Nursery and Field Response of Sissoo Plants (*Dalbergia sissoo*) to *Rhizobium* Inoculation

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The present research work aims to demonstrate the rehabilitation of a degraded forest land by afforestation of sissoo plants (*Dalbergia sissoo* Roxb.) inoculated with nitrogen fixing bacteria sp. In this study, effects of the three different *Rhizobium* isolates from three localities such as i. *Kotre-isolate*, ii. *Pokhara-isolate* and iii. *Syangaza-isolate* were assessed both in nursery and in the field. It was noted that the growth and biomass increment of seedlings in nursery as well as in the field after rhizobial inoculation were significantly high compared to control one. Among these 3-isolates, *Kotre-isolate* was found to be superior to others. Soil improvement around the root of inoculated seedlings was remarkable high. Nitrogen content of the soil increased in the range of 20–40 % compared to control (only 10–20 %). *Kotre-isolate*, in general, caused more soil improvement than the other isolates. The nutrient content in the green foliage, particularly nitrogen, increased in the range of 30–50 %, compared to control one. The considerable increase in nutrients content of soil as well as in the foliage indicates the improvement in the quality of site.

Keywords *Rhizobium, Dalbergia sissoo,* nodules, rehabilitation, soils, nitrogen-fixation
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1 Introduction

Due to increasing population in tropical region, the wood-demand has also increased with reduced land for forest production. The present natural forest trees will never be able to yield wood sufficient to meet the increasing demand for wood products. Fortunately, for the region there are many fast growing species that could be grown intensively to produce more wood than can be produced under traditional forestry.

However, it is not easy to manage degraded tropical forests. Tropical forests, despite their biological richness, are fragile and less able to recover from severe repeated human disturbances than temperate forests. This is due to the fact that the soil in tropical forests is poor and much larger percentage of nutrients is available in the biomass. Once the tropical forest is removed, the soil becomes degraded. On the contrary, in the temperate zone, a large portion of organic matter and nutrients is at all times in the soil. Therefore, when a temperate forest is removed, the soil retains nutrients and the degradation of soil will be less (Kira and Shidei 1967)

The present study is concerned with the reclamation and rehabilitation of a degraded forest lands of Khairinitar (about 26 km north-east of the Pokhara valley). This area has been completely deforested and the present reforestation program has been very difficult due to its poor soil. The chemical fertilizers, being environmentally unfriendly and also very expensive, do not solve the critical problem of forest ecosystem stability at a high level of long-term. A new technology is obviously needed. One approach to new technology is through utilizing biological nitrogen fixation, a product of symbiotic associations between certain plants and microorganisms, both free living and symbiotically living types. Free-living N_2-fixers develop slowly because of relatively limited habitat and energy sources. In contrast, symbiotically living microorganisms provide relatively large amounts of nitrogen, particularly those which form root nodules associations between plants and *Rhizobium* sp. of bacteria (Tarrant 1983). Thus, symbiotically living *Rhizobium* and their plant hosts are of great interests for possible silvicultural uses.

In this work, a fast growing tree sp. *Dalbergia sissoo* has been taken into consideration for fullfilment of the above mentioned objectives. This is a multipurpose tree species (MPTS), easy to plant and manage, and has a wide range of end-uses. It is the most popularly grown native MPTS (especially for its quality timber) in the Terai as well as in mid-hills of Nepal (White 1990). The overall goal of this study is the plantation of the fast growing leguminous species *D. sissoo* and to examine the effects of rhizobial inoculation on their biomass increment and soil improvement. Therefore, this will be very useful in solving the critical problems of reforestation program of degraded sites.

2 Methodology

2.1 Study Area

The experimental site has been selected at Khairinitar about 26 km north-east from the Pokhara valley, situated in the Central Development Region of Nepal. The average monthly maximum temperature is about 26°C and average monthly minimum temperature of about 15°C. The average annual rainfall (monsoon rainfall) is of about 1500 mm. The experimental plot lies along the Seti river. The deep gorges, high mountain steep walls along the Seti river and irregular stream profiles are some of the important topographical features of the study area. The soil is “Lithosols”. The site is highly degraded with stony and highly porous soil virtually unsuitable for cultivation and the nutrients status is very poor.

