NITRATE REDUCTASE ACTIVITY AND NITROGEN CONTENT IN RELATION TO SEED SOURCE VARIATION IN *DALBERGIA SISSOO* SEEDLINGS

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SINGH, N. & POKHRIYAL, T. C. 2005. Nitrate reductase activity and nitrogen content in relation to seed source variation in *Dalbergia sissoo* seedlings. *In vivo* nitrate reductase activity and total reduced nitrogen content were estimated in different plant parts, i.e. leaf, stem, root and nodule, of *Dalbergia sissoo* seedlings. These seedlings were raised from six different seed sources, viz. Sibsagar (Assam), Chiriyapur (Uttaranchal), Tulsipur-Gonda (UP-East), Hissar (Haryana), Simblewala (Jammu and Kashmir) and Kankai (Nepal), from across a distributional range in India and Nepal and assessed at the Forest Research Institute, Dehra Dun, India. The Gonda seed source showed the highest nitrate reductase activity and nitrogen content followed by seed sources from Nepal, Chiriyapur, Jammu, Hissar and Assam. Assam seed source was observed to be the poorest in nitrate reductase activity as well as total nitrogen content. Among different plant parts, maximum nitrate reductase and nitrogen contents were recorded in root nodules followed by leaves, roots and stems. The maximum variation among seed sources was recorded during the rainy season in September. The statistical analysis of various characteristics of the six *D. sissoo* seed sources provides a base for the selection of provenances using more evenly distributed samples from the whole range of the species.

Key words: Nitrate reductase – nitrogen – seedlings – seed source – variation – *Dalbergia sissoo*

SINGH, N. & POKHRIYAL, T. C. 2005. Aktiviti nitrat reduktase dan kandungan nitrogen dalam anak benih *Dalbergia sissoo* berdasarkan variasi sumber biji benihnya. Aktiviti nitrat reduktase *in vivo* dan kandungan nitrogen dianggar dalam bahagian berlainan anak benih *Dalbergia sissoo* iaitu daun, batang, akar dan nodul. Anak benih ini berasal dari enam sumber berlainan iaitu Sibsagar (Assam), Chiriyapur (Uttaranchal), Tulsipur-Gonda (UP-Timur), Hissar (Haryana), Simblewala (Jammu dan Kashmir) dan Kankai (Nepal) dan dinilai di Institut Penyelidikan Perhutanan, Dehra Dun, India. Sumber ini mewakili julat taburan di India dan Nepal. Sumber biji benih dari Gonda menunjukkan aktiviti nitrat reduktase dan kandungan nitrogen yang tertinggi diikuti oleh sumber dari Nepal, Chiriyapur, Jammu, Hissar dan Assam. Sumber biji benih Assam menunjukkan aktiviti nitrat reduktase dan kandungan

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nitrogen yang terendah. Aktiviti nitrat reduktase dan kandungan nitrogen adalah maksimum dalam nodul, diikuti oleh daun, akar dan batang. Variasi maksimum antara sumber biji benih dicatat semasa musim hujan pada bulan September. Analisis statistik ciri-ciri enam sumber biji benih *D. sissoo* menyedikan asas bagi pemilihan provenans menggunakan sampel yang ditabur dengan seragam daripada spesies yang dikaji.

Introduction

Developing countries like India could not draw any substantive benefit from tree improvement research programmes which aim at development and dissemination of genetically superior germplasm to meet the basic requirements for fuel, fodder and timber in the present context of increasing bovine and human populations. However, screening and evaluation of different populations of tree species for adaptability and growth characteristics deserve greater attention from researchers. There is a need to emphasize the development of genetically superior germplasm for different agro-ecological zones and *ex situ* and *in situ* conservation of the available diverse germplasm for the future use in tree improvement programmes. The importance of genetic variation studies and provenance testing in tree improvement is well known (Pryor 1963, Wright 1976). Variation in seed and seedling traits and its significance in seed source studies have been documented in a number of tree species such as *Pinus caribaea* (Venator 1974), *Eucalyptus globulus* (Kirkpatrick 1975) and *Dalbergia sissoo* (Sidhu 1997).

It is well known that most forest soils in India are generally deficient in nitrogen. Therefore the screening of efficient nitrogen-utilizing multipurpose tree species can be a useful and versatile tool for improving and maintaining the fuel, fodder and timber productivity under various afforestation programmes. Nitrate is considered to be the primary source of nitrogen available in the soil and nitrate reductase is the key enzyme responsible for the reduction of nitrate to nitrite in the plant cell (Bray 1983). The reduction of nitrate to nitrite through nitrate reductase is well documented (Beevers & Hageman 1969). This enzyme is considered to be one of the limiting factors for growth and development and protein production in plants.

A continuous increasing demand for utilizable biomass from managed ecosystems due to the present increasing trend of population in many developing countries has drawn attention towards the fast-growing tree species for various afforestation programmes. *Dalbergia sissoo* is a well-known multipurpose tree species that produces quality timber, fuel, fodder and also fixes nitrogen. It is native to India, Pakistan and Nepal. The main distribution zone in India is the subHimalayan region from the Indus river to Assam and in the Himalayan valley up to an elevation of 900 m and sometimes up to 1500 m.

