NITROGEN FIXATION AND NODULATION BEHAVIOUR IN RELATION TO SEED SOURCE VARIATIONS IN *DALBERGIA SISSOO* SEEDLINGS

N. Singh & T.C. Pokhriyal*

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

Received March 2000

SINGH, N. & POKHRIYAL, T. C. 2002. Nitrogen fixation and nodulation behaviour in relation to seed source variations in *Dalbergia sissoo* seedlings. Nitrogen fixing abilities of six *Dalbergia sissoo* seed sources, namely, Sibsagar (Assam), Tulsipur-Gonda, Chiriyapur, Hissar (Haryana), Simblewala (Jammu and Kashmir) and Kankai (Nepal), from the entire distribution range of this species in India and Nepal were studied at the Forest Research Institute, Dehra Dun (32° 20' 40'' N latitude, 77° 52' 12'' longitude), India from 1997 to 1999. Significant differences in nodulation behaviour, nitrogenase activity and soil nitrogen accretion were observed between different seed sources. Maximum nodule biomass and nitrogen fixation were recorded in Gonda seed source followed by Nepal, Chiriyapur, Jammu, Hissar and minimum in Assam. A positive correlation was noticed between nodule number and its biomass with nitrogen fixation ability. The differences were discernible and pronounced only during the second year of growth. These are important to exploit the genetic variability among different seed sources of *D. sissoo* in amelioration of degraded sites.

Key words: Nitrogen fixation - nodulation behaviour - seed source - variations - *Dalbergia sissoo*

SINGH, N. & POKHRIYAL, T. C. 2002. Kaitan pengikatan nitrogen dan tingkah laku penodulan dengan perubahan sumber biji benih anak benih *Dalbergia sissoo*. Keupayaan pengikatan nitrogen enam sumber biji benih *Dalbergia sissoo* iaitu Sibsagar (Assam), Tulsipur-Gonda, Chiriyapur, Hissar (Haryana), Simblewala (Jammu dan Kashmir) dan Kankai (Nepal), daripada keseluruhan julat taburan spesies di India dan Nepal, dikaji di Institut Penyelidikan Perhutanan, Dehra Dun, India (latitud 32° 20' 40", longitud 77° 52' 12") dari tahun 1997 hingga 1999. Perbezaan bererti dalam tingkah laku penodulan, aktiviti nitrogenase dan pengakretan nitrogen tanih dicerap di dalam sumber biji benih yang berbeza. Biojisim nodul dan pengikatan nitrogen maksimum dicatatkan dalam sumber biji benih Gonda, diikuti Nepal, Chiriyapur, Jammu dan Hissar manakala sumber dari Assam adalah minimum. Terdapat korelasi positif antara bilangan nodul dan biojisim dengan keupayaan pengikatan nitrogen. Perbezaan dapat dilihat dengan jelas hanya pada tahun kedua pertumbuhannya. Keputusan ini penting bagi mengeksploitasi variasi genetik dalam sumber biji benih *D. sissoo* bagi pembaikan tapak ternyah gred.

*Author for correspondence. E-mail: pokhriyaltc@icfree.org

Introduction

The differences in the growth and development processes during succession have taken a long time to evolve many types of mutations, which may have spontaneously occurred in trees due to eco-climatic conditions. Geographical variations influence genetic and phenotypic expressions. The knowledge of genetic variation within species is essential for developing effective tree improvement and breeding strategies. Tree improvement programme starts with the scanning of available variations in the distributional range of the species and delineation of provenance/seed source capable of providing the best adaptable trees (Callaham 1964, Wright 1976, Suri 1984). Seed source studies are needed for the screening of naturally available genetic variations to utilise the best material for obtaining maximum productivity and further breeding work (Shiv & Banerjee 1986). The most successful tree improvement programmes are those in which proper seed source and provenance are used (Zobel & Talbert 1984).

