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Nitrogen Fixation Activity of Twenty Leguminous Tree Species in Eastern Nepal

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Abstract

Nitrogen fixing trees (NFTs) are being widely adopted for multipurpose uses because of their unique ability to fix atmospheric nitrogen which helps in restoration and maintenance of soil fertility. The forests in Eastern Nepal have been under continuous degradation and are in need of remedial measures to restore their productivity. A feasible solution to rejuvenate the sites is through the introduction of indigenous and locally suitable NFTs. Therefore, a study was conducted to observe nodulation and nitrogenase activity in twenty leguminous trees, with potential for use in such programmes. The study was primarily based on seedlings raised in the nursery at Biratnagar. Locally collected seeds were used in the nursery. A great variation in the production of nodules and nitrogenase activity of the species even within the same genus was recorded. Maximum (818.3) active nodules were observed in *Albizia julibrissin* and minimum (33.9) in *Samanea saman*, however, the highest (26206.0 n moles) total nitrogenase activity occured in *Sesbania grandiflora* and lowest (10.0) in *Samanea saman.* Similarly, maximum (202.2) inactive nodules were recorded in *Sesbania grandiflora* and minimum (4.1) in *Acacia auriculiformis.* The variations recorded in the study are regarded to be influenced by climatic as well as physio-chemical factors.

Keywords: Altitude, Degeneration, Nodulation, Nodules, Symbiosis.

Introduction

Biological nitrogen fixation (BNF) is a cheap renewable N-source, essential to the sustainable productivity of afforested sites. The importance of BNF by *Rhizobium* species in symbiosis with leguminous trees is well known. They have been successfully used for fuel, fodder, green manure, shade or, companion crops in many of ways for decades (Davey and Wollum 1984). Their use has also been extended in establishment of crops, ornaments and promote soil regeneration (NFTA 1989). In the recent past, considerable attention has been given to fuel and fodder production through the use of leguminous NFTs. Which are often preferred for commercial and agro-forestry plantations because of their ability to reduce gaseous nitrogen to ammonia and to grow fast over other species (MacDicken 1994).

Root nodulation and nitrogen fixation activity in trees are governed by many factors. Gibson and Jordan (1983) reported that they are significantaly influenced by climatic conditions. The rate of photosynthesis is also said to be closely related to nodulation and nitrogenase activity. A significant positive correlation between net photosynthesis and nitrogenase activity has been reported by Gordon and Wheeler (1978). Similarly, Pizelle (1984) reported that nodulation and nitrogenase activities are also influenced by seasonal variations. However, much more is to be known about the periodicity of these activities. So far, over 600 woody species, majority of them legumes, are presently known to fix nitrogen (Halliday and Nakao 1982). In the case of eastern Nepal, documented information on nitrogenase activity of tree species is not available, thereforethe objective of this study was to observe nodulation and nitrogen fixation activity of twenty leguminous trees, so that, these species could be used in the afforestation programmes.

Methodology

The study primarily based on earthenpot culture, included 1 species from Caesalpinioideae, 9 Mimosoideae and 10 Papilionoideae. However, small and uniform seedlings of 7 species grown at various altitudes were also utilized. The natural seedlings studied were *Acacia catechu* (150m), *Albizia lebbeck*

(200m), *Butea monosperma* (100m), *Calliandra calothyrsus* (300m), *Dalbergia latifolia* (300m), *Erythrina arborescens* (1850m) and *Ougeinia oojeinensis* (160m). Seeds of these species were not viable during the establishment of the nursery, hence, nodules from their seedlings growing under field condition were collected. The nursery was established at the Regional Forest Directorate Office, Biratnagar. Seeds of the rest 13 species were collected at 70 to 1850m altitudes within this part of Nepal and were sown in the nursery. Forest soil was used in the nursery beds and earthenpots. The well sieved soil and sand proportion was mixed in a ratio of 3:1 respectively. Seeds were soaked in water for a few hours and directly sown in the beds. The seed beds were covered with rice-straws and daily watering was done until germination was completed. Weeding was also done when necessary. Well germinated seedlings were transplanted in 20.0cm diameter earthenpots. About 6.5kg sieved, and sand mixed forest soil was used in each pot. Watering, and weeding was done when required. The earthenpot culture study lasted for one year and nodule collection was carried out in rainy season. The main object of the study was to examine nodulation and nitrogen fixing activity at the seedling stage under the climatic conditions in east Nepal, therefore, no other treatment was conducted.

