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Biological N_2 fixing capacity of *Gliricidia sepium* and *Calliandra calothyrsus* under different agroclimatic conditions

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ABSTRACT

Biological nitrogen fixing capacity of *Gliricidia sepium* and *Callianndra calothyrsus* was investigated under field conditions in two contrasting locations in the Mid- country Intermediate and Mid-country Wet Zones of Sri Lanka at Nillambe and Matale respectively using the ^{15}N isotope dilution method. Two non nodulating tree legume species *Senna siamea* and *Senna spectabilis* were used as reference crops. Under Matale conditions, all the species performed well. The highest above ground biomass and total N content were found in *S. spectabilis* (5622g and 126.97gN plant^{-1}). *C. calothyrsus* produced a higher biomass and contained more N than *G. sepium*. However, under Nillambe conditions, total biomass production was extremely low in comparison to Matale and the highest biomass recorded, by *C. calothrsus* was only 197g plant^{-1}. Low biomass production under these conditions is attributed to the moisture stress and strong windy conditions prevailed during the growth period. Under Matale conditions no significant (P=0.01) difference was found between the two reference crops and estimated average Pfix values for *G. sepium* and *C. calothysus* were 56 and 42%, respectively. Total amount of N_2 fixed by above ground parts of *C. calothyrsus* (33g N plant^{-1}) was greater than *G. sepium* (26g plant^{-1}). The values are equivalent to 110 and 88kg N ha^{-1} (3333 plants ha^{-1}). Under Nillambe conditions Pfix values for both fixing species were higher than at Matale but total N fixed were limited to the maximum of 6.0 and 9.2 kg N ha^{-1} yr^{-1} for *G. sepium* and *C. calothyrsus*, respectively. Performance of two reference species was also significantly (p=0.01) different at Nillambe. Overall results suggest that the performance of N_2 fixing trees varies with the agroclimatic conditions and *S. siamea* as well as *S. spectabilis* appear to be equally suitable as reference plants for estimation of BNF potential of *G. sepium* and *C. calothyrsus* under the conditions of this study.

Keywords: multipurpose trees, isotope dilution technique, reference crops, Sri Lanka, dry matter yield.

INTRODUCTION

Low productivity associated with degradation of soil physical, chemical and biological properties resulting from the adoption of unprotected cultivation practices is one of the major problems in mid country of Sri Lanka. Unpredictable rainfall patterns have further aggravated the problem. With the increase in cost of fertilizer and relatively low prices received for the agricultural products, most of the small scale farmers have deviated from the practice of manuring their crops. This situation has compelled to find alternatives for inputs and conservation of the resources. In respect to Nitrogen fertilizers, use of biological N_2 fixation process associated with living organisms has a great potential (Peoples and Craswell 1992; Liyanage *et al.* 1994; Mc Donagh *et al.* 1995). The terrestrial flux of N from of biological N_2 fixation has been estimated to range from 129-170x10^6 tN/yr (Burns and Hardy 1975; Paul 1988) and this is several fold higher than input of N from N fertilizer (65x106 t N/yr; Paul 1988).

Multipurpose trees are used for purposes such as timber, fuel wood shade and support for other crops, to reduce water table, to prevent leaching losses, recycle nutrients from depth, or reclaim and ameliorate the soil through litter fall, root turnover or incorporation of prunings (Garrity 1995). Perennial crop based agro forestry systems are common in Sri Lanka and widely accepted due to low productivity of the land. Black pepper, coffee and cocoa based agro forestry systems are common, especially in the mid country region in Sri Lanka and *Gliricidia sepium* (Gliricidia) is the most common leguminous tree species found in these systems. It is widely used as a shade tree for cocoa, coffee, tea and many other crops and as a support and shade tree for black pepper. Peoples *et al.* (1996) highlighted the importance of looking for alternative tree species for agro forestry systems, taking soil acidity and pest susceptibility of *Leucaena leucocephala* as an example. It is essential to evaluate and identify other alternative species for Gliricidia, though it has the ability to produce high biomass and good quality fodder as well as green manure. Several workers have reported *G. sepium* as a high N_2-fixer (Awonaike *et al.* 1992; Sanginga 1992; Liyanage *et al.* 1994) and recent work has proved equally good performances of *Calliandra calothyrsus* (Herridge *et al.* 1996; Peoples *et al.* 1996). Biomass production of Calliandra is higher than Gliricidia and leaf to stem ratio (0.89) also is significantly higher than that of Gliricidia grown under mid country conditions of Sri Lanka (Gunarathne and Heenkenda 1995).