Biological nitrogen fixation is a cheap, renewable source essential for the sustainable productivity of afforested sites. Nodulation and nitrogen fixing activity of trees are significantly influenced by climatic conditions (Gibson & Jordon 1983). The amount of nitrogen fixed depends on the seasonal pattern of the nitrogenase activity (Pizzelle 1984, Pokhriyal *et al.* 1990,1991, Chaukiyal *et al.* 2000). Significant differences in growth, nodulation and nitrogen fixation are observed between clones of *Casuarina equisetifolia* (Sougoufara *et al.* 1987). Significant differences are also reported in seedling growth and nitrogen fixing ability among different provenances (Sanginga *et al.* 1990, Beniwal *et al.* 1995). Chaukiyal *et al.* (1999) reported seasonal variation effects on the nodulation and nitrogen fixation behaviour in eight *Acacia* species.

Dalbergia sissoo, a multipurpose tree crop, has been recognised for the production of fuel wood, timber and forage in plantation programmes and agroforestry systems in arid and semi-arid regions. Apart from its ability to fix atmospheric nitrogen, this species is able to release a considerable amount of nitrogen rich organic biomass which decomposes quickly and improves the nutrient status of the soil. Selection of the higher nitrogen fixing provenances would therefore greatly increase the usefulness of *D. sissoo* in plantation programmes, particularly on degraded wastelands. However, not much studies have been conducted in this aspect. Therefore, in the present study an attempt has been made to investigate the differences in the nitrogen fixation behaviour of *D. sissoo* seedlings raised from different seed sources distributed over its natural distribution range.

Materials and methods

The experiment was conducted at the Botany Division, Forest Research Institute, Dehra Dun (situated between 30° 20' 40" N latitude, 77° 52' 12" E longitude) from 1997 to 1999. Seeds were collected from six different sources (five from India and one from Nepal), covering almost the entire distributing range of *D. sissoo,* namely, Sibsagar (Assam), Tulsipur-Gonda (Uttar Pradesh-East), Chiriyapur (Uttar Pradesh-West), Hissar (Haryana), Simblewala (Jammu and Kashmir) and Kankai (Nepal). Geographical details of each seed source are given in Table 1.

Table 1 Geographic location of *Dalbergia sissoo* seed sources

Seed source	State/ country	Latitude N (°)	Longitude E (°)	Altitude (m asl)	Rainfall mm/year^{-1}
Chiriyapur	U.P.-West	29.60	76.40	454	1166.00
Kankai	Nepal	27.30	85.20	121	1125.00
Hissar	Haryana	28.90	76.60	178	567.00
Sibsagar	Assam	27.00	94.60	97	2504.30
Simblewala	J & K	32.30	74.80	324	978.00
Gonda (Tulsipur)	U.P.-East	27.10	81.90	110	1294.00

J & K = Jammu and Kashmir
U.P. = Uttar Pradesh

Well-sieved soil mixture (soil, sand and farm yard manure in 2:1:1 ratio) weighing 5 kg was filled in each earthern pot (30-cm diameter). A total of 1000 pots were prepared and numbered. Germinated seeds were transferred into the pots. Seedlings were uprooted randomly at monthly intervals for visual observations to record the nodule formation in different seed sources. The nodules were separated with the help of regulated water pressure to minimise root damages. Three replicates were taken for each treatment. Periodical observations on nitrogenase activity and the amount of nitrogen fixed by individual plant raised from different seed sources were determined at monthly intervals for the period of two years by acetylene-ethylene reduction assay as described by Hardy *et al.* (1968). Approximately, one gram nodules with 10% acetylene in rubber serum stoppered vials were incubated at 30 °C for one hour in a shaking water bath. The reduction of acetylene was measured by injecting 1 ml of gas mixture of enzyme assay into a Porapack-N column (1.8 m in length, 3.12 OD and 80-100 mesh) of CIC gas chromatograph using nitrogen as the carrier gas. Ethylene produced was quantified with reference to standard ethylene gas supplied by EDT Research, England. The nitrogenase activity was expressed as μmoles C_2H_2 reduced per gram and per plant per hour and nmols N fixed per plant per day (Hardy *et al.* 1968).

