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Seasonal Variation in Leaf Physiology and its Relationship with Nitrogen Dynamics in *Albizia amara* Boivin

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Abstract: A study on seasonal variation in leaf physiological parameters and dynamics of nitrogen metabolism was conducted in *Albizia amara*, a fodder-yielding, drought-resistant and fire-hardy tree of the arid and semi-arid regions of India, in National Research Centre for Agroforestry, Jhansi. Rate of transpiration and resistance to diffusion by stomata of the leaves of *Albizia amara* were inversely related and showed significant seasonal variation. Higher leaf temperatures were observed in summer season (March to June). Relative water content during winter months (November to February) and monsoon (July to October) were significantly higher than that in the summer. High leaf transpiration rates reduced relative water content significantly. Total nitrogen did not exhibit a distinct linear relationship with nitrate reductase activity, although they were directly related. Significant variations in nitrate reductase activity due to season were observed. Nitrate reductase activity showed distinct relationships with diffusive resistance, transpiration rate and relative water content. Summer season showed significant difference in all the parameters recorded in comparison with that in winter and monsoon.

Key words: *Albizia amara*, season variation, nitrate reductase activity.

Albizia amara is a tree recommended for agroforestry in semi-arid and arid regions of India. The regions are typically characterized by hot, dry winds and low and erratic rainfall. Intense solar radiation and dry, high speed winds, locally called "loo" are common in these regions throughout the summer months (Shankarnarayan *et al.*, 1986). Livestock production, as an alternative method of agriculture, is practised extensively in these regions. Trees which can withstand harsh environments are important sources of fodder and feed for livestock. *Albizia amara* is one such tree which is not only drought- and fire-hardy, but also gives high quality fodder, growing appreciably well in regions with annual rainfall range of 500 to 700 mm, and withstanding temperatures upto 47°C (Rao, 1993). Since crude protein content is a crucial parameter in assessment and recommendation of fodder tree in dry areas (Roy, 1991), nitrogen metabolism as well as synthesis and transport of assimilates to leaves, and its relationship with seasonal changes in environment, offer wide scope for research on this tree.

Nitrate Reductase Activity (NRA), nitrate content in trees and their role in nitrate assimilation are well documented (Campbell, 1988; Stadler and Gebaver, 1992). Protein synthesis and NRA have been shown to be influenced by water status. It is also known that NRA directly regulates nitrogen flux in leaves (Barber and Solomonson, 1990). Studies on seasonal

Table 1. Monthly variation in leaf physiological parameters and nitrogen dynamics in Albizia amara

Month	RWC (%)	Transpiration rate (mg $m^{-2}s^{-1}$)	Diffusive resistance (s cm^{-1})	T_{leaf} (°C)	Total N (%)	Crude protein (%)	NRA (μmNO_2 h^{-1} g^{-1})
Mar	63.91	30.01	8.31	30.32	1.326	8.29	6.238
Apr	60.83	35.73	6.83	34.18	1.303	8.14	6.118
May	57.08	36.03	6.67	35.71	1.305	8.16	6.087
Jun	58.09	36.17	6.10	36.32	1.325	8.29	6.093
Jul	71.10	24.32	9.72	34.83	1.394	8.71	6.212
Aug	78.74	23.06	10.03	30.42	1.390	8.69	6.231
Sept	77.73	24.54	9.81	30.94	1.392	8.70	6.249
Oct	76.58	25.31	9.87	30.19	1.398	8.74	6.284
Nov	78.31	24.86	9.79	29.07	1.382	8.64	6.279
Dec	73.82	25.32	9.63	27.41	1.338	8.36	6.287
Jan	67.79	24.18	9.38	27.53	7.340	8.38	6.241
Feb	64.86	27.38	9.1	28.96	1.357	8.48	6.237
CD (P=0.05)	7.234	8.482	1.832	8.014	0.0532	0.327	0.1337

variation in NRA (Balasimha, 1982), and its relationship with nitrogen balance and total nitrogen concentration (Sarjala *et al.*, 1987; Pietilainen *et al.*, 1991; Downs *et al.*, 1993) have shown results in the direction of assessing the physiological status of trees. The present study was taken up to understand and elucidate dynamics of nitrogen metabolism under different leaf water status and temperature conditions, essentially governed by seasons prevailing in the Bundelkhand region of India.

