in combination under various ratios resulted in significant enhancement ($P < 0.01$ to < 0.001) of plant growth compared to the control (Table 2). These fungal strains yielded maximum shoot dry biomass (46.30 ± 4.01 g), number of leaves (140.56 ± 13.43), and shoot height (145.36 ± 5.14 cm) among all treatments. The quantity of inoculum did not affect the plant height and biomass substantially. However, the number of leaves increased due to high inoculum density of *Penicillium chrysogenum* when used in combination with *Aspergillus* sp. (1) in 1 : 2 ratio. It was also noted that combined application of *P. chrysogenum* and *Aspergillus* sp. (1) exhibited the best result in the case of number of leaves of seedlings as compared to other treatments. All fungal-inoculated seedlings showed better quality (mean QI 2.46) and comparatively

more growth (mean RGR $0.46 \text{ mg g}^{-1} \text{d}^{-1}$) than the uninoculated control (mean QI, 1.48 and mean RGR $0.25 \text{ mg g}^{-1} \text{d}^{-1}$).

Discussion

Nodulated legumes generally have a high requirement for phosphorus to generate ATP which is required for nitrogenase function. It has been observed that growth and nodulation in *Dalbergia sissoo* increased by the use of phosphate fertilizers (Huda *et al.* 2007). Most of the tropical soils are phosphate fixing, use of mineral solubilizers of microbial origin may make phosphorus available to the host plants. Microbial inoculants are also found to be useful in enhancing growth of *Dalbergia sissoo* Roxb. seedlings grown under stress conditions (Bisht *et al.* 2009; Dabas & Kaushik 1998). Seedlings of *Dalbergia sissoo* inoculated with different phosphate solubilizers and iron ore leaching fungi also exhibited good growth in terms of plant height, biomass and plant

parts compared to uninoculated control. Bacterial strains performed poorly in improving plant health grown under this experiment. Increase in plant height of *D. sissoo* seedlings over uninoculated control was indicative of the potential effect of these inoculants. *Penicillium chrysogenum* and *Aspergillus* sp. (1) are observed to be effective inoculants for this tree species influencing the dry biomass of leaves and total shoot dry biomass. The combination of *Penicillium chrysogenum* and *Aspergillus* sp. (1) in equal proportions was the most effective inoculant. *P. chrysogenum* was found to be superior in influencing height than other organisms. Fungal inoculation also increased the number of branches in seedlings. Enhancement in number of branches may facilitate the development of a good tree crown. Jankiewicz & Stecki (1976) reported that branching pattern indicates the type and form of tree crown.

Knowledge on physiological variables like RGR, NAR and LAR could be useful tools for assessing the growth and development of seedlings. NAR expresses the capacity of the plant to increase dry weight through the area of its assimilatory surface (Tewari *et al.* 2006). It appeared that differences in growth rates of *D. sissoo* Roxb. under present experimental conditions were probably due to differences in NAR and LAR.

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