Total reduced nitrogen was estimated in nodules and soil by the method of Jackson (1967). Organic particle-free soil samples from six seed sources and control (without plants) were collected at six monthly intervals for soil nitrogen accretion analysis. The data collected were subjected to analysis of variance to determine the contribution of each seed source. Two years pooled data were used for two-way ANOVA to see the difference between sampling period and treatment.

Results

Overall, an increase in nodule number and biomass per plant was observed from February to September. This was followed by a sharp decline during the winter months in both years. Maximum nodule number and the biomass per plant were recorded in Gonda seed source and minimum in Assam (Table 2). However, no nodules were recorded in January and February in all the six seed sources and the differences between nodule number per plant were only distinct in the second year of this experiment. In September, maximum (2.44 g) nodule dry weight per plant was recorded in seeds from Nepal and minimum (0.30 g) from Assam (Figure 1). However, pooled analysis also exhibited significant differences between different seed sources.

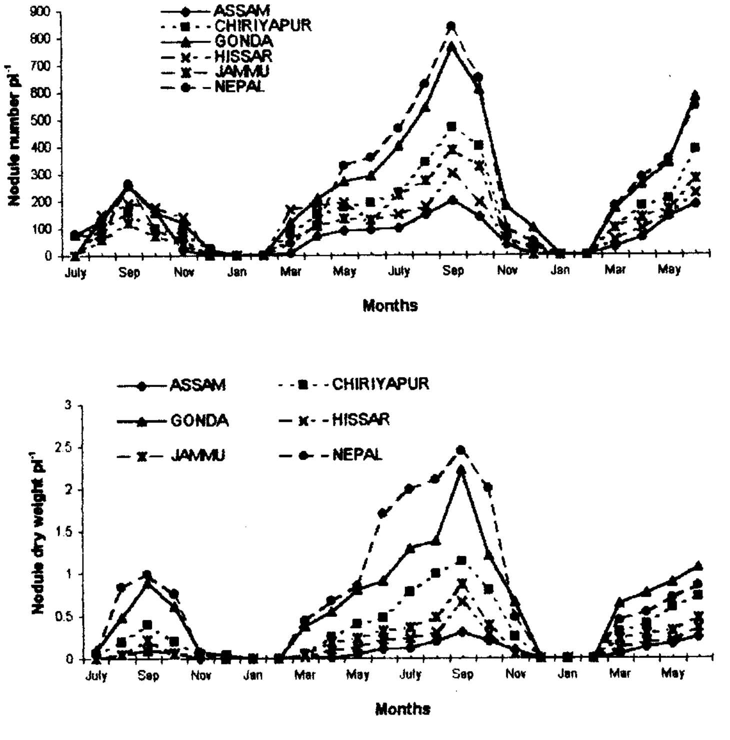

Figure 1 Seasonal variation effects on nodule number and dry weight per plant in *Dalbergia sissoo* seedlings raised from different seed sources

An increase in the nitrogenase activity was recorded from July to September followed by a decline until February. A small peak was observed in April with the emergence of new nodules in spring (Figure 2). In general, nitrogenase activity showed similar pattern in both years. Gonda seed source performed best and Assam poorest. Differences in total nitrogenase activity were discernible only in September and no activity was recorded in January and February.

Table 2 Statistical analysis of different characters recorded in *Dalbergia sissoo* seedlings raised from different seed sources