2.2 Experimental Description

2.2.1 Experimental Design

The experimental design is a Randomized Complete Block Design (RCBD) with 8 blocks (replication) (Fig. 1). The size of each block was 252 m^2 (18 × 14 m). In this way, total area of experimental plot was 2016 m^2 (8 blocks × 252 m^2). Furthermore, each block consisted of 3 sub-blocks (16 × 4 m), each with 4 plots (each 4 × 4 m) i.e. each block comprised of 12 plots. Hence, the total number of plots in the whole experimental area were 96 plots (12 × 8 blocks). Each block consisted of four treatment (three strains of *Rhizobium* and one control) randomly assigned within each sub-block. Control received no rhizobial treatment.

2.2.2 Experimental Lay-out

In the experimental plot, tree spacing was fixed to be 1 × 1 m (Fig. 1). Each plot consisted of 5 rows spaced 1 m apart. Each row consisted of 5 trees also 1 m apart; 5 rows × 5 trees = 25 trees/plot. The outer rows of each plot were buffer trees and the interior 9 trees (3 × 3 rows) have been used for data collection (samples meas-

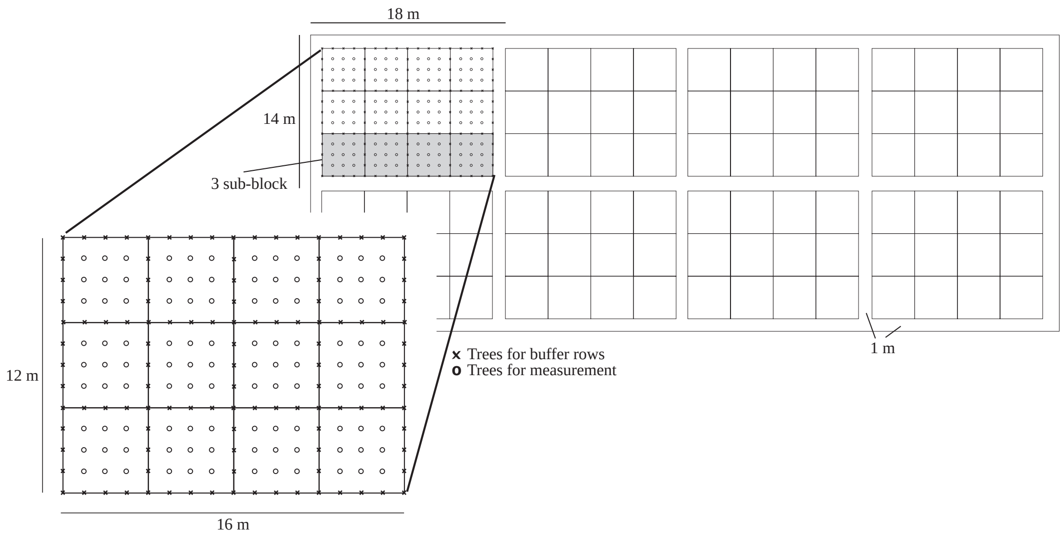

Fig. 1. Experimental design. The experimental design is a Randomized Complete Block Design (RCBD) with 8 blocks (replication)

ured). The number of plants (including buffer rows) in one block were 13 × 17 plants = 221 plants. In this way, total number of plants in the whole experimental plot were 221 plants × 8 blocks = 1768 plants.

2.3 Production of *Rhizobia*-Inoculant

The effective brown nodules under the *D. sissoo* trees were collected from 3 regions of Pokhara: (i) Nursery at Pokhara (ii) Kotre and (iii) Syangaza. Rhizobial isolation from nodules was performed using the methods of Vincent (1970). The collected nodules were surface sterilized. The sterilized nodules were aseptically crushed into small glass vial and a loopful of the breis was transferred to sterilized Yeast Extract Mannitol Broth (10 ml) as inoculant. The chemicals used in the present study were of analytical grades obtained from BDH (Merk and Sigma, USA). Yeast Mannitol growth media was prepared according to Vincent (1970).