In recent past, a few studies has been reported on the nodulation, nitrogen fixation potential and growth characteristics of *D. sissoo* in India and elsewhere (Rehman & Husain 1986, Sidhu 1997, Singh & Pokhriyal 2000, 2001, 2002). Significant differences in nodulation behaviour, nitrogenase activity and soil nitrogen accretion in different seed sources of *D. sissoo* were reported by Singh and Pokhriyal (2002). However, no systematic studies have yet been reported on the variations of nitrogen utilization pattern in the trees raised from different seed sources. In this report, an attempt is made to examine the effect of seasonal variations on nitrate reductase activity and total reduced nitrogen content in *D. sissoo* seedlings raised from six different seed sources in India and Nepal.

Materials and methods

The experiment was conducted at the Plant Physiology Branch, Botany Division, Forest Research Institute, Dehra Dun (30°20'40" N, 77°55'12" E; 640 m asl). Seeds from six different sources across the distribution range of *D. sissoo*, viz. Sibsagar (Assam), Chiriyapur (Uttaranchal), Gonda-Tulsipur (UP-East), Hissar (Haryana), Simblewala (Jammu and Kashmir) and Kankai (Nepal), were procured from the Social Forestry Division, Seed Testing Laboratory and Plant Physiology Branch of the FRI, Dehra Dun. The experimental details of the sampling procedure, etc. have already been described elsewhere by Singh and Pokhriyal (2000, 2001, 2002).

Three replicates were taken from each seed source treatment and *in vivo* nitrate reductase (NR) activity was analysed in duplicate periodically at monthly intervals in different plant parts, viz. leaf, stem, root and nodule, as earlier described by Klepper *et al.* (1971). The plant parts were separated and washed thoroughly in tap water followed by distilled water. Finally, excess moisture was removed with the help of tissue paper before measuring the fresh weight. The procedure adopted for the NR assay was similar to that earlier described by Pokhriyal and Raturi (1985) for *D. sissoo*. The amount of nitrite produced during enzyme activity was determined by the method described by Evans and Nason (1953). Total reduced nitrogen was estimated in the tissues of leaf, stem, root and nodule by Nesler's method (Jackson 1967).

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 differences between sampling period and treatments.

Results

Leaf and nodule nitrate reductase (NR) activity exhibited two sharp peaks throughout the year, i.e. a small and large peak were observed in the months of August and April respectively in two consecutive seasons. Variation in NR activity and nitrogen among different seed sources was only discernible during the period of maximum activity, i.e. April and August. The Gonda seed source exceeded all other seed sources throughout the growing season in NR activity. Overall total NR activity in Gonda seedlings was significantly ($p = 0.001$) higher than in other seed sources, where Assam's performance was the poorest. Summer and rainy seasons proved to be favourable months for leaf NR activity (Table 1). Maximum NR (4439.6 n moles NO_3^- reduced g^{-1} fresh wt h^{-1}) and minimum NR (1229.0 n moles NO_3^- reduced g^{-1} fresh wt h^{-1}) activities were recorded in Gonda and Assam seed sources respectively in April (Figure 1).

Table 1 Statistical analysis of monthly and pooled values observed for nitrate reductase (n moles NO_3^- reduced g^{-1} fresh wt. h^{-1}) activity in different plant parts, i.e. leaf, stem, root and nodule, in *Dalbergia sissoo* seedlings raised from different seed sources

Sl. No.	Character	Source of variation	Bar diagram of mean values												C. D.	Level of significance
1	Leaf NR	Seed source	ASSAM 370		HISSAR 586		CHIRIYAPUR 716		NEPAL 913		GONDA 1238		JAMMU 11200		-	ns
		Month	JAN	FEB	DEC	NOV	MAY	OCT	SEP	JUN	JUL	MAR	APR	AUG		
			-	-	172	214	343	390	720	883	906	1420	2250	22748	-	ns
2	Stem NR	Seed source	ASSAM 159		HISSAR 197		JAMMU 252		CHIRIYAPUR 304		NEPAL 389		GONDA 557		45	***
		Month	JAN	DEC	NOV	FEB	OCT	SEP	MAY	JUN	JUL	MAR	AUG	APR		
			72	75	78	88	166	259	357	360	385	413	442	1019	84	***
3	Root NR	Seed source	ASSAM 251		JAMMU 386		HISSAR 404		CHIRIYAPUR 504		NEPAL 849		GONDA 892		204	*
		Month	JAN	FEB	DEC	NOV	OCT	JUN	JUL	SEP	MAY	MAR	AUG	APR		
			141	169	197	260	326	426	440	463	601	714	927	1908	384	***
4	Nodule NR	Seed source	ASSAM 536		HISSAR 752		JAMMU 920		CHIRIYAPUR 1401		NEPAL 1747		GONDA 2047		151	***
		Month	JAN	FEB	DEC	NOV	OCT	SEP	AUG	MAY	JUN	MAR	JUL	APR		
			-	-	211	432	704	918	1199	1204	1730	1911	2224	4327	284	ns

*p = 0.05, ***p = 0.001, ns = not significant, bar = treatments under bar are homogenous.