Character	Source of variation	Bar diagram of mean values												C. D.	Level of significance
Nodule number	Seed source	ASSAM		JAMMU		HISSAR		CHIRIYAPUR		NEPAL		GONDA			
		79.00		111.01		115.29		143.86		226.69		232.93		42.66	***
	Months	Jan.	Feb.	Dec.	Nov.	May	July	April	Mar.	Aug.	Oct.	June	Sept.		
		00.00	00.00	22.10	76.80	93.10	143.40	155.80	212.90	229.40	254.20	279.40	350.00	80.65	***
Nodule dry wt. pl^{-1}	Seed source	ASSAM		HISSAR		JAMMU		CHIRIYAPUR		NEPAL		GONDA			
		0.08		0.13		0.19		0.34		0.61		0.78		0.13	***
	Months	Feb.	Jan.	Dec.	Nov.	Mar.	Apr.	May	July	Oct.	Aug.	June	Sept.		
		0.00	0.01	0.02	0.15	0.22	0.35	0.44	0.50	0.55	0.59	0.60	0.81	0.25	***
N_2ase activity g^{-1} fresh wt. h^{-1}	Seed source	ASSAM		HISSAR		JAMMU		CHIRIYAPUR		NEPAL		GONDA			
		133		205		251		313		385		637		48	***
	Months	Jan.	Feb.	Dec.	June	July	May	Mar.	Nov.	Aug.	Oct.	Apr.	Sept.		
		00	00	82	173	174	263	278	288	460	465	619	1060	91	ns
N_2ase activity pl^{-1} h^{-1}	Seed source	ASSAM		HISSAR		JAMMU		CHIRIYAPUR		NEPAL		GONDA			
		100		226		302		353		895		8394		-	ns
	Months	Jan.	Feb.	Dec.	Nov.	Mar.	July	Jun	Oct.	Apr.	Aug.	Sep.	May		
		00.00	00.00	14	90	204	242	457	731	761	1159	3790	13 444	-	ns
µg N fixed pl^{-1} day^{-1}	Seed source	ASSAM		HISSAR		JAMMU		CHIRIYAPUR		NEPAL		GONDA			
		23.81		53.87		71.22		126.87		208.11		478.28		144.30	***
	Months	Jan.	Feb.	Dec.	Nov.	Mar.	July	May	June	Apr.	Oct.	Aug.	Sept.		
		0.00	0.00	3.60	25.30	51.10	57.40	140.10	159.50	166.70	169.30	274.50	876.90	-	***
Soil nitrogen accretion (kg ha^{-1})	Seed source	GONDA		NEPAL		CHIRIYAPUR		JAMMU		HISSAR		ASSAM	CONTROL		
		271.20		231.60		199.80		194.20		162.50		121.10	38.90	53.30	***
	Months	6		12		18		24							
		51.48		163.65		207.56		288.92						31.50	***

Journal of Tropical Forest Science 14(2): 198–206 (2002)

The amount of nitrogen fixed from October to March was almost negligible (Table 2). Between the six seed sources, maximum and minimum values of nitrogen fixed per plant per day were recorded in the case of Gonda and Assam respectively during the peak period, i.e September, in the second year of growth. The amount of soil nitrogen (kg h^{-1}) estimated during the two years was maximum in Gonda followed by Nepal, Chiriyapur, Jammu, Hissar and Assam (Table 2). All provenances showed higher values of soil nitrogen compared with the control.

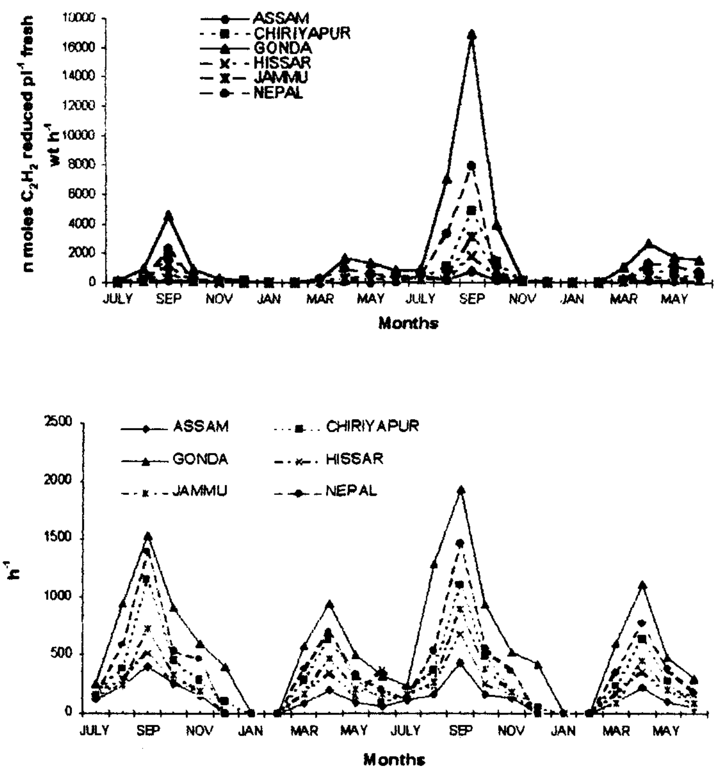

Figure 2 Seasonal variation effects on nitrogenase activity in *Dalbergia sissoo* seedlings raised from different sources