Materials and Methods

Five full-grown trees of 7 to 8 years of age, with full canopy cover, were randomly selected from the farm of NRC for Agroforestry, Jhansi. The selected trees were not pruned and no irrigation was applied. Leaf physiological parameters, viz., transpiration rate (E), stomatal diffusive resistance (G_s) and leaf temperature (T_{leaf}) were measured using steady state porometer, model LI-1600. Relative water content (RWC) was measured gravimetrically in the laboratory in detached leaves within 15 minutes of detachment. Total nitrogen (TN) content was estimated according to AOAC (1990); crude protein (CP) was expressed as a factor of TN. Nitrate reductase (EC 1.6.6.1) enzyme activity was measured as described by Hageman and Hucklesby (1971). Assay conditions followed were according to Pereira-Netto *et al.* (1989); incubation time for nitrite release was 30 to 60 minutes; buffer pH was 6.5; nitrate concentration was 50 mM and incubation temperature was 32°C. One pinnately compound leaf was collected from each of the first, third and fifth branches of the tree. Leaflets were removed, pooled, weighed and used for NRA and TN analysis. Observations thus obtained were taken as representative data for the tree. Data from each of the five trees (n=5) were considered as replicates. Statistical analysis was done

Table 2. Pearson correlation matrix of leaf physiological parameters and nitrogen status in Albizia amara

Parameter	RWC	E	G_s	T_{leaf}	TN	CP	NRA
RWC	1.000						
E	-0.887**	1.000					
G_s	0.910**	-0.986**	1.000				
T_{leaf}	-0.587	0.712	-0.721	1.000			
TN	0.862*	-0.820*	0.830	-0.306	1.000		
CP	0.862*	-0.821*	0.830	-0.308	1.000	1.000	
NRA	0.851*	-0.890**	0.921**	-0.862*	0.670	0.670	1.000

** Significant at P = 0.01; * Significant at P = 0.05.

using General Linear Model (Wilkinson *et al.*, 1996) for analysis of variance in completely randomized design. Critical difference was used to compare treatments (months). Pearson's correlation matrix was worked out to compare interrelationship between parameters. Confidence ellipse method was used to depict seasonal flux in parameters. Observations were recorded from March 1997 to February 1998. NRA and TN content were analyzed at monthly intervals while other observations were recorded thrice every month.

Results and Discussion

Transpiration rate and diffusive resistance conclusively followed an inverse relationship throughout the year. In May and June leaves transpired upto 36.17 mg m^{-2} s^{-1} while exhibiting incipient wilting (Table 1). Transpiration was the lowest in August, mainly due to increased stomatal resistance that was partially caused by constant film of water on the leaf surface and changes in the boundary layer dynamics near and about the stomata-atmosphere continuum. RWC ranged from 78.74% (August) to 57.08% in May. May and June showed significant difference from the rest of the months, which was due to high ambient temperature of 47 to 49°C. Post-monsoon RWC varied significantly from 76.58% in October to 64.86% in February, which can be attributed to gradual reduction in relative humidity with the onset of winter. Leaf temperature varied from 36.32°C in June to a minimum of 27.41°C in December. There was no significant difference in variation in leaf temperature between months, indicating that the tree has an adaptive mechanism to maintain leaf temperatures to support near-optimal metabolic activity.

Activity of nitrate reductase ranged between 6.08 and 6.29 µmol NO_2 $h^{-1}g^{-1}$, which is in accordance with the data of Smirnoff *et al.* (1984) and Pokhriyal *et al.* (1993). Total nitrogen content showed significant difference between months April-June and July-November, the reason being reduction in NRA in the summer months. Total nitrogen content did not exhibit a linear relation as seen in the winter months, where it was observed that even with a decrease in NRA there was a marginal increase in total nitrogen. This suggests that total nitrogen is not wholly governed by NRA and also that externally available N to the tree considerably influences NRA

