EFFECTS OF SEEDLING SOURCE VARIATION ON NITROGEN METABOLISM AND BIOMASS PRODUCTION IN *ALBIZIA LEBBEK*

A. Kaur*, A. Husen & T. C. Pokhriyal

Plant Physiology, Botany Division, Forest Research Institute, P. O. New Forest, Dehra Dun-248 006, India

Received September 2003

KAUR, A., HUSEN, A. & POKHRIYAL, T. C. 2005. Effects of seedling source variation in relation to nitrogen metabolism and biomass production in *Albizia lebbek*. Seedlings of *Albizia lebbek* were collected from five different Forest Department nurseries, namely, the forest divisions from Barkot and Lalpani in Dehra Dun; Kalsi in Chakrata; Kiratpur Sahib in Roopnagar (Punjab); and Lalkuan in Haldwani to study the changes in some growth parameters, mineral nutrients (nitrogen, phosphorous and potassium) in different parts of the seedlings and *in-vivo* nitrate reductase and nitrogenase activity. Overall highest and lowest growth, biomass production and nitrogenase activity were recorded in Barkot and Kalsi sources respectively. The reverse trend was observed for the number of nodules per plant and nitrate reductase activity. The highest and the lowest contents of nitrogen and phosphorous in leaves and stem were recorded in Kalsi and Kiratpur Sahib seedlings respectively. Barkot source exhibited the highest contents of total nitrogen and total phosphorous per seedling whereas total potassium content per seedling was highest in seedlings from Kiratpur Sahib. The physiological changes in the seedling behaviour may be attributed to the variation in the origin of seed and the prevailing edaphic conditions, including rhizospheric microflora.

Key words: Nitrogenase – nitrate reductase – nitrogen – phosphorous – potassium

KAUR, A., HUSEN, A. & POKHRIYAL, T. C. 2005. Kesan variasi tempat asal anak benih terhadap metabolisme nitrogen dan penghasilan biojisim *Albizia lebbek*. Anak benih *Albizia lebbek* dikumpul daripada lima tapak semaian Jabatan Hutan yang berbeza iaitu dari Barkot dan Lalpani di Dehra Dun, Kalsi di Chakrata, Kiratpur Sahib di Roopnagar (Punjap) dan Lalkuan di Haldwani. Tujuannya ialah untuk mengkaji perubahan dalam sesetengah parameter pertumbuhan, nutrien mineral (nitrogen, fosforus dan kalium) dalam bahagian anak benih yang berbeza serta aktiviti nitrat reduktase dan nitrogenase *in-vivo*. Secara keseluruhan, bacaan tertinggi dan terendah bagi pertumbuhan, penghasilan biojisim dan aktiviti nitrogenase dicerap masing-masing dalam anak benih dari Barkot dan Kalsi. Trend sebaliknya diperhatikan untuk bilangan nodul bagi setiap anak benih dan aktiviti nitrat reduktase. Kandungan tertinggi nitrogen dan fosforus didapati dalam anak benih yang berasal dari Kalsi sementara yang terendah, dari Kiratpur Sahib. Anak benih Barkot menunjukkan kandungan jumlah nitrogen serta jumlah fosforus yang tertinggi bagi setiap anak benih. Bacaan tertinggi bagi jumlah kalium bagi setiap anak benih adalah dalam anak benih Kiratpur Sahib. Perubahan fisiologi dalam anak benih dikaitkan kepada variasi tempat asal anak benih serta keadaan edafik sedia ada, termasuklah mikroflora rizosfera.