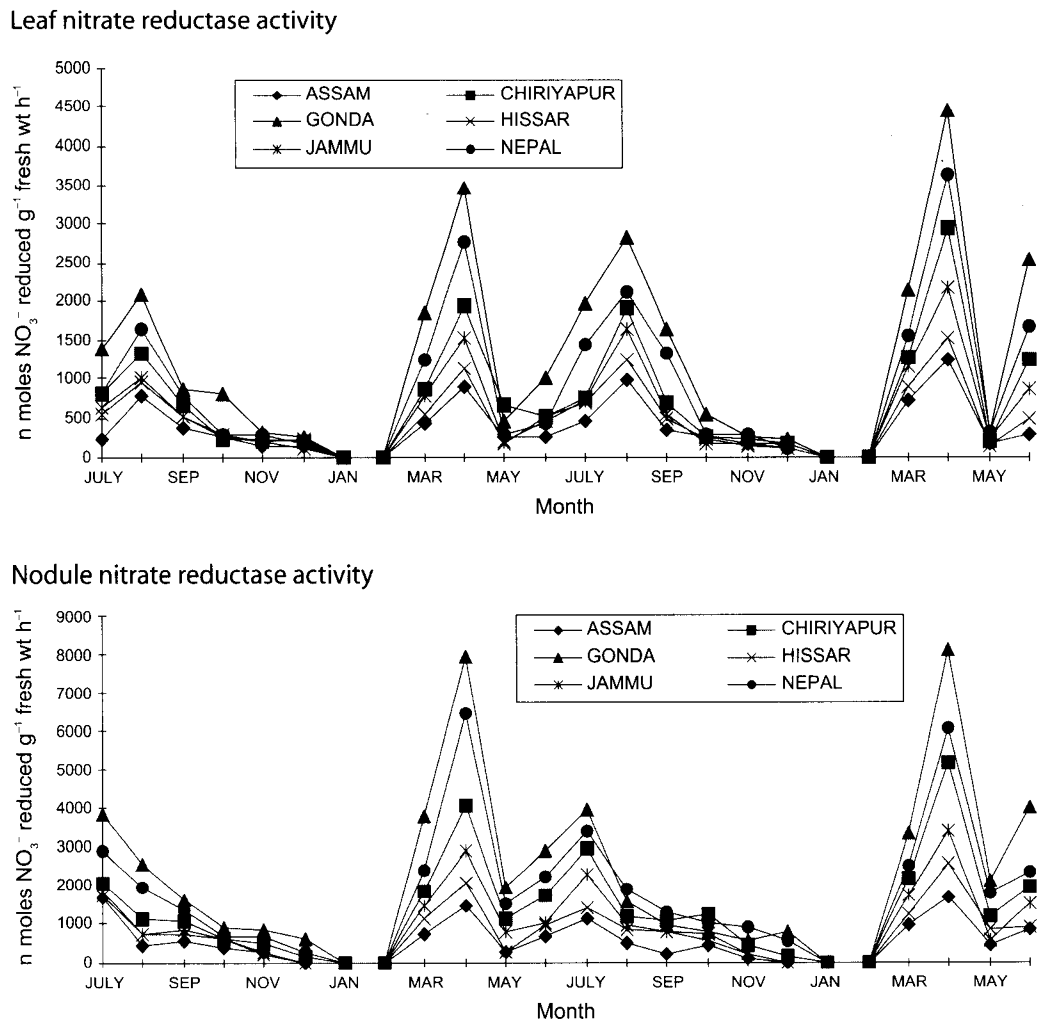
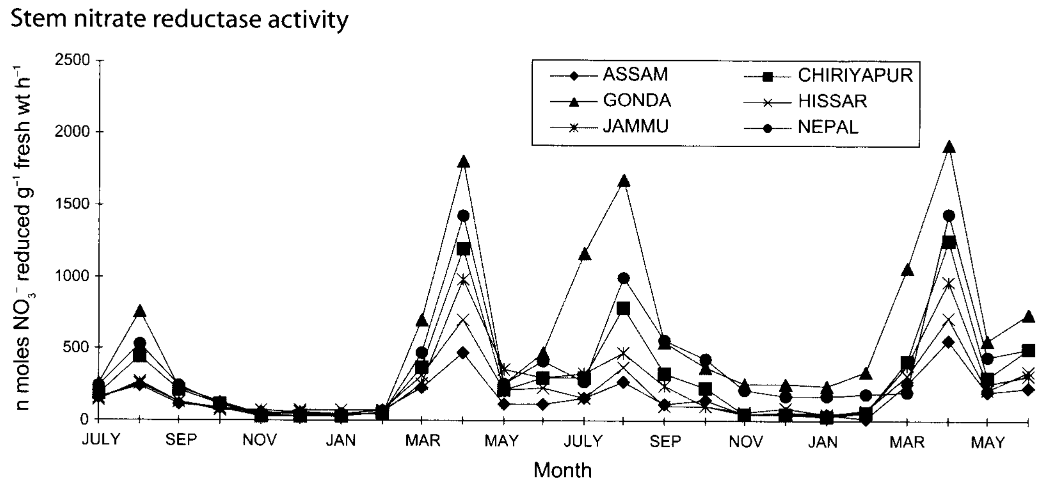