Use of isotope dilution technique has a wide acceptance in N_2 fixation studies but several methodological issues are to be solved for tree crops. Selection of a suitable reference crop is of prime importance since the values estimated primarily depend on the root activity and growth pattern of the reference crop (Danso and Kumarasinghe 1990; Parrotta *et al.* 1994; Peoples *et al.* 1996). Use of more than one reference crop may increase the precision of the results over a single reference crop, due to inherent variation of the root distribution patterns, growth rates of the reference crops and influences on the mineralization of organic N (Boddey *et al.* 1990; Witty 1983; Witty and Giller 1991). Present study was undertaken to estimate the biological N_2- fixing capacity of *Gliricidia sepium* and *Calliandra calothyrsus* under different agro ecological conditions in Sri Lanka. Two non-nodulating tree legumes, *Senna siamea* and *Senna spectabilis* were used as non N_2- fixing reference species.

MATERIALS AND METHODS

Seeds of *Gliricidia sepium* (Jacq.) Walp. (Gliricidia), *Calliandra calothyrsus* Meissn. (calliandra) and non-N_2-fixing shrub legumes *Senna siamea* (Siamea) and *Senna spectabilis* (Spectabilis) were grown in 15x21 cm polyethylene bags filled with equal parts of top soil, coir dust and sand. A slurry of the crushed nodules (5g fresh nodules in 100ml of water) obtained from field grown Gliricidia and Calliandra was applied to bags containing N_2 -fixing plant at the rate of 5ml per bag. Calliandra and Spectabilis seeds were scarified before planting with concentrated H_2SO_4 for 8 and 15 minutes, respectively, and thoroughly washed in running water. Potted plants were kept inside a nursery shed providing adequate water until planting out in the field.

Two field sites were selected for the experiment from the mid country region of Sri Lanka, with contrasting agro ecological conditions. The first site was at the main research station of the Department of Export Agriculture at Matale (07° 30N'., 80°. 5'E., 357m amsl) and the second site was located at one of the sub-stations at Nillambe (07° 10'N., 80° 40'E., 540m amsl). Annual rainfall during the study period for Matale was 2857mm and 2418mm for Nillambe. Soil properties of the sites are given in Table1. Matale site is flat and has been used for growing cocoa for a long period before it was abandoned for the last 20 years. Nillambe site is sloping and has been used for tea cultivation and subsequently for growing annual crops for about 15 years with frequent ploughing.

Five weeks old tree seedlings were field planted in weed free plots in rows 2.0m apart and 1.5m between plants in 6x6m plots during the rainy season in early June 1993. Each plot consisted of 12 plants and replicated three times in a randomized complete block design. Within each plot, 3x4m isotope sub-plot was demarcated to cover two center plants. To limit lateral migration of labeled N and to prevent the horizontal spread of roots, 50 cm deep trenches were dug and lined with two layers of thick polythene sheet. One month after the field planting, 100 and 85 kg ha^{-1} of K_2O and P_2O_5 respectively were applied as muriate of potash and triple super phosphate to all the plots and incorporated in to the soil.