Discussion

Remarkable changes in nodulation behaviour, nitrogenase activity and nitrogen content were noticed in *D. sissoo* seedlings raised from different seed sources. Overall, Gonda seed source performed better compared with others. Variations in nodulation pattern have been reported for provenances of *Casuarina* (Dawson *et al.* 1989) and *Gliricidia sepium* (Sanginga *et al.* 1991). Nodulation behaviour as a seed source dependent character has also been described in *D. sissoo* (Devagiri 1997). From our study the seedlings raised from Gonda, Chiriyapur and Nepal showed faster growth and more number of nodules per plant compared with the other three seed sources. This may be due to the inherent potential of these seed sources to develop efficient host-parasite relationship. Similar variations in the nodulation behaviour is reported in twelve different tree species (Himmat 1997). However, in the present study, none of the characters show any discernible geographical trend and the variations observed are sporadic.

Nitrogenase activity showed a wide variation in different seed sources of *D. sissoo*. Maximum value was recorded in Gonda followed by Nepal and Chiriyapur. Poor growth in Assam seed source may be attributed to poor nodulation. Wide variations in nitrogenase activity is also observed in *Acacia albida* provenances (Sniezko 1987, Sanginga *et al.* 1990). Close variation between acetylene reduction rate of field grown soybean with air temperature and environmental variables has been established (Sloger *et al.* 1975). The low nitrogenase activity in newly formed nodules during hot summer is supported by Kuo and Boersoma (1971), Sprent (1972), Pokhriyal *et al.* (1990) and Uniyal (1997). Availability of photosynthate is one of the major factors controlling the rate of nitrogen fixation (Hardy & Havelka 1976). A significant positive correlation between net rate of photosynthesis, nodule biomass per plant and nitrogenase activity has been established in *Alnus glutinosa* (Gordon & Wheeler 1978). Comparatively, fast growth, leading to a larger photosynthetic area of seedlings, exhibited by Gonda seed source in the present study may be a possible reason for the generation of more ATP and nitrogenase activity. The poor growth of Assam, Hissar and Jammu seedlings may be responsible for the limited supply of photosynthates for nodule growth and nitrogen fixation as already discussed above. Environmental factors influencing the symbiotic nitrogen fixation in plants have been reviewed by Havelka *et al.* (1982) and Dixon and Wheeler (1983). The variations in nitrogen fixation and soil nitrogen content was the obvious reflection of differences in the nitrogen utilisation behaviour of different seed sources. Gonda seed source with higher nitrogen fixing ability added maximum N into the soil compared with other seed sources. The contribution from leguminous nitrogen fixing plant systems vary from 100–700 kg N ha^{-1} $year^{-1}$ but the average values are 100–200 kg N ha^{-1} (Donald 1960). The amount reported in non-leguminous trees ranges from 15–350 kg N ha^{-1} $year^{-1}$ in *Myrica cerifera* and *Alnus rubra* (Silver & Mague 1970, Newton *et al.* 1968). Densly-managed *C. equisetifolia* and *Leucaena leucocephala* stands have fixation potentials exceeding 250–500 kg N ha^{-1} $year^{-1}$ (Dommergues *et al.* 1984). It was also reported that soil nitrogen accretion varies between 179–336 kg ha^{-1} y^{-1} in some nitrogen fixing leguminous and non leguminous plants (Himmat & Pokhriyal 1998). The extent of nodulation therefore can be considered as a suitable factor for better shoot growth as reported by Schwintzer and Lancelle (1983). Although some seed sources may be genetically capable of supporting high nitrogen fixation, this could be further exploited by suitable management practices.

