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Evaluation of the Symbiotic Performance of Rhizobia Biochemical Mutants in Legume Trees

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Abstract: Nodulation potential, nitrogen fixation efficiency (nitrogenase activity) and biomass yield in response of *Leucaena leucocephala* and *Sesbania sesban* to inoculation with auxotrophic mutants of fast growing *Rhizobium* strains was explored in short-term field trials. All the strains formed nodules and fixed nitrogen in both hosts with some relative differences. The diversity of rhizobia that form symbioses with the roots of both hosts, an economically important leguminous tree species, was examined by inoculating seedling root zones with samples of auxotrophic mutants of rhizobia derived from both mutagens, acridine and ascorbic acid. Nitrogen fixation, total nitrogen accumulation, and plant growth varied significantly among both hosts seedlings inoculated with the representative isolates. All auxotrophic mutants derived from the parental strain FFAMU-8, stimulated chlorophyll (a) formation in *Sesbania sesban,* relative to the negative control. Although, three of auxotrophic mutants, asc_2-FFAMU-8, asc_3-FFAMU-8 and asc_4-FFAMU-8 affected to significantly increase chlorophyll (b) formation than their negative and positive control plants. Both auxotrophic mutants, asc_3-FFAMU-8 and asc_3-ARCG-10 affected to significantly greater below ground biomass components in *Leucaena leucocephala* than their in the positive control plants. In addition, some of auxotrophic mutants derived from the strain FIRT-27 affect to significantly increase woody, aerial and root biomass over the positive control of *Sesbania sesban.* Some of auxotrophic mutants appeared reliable ranking for nodule development/plant biomass among both hosts. Many of significant correlations were obtained among both tree legumes between nodulation, nitrogen fixation parameters with plant growth criterion. The results support the use of efficient rhizobial strains to inoculate woody legumes for improving plant survival and biomass development.

Key words: Auxotrophic mutants, acetylene reduction, biomass development, correlation coefficient, *Leucaena leucocephala,* nitrogen fixation, nodulation, *Sesbania sesban*

Introduction

Nitrogen fixing trees (NFTs), like their herbaceous counterparts, may enhance soil fertility through the return of N-rich litter if nodulated with effective rhizobia. The integration of NFTs in crop production systems is seen as a viable alternative to N fertilizer application, especially for the resource poor farmers in the tropics (Nyamai, 1992). In addition, they can also provide protein (seed and leaves) for man and livestock (Topps, 1992). The formation of nitrogen-fixing root nodules on legumes by rhizobia is a complex developmental process that involves constant communication between the partners. Products of the phenylpropanoid biosynthetic pathways, flavonoids (Rolfe, 1988), have been widely identified in leguminous plants as being involved in the initial signalling steps between plant and rhizobia, including chemotaxis towards a potential host (Kape *et al.*, 1991) and induction of the bacterial nodulation (nod) genes which are necessary to elicit a corresponding signal to the plant from the bacterium (Dahiya, 1991).

Globally, tree legumes are an important source of timber, fuel and fodder. Their ability to fix N_2 in association with *Rhizobium, Bradyrhizobium* and *Azorhizobium* bacteria means they can meet their N requirements directly from symbiosis. Additionally, they can improve the N fertility of soils in which they grow through release of symbiotic N from decomposing organic residues. Consequently, tree legumes play a vital role in rehabilitation of degraded and marginal soils and restoration of nutrient fertility in fields exhausted by intensive cultivation. The objective of the present study, therefore, were II) to evaluate the ability of the auxotrophic mutants of *Rhizobium* spp. derived from acridine and ascorbic acid to renodulate *Leucaena leucocephala* and *Sesbania sesban,* and (ii) to determine whether inoculation actually increases biomass of tree legumes.

Materials and Methods

Bacterial strains and media: *Rhizobiam* strains and their auxotrophic mutants induced which used in the present study are listed in Table 1.

Yeast extract mannitol medium (YEM) was used for culture maintenance according to Vincent (1970). Tryptone yeast extract medium (TY) *was* used as a complete medium to ensure the independence of mutations according to Beringer (1974). Evaluation of the symbiotic performance of auxotrophic mutants in pots experiment: Pots were filled with sand and then washed with hydrochloric acid for 24 hours, after that with tap water for 48 hours. The gars were sterilized with chloroform 0.25%, then washed three times with tap water until all chloroform disappear from all pots. Five sterilized *Sesbania sesban* and *Leucaena leucocephala* seeds were cultivated in each of three replicate systems and thinned to three seedlings after 15 days of growth (Vincent, 1970). Then they were inoculated with three ml of rhizobial broth culture per plant. The plants were irrigated using free nitrogen nutrient solution of Bond's modified Arone's stock mixture (Allen, 1959). Plants were harvested after three months of planting and growth was measured as plant height, stem diameter and plant total biomass. Nodules, roots and shoots were oven dried at 70°C prior to dry matter determination. The plant samples were finely ground and weighed out. Total N in each sample was analyzed following kjeldahl digestion, and N_2 fixation measured as the difference between total N in inoculated (nodulated) plant and total N in uninoculated (non-nodulated) plants. Inoculated and uninoculated *Sesbania sesban* and *Leucaena leucacepala* plants together with controls were also harvested and their root stems were observed for nodule formation and nodule number per plant.

Nodule numbers and plant dry weight: The plants of three replicates each containing two plants from tube method experiment, and three plants from pot system experiment were removed and washed by tap water. Then the number of nodules per plant was calculated for both *Sesbania sesban* and *Leucaena leucocephola.* The plants were dried at room temperature for two days, then oven dried at 70°C for two days and put in the desiccator before measurement of dry weight. The dry weight per plant was calculated for each inoculation treatment.

Photosynthetic activity: Chlorophyll a, b and total chlorophyll were determined after 13 weeks of planting using a spectrophotometric method, for measuring optical density (OD) according to Mackinney (1941).

Determination of nitrogen content in plant: Nitrogen content in dried plant materials was determined by the wet digestion of dried and finely pulverized plant material using the macrokjeldahl method (Jackson, 1958).

Nitrogenase activity: N2(C2H2)-fixation potential of root nodules was estimated by the acetylene reduction assay according to Hardy *et al*. (1968) and Dart *et al*. (1972).

Statistical analysis: Data were subjected to statistical analysis of variance using the Statistical Analysis System (SAS). When analysis of variance showed significant effect between treatments, the least significant difference (LSD) test was applied to make comparisons among the means at 0.05 and 0.01 levels of significance (Steel and Torrie, 1980).

Results and Discussion

Effect of inoculation with *Rhizobium* on chlorophyll content: The effect of several inocula of *Rhizobium* auxotrophic mutants on the chlorophyll content in *Sesbania sesban* and *Leucaena leucocephala* are shown in Table 2 and 3, respectively. As shown from Table 2, relative to the control, all auxotrophic mutants, derived from parental isolate $FFAMU_8$, stimulated chlorophyll (a) content. In addition, three auxotrophic mutants, asc_2 $FFAMU_8$, asc_3-$FFAMU_8$ and asc_4-$FFAMU_8$; were significantly increase chlorophyll (b) content than their negative and positive control plants. Non of auxotrophic mutants showed significant increase in total chlorophyll content, if comparison with the positive control; but all of them showed significant increase in total chlorophyll content, if comparison with the negative control.