Commencing from the third month after

Table 1. Soil characteristics of experimental sites

Depth	Texture	pH (1.5 soil: water)	Total N (%)	Organic C (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})	CEC (me q/ 100g)
Matale							
0-30cm	sandy clay loam	5 28	0.263	2.82	51	55	38.91
30-45cm	sandy clay loam	5.06	0.211	1.86	14	34	28.66
Nillambe							
0-30cm	sandy loam	6.10	0.141	1.23	30	32	22.10
30-45cm	sandy clay loam	5.82	0.096	0.89	24	29	23.42

planting (map),, 10.39 atom % ^{15}N ammonium sulfate was applied as a solution in water in three equal splits (3,6 &9 map), to the isotope sub-plots using an atomizer at the rate of 20kg N ha^{-1}. Split applications of isotope was practiced rather than a single large applications to minimize errors in determination of N_2 fixation associated with rapid decline in $^{15}N/^{14}N$ ratio in plant available soil N (Witty 1983). After each application, plots were watered thoroughly using a watering can. Plots were kept weeds free by hand weeding and weeds removed from the isotope sub plots were allowed to decompose within the plot.

At the end of 12 months after field planting, above ground parts of the fixing and no fixing reference trees were carefully harvested at 3cm from the base and separated in to leaves and stem. Wet weight of them were recorded and twigs and trunk were cut into small pieces (<3cm) and mixed thoroughly. Sub samples from leaves and stems were dried at 70°C to a constant weight, and subsequently used for the determination of N content and ^{15}Na.e. Total N content was determined by micro Kjeldahl method and ^{15}N a.e. was determined on VG-Isogas mass spectrometer. Nitrogen fixation for individual plant parts (leaves and stems) as well as for above ground parts were calculated using the isotope dilution equation (Freed and Middelboe 1977). Weighted atom % ^{15}N excess (WAE) was used for the calculation of the N_2 fixation by whole above ground parts (Fried *et al.* 1983).

RESULTS

Dry matter yield

Total dry matter production of both N_2- fixing and reference crop species were significantly higher under Matale conditions over Nillambe. Poor growth at Nillambe may be attributed to strong wind experienced from June to August and January to March period (Table2). Although the total rainfall during the growth period was not a limiting factor, strong wind and low soil water retention capacity in Nillambe have limited the amount of available water for the plants during short dry spells. Moreover, ploughpan developed for over a period of 10 years may have limited the root penetration to the lower horizons. Non fixing reference crop *S. spectabilis* showed a profuse growth at Matale and produced significantly (P=0.01) higher leaf and stem biomass over both fixing trees and the other reference crop, Siamea. Liyanage *et al.* (1994) also observed a similar trend with *S. siamea* over *G. sepium.* However, at Nilambe the growth of Calliandra was superior to the other crops though leaf biomass did not show any significant difference among the species, but stem biomass of Calliandra was significantly (P=0.05) higher than in Spectabilis.

N yield

Leaf and stem N% of Gliricidia and Calliandra were significantly (P=0.01) higher than the reference species under Matale conditions but under Nillambe conditions only Gliricidia indicated a significantly (P=0.01) higher leaf N% over the others and the highest stem N% was found in Spectabilis. The highest total leaf and stem N yields were found in Spectabilis but the value for Calliendra leaves did not differ significantly (P=0.05) from that of Matale condition (Table 2). In contrast to the results at Matale, total N yields are very low despite similar N% found in plants. Calliandra recorded the highest

Table 2. Above ground dry matter, N% and N yield and their distribution in leaves and stem

