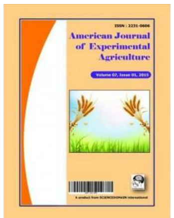

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Evaluation of Seven Forage Legumes for Biological Nitrogen Fixation (BNF) and Their Effects on *Amaranthus cruentus* in a Fluvisol (River Sand)

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Authors' contributions

This collaborative work was carried out by both authors. Author SAO designed the research, analyzed the data and wrote the first draft. Author EJF supervised data collection and revised the first and final drafts. Both authors read and approved the final manuscript.

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ABSTRACT

A six month screen house experiment was conducted at the Department of Crop Science, University of Benin, Benin City to assess seven forage legumes for N_2 fixation and their effects on *Amaranthus cruentus* in a fluvisol (river sand). *Cajanus cajan, Centrosema pascuorum, Leucanea leucocephala, Peuraria phaseoloides, Stylosanthes guianensis, Stylosanthes hamata* and *Lablab purpureus* were fitted into a completely randomized design with three replications. All seeds except *Lablab purpureus* were scarified and treated with benlate (50% benomyl) before sowing in river sand. Measurements taken at 4- week intervals were root length (cm), root fresh weight (g) and root dry weight (g). At 8 weeks after sowing (WAS), number of nodules, number of effective nodules, nodules fresh weight and nodules dry weight were measured. Shoot and soil nitrogen (g kg^{-1}), leaf chlorophyll index and carbon: nitrogen ratio was assessed at 12 WAS. *Amaranthus cruentus* followed legumes in sequence and number of days to emergence, plant height, number of leaves, root length, fresh weight of leaves, stems and roots (g) including dry weight of leaves, stems and roots (g) were assessed at 4 WAS. The seven forage legumes accumulated substantial quantities of nitrogen in their shoot (30.5–40.9 g kg^{-1}) and also fixed considerable quantities of nitrogen in the soil (3.2–6.3 g kg^{-1}). *Centrosema pascuorum* recorded the highest shoot nitrogen

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(40.9 g kg^{-1}) whereas *Stylosanthes hamata* fixed the highest quantity of soil nitrogen (6.3 g kg^{-1}). *Leucanea leucocephala* furnished the best (p = 0.05) root variables while *Stylosanthes hamata* exhibited the best root nodule characteristics. Carbon: nitrogen ratio ranged from 2.6 to 13.3. Amaranth seeds emerged within 2–7 days after sowing. Growth and yield of amaranth was significantly better in the Lablab-amaranth than other sequences. These positive responses indicate their usefulness for biological nitrogen fixation, forage production and soil fertility improvement. Lablab-amaranth sequence should be developed further for increased vegetable consumption.

Keywords: Centrosema pascuorum; crop rotation; Lablab purpureus; soil fertility; vegetable production.

1. INTRODUCTION

Legumes provide high quality protein food and feed, improve soil fertility and increase soil organic matter [1]. They fix 50–400 kg ha^{-1} of N yearly [2], suppress weeds [3] and control soil erosion from wind and water. Amount of nitrogen fixed depends on species, total biomass and percentage of nitrogen in plant tissues [4]. However, the availability of biologically fixed nitrogen to a subsequent crop may differ among legume species [5]. The optimum C: N ratio for rapid legume decomposition ranges between 15:1 and 25:1 [6].

Biological Nitrogen Fixation (BNF) may be quantified using acetylene reduction, xylem ureide analysis, labeled (^{15}N) isotope or nitrogen difference techniques [7]. An extension of the N-difference method whereby legumes are grown in a nutrient deficient medium maybe called the N-accumulation method. All nitrogen accrued in plant shoot and growth medium are presumed fixed by legume. Seven forage legumes were introduced into the University of Benin for research purposes. Their evaluation will enable informed decisions about suitable roles. Legume-vegetable sequence may reduce production overhead occasioned by high cost of inorganic and animal fertilizers. The objective of the study was to compare the 7 legumes on the basis of nitrogen fixation and their effects on a subsequent vegetable crop in river sand.