The nodules produced on the root-hairs or, roots of individual seedling were separated, washed in running water, kept in filter papers to absorb excessive moisture, nodule number counted, weighed, kept in the rubber serum stoppered 30ml glass incubation vials, and preserved for their enzyme estimation at Plant Physiology Laboratory, Forest Research Institute, Dehra Dun, India. Nitrogenase activity was determined by estimating acetylene-ethylene reduction assay as described by Hardy *et al.* (1968) and Pokhriyal *et al.* (1987). One gram nodule was taken in the rubber serum stoppered incubation vial containing ten percent acetylene in air and the vial incubated in shaking water bath for one hour at 30° C. The vial was placed into ice filled beaker to stop reaction. The ethylene formed due to the reduction of acetylene was measured by injecting 1ml of the gas mixture into a Porapak-N column of CIC Gas Chromatography Model using nitrogen as carrier gas.

Results

Among twenty leguminous tree species, maximum (818.3) active nodules were recorded in *Albizia julibrissin* and minimum (33.9) in *Samanea saman*, whereas, maximum (202.2) inactive in *Albizia julibrissin* and minimum (4.1) in *Acacia auriculiformis*. Maximum (6.3g) nodule fresh weight was in *Albizia julibrissin* and minimum (0.4g) in *Calliandra callothyrsus, Samanea saman*, similarly, maximum (1.6g) dry weight in *Albizia julibrissin* and minimum (0.1g) in *Acacia catechu, Calliandra calothyrsus, Samanea saman* (Table 1).

Maximum (10197.0 n moles) nitrogen fixation activity per gram fresh weight per hour was in *Sesbania grandiflora* and minimum (4.0) in *Albizia odoratissima*, whereas, maximum (303.0 n moles) per nodule per hour in *Sesbania grandiflora* and minimum (0.1) in *Albizia odoratissima*. However, maximum (26206.0 n moles) total nitrogenase activity per plant per hour occured in *Sesbania grandiflora* and minimum (10.0) in *Samanea saman* (Figure 1).

Discussion

Nodule development is the summation of a number of physiological and biochemical processes. Formation of nodule and nitrogenase activity in trees vary from one species to another. These activities even vary within the same species in different localities and seasons. Some species nodulate significantly in summer while some in rainy season. Among twenty species, different pattern of nodulation and nitrogen fixation behaviour was obtained. The nodules recorded in *Albizia julibrissin, Butea monosperma* and *Dalbergia latifolia* seem to be higher, however, indicate that they nodulate significantly during rainy season. The marked variation in nodule biomass among different species compares fairly well with an earlier study conducted by Pokhriyal *et al.* (1996). The degeneration of nodules is a gradual phenomenon in NFTs, but nothing is known precisely about the exact physiological reasons for this process. However, nodule formation, growth and decay are regarded to be continuous processes in all nitrogen fixing species. Active and inactive nodules observed on all the trees examined seem to support this notion.

A great variation of nitrogen fixation activity was also observed. However, it was not recorded to be increasing or, decreasing as nodule number was increased or, decreased. Maximum nodules were recorded in *Albizia julibrissin*, but maximum nitrogen fixation activity per gram, or, per nodule, or, per plant was observed in *Sesbania grandiflora* with less nodules. Thus, nitrogen fixing activity was not significantly effected by variation in nodule quantity. The rate of root nodule initiation, formation,