In respect of relative increase in total chlorophyll content (a + b), all of auxotrophic mutants and their parental wild type isolate showed significant relative increase than uninoculated plants. On the other hand, there is no significant increase in total chlorophyll content was obtained by any of auxotrophic mutants induced from $ARCG_{io}$ if compared with their positive and negative control. The results obtained here concerning the effect of *Rhizobium* inoculation on the significant increase of chlorophyll formation indicated that nitrogen fixation by *Rhizobium* wild type and auxotrophic mutants has a stimulatory effect on chlorophyll formation. This are in agreement with Gupta *et al*. (1992), who reported that NO_2 has a stimulatory effect on photosynthesis (PN).

The results obtained in Table 3 did not show any significant differences between treatments with different *Rhizobium* inoculation for chlorophyll content in *Leucaena leucocephala.* This indicated that there were no clear differences between treatments in the mechanisms of chlorophyll formation. Sims *et al*. (1998) reported that nitrogen supply primarily affected photosynthetic capacity per unit volume of tissue, whereas photosynthetic capacity per unit nitrogen was significantly affected only by nitrogen supply. Treshow and Anderson (1989) reported that high concentration of NO_2 (above 0.5 μmol mol^{-1}) are known to inhibit plant growth. Low levels (up to 0.2 μmol mol^{-1}) of NO_2, on the other hand, increase the rate of photosynthesis, N and chlorophyll both in soybean [*Glycine max* (L.) Merr.] (Sabaratnam and Gupta, 1988) and in black turtle bean *(Phaseolus vulgaris* L.) (Sandhu and Gupta, 1989).

Biomass production: Table 4 shows that belowground biomass components in *Sesbania sesban* was significantly greater after the plants were inoculated with both auxotrophic mutants asc-3 FFAMU-8 and asc-3 ARCG-10, if compared with the positive control. The plants inoculated with different auxotrophic mutants showed insignificant differences for the aboveground biomass components. The present results are in agreement with Lal and Khanna (1996a), who found that biomass yield of all inoculated plants of *L. leucocephala* at the end of five years showed a significant increase over the uninoculated plants, among the three strains tested, one strain gave the maximum response (45% more dry matter) in biomass production and w as followed by another strain, which showed 27% increase over the negative control (uninoculated plants). The results summarized in Table 5 demonstrated that some of the auxotrophic mutants derived from the isolate HRI-27 revealed a significant increase over the positive control plants for woody, aerial and root biomass. This indicated that these auxotrophic mutants may efficient nitrogen fixers resulting in higher biomass production of *Leucaena leucocephala,* whereas the others proved to be a poor nitrogen fixers (Lal and Khanna, 1996a). These observations are in agreement with previous reports on inoculation of woody legumes with selected rhizobial strains, which showed shoot biomass yield was the major criterion in the selection of rhizobial inoculants. Short-term observation on the effect of *Rhizobium* on biomass yield of tree legumes do not give a true picture, a long-term study is essential to assess the potential of *Rhizobium* in increasing the biomass yield of tree legumes. The obtained results are in accordance with Lal and Khanna (1996b), who found that in nursery and short-term field trials of *Acacia nilotica* and *Leucaena leucocephala* the inoculation of these tree legumes with specific and effective strains of *Rhizobium* spp. had a positive effect on tree biomass (expressed as total dry matter). Shoot biomass yield was the major criterion in the selection of rhizobial inoculants.

Nodulation of woody legumes: Results in Table 6 show that auxotrophic mutants of rhizobia capable of eliciting nodules more than 40 per plant by both isolates derived from strains FFAMU-8 and ARCG-10. Auxotrophic mutant acr-1

Table1: Source of *Rhizobium* strains used in the present study

Legumes-host	Location	Designation of rhizobia
Sesbania sesban	Fac. Of Art., Minia Univ.	FFAMU-8
Sesbania sesban	Agric., Res. Center, Giza	ARCG-10
Leucaena leucocephala, Lam.	National Res. Center, Dokki	NRC-19
Leucaena leucocephala, Lam.	Hort. Res. Inst., Agric. Res. Center	HRI-27

Table 2: Effect of inoculation with *Rhizobium* biochemical mutants on chlorophyll content (mg/g fresh weight) in *Sesbania sesban*

	FFAMU-8 parental isolate				Inoculum	ARCG-10 parental isolate			
Inoculum	Chl. (a)	Chl. (b)	Total Chl.	Relative increase in Chl content		Chl. (a)	Chl. (b) (b)	Total Chl.	Relative increase in Chl content
Uninoculated (NC)	1.8262	0.516	2.342	0.0	Uninoculated (NC)	2.632	0.838	3.47	0.0
Parental strain (PC)	3.038	0.496	3.529	50.735	Parental strain (PC)	2.863	0.868	3.731	1.819
acr1- ARCG -10 (phe^{-})	3.015	0.501	3.516	50.129	acr1-FFAMU-8 (sys^{-})	2.633	0.869	3.502	1.876
acr2-ARCG -10 (gly^{-})	3.051	0.511	3.562	51.858	acr2-FFAMU-8 (ala^{-})	2.654	0.874	3.528	1.712
acr3-ARCG -10 (oth)	3.044	0.511	3.555	51.502	acr3-FFAMU-8 (trp^{-})	2.653	0.845	3.498	1.172
acr4-ARCG -10 (arg^{-})	3.049	0.525	3.574	52.320	acr4-FFAMU-8 (rev)	2.675	0.886	3.561	1.54
asc1-ARCG -10 (met^{-})	3.027	0.505	3.532	50.550	asc1-FFAMU-8 (trp^{-})	3.027	0.505	3.532	1.352
asc2-ARCG -10 (glu^{-})	2.612	0.889	3.501	49.222	asc2-FFAMU-8 (met^{-})	2.632	0.874	3.506	1.068
asc3-ARCG -10 (pyrid^{-})	2.615	0.856	3.471	48.915	asc3-FFAMU-8 (arg^{-})	2.664	0.849	3.513	1.471
asc4-ARCG -10 (sys^{-})	2.769	0.836	3.605	53.634	asc4-FFAMU-8 (leu^{-})	2.665	0.890	3.555	2.490
F-test	**	**	**	**	F-test	**	**	NS	NS
0.05	0.133	0.051	0.157	6.756	0.05	0.112	0.075		
L.S.D. 0.01	0.182	0.070	0.216	9.266	0.01	0.154	0.102		

Chl. = Chlorophyll ** = Significant at 0.01 of probability level. NS = Non significant. rev = Revertant to wild type. oth = Other requirements. acr and asc = Auxotrophic mutants derived from the treatment with acridine and ascorbic acid, respectively. NC, PC = negative and positive control, respectively.