	Dry weight (g $plant^{-1}$)			N%		N Yield(g $plant^{-1}$)		
	Leaves	Stems	Total	Leaves	Stems	Leaves	Stems	Total
Matale								
G. Sepium	832.1	928.7	1760.9	3.3	2.2	27.4	20.5	47.8
C. Calothyrsus	1465.1	1458.9	2924.0	3.2	2.2	46.5	32.2	78.7
S. siamea	932.9	1038.8	1971.7	2.3	1.9	21.3	14.8	36.1
S. Spectabilis	2871.2	2750.8	5622.0	2.6	1.4	75.8	51.1	127.0
Significance	***	***	ND	***	***	**	***	ND
LSD(p=0.05)	904.5	836.7		0.3	0.2	29.7	17.0	
CV%	29.7	34.7		4.4	4.3	34.7	28.6	
Nillambe								
G. Sepium	55.6	63.1	118.7	3.4	1.3	1.9	0.8	2.7
C.calothyrsus	113.5	83.7	197.2	2.7	1.1	2.9	0.9	3.8
S. Siamea	103.7	60.7	164.4	2.4	1.1	2.4	0.7	3.1
S. Spectabilis	93.1	47.7	140.8	2.6	1.8	2.4	0.9	3.3
Significance	NS	*	ND	***	***	NS	NS	ND
LSD(p=0.05)		34.6		0.4	0.2			
CV%		26.6		9.3	35.2			

NS- not significant ND- not determined

leaf and stem N at Nillambe though the values are statistically not significant.

Atom% ^{15}N

Atom% ^{15}N enrichment in leaves and stems as well as calculated weighted average of both the fixing tree species *G. sepium* and *C. calothyrsus,* were significantly lower than the reference species confirming that fixing trees do not rely on soil and applied nitrogen at both locations (Table 3). However, leaf atom% ^{15}N excess in *S. siamea* was significantly (P=0.01) higher than in *S.spectabilis* under Nillambe conditions, indicating that the pattern of soil N absorption differs between two reference species. Lower atom % ^{15}N distribution could be found in leaves than in stems at both locations expect in *S. siamea* at Nillambe, which is the highest value recorded. Under Matale conditions, *G. sepium* gave lower enrichment values than *C. calothyrsus* but the reverse was true under Nillambe conditions.

N_2 Fixation

Percentage of N derived through fixation (Pfix) by *G. sepium* and *C. Calothyrsus* in above ground parts, calculated on the basis of two reference crops are given in Table 4. Estimated values for leaves and stems based on atom% ^{15}N excess in individual parts and for whole above ground parts based on weighted atom excess(WAE) are given separately. Under Matale conditions, *G. sepium* and *C. calothyrsus* have derived 52.8-58.3% and 38.4-.45.6% of N, respectively, in above ground plant parts from fixation. Comparatively higher percentage of leaf N than stem N has been derived from fixation. Based on Pfix values, *G. sepium* has a significantly (P=0.01) higher ability to obtain its N requirement through fixation than *C. calothyrsus*. Although *S. siamea* estimated a higher value of Pfix than *S. spactabilis*, no significant difference was observed between them for the cumulative Pfix values either for Gliricidia or Calliandra. Pfix values based on *S. siamea* for leaves of both the fixers and for stems of Caliandra were significantly (P=0.05) higher. Total amount of N^2- fixed by Calliandra is higher than Gliricidia due to high biomass and total N content but the difference is not statistically significant. During the first year of growth, Calliendra fixed 30.32-35.72g N plant^{-1} (100-119kg ha^{-1}) while Gliricidia fixed 24.81-27.77g plant^{-1} (83-93kg ha^{-1}). In both the cases 58-61% of the fixed N of above ground parts were found in leaves.

Situation at Nillambe was quite different from Matale and Pfix was higher for both Gliricidia and Calliandra ranging from 51.1-70.2% and 36.1-72.2%, respectively. Pfix values were highly significant for the reference crops but no difference could be found between two N_2- fixers either for whole above ground parts or individual plant parts with respect to a single reference crop. Despite high Pfix values, the amount of N_2 fixed was extrermely low for both fixing species due to poor biomass production. Total amount of N_2 was also highly significant between two fixers as well for the values based on two reference crops. Highly variable N- fix values observed for Gliricidia (0.95-1.78g N plant^{-1} or 3.17-5.96kg N ha^{-1}) and for Calliandra (1.80-2.76g N plant^{-1}or 6.00-9.21kg ha^{-1}) suggest the uncertainty of the values obtained under the conditions, where plant growth is arrested due to