Email: ajeetkaur2000@yahoo.com

Introduction

Scarcity of fuelwood, fodder and food for man and animals, along with increased erosion and decreased fertility of soil are among the major problems shared by many developing countries. Fast-growing leguminous trees and shrubs are now being planted on a large scale to provide fuelwood, construction material, fodder and nitrogen-rich biomass for improving soil fertility. When non-nitrogen fixing trees are intercropped with nitrogen-fixing leguminous trees, soil nitrogen availability and growth have been reported to be enhanced (Du Cros *et al.* 1984). *Albizia lebbek* is a fast-growing leguminous tree with nitrogen fixation and assimilation capabilities. It is grown as a nurse tree in tea and coffee plantations and the shed leaves also make good fertilizer. The foliage of this tree is lopped for nutritious fodder and the timber is used for construction of houses and furniture. Its bark contains tannins, saponins and reddish brown gum. *Albizia lebbek* is extensively planted as an avenue tree, wind breaks and shelter belts. It grows in a variety of soils and climates and is fairly resistant to salt, drought and frost. Chauhan and Pokhriyal (2001, 2002) reported an increase in the overall growth and nitrogen fixation potential of *A. lebbek* seedlings inoculated with *Rhizobium* and arbuscular mycorrhiza. Pokhriyal *et al.* (1987) reported higher plant height, nodule biomass and nitrogenase activity in *A. lebbek* seedlings compared with *Acacia* and *Dalbergia*.

Nitrogenase and nitrate reductase activities are estimates of N_2 fixation and assimilation efficiencies and have a direct bearing on the growth of plants. These enzymes, together with macronutrients such as phosphorous, potassium and nitrogen, play an important role in nitrogen metabolism. Not much work has been done to delineate the relationships between these elements in the metabolism of nitrogen. Therefore, an attempt was made in the present investigation to study the status of these elements in various parts of *A. lebbek* seedlings of different sources in relation to nitrogen fixation potential.

Materials and methods

Seven- to eight-month-old *Albizia lebbek* seedlings were collected in October and November 1999 from the Forest Department nurseries, namely, forest divivisions from Barkot and Lalpani in Dehra Dun, Kalsi in Chakrata, Kiratpur Sahib in Roopnagar (Punjab) and Lalkuan in Haldwani. Uniform sized seedlings (~ 25.5 cm high) were selected from each location and kept for a period of one year in a glass house under similar environmental conditions, following complete randomized design, at the Forest Research Institute in Dehra Dun, India. The morphological observations on the various growth parameters, namely, root length (cm), shoot length (cm), collar diameter (mm), number of leaves and number of nodules were recorded in the month of October 2000. Out of 20 selected plants, four from each source were randomly taken for the study.

For biomass estimation, the different plant parts, namely, leaves, stem, root and nodules were separated and dried in the oven at 70 °C until constant weight was achieved. Total nitrogen (%) in plant and soil samples were estimated using the method described by Loomis and Shull (1937). Total phosphorus (%) and total potassium (%) in plant samples were estimated using the method described by Vogel (1961).

In-vivo nitrate reductase (NR) activity (n mole NO_3^- reduced $hour^{-1}$) was assayed as described by Klepper *et al.* (1971) but with modification. Approximately 0.5 g nodules from each plant were weighed into 30 ml culture tubes containing 6 ml of infiltration medium at 3 °C. The infiltration medium was composed of 0.35 M KNO_3 and 0.05 M phosphate buffer at pH 7.0. The tubes were evacuated using vacuum pump for about 2 min with intermittent releases of vacuum until the nodules settled down. The tubes were then incubated at 25 °C for 1 hour. The reaction was stopped by immersing the culture tubes in boiling water bath for 4 min. The nitrite (NO_2^-) produced was determined using methods described by Evans and Nason (1953). Nitrogenase (N_2ase) activity in the root nodules was determined using methods by Hardy *et al.* (1968).

Percentage data were transformed to arc sine (Anderson & McLean 1974) and remaining data were analysed without transformation. The technique of analysis of variance (ANOVA) was utilized to test the significant effect of treatments (source of seedlings) on each variable measured.

Results

The seedlings collected from different sources exhibited significant variations in shoot length, number of leaves and number of nodules produced per plant (Table 1). However, the differences among root length and collar diameter were not significant. Highest shoot length was recorded in seedlings collected from Lalpani. Kalsi seedlings had the lowest shoot length and collar diameter, but the highest number of nodules. Barkot had the lowest number of leaves and nodules per seedling.

Highest leaf dry weight was observed in Kiratpur Sahib source but for stem and nodule, Lalpani. It is interesting to note that the means for dry weights of leaves, stem, root, nodule and total weight of seedlings were lowest in the seedlings collected from Kalsi which exhibited the highest number of nodules.