Table 3: Effect of inoculation with *Rhizobium* biochemical mutants on chlorophyll content (mg/g fresh weight) in *Leucaena leucocephala*

Inoculum	NRC-19 parental isolate				Inoculum	HRI-27 parental isolateInoculum			
Inoculum	Chl. (a)	Chl. (b)	Total Chl.	Relative increase in Chl content		Chl. (a)	Chl. (b) (b)	Total Chl.	Relative increase in Chl content
Uninoculated (NC)	0.104	0.832	0.436	0.0	Uninoculated (NC)	0.107	0.832	0.936	0.0
Parental strain (PC)	0.161	1.063	1.224	30.74	Parental strain (PC)	0.138	0.795	0.933	0.32 (-)
acr1 -NRC-19 (trp^{-})	0.192	0.747	0.939	0.320	acr1-HRI-27 (rev)	0.190	0.820	1.010	7.905
acr2-NRC-19 (val^{-})	0.100	1.140	1.240	32.47	acr2-HRI-27 (lys^{-})	0.274	0.561	0.835	10.79 (-)
acr3-NRC-19 (arg^{-})	0.132	1.105	1.237	32.158	acr3-HRI-27 (val^{-})	0.158	0.989	1.147	22.54
acr4-NRC-19 (glu^{-})	0.109	1.390	1.499	60.149	acr4-HRI-27 (oth)	0.212	0.836	1.048	11.965
asc1- NRC-19 (trp^{-})	0.104	0.819	0.923	1.3381	asc1-HRI-27 (asp^{-})	0.196	0.606	0.802	14.31(-)
asc2-NRC-19 (ade^{-})	0.259	0.716	0.975	4.166	asc2-HRI-27 (glu^{-})	0.181	0.943	1.124	20.08
asc3-NRC-19 (rev)	0.183	0.912	1.095	16.987	asc3-HRI-27 (oth)	0.187	0.812	0.999	6.73
asc4-NRC-19 (val^{-})	0.143	0.971	1.114	10.019	asc4-HRI-27 ((glu^{-})	0.175	0.901	1.076	14.497
F-test	NS	NS	NS	NS	F-test	NS	NS	NS	NS

NSChl. = Chlorophyll NS = Non significant. rev = Revertant to wild type. oth = Other requirements

Table 4: Cumulative biomass production (g plant^{-1}) after three months in *Sesbania sesban* tree legume inoculated with *Rhizobium* wild type and biochemical mutants

Inoculum	FFAMU-8 parental isolate					Inoculum	ARCG-10 parental isolate				
	Above ground		Total biomass		Total dry Weight		Above ground		Total biomass		Total dry Weight
	Leaves	Woody	Aerial	Root			Leaves	Woody	Aerial	Root	
Uninoculated (NC)	4.587	8.516	13.079	7.802	20.281	Uninoculated (NC)	4.567	8.512	13.059	7.802	20.881
Parental strain (PC)	7.723	10.498	18.821	16.827	35.108	Parental strain (PC)	8.459	11.070	17.539	11.240	28.779
acr1-FFAMU-8 (sys^{-})	6.033	9.416	15.449	18.030	33.479	acr1-ARCG -10 (phe^{-})	7.072	10.91	17.982	13.713	31.701
acr2-FFAMU-8 (ala^{-})	8.545	13.832	22.377	10.823	45.20	acr2-ARCG -10 (gly^{-})	4.054	13.901	17.955	24.389	42.344
acr3-FFAMU-8 (trp^{-})	8.268	14.566	22.824	15.342	38.176	acr3-ARCG -10 (oth)	5.911	8.92	14.831	14.954	29.785
acr4-FFAMU-8 (rev)	7.96	12.911	20.871	10.491	31.362	acr4-ARCG -10 (arg^{-})	18.181	9.703	27.884	9.341	37.315
asc1-FFAMU-8 (trp^{-})	9.685	14.665	24.350	13.312	37.662	asc1-ARCG -10 (met^{-})	7.711	16.747	24.458	18.088	42.540
asc2-FFAMU-8 (met^{-})	4.896	10.206	15.102	6.231	21.333	asc2-ARCG -10 (glu^{-})	6.057	10.607	16.664	16.298	32.962
asc3-FFAMU-8 (arg^{-})	8.112	7.758	15.870	27.26	43.131	asc3-ARCG -10 (pyrid^{-})	25.095	28.688*	53.763	26.762**	80.525
asc4-FFAMU-8 (leu^{-})	11.993	18.343	30.336	19.414	49.750	asc4-ARCG -10 (sys^{-})	4.792	12.610	17.402	12.93	** 30.332
F-test	*	*	NS	**	**	F-test	*	*	NS	**	*
0.05	6.027	8.715		7.547	5.040	0.05	4.242	9.985		4.579	23.304
L.D.S.						L.D.S.					
0.01	8.265	16.102		10.35	6.912	0.01	5.818	113.694		6.279	31.960

* = Significant at 0.05 of probability level. ** = Significant at 0.01 of probability level.
NS = Non significant. rev = Revertant to wild type. oth = Other requirements.

Table 5: Cumulative biomass production (g plant^{-1}) after three months in *Leucaena leucocephala* tree legume inoculated with *Rhizobium* wild type and biochemical mutants

Inoculum	NRC-19 parental isolate					Inoculum	HRI-27 parental isolate inoculum				
	Above ground		Total biomass		Total dry Weight		Above ground		Total biomass		Total dry Weight
	Leaves	Woody	Aerial	Root			Leaves	Woody	Aerial	Root	
Uninoculated (NC)	5.2	5.63	10.83	14.22	15.04	Uninoculated (NC)	5.20	5.63	10.83	14.28	25.04
Parental strain (PC)	29.57	16.96	46.33	20.54	67.07	Parental strain (PC)	13.50	19.63	33.13	20.10	54.23
acr1-NRC-19 (trp$^-$)	9.04	10.66	19.70	18.20	37.90	acr1-HRI-27 (rev)	10.70	23.13	33.83	16.03	49.60
acr2-NRC-19 (val$^-$)	7.56	15.80	23.36	17.83	39.37	acr2-HRI-27 (lys$^-$)	12.96	15.70	28.66	25.70	54.36
acr3-NRC-19 (arg$^-$)	6.27	15.80	23.36	17.83	39.37	acr3-HRI-27 (val$^-$)	14.30	24.96	39.26	25.70	54.36
acr4-NRC-19 (glu$^-$)	12.23	15.83	28.06	23.63	51.70	acr4-HRI-27 (oth)	28.56	15.5	44.06	16.40	42.76
asc1- NF1C-19 (trp$^-$)	7.17	12.26	19.43	11.96	31.40	asc1-HRI-27 (asp$^-$)	16.72	8.48	25.20	18.56	43.76
asc2-NRC-19 (ade$^-$)	3.33	8.3	11.63	17.33	28.96	asc2-FIRI-27 (glu$^-$)	12.40	15.56	27.96	28.26	56.23
asc3-NRC-19 (rev)	16.26	18.8	35.06	25.20	61.26	asc3-FIRI-27 (oth)	13.47	9.43	22.90	18.20	41.10
asc4-NRC-19 (val$^-$)	10.67	11.53	22.20	15.16	35.36	asc4-FIRI-27 (glu$^-$)	3.70	6.00	9.70	15.40	25.10
F-test	**	**	*	**	**	F-test	NS	**	**	*	**
0.05	6.678	3.087	9.770	8.169	20.16	0.05		6.81	10.876	7.183	14.522
L.S.D.						L.S.D.					
0.01	9.168	4.233	13.400	11.203	27.648	0.01		9.34	14.916	9.851	19.917

* = Significant at 0.05 of probability level. ** = Significant at 0.01 of probability level. NS = Non significant.
rev = Revertant to wild type. oth = Other requirements.