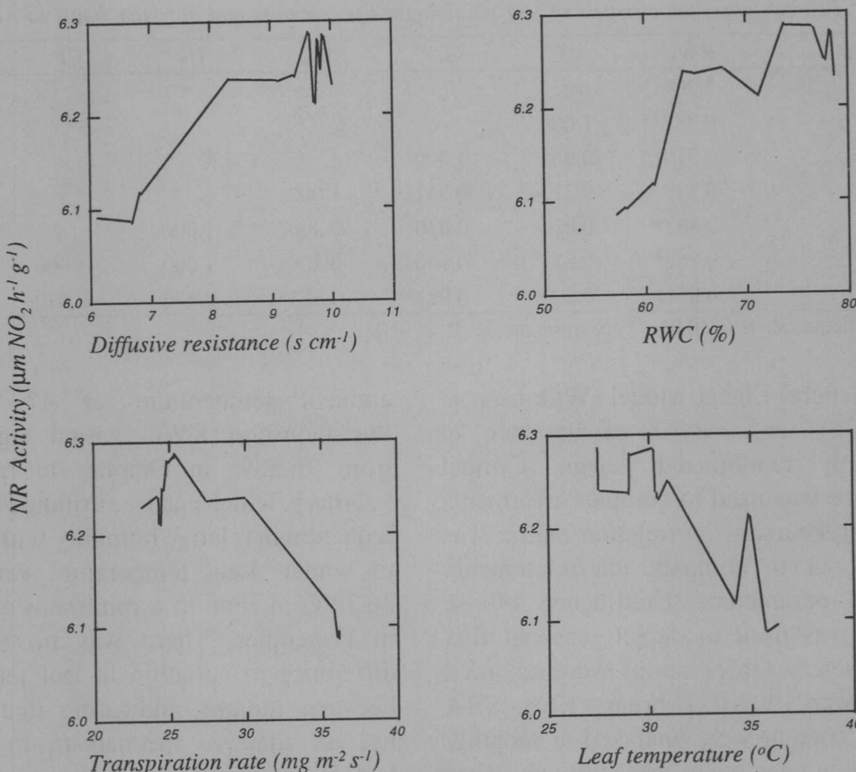

Fig. 1. Effect of leaf physiological parameters on NR activity in Albizia amara.

in trees (Lee and Titus, 1992; Wu-BingHau *et al.*, 1994). There was significant difference in NRA of months May and June and the rest of the months, due to high temperature and low water status. This is in agreement with the observations of Alcaraz *et al.* (1979) and Zhow and Su (1988). Pearson correlation matrix of all the parameters (Table 2) shows significant positive correlation (P=0.01) between NRA and diffusive resistance and between RWC and diffusive resistance. This suggests the role of stomata in water retention and metabolic activity. Transpiration rate exhibited significant negative relationship with both diffusive resistance and NRA. High transpiration rates reduced RWC significantly. NRA showed clear trends with variation in diffusive resistance, transpiration rate and RWC (Fig. 1). Although leaf temperature did not show a clear trend, optimum temperature for maximum NRA can be deduced as 28 to 30ºC. The sudden spur of activity at 30ºC can be explained by further studying the enzyme kinetics at different temperatures. Overall seasonal flux of all parameters recorded is shown in Fig. 2 by clubbing months March to May and June as summer, July to September and October as monsoon and November,

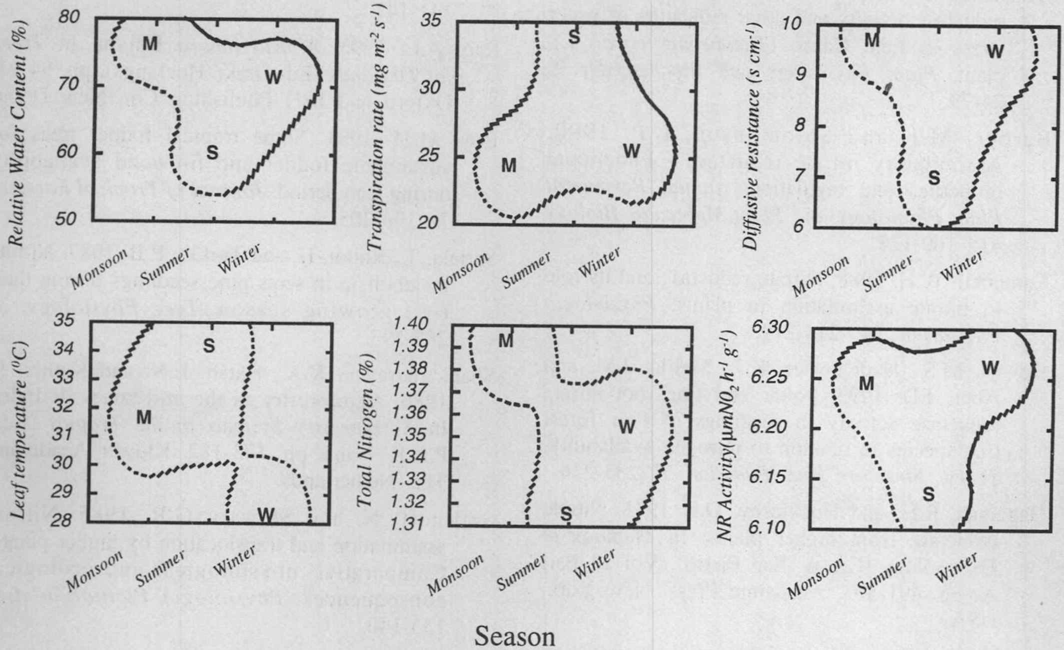

Fig. 2. Seasonal flux in leaf physiological parameters and nitrogen dynamics depicted as confidence ellipse in Albizia amara.

December, January and February as winter. It is seen that monsoon and winter values of all the parameters are at par, except for leaf temperature and total nitrogen. Similar trend has been reported by Pietilainen *et al*. (1991). This indicates that the effect of external environment (ambient temperature and, possibly, nutrient addition) is pronounced in all the seasons as against the notable effect of only summer in the rest of the parameters.