Table 3. ^{15}N atom % excess in different plant parts and mean weighted ^{15}N atom % excess (WAE) for the whole plant

Treatment	Atom% 15N excess		WAE
	Leaves	Stems	
Matale			
G. sepium	0.0086	0.0131	0.0104
C. calothyrsus	0.0121	0.0154	0.0136
S. Siamea	0.0229	0.0284	0.0251
S.spectabilis	0.0194	0.0252	0.0221
Significance	***	***	***
LSD(p=0.05)	0.0028	0.0029	0.0023
CV%	8.97	7.16	6.56
Nillambe			
G.sepium	0.0793	0.0902	0.0821
C.calothyrsus	0.0603	0.0797	0.0650
S.siamea	0.2797	0.1299	0.1356
S.spectabilis	0.1240	0.1360	0.1268
Significance	***	**	**
LSD(P=0.05)	0.0823	0.0381	0.427
CV%	30.33	34.21	33.16

Table 4. Estimates of the proportion (Pfix) and amounts of N derived from N_2 fixation in leaves, stems and whole above ground parts of *G. sepium* and *C. Calothyrsus* at two locations. Estimated values based on *S. siamea* and *S. spectabilis* given against R1 and R2, respectively.

Treatment		Pfix %			Amount of N fixed (g/plant)		
		Leaves	Stems	Whole plant[a]	Leaves	Stems	Wholelant[a]
Matale							
G. sepium	-R1	62.6± 1.2	53.6± 5.0	58.3±2.6	17.11±3.16	10.83±2.51	27.77±5.61
	-R2	47.2±0.8	45.6±3.2	52.8±3.0	15.28±2.82	9.99±2.11	24.81±4.32
C. calothysus	-R1	55.8±1.7	49.5±0.7	45.6±3.3	21.92±0.85	14.63±2.31	35.72±0.92
	-R2	37.5±2.8	38.8± 1.4	38.4±2.0	17.47±1.29	12.40±1.74	30.22±2.29
Significance		***	**	***	NS	NS	NS
LSD(p=0.05)		6.8	10.1	9.4			
CV%		6.74	10.88	9.63			
Nillambe	-R1	70.2±5.8	52.4±15.8	67.7±4.9	1.32±0.16	0.33±0.14	1.79±0.11
G. sepium	-R2	51.1±5.6	30.6±8.8	35.4±2.9	0.24±0.04	0.10±0.01	0.95±0.15
C. Calothyrsus	-R1	77.2±4.0	56.0±4.0	74.0±5.1	2.23±0.39	0.46±0.12	2.76±0.38
	-R2	36.1±3.8	38.1±10.8	48.5±4.7	0.37±0.09	0.12±0.01	1.80±0.02
Significance		***	*	***	***	*	***
LSD(p=0.05)		18.5	20.0	14.0	0.572	0.299	0.412
CV%		15.81	22.65	12.45	27.57	59.32	11.29

[a] calculation based on WAE

stress conditions.

DISCUSSION

Isotope dilution technique was used in this study for the quantification of nitrogen fixation in field based comparison of the ^{15}N isotope enrichment of fixing and non-fixing reference plants grown on isotopically enriched soil. Calculation of N_2 fixation using this method based on the assumption that N uptake from the soil by reference plants has the same isotopic composition as the N uptake by the N_2 fixing plant, though this is not always met (Witty and Giller 1991). Discrepancies in N_2 fixing values estimated for a given crop using different reference crops often arise as a result of disimilarities in the $^{15}N/^{14}N$ uptake from soil in which the $^{15}N/^{14}N$ ratio declines rapidly. Also errors attributable to reference crop have greater effect at lower than at higher levels of N_2 fixation (Danso and Kumarasinghe 1990; Witty and Giller 1991). In this study, fairly accurate estimates could be obtained for both fixing species under Matale conditions where environmental factors were favorable for fixing and reference crops. Gliricidia and Calliandra were found to be high potential N_2-fixers under this condition with the capacity to fix nearly 100kg of atmospheric N_2 ha^{-1} yr^{-1}. However, under Nillambe conditions, Gliricidia as well as Calliriandra did not perform well, due to the fact that biomass production was extremely restricted by stress, despite higher Pfix values observed than at Matale. Higher Pfix values may be attributed to low N status of the soil (Witty and Giller 1991 ; Danso *et al*. 1992) and higher ^{15}N enrichment of both the N_2-fixers and reference plants confirmed the accumulation of a greater proportion of applied fertilizer.