Table 6: Nodulation and relative symbiotic effectiveness of *Rhizobium* sp. (*Sesbania sesban*) wild type and auxotrophic mutants

Inoculum	FFAMU-8 parental isolate Nodule/plant					Inoculum	ARCG-10 parental isolate Nodule/plant inoculum				
	Nodule No.	Dry wt. (mg)	Acetylene reduction (µmol h^{-1})	Plant height (cm)	Stem diameter (cm)		Nodule No.	Dry wt. (mg)	Acetylene reduction (µmol h^{-1})	Plant height (cm)	Stem diameter (cm)
Uninoculated (NC)	0.0	0.0	0.0	81.33	0.333	Uninoculated (NC)	0.0	0.0	0.0	81.33	0.333
Parental strain (PC)	68.33	0.774	11.340	86.33	0.933	Parental strain (PC)	42.00	0.503	9.1359	94.00	0.466
acr1-FFAMU-8 (sys$^-$)	135.00	0.383	22.177	96.00	0.933	acr1-ARCG-10 (phe$^-$)	45.33	0.35	10.7710	98.33	0.600
acr2-FFAMU-8 (ala$^-$)	56.33	0.500	47.16	115.66	0.533	acr2-ARCG-10 (gly$^-$)	90.33	0.733	10.0150	102.33	0.700
acr3-FFAMU-8 (trp$^-$)	74.66	1.133	18.771	116.33	0.933	acr3-ARCG-10 (oth)	101.33	0.666	29.691	91.00	0.533
acr4-FFAMU-8 (rev)	71.00	1.330	18.020	120.00	0.933	acr4-ARCG-10 (arg$^-$)	110.00	0.52	13.5834	102.33	0.700
asc1-FFAMU-8 (trp$^-$)	74.66	0.583	38.178	104.00	0.566	asc1-ARCG-10 (met$^-$)	65.66	0.343	15.7704	105.00	0.400
asc2-FFAMU-8 (met$^-$)	61.66	1.85	23.620	118.00	0.433	asc2-ARCG-10 (glu$^-$)	80.33	1.223	11.5899	101.00	0.500
asc3-FFAMU-8 (arg$^-$)	71.66	1.063	11.743	101.00	0.600	asc3-ARCG-10 (pyrid$^-$)	90.00	0.433	22.8870	143.00	0.833
asc4-FFAMU-8 (lev$^-$)	60.00	0.510	40.379	114.66	0.733	asc4-ARCG-10 (sys$^-$)	99.00**	0.770	37.696	100.00	0.900
F-test	*	*	*	**	*	F-test	**	**	**	NS	**
0.05	50.15	0.581	19.732	16.292	0.369	0.05	33.614	0.411	14.617		0.156
L.S.D.						L.S.D.					
0.01	68.78	0.797	27.129	22.344	0.507	0.01	46.099	0.564	20.047		0.215

* = Significant at 0.05 of probability level. ** = Significant at 0.01 of probability level. NS = Non significant. rev = Revertant to wild type. oth = Other requirements.

Table 7: Nodulation and relative symbiotic effectiveness of *Rhizobium* sp. (*Leucaena leucocephala*) wild type and auxotrophic mutants

Inoculum	NRC-19 parental isolate Nodules/plant					Inoculum	HRI-27 parental isolate Nodules/plant				
	Nodule No.	Dry wt. (mg)	Acetylene reduction (µmol h^{-1})	Plant height (cm)	Stem diameter (cm)		Nodule No.	Dry wt. (mg)	Acetylene reduction (µmol h^{-1})	Plant height (cm)	Stem diameter (cm)
Uninoculated (NC)	0.0	0.0	0.0	57.33	0.40	Uninoculated (NC)	0.0	0.0	0.0	57.33	0.40
Parental strain (PC)	41.00	0.41	3.076	79.66	0.50	Parental strain IPC)	36.00	0.26	37.588	91.00	0.77
acr1-NRC-19 (trp$^-$)	59.00	0.97	22.083	104.66	0.66	acr1-HRI-27 (rev)	107.00	1.73**	25.855	82.33	0.78
acr2-NRC-19 (val$^-$)	34.00	0.36	28.300	92.66	0.73	acr2-HRI-27 (lys$^-$)	53.00	0.76	13.577	115.00	0.91
acr3-NRC-19 (arg$^-$)	51.00	0.36	28.142	104.68	0.52	acr3-HRI-27 (val$^-$)	61.00	0.73	17.300	141.00	0.80
acr4-NRC-19 (glu$^-$)	70.00	0.76	12.611	91.66	0.63	acr4-HRI-27 (oth)	102.00	1.73	9.865	104.00	0.55
asc1-NRC-19 (trp$^-$)	45.00	0.60	19.935	92.00	0.53	asc1-HRI-27 (asp$^-$)	79.00	1.43	5.511	97.33	0.45
asc2-NRC-19 (ade$^-$)	75.00	0.96	8.234	83.33	0.63	asc2-HRI-27 (glu$^-$)	25.00	0.13	61.850	118.66	0.51
asc3-NRC-19 (rev)	127.0	1.90	16.832	80.00	0.78	asc3-HRI-27 (oth)	34.00	0.47	37.902	91.33	0.55
asc4-NRC-19 (val$^-$)	48.00	0.76	8.024	71.66	0.68	asc4-HRI-27 (glu$^-$)	34.00	0.56	26.400	76.00	0.53
F-test	**	**	**	NS	NS	F-test	*		**	NS	NS
0.05	19.13	0.56	4.032			0.05	37.00	0.77	3.618		
L.S.D.						L.S.D.					
0.01	26.24	0.77	5.529			0.01	50.75	1.060	4.962		

* = Significant at 0.05 of probability level. ** = Significant at 0.01 of probability level. NS = Non significant. rev = Revertant to wild type. oth = Other requirements.

FFAMIJ-8 revealed significant increase in number of nodules per plant. In addition, most of auxotrophic mutants derived from ARCG-10 shows significant increase in number of nodules per plant. Sanginga *et al.* (1991) reported a wide range of nodulation among provenances of inoculated *Gliricidia sepium* in Austrian soils. The present study clearly demonstrates the ability of *Rhizobium*auxotrophic mutants induced to survive and renodulate under field conditions and their effect on biomass yield of tree legumes. Shoot biomass yield was the major criterion in the selection of rhizobial inoculants. Short-term observation on the effect of *Rhizobium* on biomass yield of tree legumes do not give a true picture; a long-term study is essential to assess the potential of *Rhizobium* in increasing the biomass yield of tree legumes. In *Sesbania sesban,* plants inoculated with auxotrophic mutant asc-4 ARCG-10 gained maximum acetylene reduction, possibly because of high nitrogenase activity.

Table 8: Nitrogen fixation abilities of *Rhizobium* wild type and auxotrophic mutants at 13 week-old *Sesbania sesban* plants and corresponding estimates of annual N_2-fixation per hectare

	FFAMU-8 parental isolate				ARCG-10 parental isolateInoculum N_2 fix.		
Inoculum	N_2 fix. (mg/plant)	Relative increase in N_2 fix.	N_2 fix. (kg/ha)	Inoculum	N_2 fix. (mg/plant)	Relative increase in N_2 fix.	N_2 fix. (kg/ha)
Uninoculated (NC)	52.573	0.0	39.429	Uninoculated (NC)	52.573	0.0	39.429
Parental strain (PC)	108.27	105.942	81.202	Parental strain (PC)	99.750	89.736	74.812
acr1-FFAMU-8 (sys$^-$)	123.977	135.818	92.982	acr1-ARCG -10 (phe$^-$)	111.894	112.561	83.920
acr2-FFAMU-8 (ala$^-$)	67.973	29.292	50.979	acr2-ARCG -10 (gyl$^-$)	126.637	140.878	94.977
acr3-FFAMU-8 (trp$^-$)	94.497	79.744	70.872	acr3-ARCG -10 (oth)	78.368	49.065	58.776
acr4-FFAMU-8 (rev)	74.495	41.698	55.871	acr4-ARCG -10 (arg$^-$)	120.148	128.535	90.111
asc1-FFAMU-8 (trp$^-$)	99.27	88.823	74.452	asc1-ARCG -10 (met$^-$)	116.490	121.577	87.367
asc2-FFAMU-8 (met$^-$)	61.993	17.71	42.93	asc2-ARCG -10 (glu$^-$)	92.124	75.344	69.138
asc3-FFAMU-8 (arg$^-$)	151.302	187.79	113.476	asc3-ARCG -10 (pyrid$^-$)	215.887	311.25	155.241
asc4-FFAMU-8 (leu$^-$)	215.069	309.03	193.44	asc4-ARCG -10 (sys$^-$)	92.290	75.546	69.217
F-test	**	**	**	F-test	NS	*	NS
0.05	89.380	45.36	67.035	0.05		47.33	
L.S.D				L.S.D			
0.01	122.579	62.208	91.394	0.01		64.916	

*= Significant at 0.05 of probability level. ** = Significant at 0.01 of probability level. NS = Non significant.
rev = Revertant to wild type. oth = Other requirements.