References

BENIWAL, R. S., TOKY, O. P. & SHARMA, P. K. 1995. Genetic variability in symbiotic nitrogen fixation between provenance of *Acacia nilotica* (L.) Wild. ex del. *Genetic Resources and Crop Evolution* 42: 7–13.

CALLAHAM, R. Z. 1964. Provenance research: investigation of genetic diversity associated with geography. *Unasylva* 18: 40–50.

CHAUKIYAL, S. P., HIMMAT, S. K. C. & POKHRIYAL, T. C. 1999. Effects of seasonal variations on nodulation and nitrogen fixation behaviour in some *Acacia* species. *Indian Forester* 7: 112–119.

CHAUKIYAL, S. P., SHEEL, S. K. & POKHRIYAL, T. C. 2000. Effects of seasonal variation and nitrogen treatments on nodulation and nitrogen fixation behaviour in *Pongamia pinnata*. *Journal of Tropical Forest Science* 12(2): 357–368 .

DAWSON, J. O., KOWLASKI, D. G. & DART, P. J. 1989. Variation with soil depth, topographic position and host species in the capacity of soil from an Australian locale to nodulate *Casuarina* and *Allocasuarina* seedlings. *Plant and Soil* 118: 1–11.

DEVAGIRI, G. A. M. 1997. Evaluation of seed source variation in seed and seedling traits in *Dalbergia sissoo* Roxb. Ph.D. thesis, Deemed University, F.R.I., Dehra Dun. 189 pp.

DIXON, R. O. D. & WHEELER, C. T. 1983. Biochemical physiological and environmental aspects of symbiotic nitrogen fixation. Pp. 55–87 in Gordon, J. C. & Wheeler, C. T. (Eds.) *Biological Foundations and Applications.* The Hague, Nijhaf.

DOMMERGUES, Y. R., DIEM, H. G., GAUTHIER, D. L., DREYFUR, B. L. & CORNET, F. 1984. Nitrogen fixing trees in the tropics: potentialities and limitations. Pp. 7–15 in Vieger, C. & Newton, W. E. (Eds.) *Advances in Nitrogen Fixation Research.* Martinus Nijhoff / Dr. W Junk Publ., The Hague.

DONALD, C. M. 1960. The impact of cheap nitrogen. *Australian Institute of Agricultural Science* 26 :319–338.

GIBSON, A. H. & JORDON, D. C. 1983. Ecophysiology of nitrogen fixing systems. Pp. 301–390 in Lange *et al.* (Eds.) *Physiological Plant Ecology Response to Biochemical Environment.* Published Spring-Verlag, Berline, New York.

GORDON, J. C. & WHEELER, C. T. 1978. Whole plant studies on photosynthesis and acetylene reduction in *Alnus glutinosa. New Phytology* 80: 179–186.

HARDY, R. W. F. & HAVELKA, U. D. 1976. Photosynthate as major factor limiting nitrogen fixation by field grown legumes with emphasis on soybean. In Nutman, P. S. (Ed.) *Symbiotic Nitrogen Fixation in Plants* 7: 421–439.

HARDY, R. W. F., HOLSTEN, R. D., JACKSON, E. K. & BURNS, R. 1968. The acetylene-ethylene assay for nitrogen fixation laboratory and field evaluation. *Plant Physiology* 43: 1195–1207.

HAVELKA, U. O., BOYLE, M. G. & HARDY, R. W. F. 1982. Biological N fixation. Nitrogen in agricultural soils. *Agronomy* 22: 365–413.

HIMMAT, S. K. C. 1997. Survey, identification and distribution of leguminous and non-leguminous nitrogen fixing plants vis-a-vis nodulation behaviour in relation to altitudinal variations from terai to tree-line in the forests of Eastern Nepal. Ph.D. thesis, Deemed University, F.R.I., Dehra Dun. 274 pp.

HIMMAT, S. K. C. & POKHRIYAL, T. C. 1998. Effects of some leguminous and non-leguminous fixing herb, shrub, climber and tree species on soil nitrogen accretion. *Annals of Forestry* 6: 119–122.

JACKSON, M. L. 1967. *Soil Chemical Analysis.* Prentice Hall of India , Private Limited, New Delhi. 249 pp.