2. MATERIALS AND METHODS

The screen house study was conducted for 6 months (April- September, 2014) at the Teaching and Research farm of the University of Benin to evaluate 7 forage legumes for ability to fix atmospheric nitrogen in a nutrient deficient fluvisol (river sand). After cropping, their effects on *Amaranthus cruentus* were also measured. *Cajanus cajan, Centrosema pascuorum, Leucanea leucocephala, Pueraria phaseoloides, Stylosanthes guianensis, Stylosanthes hamata* and *Lablab purpureus* were fitted into a Completely Randomized Design (CRD) as treatments with 3 replications. River sand was thoroughly washed with un-chlorinated underground water before air-drying for 2 days. The soil was analyzed for routine physical and chemical analysis before while nitrogen was analyzed after the experiment [8].

Legume seeds were treated with a fungicide (Benlate, 50% Benomyl) before sowing. Except *Lablab purpureus*, seeds were manually scarified with rough sand paper to promote early germination and seedling emergence. Seeds were drilled into 2 cm deep furrow spaced 2 cm apart in the respective plots (plastic bowls) and covered lightly with sand. Plots were watered twice daily (morning and evening) with underground water. Weeds occurred sparsely within plots and were hand- picked intermittently. Incidence of pest and disease infestations was monitored but there was no drastic occurrence of either. Eleven variables appraised were shoot nitrogen (g kg^{-1}), soil nitrogen (g kg^{-1}), leaf chlorophyll index, carbon: nitrogen ratio, root length (cm), root fresh weight (g), root dry weight (g), number of nodules (NON), number of effective nodules (NEN), nodule fresh weight (g) and nodule dry weight (g). Nitrogen (N) concentration was determined by the micro Kjeldah method [9]. Leaf chlorophyll index of plants was obtained with an automated chlorophyll meter. Carbon: nitrogen ratio was calculated with the equation: C: N ratio = 40% / Nitrogen (%) [4]. Effectiveness of nodules was determined by visual observation. Nodules were detached from roots and excised with a razor blade. Effective nodules possess leghaemoglobin with a red colored interior whereas ineffective nodules have white and spent nodules have green interiors [10].

After cropping for three months, plots were rid of all remaining plant materials and tilled before sowing amaranth seeds. At 4 WAS, amaranth variables assessed were Number of Days to Emergence (NDTE), Plant Height (PH), Number of Leaves (NOL), Root Length (RL), fresh weight of leaves, stems and roots including dry weight of leaves, stems and roots. The data collected were subjected to analysis of variance (ANOVA) with SAS software [11]. The means were separated using the Least Significant Difference (LSD) method at 5% level of probability.

3. RESULTS AND DISCUSSION

In Table 1, the river sand was slightly acidic in reaction (pH 6.7). It was devoid of nitrogen, low in organic carbon (0.2 g kg^{-1}) while available P (18.18 mg kg^{-1}) was in the medium range (Table 1). In view of the absence of nitrogen and medium rating of available phosphorus [12], the assumption underlying the nitrogen accumulation technique is valid. This means that the nitrogen measured in legume shoots and river sand were products of N_2-fixation. The seven forage legumes fixed different quantities of nitrogen even though the soil was limited in nutrients and seeds were not inoculated with rhizobia. This is an attestation to their promiscuousness since effective nodules were produced in soil in which legumes had never been grown. This also infers that with phosphorus application these legumes may fix considerably larger quantities of nitrogen. Phosphorus increased N_2-fixation and grain yield of succeeding wheat by 20% [13], through root proliferation, nodule formation and energy transformations. In practical terms, these legumes can increase soil fertility and control soil erosion [14], which has devastated a large expanse of agricultural lands in humid rainforest regions.

Table 1. Physical and chemical properties of the river sand

Variables	Value
Texture class	**Sand**
p H (H_2O)	6.70
Organic carbon (g kg^{-1})	0.20
Total nitrogen (g kg^{-1})	0.00
Available phosphorus (mg kg^{-1})	18.18
Exchangeable bases (cmol kg^{-1})	
Potassium	0.21
Calcium	1.20
Magnesium	0.30
Sodium	0.37
Cation exchange capacity	2.40