Figure 1 Changes in leaf and nodule nitrate reductase activity in *Dalbergia sissoo* seedlings raised from different seed sources

Stem and root NR activities followed almost similar trends to those of leaf NR activity. A large peak was observed in April and a smaller peak in August. Highest stem and root NR activities were recorded in Gonda seed source in April during both years (Figure 2). Statistical analysis showed that Gonda seed source was significantly greater than other seed sources in NR activity and minimum activity was recorded in Assam (Table 1). Overall, the Gonda seed source showed the highest total NR activity followed by Nepal, Chiriyapur, Jammu, Hissar and (lowest) Assam (Figure 3). Total NR activity per plant showed a small initial rise during the rainy season and remained low during the entire winter season (Table 2). An increase in the total NR activity was noticed from February to a maximum value in April and again another small peak in August the following year.

In both years, an increase was recorded in leaf and nodule nitrogen (N) percentage from July to September followed by a decline up to January (Figure 4). Maximum (4.1%) and minimum (3.1%) leaf nitrogen were recorded in Gonda



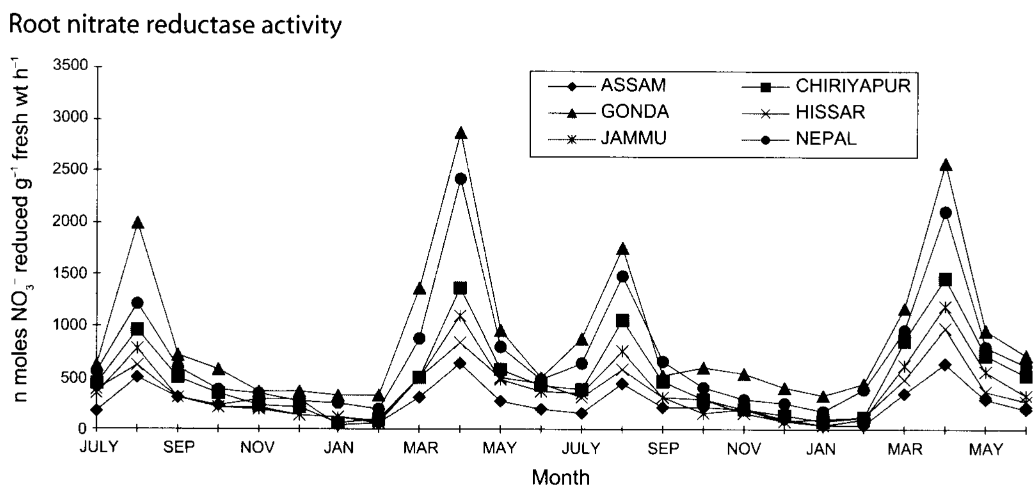


Figure 2 Changes in stem and root nitrate reductase activity in *D. sissoo* seedlings raised from different seed sources

and Assam seed sources in September. The statistical analysis of pooled values also revealed that all seed sources differed significantly in their N content on both a percentage and a total amount per plant basis. The Gonda seed source was significantly ($p = 0.001$) greater than all others in N content and was similar to Nepal, Chiriyapur and Jammu seed sources. Among months, September had the highest N contents (Table 3). During the second year in April, maximum (5.2%) nodule nitrogen was observed in Gonda followed by Nepal (3.7%), Jammu (3.5%), Hissar (3.5%), Chiriyapur (3.0%) and Assam (3.2%). Stem and root N followed a different trend than that in the leaves. Nitrogen content was low during July and followed an upward trend till February. The lowest nitrogen contents were observed in March during both years. The highest (4.1%) and lowest (2.9%) stem nitrogen contents were recorded in Gonda and Assam in February (Figure 5). The nitrogen content in the root also followed an almost similar trend to that in the stem but no sharp peak was noticed. However, N content remained high during September