Highest and lowest N contents in leaf, stem and root were recorded in Kalsi and Kiratpur Sahib sources respectively (Table 2). In nodules, the highest and the lowest N values were found in Lalpani and Barkot sources respectively. Kiratpur Sahib seedling showed the lowest values of P contents in leaves, stem, root and nodules. The highest P contents in leaves and stem were estimated in Kalsi seedlings. The highest percentages of K in leaves and stem were in Kalsi and Kiratpur Sahib seedlings respectively. Lalkuan seedling exhibited the highest K contents in root and nodules. The lowest K contents in leaves and stem were recorded in Barkot seedling while in root and nodules, in Lalpani and Kiratpur Sahib sources respectively. Highest soil N content was in Lalkuan and the lowest, in Kiratpur Sahib seedlings.

Table 1 Effects of seedling sources on growth and biomass parameters in *Albizia lebbek* seedlings

Source of seedlings	Root length (cm)	Shoot length (cm)	Collar diameter (mm)	Number of leaves/plant	Number of nodules/plant	Dry weight (g)				Total dry weight (g)
						Leaves	Stem	Root	Nodule	
Barkot	14.57	92.15	9.92	6.25	9.50	0.70	12.83	14.01	0.08	27.62
Lalpani	12.77	94.90	9.22	7.00	13.25	0.35	13.28	10.96	0.09	24.69
Kalsi	14.47	50.60	7.56	7.00	16.50	0.36	4.16	9.32	0.06	13.90
Kiratpur Sahib	17.20	91.25	8.08	12.50	10.50	0.79	9.66	11.16	0.08	21.69
Lalkuan	13.67	61.65	10.07	15.25	15.50	0.63	8.85	9.38	0.06	18.92
Significance level	NS	***	NS	**	**	**	***	NS	NS	**
$CD_{(0.05)}$	-	14.91	-	4.58	4.51	0.24	3.34	-	-	6.26

Note: ** and *** reflect significant variations at 1% and 0.1% level respectively.

Table 2 Effects of seedling source variations on the nitrogen (N), phosphorous (P) and potassium (K) contents in different plants parts of *Albizia lebbek* seedlings

Source of seedlings	Soil N%	Leaf N%	Stem N%	Root N%	Nodule N%	Leaf P%	Stem P%	Root P%	Nodule P%	Leaf K%	Stem K%	Root K%	Nodule K%
Barkot	0.13	2.52	1.21	1.40	3.36	0.16	0.13	0.11	0.39	0.50	0.37	0.36	5.77
	(0.036)	(0.159)	(0.110)	(0.119)	(0.184)	(0.040)	(0.036)	(0.033)	(0.062)	(0.071)	(0.060)	(0.060)	(0.243)
Lalpani	0.13	2.87	1.20	1.52	4.34	0.22	0.08	0.08	0.97	1.16	0.55	0.27	6.03
	(0.036)	(0.170)	(0.110)	(0.124)	(0.210)	(0.047)	(0.028)	(0.029)	(0.098)	(0.108)	(0.074)	(0.051)	(0.248)
Kalsi	0.11	3.22	1.74	1.59	3.92	0.28	0.15	0.10	0.41	1.28	0.49	0.62	5.65
	(0.034)	(0.180)	(0.132)	(0.126)	(0.199)	(0.053)	(0.039)	(0.032)	(0.063)	(0.113)	(0.070)	(0.078)	(0.240)
Kiratpur Sahib	0.04	2.60	1.31	1.16	3.78	0.12	0.05	0.04	0.22	0.74	1.05	0.60	1.07
	(0.019)	(0.162)	(0.115)	(0.108)	(0.196)	(0.034)	(0.022)	(0.021)	(0.047)	(0.086)	(0.102)	(0.075)	(0.104)
Lalkuan	0.18	2.66	1.26	1.40	4.06	0.17	0.15	0.12	0.50	0.96	0.42	0.86	7.23
	(0.043)	(0.164)	(0.112)	(0.118)	(0.203)	(0.041)	(0.039)	(0.034)	(0.071)	(0.098)	(0.064)	(0.092)	(0.272)
Significance level	***	***	***	*	***	***	***	***	***	***	***	***	***
$CD_{(0.05)}$	0.006	0.007	0.009	0.011	0.009	0.007	0.004	0.005	0.100	0.006	0.018	0.021	0.003

Note: *, ** and *** reflect significant variations at 5%, 1% and 0.1% level respectively.
The values within parentheses show arc $sine^{-1}$ transformed values.
CD has been calculated on arc $sine^{-1}$ transformed values.