Table 1. Active and inactive nodules, fresh and dry weight

Sl. No	Species	Location	Altitude (m)	Legume sub-family		Nodule		
					Number inactive	Active	Fresh wt (g)	Dry wt (g)
1	*Acacia auriculiformis*	Biratnagar	70	Mimosoideae	4.1 ±4.9	122.7 ±0.2	1.3 ±0.1	0.4
2	*A. catechu*	Tarahara	150	"	15.7 ±4.4	41.4 ±0.1	0.6 ±0.01	0.1
3	*Albizia julibrissin*	Biratnagar	70	"	202.2 ±211.0	818.3 ±0.4	6.3 ±0.1	1.6
4	*A. lebbeck*	Seutikhola	200	"	67.9 ±11.0	206.0 ±0.3	5.0 ±0.1	1.2
5	*A. odoratissima*	Biratnagar	70	"	27.7 ±3.4	214.0 ±0.1	4.4 ±0.01	1.1
6	*Butea monosperma*	Itahari	100	Papilionoideae	126.9 ±120.6	745.2 ±0.3	2.4 ±0.1	0.6
7	*Calliandra calothyrsus*	Dharan	300	Mimosoideae	8.9 ±10.2	106.8 ±0.06	0.4 ±0.02	0.1
8	*Dalbergia latifolia*	Dharan	300	Papilionoideae	68.4 ±217.7	731.3 ±0.6	2.3 ±0.2	0.6
9	*D. sericea*	Biratnagar	70	"	12.9 ±22.2	179.3 ±0.21	1.0 ±0.06	0.3
10	*Derris robusta*	Biratnagar	70	"	170.0 ±18.0	478.0 ±0.2	4.9 ±0.04	1.2
11	*Erythrina arborescens*	Hile	1850	"	5.0 ±7.0	61.0 ±0.6	5.0 ±0.1	1.4
12	*E. stricta*	Biratnagar	70	"	11.3 ±8.7	69.0 ±0.4	3.4 ±0.10	0.6
13	*Leucaena leucocephala*	Biratnagar	70	Mimosoideae	5.1 ±6.0	54.0 ±0.10	0.9 ±0.02	0.2
14	*Ormosia glauca*	Biratnagar	70	Papilionoideae	16.4 ±2.0	90.0 ±0.3	1.1 ±0.04	0.2
15	*Ougeinia oojeinensis*	Kane-pokhari	160	"	14.0 ±17.0	233.0 ±0.10	1.6 ±0.03	0.4
16	*Peltophorum pterocarpum*	Biratnagar	70	Caesalpinioideae	132.8 ±83.0	314.3 ±0.5	1.8 ±0.1	0.5
17	*Pithecellobium dulce*	Biratnagar	70	Mimosoideae	19.1 ±22.9	142.0 ±0.3	1.6 ±0.1	0.5
18	*Pterocarpus marsupium*	Biratnagar	70	Papilionoideae	13.1 ±28.3	159.0 ±0.6	2.9 ±0.2	0.8
19	*Samanea saman*	Biratnagar	70	Mimosoideae	9.5 ±6.0	33.9 ±0.1	0.4 ±0.02	0.1
20	*Sesbania grandiflora*	Biratnagar	70	Papilionoideae	8.9	86.3 ±16.2	2.6 ±0.5	0.6 ±0.1