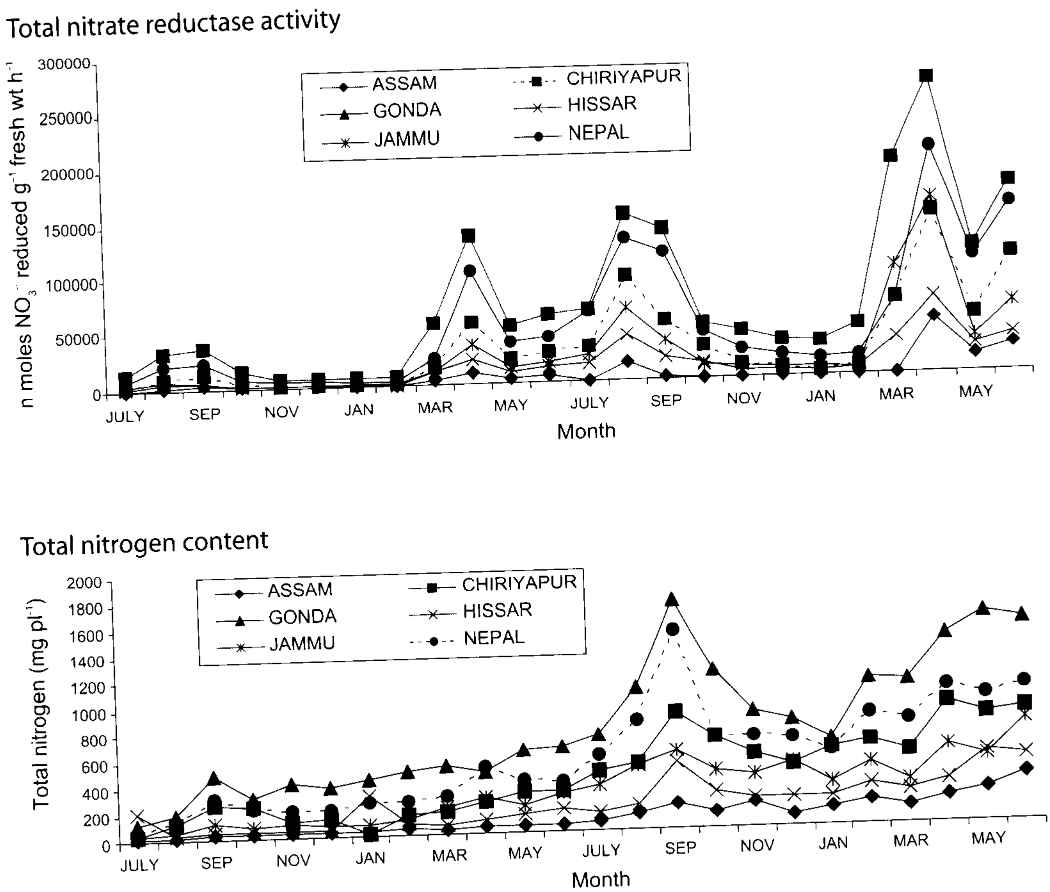


Figure 3 Changes in total nitrate reductase activity and nitrogen content in *D. sissoo* seedlings raised from different seed sources

to February in both years and was lowest in March only. An increasing trend in total nitrogen accumulation was followed throughout the period of growth. Total N content ranged between 192.4 mg (Assam) and 1741.6 mg (Gonda) per plant in September during the second year. Statistically, on the basis of average values, Gonda seed source had the highest N concentration at 0.1 percent level of significance (Table 3).

Discussion

The primary input of nitrogen in many ecosystems is through the process of biological nitrogen fixation. Nitrogen fixing plants can utilize atmospheric as well as soil nitrogen, thereby benefiting the associated vegetation regularly. The process of nitrogen utilization is a complex phenomenon since many climatic and edaphic factors influence the nitrogen availability.

Among different plant parts, viz. leaf, stem, root and nodule, overall maximum nitrate reductase (NR) activity was observed in Gonda seed source, followed by Nepal and Chiriyapur during both years. Maximum NR activity was observed in

Table 2 Statistical analysis of monthly and pooled values recorded for total nitrate reductase (n moles NO_3^- reduced pl^{-1} h^{-1}) activity in leaf, stem, root and nodule in *D. sissoo* seedlings raised from different seed sources

Sl. No.	Character	Source of variation	Bar diagram of mean values	C. D.	Level of significance
1	Total leaf NR	Seed source	ASSAM 2361, HISSAR 4215, JAMMU 5913, CHIRIYAPUR 8544, NEPAL 16516, GONDA 29696	4978	***
		Month	FEB -, JAN 34, DEC 744, NOV 2021, MAY 5287, OCT 6949, MAR 7571, JUL 12008, SEP 22052, AUG 23362, APR 23550, JUN 30852	9381	***
2	Total stem NR	Seed source	ASSAM 3364, HISSAR 5257, CHIRIYAPUR 11535, JAMMU 12610, NEPAL 17625, GONDA 34232	8141	***
		Month	JAN 3171, NOV 4494, FEB 3426, DEC 5104, OCT 6494, SEP 8924, JUL 9982, AUG 15308, JUN 19322, MAY 19713, MAR 28454, APR 44852	15343	***
3	Total root NR	Seed source	ASSAM 1401, HISSAR 4494, JAMMU 6139, CHIRIYAPUR 10330, NEPAL 17912, GONDA 27854	3304	**
		Month	JAN 3107, DEC 3447, FEB 3919, NOV 4384, JUL 5411, SEP 6490, OCT 6541, AUG 12875, JUN 16197, MAR 6472, MAY 17500, APR 39916	622.7	***
4	Total nodule NR	Seed source	ASSAM 264, HISSAR 647, JAMMU 962, CHIRIYAPUR 1667, NEPAL 2981, GONDA 4820	688	***
		Month	JAN -, FEB -, DEC 39, NOV 122, OCT 970, MAR 2168, JUL 2446, AUG 2486, SEP 2530, MAY 3240, APR 3764, JUN 4914	1297	***
5	Total NR	Seed source	ASSAM 7390, HISSAR 14613, JAMMU 25624, CHIRIYAPUR 32076, NEPAL 55034, GONDA 96602	13111	***
		Month	JAN 6312, FEB 8413, DEC 9333, NOV 10014, OCT 20954, JUL 29847, SEP 39997, MAY 45741, AUG 54031, MAR 54666, JUN 71285, APR 112082	24708	***