Total nodular NR and N_2ase activities did not differ significantly among seedlings of five different sources. However, the specific ativities (per g fresh weight) of both enzymes showed significant differences. The specific nodular NR activity was highest in Kalsi source followed by Lalkuan and Lalpani (Figure 1). The Kalsi and Barkot seedlings exhibited the lowest and the highest specific N_2ase activities respectively (Figure 2). The trend for total N content per seedling was similar with the specific N_2ase activity. Total P content per seedling was highest in Barkot followed by Lalpani seedling. The seedlings collected from Kiratpur Sahib exhibited highest total K and lowest total P contents (Figure 3).

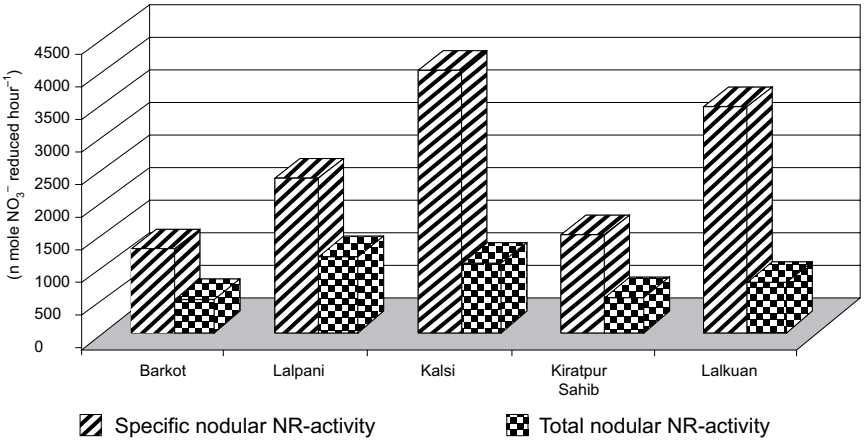

Figure 1 Changes in *in-vivo* nodular NR-activity of *Albizia lebbek* seedlings

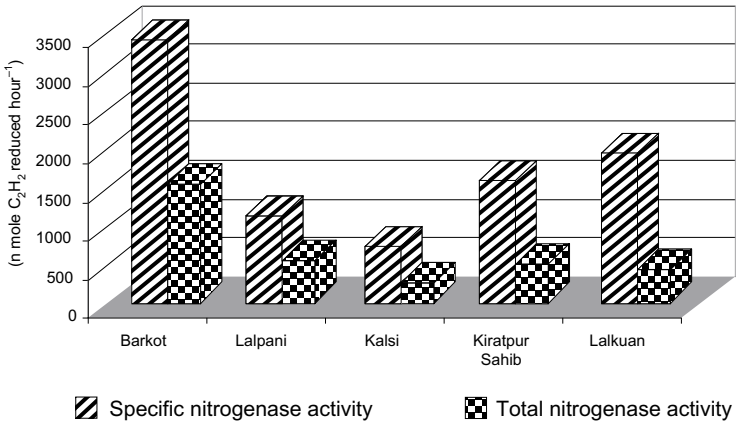

Figure 2 Changes in nitrogenase activity of *Albizia lebbek* seedlings

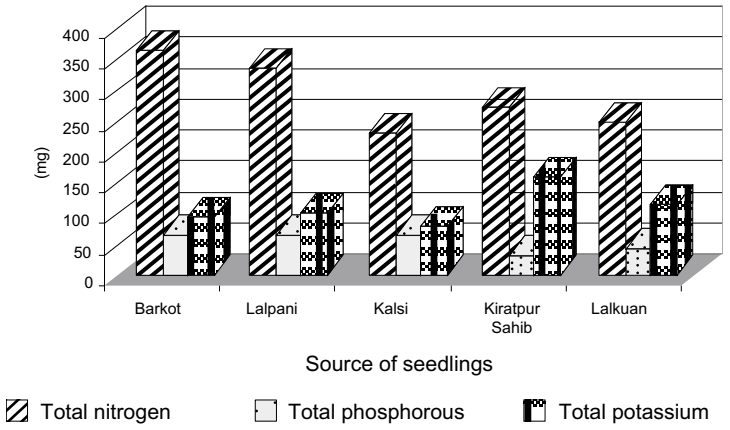

Figure 3 Changes in total nitrogen, phosphorous and potassium contents of *Albizia lebbek* seedlings

Discussion

Albizia lebbek seedlings collected from five different sources behaved differently although they were initially of almost uniform size and age and were acclimatized under similar environmental conditions for one year. This may be attributed to the prevailing edaphic conditions, i.e. soil types and existing microflora in the rhizosphere as well as the origin of seed sources/genotypes from where seedlings were collected. The genotype, status of nutrients especially N and P and population of effective nodulating rhizobia influence the extent of nodulation and nitrogen fixation. Almost similar findings have been reported by Pande *et al.* (1999) in *Dalbergia sissoo*. Inadequate rhizobial population limits the efficacy of nitrogen fixation in many tropical soils (Singleton *et al.* 1992). Rhizobial strains have been reported to influence shoot biomass and N content significantly (Somasegaran *et al.* 1990). In the present investigation the seedlings from Barkot performed the best in terms of biomass production and the rest of the growth parameters studied, except for numbers of leaves and nodules per plant, which were lowest in this source. The seedlings from Kalsi performed the poorest in terms of total biomass production but had the highest number of nodules. These results are contradictory to those reported by Singh and Pokhriyal (2002) who related poor growth with poor nodulation in seedlings.