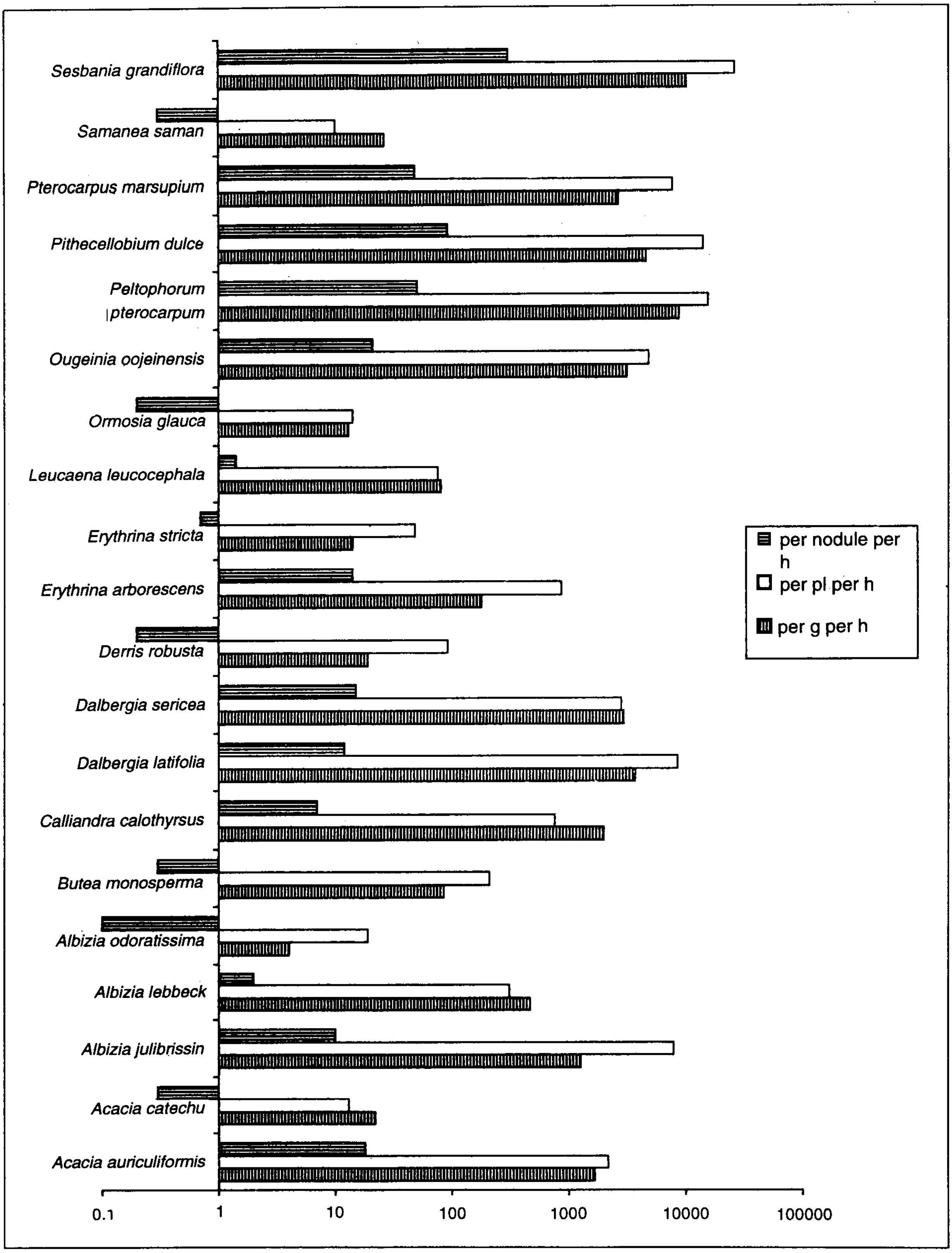

Figure 1. Nitrogenase (n moles acetylene reduced) activity g^{-1} h^{-1}, pl^{-1} h^{-1}, $nodule^{-1}$ h^{-1}

growth, and nitrogen fixation in various species vary as they have different maximum, optimum, and minimum temperatures (Gibson 1977, Gibson and Jordan 1983). Physiological processes like photosynthesis also effect nodulation and nitrogenase activity to a great extent. Higher photosynthetic rates ensure larger levels of transport of metabolites like melate and sugar, and consequently provide more energy available for nitrogen fixation at more favourable conditions (Lawrie and Wheeler 1975). K.C. (1997) has also reported that under the same climatic conditions, among leguminous herb, shrub, climber and tree species, highest nitrogenase activity was recorded in trees which had comparatively higher photosynthesis rate than other habits.

Conclusion

In the present study, it can be concluded that the variations in nodulation, degeneration and nitrogenase activity may be due to interactions of the physiological and biochemical processes of the species with climatic conditions.

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