For solid medium 1.5 % agar was used. Gram staining (Vincent 1970) of the cultured isolates was done to provide information as presumptive tests of the isolates.

The authentication of the isolates was performed using the methods of Somasegaran and Haven (1985). Healthy seeds of *D. sissoo* were collected and were surface sterilized so as to remove the surface contaminants. The broth culture of thus authenticated *Rhizobium* was used for *D. sissoo* seeds inoculant for the experiment.

2.4 Seedlings Production in Nursery and their Plantation in Field

The control as well as inoculated seeds with *Rhizobium* were grown in nursery in plastic pots. The heights of the seedlings were measured per month in nursery for 10 months.

10 months old nursery seedlings (both inoculated and non-inoculated) were planted at the spacing of 1 × 1 m in a complete randomized block described as above. After plantation, the shoot heights of all treated and control seedlings were measured per year.

2.5 Soil and Plant Samples Collection and Their Chemical Analysis

8 soil samples from each block (before and after plantation) were taken from the experimental

plots at points randomly selected and these were analyzed for pH, texture, N, P, and K by standard methods. Green foliage samples of the seedlings of both inoculated and control were collected and analysed for its different major nutrients such as N by Microjehldahl, P by Photometer, K and Ca by Atomic Absorption Spectrophotometer.

For dry weight biomass estimation, 10 seedlings from each treatment were taken out from the field and made dry in oven at 60°C. Similarly, nodules from 10 seedlings of each treatment was extracted and their number and fresh weight was estimated.

3 Results and Discussion

3.1 Shoot Height Increment in Nursery

Table 1 presents the data of shoot height measurements of seedlings in the nursery. It is evident from this table that there are prominent differences in the seedlings height of control and those of treated (i.e. inoculated) seedlings. In the earlier stage of growth (1–6 months), the growth rate of seedlings was not so prominent but after 6 months duration, there was a sudden increase in the growth of seedlings (after 10 months) up to 4–5 times the growth in the beginning. This may be attributed to the abundance of lesser population of *Rhizobium* in the beginning period than in the later stages of growth. Furthermore, Table 1 also indicates that the *Rhizobium* isolate of Kotre region is superior to others (Pokhara- and Syangaza-isolates).

3.2 Shoot Height Increment after Plantation in the Field

Mean shoot heights measured after one year planting are given in Table 2. This table indicates the similar pattern of results as those in nursery. Here, it is also noted that Kotre-isolate of *Rhizobium* shows the highest mean heights (47.4 cm) of seedlings compared to others (Pokhara-isolate = 42.7 and Syangaza-isolate = 46.9 cm). The range of percentage height increment is 30 % (control) to 58 % (Kotre-isolate). This range of increase in height of seedlings is less than that in the nursery, because the growth rate in the earlier period of seedlings development is greater than that in the older age.

From Table 2, it is evident that after 2.5 years planting the increment of seedlings height of inoculated seedlings ranged from 123–196 % compared to control of 98 %. Again, the *Kotre*-isolate was found to be superior to other isolates. Furthermore, results of ANOVA clearly indicate the significant treatment differences at 5 % level. This means there are differences in the growth of seedlings in the different treatments.

Table 1. Mean shoot heights (in cm) of both control and inoculated *D. sissoo* seedlings (1–10 months old) in nursery (n = 51, $\pm\sigma_{\bar{x}}$, Standard error of Means).

Treatments	Shoot heights 1 month	3 month	6 month	10 month
1. Control	4.9 (±1.3)	5.1 (±1.8)	5.8 (±1.4)	20.4 (±4.2)
2. Kotre-isolate	4.9 (±1.5)	5.3 (±2.0)	8.6 (±3.0)	30.0 (±4.1)
3. Pokhara-isolate	4.9 (±1.8)	5.7 (±1.7)	6.9 (±3.4)	28.9 (±3.6)
4. Syangaza-isolate	4.4 (±1.7)	5.1 (±1.6)	6.3 (±2.1)	31.3 (±4.8)

Table 2. Mean shoot heights (in cm) of both control and inoculated *D. sissoo* seedlings (1–2.5 years old) plantation in field (n = 216, $\pm\sigma_{\bar{x}}$, Standard error of means).