Kalsi seedling exhibited the highest nodular NR activity and the lowest N_2ase activity. On the other hand, Barkot seedling with lowest NR activity in nodules exhibited the highest N_2ase activity. Pokhriyal *et al.* (1991) reported that *D. sissoo* complemented their N_2ase activity with nodular NR activity in order to meet its persistent nitrogen requirement from soil as well as atmosphere. The seedlings from Kalsi, with greatest number of nodules per plant, had the lowest N_2ase activity. Soil N, especially nitrate, has an inhibitory effect on the nodulation and nitrogen fixation of legume-rhizobium symbiosis (Gibson & Jordan 1983). However, no such

correlation was observed in our study. Probably, the soil N contents were well below the threshold level.

A close correlation has been reported among shoot and nodule dry weight, shoot N content and total N_2ase activity (Somasegaran *et al.* 1990). Nitrogenase activity has also been correlated with height, and aboveground dry biomass and plant growth rate (e.g. Miettinen 1989, Singh & Pokhriyal 2002). Just as N and P, K also plays an important role in nitrogen metabolism. P is required during nitrogen fixation and assimilation. K, on the other hand, helps in the uptake of NO_3^- from soil and also in the synthesis and activation of NR. In this study, seedlings from Kiratpur Sahib, which showed average growth and biomass parameters, had the lowest N and P contents in leaf, stem and root. Nodular P and K contents were also the lowest in this source. The seedlings of Kalsi source, on the other hand, exhibited higher N and P contents in leaf, stem, root and nodule. However, the total N, P and K contents were low in these seedlings, which reflect their poor response in terms of growth. The highest value of total N content per seedling in Barkot source may be attributed to its highest N_2ase activity. Total N content of seedlings in the rest of the sources showed similar patterns. Future work of this study should focus on the role of these macronutrients in N metabolism. Strain isolation from rhizospheric microflora and inoculation and N fixation studies are also other aspects to be looked into. These studies with relation to growth and development will throw light on the various aspects of nitrogen metabolism in plants.

Acknowledgement

The first author acknowledges the Council of Scientific and Industrial Research, New Delhi for financial assistance.