**p = 0.01, ***p = 0.001, bar = treatments under bar are homogenous.

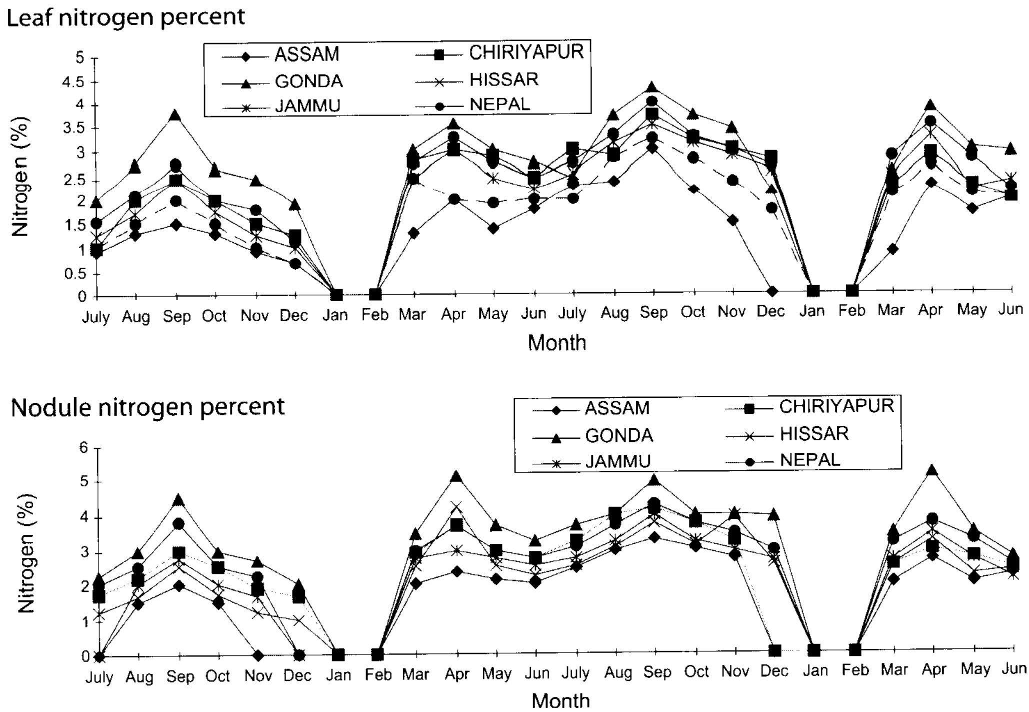


Figure 4 Changes in leaf and nodule nitrogen percent in *D. sissoo* seedlings raised from different seed sources

nodule followed by leaf, root and stem. Total NR activity was highest during the rainy months followed by summer and winter months. Almost similar results were reported earlier by Pokhriyal *et al.* (1991) in *D. sissoo* and Chaukiyal and Pokhriyal (1996) in *Pongamia pinnata.* Two distinct peaks, one during the rainy and a second in the summer season, were observed for NR activity in the seedlings of the six different seed sources. Almost similar results have been reported earlier in *Acacia catechu* and *D. sissoo* by Pokhriyal *et al.* (1990, 1991). An increase in the NR activity in *D. sissoo* during March and April may be due to low water potential resulting in the suppression of root hair infection and further nodule development leading to a reduction in total nitrogenase (Worral & Roughly 1976). Nitrogen requirement of *D. sissoo* nodules thus might be compensated by the enhanced NR activity during this period as suggested by Pokhriyal *et al.* (1990).

Among different plant parts, maximum (5.5%) nitrogen content was observed in nodule followed by leaf (4.1%) during July in Gonda seed source. However, maximum stem nitrogen content was recorded during winter. Almost similar observations were also reported in *P. pinnata* (Chaukiyal & Pokhriyal 1996) and in *D. sissoo* (Uniyal 1997). The differences in nutrient absorption of different seed sources may be attributed to inherent variation as reported by Beniwal *et al.* (1995). The concentration of leaf nitrogen markedly declined up to senescence phase. This may be due to the translocation of nitrogen to the woody parts of the plants thus causing an increase in the N content of stem during winter. Similar results were earlier reported by Day and Monk (1977), Ralhan and Singh (1987), and