Treatments	Shoot height After 1 year	After 2.5 years
1. Control	26.5 (30 %) (±4.1)	52.5 (98 %) (±5.1)
2. Kotre-isolate	47.4* (58 %) (±8.1)	140.5* (196 %) (±25.3)
3. Pokhara-isolate	42.7* (48 %) (±3.9)	95.3* (123 %) (±10.1)
4. Syangaza-isolate	46.9* (50 %) (±8.4)	115.8* (146 %) (±23.2)

* = ANOVA-test showing significant treatment difference from control at 5 % level of significance
In parenthesis: Percentage of shoot height increment

Table 3. The mean fresh and dry weight biomass (in g) of both control and inoculated *D. sissoo* seedlings (6 months old) in nursery (n = 10, $\pm\sigma_{\bar{x}}$, Standard error of means).

Treatments	Fresh wt. (F)		Dry wt. (D)		D:F	
	Stem	Leaves	Stem	Leaves	Stem	Leaves
1. Control	9.1 (±2.1)	20.5 (±8.2)	3.2 (±1.3)	4.8 (±2.7)	0.35	0.23
2. Kotre-isolate	11.8 (±4.6)	22.7 (±7.3)	5.3 (±2.6)	8.5 (±3.3)	0.45	0.37
3. Pokhara-isolate	11.5 (±5.5)	21.4 (±6.8)	4.4 (±1.7)	6.8 (±2.8)	0.38	0.32
4. Syangaza-isolate	11.4 (±5.2)	21.0 (±5.9)	4.4 (±1.8)	7.0 (±2.5)	0.39	0.33

Table 4. Mean fresh weight biomass and no. of nodules in *D. sissoo* seedlings (6 months old) in 4-rhizobial isolates and control treatment in nursery (n =10, $\pm\sigma_{\bar{x}}$, Standard error of means).

Treatments	No. of nodules/ seedling	Fresh wt. of nodules/seedling
1. Control	66 (±10)	0.70 (±0.11)
2. Kotre-isolate	112 (±25)	1.8 (±0.31)
3. Pokhara-isolate	108 (±20)	1.1 (±0.22)
4. Syangaza-isolate	105 (±21)	1.4 (±0.28)

3.3 Biomass and Nodulation in Nursery

From Table 3, it is apparent that the ratio of dry weight (D) to fresh weight (F) of stem and leaves of seedlings were higher in inoculated than in the non-inoculated; indicating the higher biomass production in inoculated seedlings. Similarly, in the term of root nodulation of seedlings, the inoculated seedlings showed a higher production of nodules over the control (Table 4). Similar results have been obtained by Daft and El-Giami (1975) in bean seedlings (*Phaseolus vulgaris*, var. Canadian Wonder). In all these cases, Kotre isolate shows again its superiority over the other isolates.

3.4 Nutrients Status of Soils and Plants Before and After Plantation

The preliminary survey of soil analysis before plantation (Table 5) indicates a suitable pH range (pH 6) for proper plant growth but the nutrients content (such as N, P and K) of soils are very poor indicating a highly degraded stage of soil condition. After plantation, the improvement in the nutrients status of soil (especially nitrogen) in the root region of different rhizobial inoculated seedlings was prominent in the range of 50–80 %, compared to control of only 20–40 % (Table 5).

The nutrients status of green leaves before and after plantation of seedlings is shown in Table 6. From this table, it can be concluded that after plantation, the plants contained prominent higher amount of major nutrients in the case of inoculated seedlings than in the control one. However, results of ANOVA test indicate the significant treatment differences at 5 % level only for the Kotre-isolate.