Table 9: Nitrogen fixation abilities of *Rhizobium* wild type and auxotrophic mutants at 13 week-old *Leucaena leucocephala* plants and corresponding estimates of annual N_2-fixation per hectare

	NRC-19 parental isolate				HRI-27 parental isolate inoculum		
Inoculum	N_2 fix. (mg/plant)	Relative increase in N_2 fix.	N_2 fix. (kg/ha)	Inoculum	N_2 fix. (mg/plant)	Relative increase in N_2 fix.	N_2 fix. (kg/ha)
Uninoculated (NC)	63.720	0.0	31.856	Uninoculated (NC)	63.720	0.0	31.856
Parental strain (PC)	226.599	255.616	113.288	Parental strain (PC)	199.276	212.736	99.628
acr1-NRC-19 (trp$^-$)	173.264	171.914	86.623	acr1-HRI-27 (rev)	131.557	106.461	65.771
acr2-NRC-19 (val$^-$)	164.447	156.077	82.215	acr2-HRI-27 (lys$^-$)	177.736	178.932	88.859
acr3-NRC-19 (arg$^-$)	156.989	146.373	78.486	acr3-HRI-27 (val$^-$)	167.119	162.270	83.551
acr4-NRC-19 (glu$^-$)	163.236	156.177	81.609	acr4-HRI-27 (oth)	201.830	216.74	100.904
asc1-NRC-19 (trp$^-$)	93.415	46.602	46.702	asc1-HRI-27 (asp$^-$)	153.197	140.390	76.580
asc2-NRC-19 (ade$^-$)	102.543	60.075	51.266	asc2-HRI-27 (glu$^-$)	208.618	227.39	104.298
asc3-NRC-19 (rev)	232.822	265.38	116.399	asc3-HRI-27 (oth)	155.367	143.827	77.675
asc4-NRC-19 (val$^-$)	144.939	127.462	72.462	asc4-HRI-27 ((glu$^-$)	80.697	26.643	40.344
F-test	NS	**	NS	F-test	NS	**	NS
0.05		89.04		0.05		76.403	
L.S.D				L.S.D			
0.01		122.112		0.01		104.781	

*= Significant at 0.05 of probability level. ** = Significant at 0.01 of probability level. NS = Non significant.
rev = Revertant to wild type. oth = Other requirements.

These observation are in agreement with previous reports on inoculation of woody legumes with selected rhizobial strains, which showed increased survival percentage in seedlings and greater biomass production in all inoculated trees (Herrera *et al*., 1993; Galiana *et al*., rhizobial inoculants. Short-term observation on the effect of *Rhizobium* on biomass yield of tree legumes do not give a true picture, a long-term study is essential to assess the potential of *Rhizobium* in increasing the biomass yield of tree legumes. In *Sesbania sesban,* plants inoculated with auxotrophic mutant asc-4 ARCG-10 gained maximum acetylene reduction, possibly because of high nitrogenase activity. These observation are in agreement with previous reports on inoculation of woody legumes with selected rhizobial strains, which showed increased survival percentage in seedlings and greater biomass production in all inoculated trees (Herrera *et al*., 1993; Galiana *et al*., 1994).

As shown from the results presented here the auxotrophic mutants inoculated woody legume, *Sesbania sesban,* differed significantly in their effect on plant heights and stem-diameter. Most of auxotrophic mutants derived from FFAMU-8 showed significant increase in plant heights due to effective nodulation. In addition, some of auxotrophic mutants derived from ARCG-10 showed significant increase in stem-diameter, suggesting effectiveness in nodulation. The pattern of plant height and stem diameter obtained in this study are consistent with published data. Where there is no history of the presence of a tree legume in a particular locality, native bacteria capable of nodulating that species are likely to be few or completely absent (Sanginga *et al*., 1985). Total numbers of nodules and nodule occupancy data, after inoculation *L. leucocephala* with strains of *Rhizobium sp. (Leucaena)* and their auxotrophic mutants, are shown in Table 7. The results revealed that many of auxotrophic mutants derived from both isolates show significant increase in nodule number, nodule dry weight and nodulation index. The woody legume tested in this study revealed that auxotrophic mutants tested in this investigation did not differed significantly in both average weight / nodule and acetylene reduction. The present results are in agreement with Masutha *et al*. (1997), who reported that nodule effectiveness is often associated with higher N_2 fixation, the nodulation data obtained in their study did not necessarily correlate with the amounts of N_2 fixation. The pattern of nodulation and N_2 fixation obtained in this study are consistent with published data.

Of the woody legume tested here the obtained results revealed insignificant differences in plant heights and stem-diameter among both isolates NRC-19 and HRI-27. The results have proved to be the fastest-growing *Leucaena leucocephala* and *Sesbania sesban* in terms of plant height, stem diameter growth. So, although it has been suggested that fast-growth in woody legumes is not necessarily an index of N_2 fixation in the species (Danso *et al*., 1992), in the study of Masutha *et al*. (1997) all variables of plant growth have correlated with N_2 fixation. If however

Table 10: Correlation coefficient (r) between plant estimates of N_2 fixation for *Sesbania sesban* grown in sterilized sandy soil

		Chl. (B) chl.	Total DW	Leaves DW	Woody DW	Aerial DW	Root DW	Total DW	Root/shoot ratio	Nodule No.	Nodule. O .W.
Chl.	I	0.070	0.901**	0.257	0.456	0.497	0.224	0.516	0.037	0.612	0.569**
	II	0.021	0.034	0.173	0.082	0.141	0.554	0.083	0.091	0.023	0.627
Chl. Ib)	I		0.162	0.310	0.116	0.036	0.343	0.115	0.672	0.197	0.180
	II		0.084	0.286	0.136	0.095	0.135	-0.147	0.343	0.120	0.308
Total chl.	I			0.403	0.243	0.522	0.353	0.579	0.341	0.675	0.532
	II			0.023	0.369	0.489	0.202	0.110	0.138	0.028	0.668
Leaves DW	I				0.813**	0.930**	0.474	0.078	0.272	0.081	0.420
	II				0.700	0.934	0.358	0.813**	0.589	0.360	0.137
Woody DW	I					0.809	0.047	0.572	-0.049	0.283	0.283
	II						0.504	0.959	-0.227	0.235	0.070
Aerial OW	I						0.154	0.739**	0.062	0.015	0.377
	II						0.605	0.710**	0.457	0.322	0.115
Root DW	I							0.669**	0.594	0.493	0.434
	II							0.815	0.215	0.407	0.272
Total OW	I								0.206	0.187	0.410
	II								0.252	0.375	0.102
Root/shoot ratio	I									0.307	0.102
	II									0.114	0.360
Nodule No.	I										0.324
	II										0.624
Nodule D.W.											