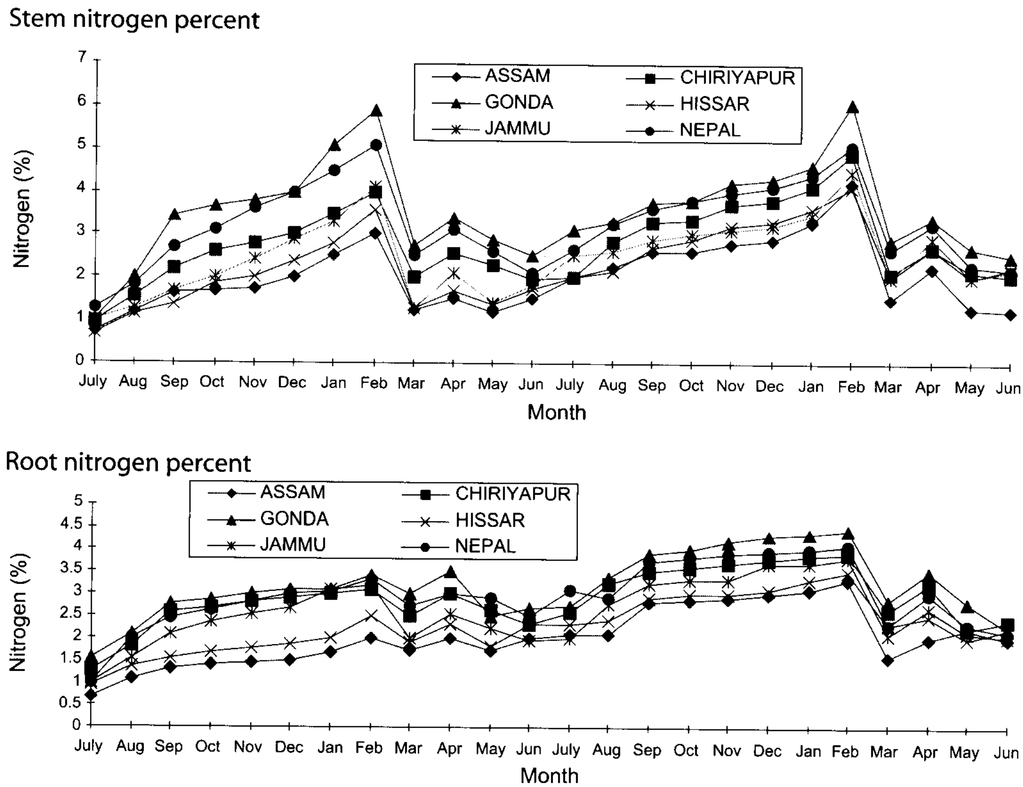


Figure 5 Changes in stem and root nitrogen percent in *D. sissoo* seedlings raised from different seed sources

Verma and Mishra (1989). There are few studies reported on the seasonal variation of nutrient concentration in woody vegetation (Grigal *et al.* 1976).

In this current study low N concentration was recorded in both stem and root during the period of rapid growth and an increase in the winter season. The increase in N concentration at the time of leaf fall may be attributed to the translocation of N from leaves back to stem and root prior to leaf fall. The amount was higher during autumn, i.e. November to February, and decreased progressively with the emergence of new leaf flushes during the onset of spring season. Gonda seed source showed the maximum nitrate reductase activity and total nitrogen content (42% and 33% respectively) and Assam the minimum (3% and 4%) over other seed sources (Figure 6).

Variation within the *D. sissoo* seed source could be due to the fact that the species grows over a wide range of rainfall, temperature, altitude and soil types in India. Thus the population will experience marked differences in selective pressure. Such variation in relation to habitat has been reported in *Albizia lebbeck* (Kumar & Toky 1993), *Acacia mangium* (Salazar 1989), *A. nilotica* (Ginwal *et al.* 1995) and *D. sissoo* (Sidhu 1997 & Devagiri 1997). Beniwal *et al.* (1995) also reported that in some provenance trials of *A. nilotica* the variation in nitrogenase activity was attributed to its inherent behaviour, i.e. some provenances may have higher nitrogen utilization potential compared with others. Singh and Pokhriyal (2000) also reported that the biomass accumulation pattern in individual plant parts differs significantly among six different seed sources in *D. sissoo* seedlings.

Table 3 Statistical analysis of monthly and pooled values estimated for nitrogen (percent and mg pl^{-1}) contents in leaf, stem, root and nodule in *D. sissoo* seedlings raised from different seed sources

Sl. No.	Character	Source of variation	Bar diagram of mean values												C. D.	Level of significance
1	Leaf N (%)	Seed source	ASSAM 1.3		HISSAR 1.7		JAMMU 2.0		CHIRIYAPUR 2.1		NEPAL 2.2		GONDA 2.5		0.48	***
		Month	JAN -	FEB -	DEC 1.6	JUL 1.9	NOV 2.1	JUN 2.3	MAR 2.3	MAY 2.2	OCT 2.5	AUG 2.5	APR 3.0	SEP 3.0	0.91	***
2	Leaf N (mg pl^{-1})	Seed source	ASSAM 25.5		HISSAR 40.3		JAMMU 72.1		CHIRIYAPUR 72.3		NEPAL 106.3		GONDA 151.8		48.32	***
		Month	JAN -	FEB -	MAR 43.9	DEC 44.8	JUL 50.4	NOV 69.1	AUG 107.4	MAY 122.6	OCT 128.0	JUN 152.8	SEP 201.6	APR 315.1	91.36	***
3	Stem N (%)	Seed source	ASSAM 1.7		HISSAR 2.0		JAMMU 2.3		CHIRIYAPUR 2.3		NEPAL 2.7		GONDA 3.0		0.49	***
		Month	JUL 1.7	MAY 2.0	MAR 2.0	OCT 2.1	AUG 2.1	JUN 2.2	NOV 2.3	DEC 2.5	APR 2.6	SEP 2.7	JAN 2.7	FEB 3.2	0.93	**
4	Stem N (mg pl^{-1})	Seed source	ASSAM 51.0		HISSAR 98.8		JAMMU 145.0		CHIRIYAPUR 195.1		NEPAL 253.0		GONDA 392.1		62.74	***
		Month	JUL 85.5	AUG 92.6	OCT 120.7	NOV 152.4	SEP 156.4	DEC 170.3	MAR 193.9	JAN 230.3	MAY 239.7	JUN 254.6	FEB 280.6	APR 292.7	118.62	**
5	Root N (%)	Seed source	ASSAM 1.6		HISSAR 1.7		JAMMU 1.9		CHIRIYAPUR 2.2		NEPAL 2.5		GONDA 2.6		0.48	***
		Month	DEC 1.6	JAN 1.8	NOV 1.8	FEB 1.8	JUL 1.9	JUN 2.1	OCT 2.2	MAR 2.2	AUG 2.3	MAY 2.3	APR 2.6	SEP 2.6	0.91	*