The ranges of nutrients increase for the leaves of inoculated seedlings (after plantation, 2.5 years old) were 50–75 % of N, and 20–50 % of P, K and Ca, as compared with control which was 20–30 % in all nutrients (compare Table 6). In ANOVA-test, only nitrogen in Kotre-isolate showed significant difference from the control. There were no significant differences in other treatments.

Table 5. Soil nutrients-status before plantation and after 2.5 years growth of *D. sissoo* seedlings in 4-treatments (n = 10, $\pm\sigma_{\bar{x}}$, Standard error of means).

Treatments	Nutrients in soil N %	P kg/ha	K kg/ha	% of nutrients increase N	P	K
1. Before plantation	0.19 (±0.03)	6.8 (±1.23)	212 (±41)	–	–	–
2. After plantation						
a. Control	0.27 (±0.05)	8.5 (±1.33)	260 (±51)	42	24	23
b. Kotre-isolate	0.35* (±0.11)	11.3* (±3.50)	358* (±66)	84	66	70
c. Pokhara-isolate	0.30 (±0.06)	9.8 (±1.05)	314 (±80)	58	44	49
d. Syangaza-isolate	0.31 (±0.09)	10.8 (±2.47)	327 (±29)	63	59	55

* = ANOVA-test showing significant treatment difference from control at 5% level of significance

Table 6. Nutrients-status of green leaves (in mg/g dry wt.) before plantation and after 2.5 years growth of *D. sissoo* seedlings in 4-treatments (n = 216, $\pm\sigma_{\bar{x}}$, Standard error of means).

Treatment	Nutrients (mg/g) N	P	K	Ca
Before plantation (6 months old)	20.1 (±5.30)	2.3 (±0.06)	12.3 (±2.90)	20.1 (±5.53)
After plantation (2.5 years old)				
1. Control	26.3 (±6.20)	2.6 (±0.08)	15.4 (±3.89)	26.1 (±7.33)
2. Kotre-isolate	34.3* (±8.11)	3.3 (±0.04)	18.6 (±4.10)	32.1 (±7.43)
3. Pokhara-isolate	31.2 (±6.91)	2.7 (±0.04)	16.5 (±3.99)	26.3 (±7.53)
4. Syangaza-isolate	32.3 (±10.01)	2.9 (±0.08)	17.3 (±4.16)	28.8 (±7.31)

* = ANOVA-test showing significant treatment difference from control at 5% level of significance

4 Conclusions

Thus, the results for 3 rhizobial treatments show that all treatments caused significant higher rate of shoot growth than does the control. The order of performance of all the isolates, in general, was Kotre > Syangza > Pokhara > Control. Similar trend was observed in the case of soil improvement and plant nutrients uptake.

Based on the above, therefore, the rehabilitation of degraded lands of tropical forests can be followed by biological N_2-fixers. Nepal, being the mountainous country (i.e. lack of convenient transportation), the transportation of heavy chemical fertilizers from one place to another, is very difficult and expensive. Therefore, the quality biological nitrogen fixers, being very easy to be transferred from one place to another, could be of great significance for Nepal.

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References

Daft, M.J. & El-Giami, A.A. 1974. Effect of Endogene mycorrhiza on plant growth and nodulation in French Bean (Phaseolus vulgaris). New Phytol. 73, p. 1139.

Kira, T. & Shidei, T. 1967. Primary production and turnover of organic matter in different forest ecosystems of the western pacific. Japanese J. Ecol. 19: 70–87.

Somasegasan, P. & Hoven, H. 1985. Methods of Legume Rhizobium technology. University of Hawaii. p. 128–138.

Tarrant, R.F. 1983. Nitrogen fixation in N. American forestry: Research and application. In: Gordon, J.C. & Wheeler, C.T. (eds.), Biological N-fixation in forest ecosystems; Foundation and applications. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague. ISBN 90-247-2849-5. p. 261–277.

Vincent, J.M. 1970. A manual for the practical study of root nodule bacteria. IBP-Handbook 15. London, Blackwell Scientific Publications.

White, K.J. 1990. Dalbergia sissoo: An anoted Bibliography. F/FRED, Winrock International, Bangkok, Thailand.

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