The actual amount of N_2-fixed is conditioned by the plants nitrogen fixing potential and by environmental factors. This may operate in two ways, either by reducing plant growth or by direct affect on the symbiosis or both the effects (Danso *et al.* 1992). Soil moisture may affect bacterial nitrogen fixation indirectly through plant growth and directly on symbiosis. Total amount of N_2-fixed is yield dependent but % N_2 fixed is not. The stress observed on plant growth at Nillambe may have affected in two ways; moisture stress due to low water holding capacity, high evapo-traspiration due to strong wind and poor root penetration to the deeper layers due to plough pan as well as the physical stress caused by wind.

S. siamea is a suitable reference crop used in N_2-fixing studies on tree or shrub legumes in the tropics (Sanginga *et al.* 1990; Ladha *et al.* 1993; Liyanage *et al.* 1994). Accuracy of the Pfix values estimated using isotope dilution technique would largely depend on the reference crop. If the reference plant and the fixing plant differ in the time course at which they absorb N from soil, or if they sample different soil horizons, then the two crops will be utilizing pools of different ^{15}N enrichment unless there is a constant and uniform enrichment of soil N. Although this problem can be reduced by careful choice of reference crop and method of ^{15}N addition, it is seldom possible to achieve perfect experimental conditions using either of these two approaches (Witty and Giller 1991). Use of more than one reference crop would increase the likelyhood of obtaining accurate estimates (Boddy *et al.* 1990; Witty 1983).

S. spectabilis has been recently used as a reference crop for N_2-fixing studies in shrub legumes (Peoples *et al.* 1996) and in the present study both the reference crops performed equally well under Matale conditions but not at Nillambe. Pfix values and the total amount of N_2 fixed values estimated, based on *S.spectabilis* at Nillambe were much lower than those based in the *S. siamea*. This may be due to root penetration of Spectabilis beyond the isotope plots and absorbing more N from the low $^{15}N/^{14}N$ zone, in comparison to Siamea. According to Danso and Kumarasinghe (1990) error attributed to reference crop is higher at lower level than at higher level of N_2 fixation. However, under present situation high error was associated with higher rate of fixation (Pfix) but low total amount of fixation. Exclusion of roots from the study did not show the full N_2 fixing capacity of the plants. Danso and Kumarasinghe (1990) reported that roots of soybean may contain 10-12% of the fixed N of whole plant.

The overall results conclude that *G. sepium* and *C.calothyrses* have the potential to obtain 55% and 42%, respectively, of their N requirement from biological N_2 fixation. Total content of the fixed N in the above ground parts of the plants averaged to 88 and 110kg ha^{-1} yr^{-1} under favorable growing conditions. When total N input to a system is considered, total biomass production and N content are equally important as the percentage fixation. However, under moisture stress and windy climatic conditions, growth of both the species are arrested and as a result of that total N input to the system is extremely low although the percent N_2 fixation seems to be higher. The results also confirm that *S. siamea* and *S. spectabilis* are suitable reference crops for N_2 fixation studies in tree or shrub legumes. Location specific performances of Gliricidia and Caliandra suggest the importance of selecting suitable N_2 fixing species for specific conditions rather than extrapolating the results from one location to another.

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