KUO, T. & BOERSOMA, L.L. 1971. Soil water stress and root temperature effects on nitrogen fixation in soybean. *Agronomy Journal* 63: 901–904.

NEWTON, M., EL HASAN, B. A. & ZAVITKOVSHI, J. 1968. Role of red alder in Western Oregon forest succession. Pp. 73–84 in Troppe, J. M., Franklin, J. F., Tarrant, R. F. & Hasen, G. M. (Eds.) *Biology of Alder.* USDA Forest Service, PNW Forest Range Experimental Station, Portland, Oregon.

PIZZELLE, G. 1984. Seasonal variation of the sexual reproductive growth and nitrogenase activity (C_2H_2) in mature *Alnus glutinosa. Plant and Soil* 78: 181–188.

POKHRIYAL, T. C., CHAUKIYAL, S. P. & NEGI, D. S. 1991. Changes in nodular nitrogenase and nitrate reductase activity in *Dalbergia sissoo. Indian Journal of Plant Physiology* 34: 166–170.

POKHRIYAL ,T. C., NEGI, D. S., CHAUKIYAL, S. P. & BHANDARI, H. C. S. 1990. Changes in nitrogenase and nitrate reductase activity in *Acacia catechu*. *Nitrogen Fixing Tree Research Report* 8: 108–110.

SANGINGA, N. K., BOWEN, G. D. & DANDO, S. K. A. 1990. Assessment of genetic variability for nitrogen fixation between and within provenances of *Leucaena leucocephala* and *Acacia albida* estimated by ^{15}N labelling techniques. *Plant and Soil* 127: 169–178.

SANGINGA, N. K., MARIQUE, B. & HARDSON, G. 1991. Variation in nodulation and nitrogen fixation by *Gliricidia sepium/ Rhizobum* spp. symbiosis in a calcareous soil. *Biology and Fertility of Soils* 11: 273–278.

SCHWINTZER, C. R. & LANCELLE, S. A. 1983. Effect of water table depth on shoot growth, root growth and nodulation of *Myrica gale* seedlings. *Journal of Ecology* 71: 489–501.

SHIV, K. P. & BANERJEE, A. C. 1986. Provenance trials of *Acacia nilotica*. *Journal of Tree Science* 5: 53–56.

SILVER, W. S. & MAGUE, T. 1970. Assessment of nitrogen fixation in terrestrial environments in field conditions. *Nature* 227: 378–379.

SLOGER, C. BEZDICEK, D., MILBERG, R. & BOONKERD, N. 1975. Seasonal and diurnal variation in N_2 (C_2H_2)-fixing activity in field soybeans. Pp. 271–284 in Stewart, W. D. P. (Ed.) *Nitrogen Fixation by Free Living Micro-Organisms*. Cambridge University Press, Cambridge.

SNIEZKO, R. A. 1987. Range wide provenance in nodulation of *Acacia albida* seedlings. *Nitrogen Fixing Tree Research Report* 5: 20–21.

SOUGOUFARA, B., BU, H. E. & DOMMERGUES, Y. R. 1987. Improvement of nitrogen fixation by *Casuarina equisetifolia* through clonal selection. *Arid Soil Research and Rehabilitation* 1: 129–132.

SPRENT, J. I. 1972. The effects of water stress on nitrogen fixing root nodules. IV. Effects on whole plants of *Vicia faba* and *Glycine max*. *New Phytology* 71: 603–611.

SURI, S. K. 1984. Analytical study of teak provenance tests in North Raipur Division of M.P. *Indian Forester* 110: 345–363.

UNIYAL, P. 1997. Effect of innoculation and nitrogen fixation, assimilation and growth behaviour in *Dalbergia sisoo* Roxb. Ph.D. thesis, Deemed University, F.R.I., Dehra Dun. 142 pp.

WRIGHT, J. W. 1976. *Introduction To Forest Genetics*. Academic Press, New York, USA. 463 pp.

ZOBEL, B. & TALBERT, J. 1984. *Applied Forest Tree Improvement*. John Wiley and Sons, New York. 505 pp.