		Nodulation Index	Average weight of	Acetylene reduction	Nodule efficiency	Root length	Plant hieght	Stem diameter	N_2-fixation (mg/plant)	N_2-fixation (%)	Aerial protein (%)
Chl. (a)	I	0.484	0.548	0.386	0.500	0.409	0.108	0.710**	0.115	0.198	0.118
	II	0.583	0.421	0.037	0.342	0.126	0.007	0.140	0.047	0.113	0.031
Chl. (b)	I	0.036	0.171	0.314	0.038	0.293	0.341	0.357	0.447	0.321	0.321
	II	0.297	0.175	0.010	0.054	0.638	0.067	0.376	0.157	0.175	0.236
Total chl.	I	0.511	0.638	0.533	0.493	0.542	0.641	0.571	0.312	0.247	0.247
	II	0.094	0.587	0.123	0.046	0.152	0.576	0.022	0.194	0.152	0.564
Leaves DW	I	0.303	0.629	0.508	0.219	0.095	0.329	0.309	0.759**	0.204	0.662*
	II	0.418	0.285	0.268	0.484	0.323	0.817**	0.547	0.545	0.465	0.539
Woody OW	I	0.084	0.286	0.526	0.434	0.594	0.224	0.513	0.448	0.448	
	II	-0.380	0.075	0.441	0.492	0.044	0.945**	0.431	0.603	0.576	0.602
Aerial DW	I	0.072	0.443	0.862**	0.525	0.316	0.108	0.273	0.637**	0.559	0.558
	II	0.434	0.193	-0.389	0.528	0.210	0.949**	0.537	0.6.19*	0.559	0.615
Root OW	I	0.658**	0.666**	0.204	0.018	0.393	0.149	0.383	0.666**	0.605	0.605
	II	0.173	0.176	0.255	0.472	0.019	0.84**	0.209	0.649**	0.405	0.500
Total DW	I	0.297	0.680**	0.335	0.302	0.009	0.316	0.315	0.361	0.662**	0.667**
	II	0.303	0.073	0.378	0.562*	0.149	0.979**	0.481	0.693**	0.581**	0.629
Root/shoot ratio	I	0.273	0.493	0.065	0.124	0.142	0.322	0.167	0.415	0.363	0.363
	II	0.593	0.508	0.260	0.087	0.508	0.243	0.064	0.154	0.100	0.170
Nodule No.	I	0.383	0.393	0.361	0.239	0.246	0.615	0.632	0.103	0.112	0.112
	II	0.492	0.252	0.487	0.255	0.335	0.439	0.619	0.521	0.169	0.029

		Modulation index	Average weight	Acetylene reduction	Nodule efficiency	Root length	Plant height	Stem diameter	N_2-fixation mg/plant	N_2-fixation (%)	Aerial protein(%)
Nodulation index	I	0.920*	0.894**	0.034	0.389	0.105	0.397	0.662	0.117	0.014	0.014
	II	0.939**	0.666**	0.289	0.337	0.030	0.118	0.202	0.025	0.137	0.225
Aver. weight of nodule	I		0.875**	0.066	0.302	0.049	0.187	0.573	0.032	0.112	0.028
	II		0.669	0.131	0.111	0.090	0.216	0.104	0.143	0.293	0.406
Acetylene reduction	I			0.047	0.444	0.081	0.273	0.267	0.443	0.403	0.403
	II			0.240	0.473	0.067	0.027	0.110	0.327	0.611	0.626
Nodule efficiency	I				0.637*	0.461	0.467	0.284	0.046	0.166	0.167
	II				0.284	0.067	0.405	0.849**	0.151	0.120	0.126
Root length	I					0.350	0.621	0.632*	0.427	0.364	0.365
	II					0.153	0.657*	0.366	0.672**	0.031	0.031
Plant height	I						0.875**	0.009	0.177	0.264	0.264
	II						0.448	0.116	0.465	0.188	0.061
Stem diameter	I							0.066	0.090	0.010	0.010
	II							0.587	0.715**	0.493	0.562
N_2-fixation (mg/plant)	I								0.035	0.072	0.024
	II								0.376	0.151	0.043
N_2-fixation (%)	I									0.970	0.969
	II									0.909	0.916**
Aerial protein (%)	I										0.999**
	II										0.940**

*, ** Indicated significant correlation at $p<0.05$ and $p<0.01$, respectively. I = FFAMU-8
II ARCG-10. Chl = Chlorophyll D.W. = Dry weight. Aver. = Average.

establishment of effective symbiosis with auxotrophic mutants is the only consideration for species selection, then both tree legumes, are suitable for use in the agroforestry program. There is however no doubt that when dealing with agroforestry systems, criteria such as competitively of the root systems are also important in addition to an active N_2 fixation by the legume.

Nitrogen fixation: The present study clearly demonstrates (Table 8, 9) the ability of introduced *Rhizobium* isolates of *Sesbania sesban* and *L. leucocephala* to significantly increase nitrogen fixation by some of auxotrophic mutants used in plants inoculation. The results showed that the inoculation of these tree legumes with specific and effective strains of *Rhizobium* spp. had a positive effect on nitrogen fixation. This are in accordance with Herrera *et al*. (1993), who reported that inoculation of woody legumes with selected rhizobia and arbuscular

Table 11: Correlation coefficient (r) between plant estimates of N_2 fixation for *Leucaena leucocephala* grown in sterilized sandy soil

		Chl. (B) chl.	Total DW	Leaves DW	Woody DW	Aerial DW	Root DW	Total DW	Root/shoot ratio	Nodule No.	Nodule. O .W.
Chl. (a)	I	0.405	0.341	0.030	0.167	0.007	0.251	0.058	0.331	0.537	0.559
	II	0.567	0.272	0.408	0.133	0.319	0.338	0.316	0.103	0.502	0.501
Chl.	I		0.972**	0.314	0.671*	0.490	0.466	0.497	0.501	0.029	0.195
	II		0.946**	0.151	0.239	0.056	0.204	0.022	0.155	0.022	0.195
Total chl.	I			0.361	0.664**	0.434	0.584	0.575	0.438	0.145	0.062
	II			0.011	0.332	0.190	0.185	0.142	0.096	0.040	0.135
Leaves DW	I				0.519	0.624	0.159	0.884**	0.512	0.190	0.161
	II				0.321	0.809**	0.127	0.416	0.629	0.637*	0.593
Woody DW	I					0.770**	0.702*	0.791**	0.075	0.487	0.304
	II					0.816**	0.713*	0.849**	0.452	0.447	0.295
Aerial DW	I						0.633*	0.965**	0.682**	0.341	0.243
	II						0.374	0.767**	0.848**	0.691**	0.515
Root DW	I							0.807**	0.151	0.705**	0.614
	II							0.805**	0.192	0.800**	0.271
Total DW	I								0.554	0.500	0.395
	II								0.708*	0.323	0.125
Root/shoot ratio	I									0.162	0.102
	II									0.653**	0.525
Nodule No.	I										0.953**
	II										0.773**
Nodule D.W.	I										
	II										