Conclusion

Major metabolic enzyme assays and their relationship with seasonal abiotic changes in the environment are a valuable tool for monitoring and improving the physiological status of trees, which are in constant interaction with unmanaged understorey vegetation. A data base on influence of season on physiological and metabolic activity of leaves has come out from this study. Insight has been gained into the adaptive mechanism of the tree to extreme climatic changes.

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References

Alcaraz, C.F., Banet, E., Hellin, E. and Llorente, S. 1979. Nitrate nitrogen and nitrate reductase activity in verna lemon tree leaves. *Journal of Plant Nutrition* 1: 347-354.

AOAC. 1990. *Official Methods of Analysis*. 15th edition. Association of Official Analytical Chemists. Virginia, USA. 59 p.

Balasimha, D. 1982. Seasonal changes in nitrate reductase activity and other indicators of water stress in field Cacao (*Theobroma cacao* L). plant. *Plant Physiology and Biochemistry* 9: 74-79.

Barber, M.J. and Solomonson, L.P. 1990. Assimilatory nitrate reductase - Functional properties and regulation. *Annual Review of Plant Physiology and Plant Molecular Biology* 41: 109-125.

Campbell, W.H. 1988. Nitrate reductase and its role in nitrate assimilation in plants. *Physiologia Plantarum* 74: 214-219.

Downs, M.S., Nadelhoffer, K.J., Melilo, J.M. and Aber, J.D. 1993. Foliar and fine root nitrate reductase activity in seedlings of four forest tree species in relation to nitrogen availability. *Trees: Structure and Function* 7: 233-236.

Hageman, R.H. and Hucklesby, D.P. 1971. Nitrate reductase from higher plants. In *Methods in Enzymology* (Ed. A. Sen Pietro), Vol 23 Part A, pp. 491-503. Academic Press, New York, USA.

Lee, H.J. and Titus, J.S. 1992. Nitrogen accumulation and nitrate reductase activity in MM 106. Apple trees as affected by nitrate supply. *Journal of Horticultural Sciences* 67: 273-281.

Pereira-Netto, A.B., Magalhaes, A.C.M. and Pisto, H.S. 1989. Nitrate reductase activity in tropical kudzu (*Pueraria phaseoliodes*); assay optimization. *Revisto-Brasileira-de-Fisiologia-Vagital* 1: 133-137.

Pietilainen, P., Poikolanen, J. and Lahdesmaki, P. 1991. Long-term monitoring of nitrate reductase activity in the needles of *Pinus sylvestris* in the context of environmental temperature and ground frost as an indicator of nitrogen balance in N Finland. *Annales Botanici Fennici* 28: 131-143.

Pokhriyal, T.C., Chauhan, S.K., Singh, U. and Bist, G. 1993. Effects of nitrate treatment on *in vivo* nitrate reductase activity and biomass production in *Eucalyptus* seedlings. *Annals of Forestry* 1: 141-147.

Rao, A.L. 1993. *Albizia amara* Boivin. In *Trees for Drylands* (Ed. Drake Hockings), pp. 94-97. Oxford and IBH Publishing Co., New Delhi.

Roy, M.M. 1991. Some tropical fodder trees for sustainable fodder and firewood availability during lean period. *Journal of Tropical Forestry* 7: 196-205.

Sarjala, T., Raitoi, H. and Turkki, E.B. 1987. Nitrate metabolism in scots pine seedlings during their first growing season. *Tree Physiology* 3: 285-293.

Shankarnarayan, K.A., Harsh, L.N. and Kathju, S. 1986. Agroforestry in the arid zones of India. In *Agroforestry Systems in the Tropics* (Eds. P.K.R. Nair), pp. 173-182. Kluwer Academic, The Netherlands.

Smirnoff, N. and Stewart, G.R. 1985. Nitrate assimilation and translocation by higher plants: Comparative physiological and ecological consequences. *Physiologia Plantarum* 64: 133-140.

Smirnoff, N., Todd, P. and Stewart, G.R. 1984. The occurrence of nitrate reduction in the leaves of woody plants. *Annals of Botany* 54: 363-374.

Stadler, J. and Gebaver, G. 1992. Nitrate reduction and nitrate content in ash trees (*Fraxinus excelsior* L): Distribution between components, site comparison and seasonal variation. *Trees: Structure and Function* 6: 236-240.

Wilkinson, L., Hill, M., Welna, J.P. and Birkenbevel, B.K. 1996. *Systat for Windows*. Version 6. SPSS Inc., Evanston, IL, USA.

Wu-BingHau, Wu-Xun, Wang-XiaoPing, Wu, B.H. Wu, X. and Wang, X.P. 1994. Activity of nitrate reductase and its control. *China Tea* 16: 16-17.

Zhow, G.Z. and Su, M.Y. 1988. A preliminary study on nitrate reductase activity in the leaves of *Cunninghamia lanceolata*. *Scientia Silvae-Sinicae* 24: 156-161.