(continued)

(Table 3 - continued)

Sl. No.		Source of variation	Bar diagram of mean values												C. D.	Level of significance
6	Root N (mg pl^{-1})	Seed source	ASSAM 26.3		HISSAR 60.1		JAMMU 82.6		CHIRIYAPUR 139.6		NEPAL 177.7		GONDA 232.7		45.36	***
		Month	JUL 68.8	DEC 89.9	AUG 90.9	JAN 94.1	NOV 96.2	OCT 102.0	FEB 114.7	SEP 132.2	MAR 134.4	MAY 163.3	JUN 166.7	APR 193.0	85.72	**
7	Nodule N (%)	Seed source	ASSAM 1.8		HISSAR 2.0		JAMMU 2.1		CHIRIYAPUR 2.4		NEPAL 2.5		GONDA 3.4		0.77	***
		Month	JAN -	FEB -	DEC 2.2	JUN 2.5	NOV 2.5	MAR 2.8	MAY 2.8	OCT 2.8	AUG 2.9	JUL 3.1	APR 3.4	SEP 3.5	1.44	***
8	Nodule N (mg pl^{-1})	Seed source	ASSAM 1.9		HISSAR 4.3		JAMMU 5.7		CHIRIYAPUR 10.7		NEPAL 13.5		GONDA 22.2		5.32	***
		Month	JAN -	FEB -	DEC 0.3	MAR 5.7	NOV 6.1	JUL 9.9	APR 11.5	MAY 12.1	JUN 13.3	OCT 14.5	AUG 17.6	SEP 26.5	10.07	***
9	Total N (mg pl^{-1})	Seed source	ASSAM 210.2		HISSAR 433.4		JAMMU 615.9		CHIRIYAPUR 541.8		NEPAL 1136.0		GONDA 1603.2		242.30	***
		Month	JUL 236.1	DEC 305.2	JAN 305.8	AUG 313.6	NOV 323.6	FEB 346.1	OCT 371.2	MAR 371.9	APR 529.5	MAY 537.7	SEP 556.0	JUN 583.4		ns

**p = 0.01, ***p = 0.001, ns = not significant, bar = treatments under bar are homogenous.

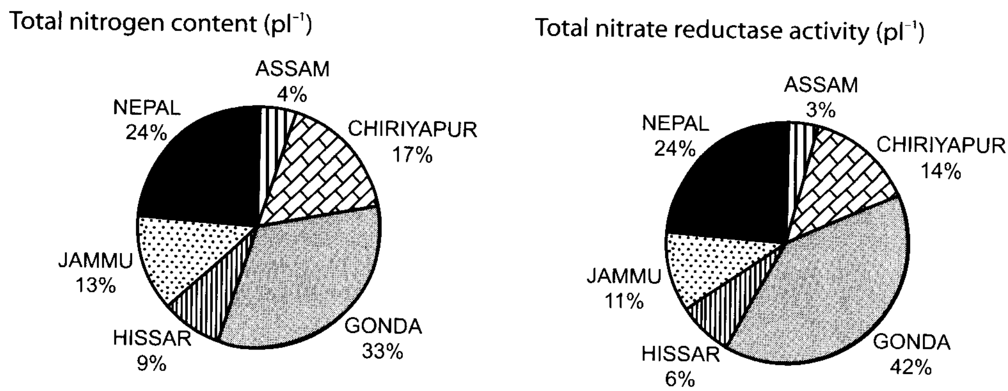


Figure 6 Pie diagrams showing percentage distribution of total nitrogen and nitrate reductase activity per plant in *D. sissoo* seedlings raised from different seed sources

The results pertaining to nitrate assimilation and nitrogen content have exhibited overall a non-significant pattern of variation. Similar trends in pods, seeds and germination behaviour in six seed sources in *D. sissoo* were also reported by Singh and Pokhriyal (2001). In general maximum nitrate reductase activity and nitrogen content were recorded in nodules during the rainy season followed by summer and winter. Among the six different seed sources, Gonda seed source was observed to have the highest NR activity and N content followed by seed sources from Nepal, Chiriyapur, Jammu, Hissar and Assam (the lowest).

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