		Nodulation Index	Average weight of	Acetylene reduction	Nodule efficiency	Root length	Plant hieght	Stem diameter	N_2-fixation Imo/plant)	N_2-fixation (%)	Aerialprotein (%)
Chi. (a)	I	0.924**	0.491	0.168	0.569	0.169	0.068	0.224	0.184	0.307	0.421
	II	0.437	0.678**	0.023	0.099	0.603	0.431	0.427	0.429	0.332	0.335
Chi. (b)	I	0.532	0.162	0.254	0.094	0.397	0.194	0.198	0.218	0.071	0.247
	II	0.156	0.366	0.417	0.381	0.322	0.123	0.168	0.074	0.038	0.023
Total chl.	I	0.082	0.055	0.239	0.135	0.400	0.238	0.285	0.393	0.165	0.154
	II	0.02	0.164	0.447	0.455	0.180	0.312	0.012	0.081	0.085	0.102
Leaves DW	I	0.182	0.181	0.181	0.002	0.600	0.122	0.039	0.711**	0.283	0.241
	II	0.420	0.274	0.128	0.033	0.135	0.474	0.074	0.725**	0.604	0 777**
Woody DW	I	0.133	0.251	0.654**	0.403	0.789**	0.474	0.428	0.721**	0.444	0.427
	II	0.051	0.028	0.028	0.271	0.794**	0.619	0.211	0.564	0.595	0.120
Aerial DW	I	0.172	0.235	0.145	0.119	0.876**	0.112	0.098	0.881**	0.386	0.353
	II	0.212	0.337	0.063	0.209	0.740	0.364	0.424	0.792**	0.259	0.174
Root DW	I	0.216	0.250	0.231	0.666**	0.580	0.101	0.528	0.702**	'0.324	0.289
	II	0.437	0.173	0.005	0.774**	0.470	0.854**	0.443	0.652**	0.031	0.028
Total DW	I	0.034	0.512	0.167	0.111	0.851**	0.726**	0.898**	0.532	0.446	0.345
	II	0.235	0.012	0.122	0.555	0.945**	0.857**	0.685**	0.796**	0.044	0.001
Rloot/shoot ratio	I	0.446	0.327	0.366	0.083	0.743**	0.218	0.291	0.413	0.346	0.331
	II	0.293	0.205	0.566	0.062	0.548	0.410	0.510	0.371	0.027	0.154
Nadule No.	I	0.670**	0.638*	0.311	0.005	0.412	0.270	0.702	0.532	0.355	0.364
	II	0.775**	0.744**	0.253	0.306	0.279	0.117	0.324	0.325	0.286	0.295

		Nodulation index	Average weight	Acetylene reduction	Nodule efficiency	Root length	Plant height	Stem diameter	N_2-fixation mg/plant	N_2 fixation (%)	Aerial protein(%)
Nodulation index	I	0.683**	0.706**	0.202	0.040	0.381	0.137	0.705**	0.469	0.045	0.393
	II	0.854**	0.789**	0.394	0.448	0.107	0.122	0.320	0.314	0.056	0.299
Aver. weight of nodule	I		0.483	0.340	0.056	0.178	0.201	0.401	0.076	0.253	0.266
	II		0.863**	0.352	0.470	0.493	0.046	0.090	0.035	0.247	0.476
Acetylene reduction	I			0.118	0.193	0.419	0.420	0.643**	0.382	0.708**	0.714**
	II			0.220	0.358	0.814	0.181	0.175	0.099	0.099	0.317
Nodule efficiency	I				0.426	0.354	0.574	0.463	0.334	0.437	0.442
	II				0.852**	0.429	0.239	0.075	0.256	0.377	0.150
Root length	I					0.234	0.818	0.031	0.367	0.715**	0.641**
	II					0.074	0.555	0.041	0.625	0.372	0.312
Plant height	I						0.204	0.362	0.731**	0.495	0.475
	II						0.177	0.439	0.120	0.277	0.354
Stem diameter	I							0.154	0.218	0.527	0.531
	II							0.483	0.726**	0.192	0.318
N_2-fixation (mg/plant)	I								0.475	0.521	0.540
	II								0.382	0.69**	0.252
N_2-fixation 1%)	I									0.951**	0.677**
	II										0.574
Aerial protein (%)	I										0.915**
	II										0.990**

***, ** Indicate significant correlation at $p<0.05$ and $p<0.01$, respectively.

1 = NRC-19 11 = HRI-27 Chl = Chlorophyll D.W. = Dry weight.

mycorrhizal fungi improves outplanting performance, plant survival and biomass development. The present study with *Sesbania sesban* and *L. leucocephala,* over 13 weeks, clearly indicated that these' tree legumes responded significantly to inoculation with some of *Rhizobium*auxotrophic mutants, they showed more nodulation, nitrogenase activity, N_2-fixation than those inoculated with wild type isolate (positive control) and uninoculated trees (negative control). The woody legumes tested in this study differed significantly in their relative increase in N_2-fixation. Although, nodule effectiveness is often associated with higher N_2-fixation also and the nodulation data obtained in this study (Table 10, 11) did not necessarily correlate with the amounts of N_2-fixation (Masutha *et al.*, 1997). The pattern of N_2-fixation obtained in this study are consistent with published data. Of the two woody legumes tested, *Sesbania sesban* and *L. leucocephala* proved to be the fastest-growing species in terms of plant height, stem diameter growth (Table 6, 7), plant biomass (Table 4, 5) and nitrogen fixed (Table 8, 9). So, although it has been suggested that fast-growth in woody legumes is not necessarily an index of N_2-fixation in these species (Danso *et al.*, 1992). On that basis, both tree legumes therefore appear to be the most promising elite Material for use in agroforestry. If however establishment of effective symbiosis with Rhizobium-auxotrophic mutants and/or indigenous soil strains is the only consideration for species selection, then both tree legumes, are suitable for use in the agroforestry program. There is however no doubt that when dealing with agroforestry systems, criteria such as competitively of the root systems are also important in addition to an active N_2-fixation by the legume. Kwesiga and Coe (1994) obtained a doubling of maize yields in Zambia in *Sesbania* follows compared with continuous unfertilized maize. Moreover, Dommergues (1987) has grouped woody legumes into low and high N_2 fixing species. However, the universality of that observation has not been tested, especially that the effective of a symbiosis can be enhanced or limited by factors unique to that particular environment.

Correlation: In respect of plants inoculated with rhizobial isolates derived from FFAMU8, it is evident from the results presented in Table 10 that chlorophyll (a) was significantly correlated with; total chlorophyll (Chl) ($r = 0.901$, $p<0.01$), nodule dry weight ($r = 0.569$, $p<0.01$) and stem diameter ($r = 0.71$, $p<0.01$). Whereas, leaves dry weight (DW) was significantly correlated with woody DW ($r = 0.813$, $p<0.01$), aerial DW ($r = 0.93$, $p<0.01$), N_2 fixation (mg/plant) ($r = 0.759$, $p<0.01$) and aerial protein % ($r = 0.662$, $p<0.05$). However, woody DW was significantly correlated with aerial DW ($r = 0.47$, $p<0.01$) and acetylene reduction ($r = 0.64$, $p<0.05$), as well as aerial DW was significantly correlated with total DW ($r = 0.739$, $p<0.01$), acetylene reduction ($r = 0.652$, $p<0.01$) and N_2 fixation (mg/plant) ($r = 0.637$, $p<0.01$). In addition, root DW was significantly correlated with total DW ($r = 0.669$, $p<0.01$), nodulation index ($r = 0.658$, $p<0.01$), average weight per nodule ($r = 0.666$, $p<0.01$) and N_2 fixation (mg/plant) ($r = 0.666$, $p<0.01$). The present results are in agreement with Dobert and Blevins (1993), who found strong correlation between; shoot and nodule dry weights, plant height with shoot DW, nodule mass and mass per nodule, nodule number with both shoot DW and nodule mass. Although, total DW was significantly correlated with average weight per nodule ($r = 0.68$, $p<0.01$), N_2 fixation ($r = 0.668$, $p<0.01$) and aerial protein % ($r = 0.667$, $p<0.01$). However, nodule number was significantly correlated with stem diameter ($r = 0.632$, $p<0.05$), as well as, nodule OW with both nodulation index, average weight per nodule and nodule efficiency. In addition, nodulation index was significantly correlated with average weight per nodule, whereas, acetylene reduction was significantly correlated with nodule efficiency, nodule efficiency with stem diameter and root length with plant height.