References

Anderson, V. L. & McLean, R. A. 1974. *Design of Experiments.* Marcel Deckker Inc., New York.

Chauhan, Y. S. & Pokhriyal, T. C. 2001. Effects of *Rhizobium* and mycorrhizal inoculation in relation to pH treatments on some morphological characters in *Albizia lebbek* Benth. *Annals of Forestry* 9(2): 314–322.

Chauhan, Y. S. & Pokhriyal, T. C. 2002. Nodulation and nitrogen fixation as influenced by *Rhizobium* and mycorrhizal inoculation in *Albizia Lebbek* (L.) Benth seedlings. *Annals of Forestry* 10(2): 243–251.

Du Cros, T. E., Jung, G. & Bariteau, M. 1984. Alder—poplar association for biomass production. *Plant and Soil* 78: 235–243.

Evans, H. J. & Nason, A. 1953. Pyridine nucleotide-nitrate reductase from extracts of higher plants. *Plant Physiology* 28: 233–244.

Gibson, A. H. & Jordan D. C. 1983. Ecophysiology of nitrogen fixing systems. Pp. 301–390 in Lange, O. L., Nobel, P. S., Osmond, C. B. & Zeigler, H. (Eds.) *Encyclopedia of Plant Physiology.* Volume 12C. Springer-Verlag, New York.

Hardy, R. W. F., Holsten, R. D., Jackson E. K. & Burns R. C. 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. *Plant Physiology* 43: 1185–1207.

Klepper, L., Flesher, D. & Hageman, R. H. 1971. Generation of reduced micotinanide adenine dinuleotide for nitrate reduction in green leaves. *Plant Physiology* 48: 580–590.

Loomis, W. E & Shull, A. C. 1937. *Methods in Plant Physiology.* Mc Graw Hill Book Co. Inc., New York.

Miettinen, P. 1989. Nitrogenase activity in *Prosopis juliflora* seedlings inoculated with *Rhizobium* isolates from eastern Kenya. *Nitrogen Fixing Tree Research Report* 7:110–111.

Pande, P. C., Chaukiyal, S. P., Pokhriyal, T. C. 1999. Nitrogenase activity vis-à-vis field testing of *Dalbergia sissoo* rhizobium isolates collected from different sources. *The Indian Forester* 125: 219–224.

Pokhriyal, T. C., Gupta, B. B., Chaukiyal, S. P. & Bhatnagar, S. K. 1991. Changes in nitrogenase activity in *Albizia lebbeck* and *Leucaena leucocephala. Indian Journal of Forestry* 14(3): 217–219.

Pokhriyal, T. C., Raturi, A. S., Pant, S. P., Pande, S. K. & Bhatnagar, S. K. 1987. Nitrogen fixation in *Albizia, Acacia, Dalbergia* and *Leucaena leucocephala. The Indian Forester* 113(5): 366–369.

Prasad, P., Uniyal, R. C., Pokhriyal, T. C. & Nautiyal A. R. 1997. Effects of soil types on nodulation and nitrogen fixation in two leguminous tree species. *Annals of Forestry* 5(1): 62–66.

Singh, N. & Pokhriyal, T. C. 2002. Nitrogen fixation and nodulation behaviour in relation to seed source variations in *Dalberga sissoo* seedling. *Journal of Tropical Forest Science* 14(2): 198–206.

Singleton, P. W., Bohlool, B. B. & Nakao, P. L. 1992. Legume response to rhizobial inoculation in the tropics: myths and realities. Pp135–155 in Lal, R. & Sanchez, P. (Eds.) *Myths and Science of Soils in the Tropics.* SSSA Special Publication No. 29. American Society of Agronomy, Madison.

Somasegaran, P., Abaidoo, R. C. & Kumaga, F. 1990. Host-*Bradyrhizobium* relationships and nitrogen-fixation in the Bambarra groundnut (*Voandzeia subterranea* (L.) Thouars nom. cons.). *Tropical Agriculture (Trinidad)* 67(2): 137–142

Vogel, A. I. 1961. *Quantitative Organic Analysis Including Elementary Instrumental Analysis.* Longmans, Green & Co. Ltd., London.