The results obtained in this study are in accordance with those reported. Who found that fixed N_2 was highly correlated with maturity ($r = 0.96$, $p<0.01$), since late-maturing lines fixed more N_2 than earlier-maturing lines. The parameters related to nitrogen fixation in *Sesbania sesban* plants inoculated with rhizobial isolates derived from ARCG 10 showed a significant correlations between; chlorophyll b with root length ($r = 0.632$, $p<0.01$), leaves DW with both woody DW ($r = 0.70$, $p<0.01$), aerial DW ($r = 0.934$, $p<0.01$), total DW ($r = 0.813$, $p<0.01$) and plant height ($r = 0.817$, $p<0.01$). Although, woody DW was significantly correlated with both aerial DW ($r = 0.909$, $p<0.01$), plant height ($r = 0.945$, $p<0.01$) and aerial protein % ($r = 0.602$, $p<0.05$). However, aerial DW showed the same trend of positive correlation with both root DW ($r = 0.605$, $p<0.05$), total DW ($r = 0.71$, $p<0.05$), plant heights ($r = 0.949$, $p<0.01$) and N_2 fixation (mg/plant) ($r = 0.619$, $p<0.05$).

In addition, root DW showed the same trend with both plant heights ($r = 0.684$, $p<0.01$) and N_2 fixation (mg/plant) ($r = 0.649$, $p<0.01$), as well as, total OW with both plant heights ($r = 0.979$, $p<0.01$), N_2 fixation (mg/plant) ($r = 0.693$, $p<0.01$) and N_2 fixation % ($r = 0.581$, $p<0.01$). On the other hand, nodule DW showed similar nature with both nodulation index ($r = 0.939$, $p<0.01$) and average weight per nodule ($r = 0.666$, $p<0.01$), as well as, nodulation index with average weight per nodule ($r = 0.669$, $p<0.01$), acetylene reduction with stem diameter ($r = 0.849$, $p<0.01$), nodule efficiency with both plant heights ($r = 0.657$, $p<0.05$) and N_2 fixation (mg/plant) ($r = 0.672$, $p<0.01$). In addition, plant heights was significantly correlated with N_2 fixation (mg/plant) ($r = 0.715$, $p<0.01$).

The results obtained herein are in agreement with those reported by Dobert and Blevins (1993), who found strongest relationship ($r = 0.85$) between nodule mass and shoot DW, although nodule number was correlated closely with shoot DW. The same authors also reported that nodule mass and often number were closely related to shoot biomass.

As shown from the results tabulated in Table 11, in the respect of plants inoculated with the isolates derived from NRC19, that the strongest relationship was existed between chlorophyll (a) and nodulation index ($r = 0.924$, $p<0.01$), chlorophyll (b) with both total chlorophyll ($r = 0.972$, $p<0.01$) and woody DW ($r = 0.671$, $p<0.05$), as well *as,* total chlorophyll with woody DW ($r = 0.664$, $p<0.01$). In addition, the same trend of significant correlation was existed between reaves DW with both total DW ($r = 0.884$, $p<0.01$) and N_2 fixation (mg/plant) ($r = 0.711$, $p<0.01$). However, woody OW showed the same trend with both aerial OW ($r = 0.77$, $p<0.01$), root DW ($r = 0.702$, $p<0.05$), total DW ($r = 0.791$, $p<0.01$), acetylene reduction ($r = 0.654$, $p<0.01$), root length ($r = 0.789$, $p<0.01$) and N_2 fixation (mg/plant) ($r = 0.721$, $p<0.01$).

Similar nature of significant correlations was obtained between aerial DW with both root DW ($r = 0.633$, $p<0.05$), total OW ($r = 0.965$, $p<0.01$), root to shoot ratio ($r = 0.682$, $p<0.01$), root length ($r = 0.876$, $p<0.01$) and N_2 fixation (mg/plant) ($r = 0.881$, $p<0.01$). Although, the same trend was existed between root OW with both total DW ($r = 0.807$, $p<0.01$), nodule number ($r = 0.705$, $p<0.01$), nodule efficiency ($r = 0.666$, $p<0.01$) and N_2 fixation (mg/plant) ($r = 0.702$, $p<0.01$). Total DW also showed the same trend with both root length ($r = 0.898$, $p<0.01$), as well as, root to shoot ratio showed the same trend with root length ($r = 0.743$, $p<0.01$).

On the other hand, nodule number was significantly correlated with both nodule DW ($r = 0.953$, $p<0.01$), nodulation index ($r = 0.67$, $p<0.01$) and average weight per nodule ($r = 0.638$, $p<0.05$), as well as, nodule DW with both nodulation index ($r = 0.683$, $p<0.01$), average weight per nodule ($r = 0.706$, $p<0.01$) and stem diameter ($r = 0.705$, $p<0.01$). However, similar nature was existed between average weight per nodule with both stem diameter ($r = 0.643$, $p<0.01$), nitrogen fixation % ($r = 0.708$, $p<0.01$) and aerial protein % ($r = 0.714$, $p<0.01$), as well as, nodule efficiency with nitrogen fixation % ($r = 0.715$, $p<0.01$) and aerial protein % ($r = 0.641$, $p<0.01$). Although, the similar nature was existed between root length with N_2 fixation (mg/plant) ($r = 0.731$, $p<0.01$).

The results obtained here are in agreement with those reported by Buttery and Dirks (1987), who found that nodule fresh weight was correlated with the rate of acetylene reduction per plant of soybean, as well as, plant weight per unit of nodule fresh weight was positively correlated with acetylene reduction rate per unit nodule fresh weight for both strains of *B. japonicum* and cultivars. The same authors also reported that *the* increase in plant dry weight is probably the best indicator of nitrogen fixing capacity (Materon and Vincent, 1980). In addition, acetylene reduction rate and nodule mass are two widely used indicators of nitrogen fixation capacity (Rys and Mytton, 1985). The most practical method for estimating nitrogen fixing ability would seem to be some combination of nodule mass and of nodule efficiency (such as acetylene reduction rate) (Buttery and Dirks, 1987). Rhizobial isolates derived from HRI-19, also revealed significant correlations between some parameters related to nitrogen fixation. The present results are in accordance with Mytton and Rys (1985), who reported that the numbers of nodules formed on white clover in the presence of nitrate was a heritable character well correlated with nitrogenase activity. Rys and Mytton (1985) suggested that both nodule formation and nitrogenase activity should be used as selection criteria in any breeding program aimed at improving fixation in the presence of combined N.

In conclusion, the selection of highly efficient strains remains one of the main tasks for inoculant procedures. In developing countries, the demand for fuel wood, timber and fodder are increasing. It is unlikely that such a demand can be met by expanding tree planting on fertile land as this is required for food crop production. Thus, extensive areas of marginal lands on which trees and shrubs can be planted are available in developing countries, to became fertile lands enough for food crop production.

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