Table 2 shows that *Centrosema pascuorum* manifested the significantly highest shoot nitrogen concentration (40. 9 g kg^{-1}) followed by *Lablab purpureus* (32.9 g kg^{-1}) whereas *Leucanea leucocephala* yielded the significantly lowest shoot nitrogen concentration (30.0 g kg^{-1}). *Stylosanthes hamata* augmented soil nitrogen (6. 3 g kg^{-1}) significantly more than other legumes that contributed between 3.2-6.1 g kg^{-1}. The largest (p = 0.05) leaf chlorophyll index (39.9) was furnished by *Centrosema pascuorum*. Carbon: nitrogen ratio ranged from 9.8–13.3 with *Centrosema pascuorum* having the lowest (p = 0.05) value. The high shoot nitrogen content which triggered the high leaf-chlorophyll index of *C pascuorum* reaffirms its suitability for ruminant nutrition. Approximately 5000 ha of *C pascuorum* cv. Calvacade cropped in Australia yearly [15] is utilized for feeding ruminants [16]. For this reason, this annual legume with high crude protein concentration should be further exploited in the rainforest zone. On the other hand, the comparatively high soil nitrogen fixation by *S hamata* is attributable to its favourable root nodule characteristics which were the conventional method of predicting the potential for Biological Nitrogen Fixation (BNF) among legumes [7]. This implies that where sophisticated measuring devices are absent, root nodule characteristics could be used to predict legume potential for BNF. Another inference is that Stylosanthes can be propagated to improve soil fertility [17].

Among legumes, *Leucanea leucocephala* exhibited the highest (p = 0.05) plant root variables (Table 3) while *Stylosanthes hamata* offered the best (p = 0.05) root nodule characteristics (Table 4). However, other legumes were generally at par in plant root variables and root nodule characteristics. The long roots of *Leucanea leucocephala* could be used to break down hard compacted soils. In Australia, lupine was used as a biological plough [18] because of its extensive rooting system. In the current study, the range for carbon: nitrogen ratio (9.8–13.1) was below the reported optimum [4]. This is probably because of the relatively short duration of vegetative growth which retarded legume dry matter and carbon accumulation. Generally, differences recorded among legumes maybe ascribed to their inherent genotypic variations. In Pakistan, mash bean (*Vigna mungo*) was significantly better than mung bean (*Vigna radiata*) in N_2 fixation [13].

The growth variables of *Amaranthus cruentus* in Table 5 shows that amaranth seeds sown into

Lablab, Leucanea and Centrosema plots emerged significantly earlier than those in the Cajanus plots which were the latest (p = 0.05) to emerge. Generally, amaranth growth was significantly better following lablab than other legumes. Similarly, yield variables of amaranth produced from previous lablab plots was significantly higher than those obtained from other legume plots (Table 6). In this study, the lablab- amaranth sequence was the most successful. The difference in emergence of amaranth seeds implies that *Cajanus cajan* may have inhibited seed germination more than the other legumes. Many plant species including legumes exhibit allelopathy [19]. *Brassica species* inhibited several germination indices of summer cereals [20]. Further studies may exonerate lablab from allelopathy. The numbers of leaves harvested were fewer than those recorded in an earlier study [21]. Differences in nutrient status and time of harvest (4 and 6 weeks after sowing in the present and former studies, respectively) may account for this variation. However, the relatively higher growth and yields recorded in the lablab-amaranth sequence suggests that lablab nitrogen was more readily available than that of other legumes. These results position lablab as a suitable green manure for vegetable production.

Table 2. Biological nitrogen fixation variables of forage legumes

Forage legume	Nitrogen (g kg^{-1})		L C index	C: N ratio
	Shoot	Soil		
Cajanus cajan	3.05e	0.32f	38.10b	13.10a
Centrosema pascuorum	4.09a	0.56c	39.90a	9.80d
Leucanea leucocephala	3.00f	0.46e	26.00c	13.30a
Peuraria phaseoloides	3.17c	0.61b	30.50e	2.60b
Stylosanthes guianensis	3.08d	0.56c	28.40f	13.10a
Stylosanthes hamata	3.08d	0.63a	23.30g	13.10a
Lablab purpureus	3.29b	0.49d	35.60d	12.20c
LSD (P=0.05)	0.018	0.013	0.015	0.175

Means in the same column followed by different letter(s) are significantly different (P= 0.05), L C=Leaf chlorophyll, C: N= Carbon: nitrogen

Table 3. Plant root variables of forage legumes

Forage legume	RL(cm)	RFW(g)	RDW(g)
Cajanus cajan	11.59b	2.41b	0.69b
Centrosema pascuorum	12.81b	2.92b	0.63b
Leucanea leucocephala	18.59a	10.42a	3.98a
Peuraria phaseoloides	12.42b	1.98b	0.53b
Stylosanthes guianensis	12.84b	1.75b	0.26b
Stylosanthes hamata	13.33b	4.24b	1.38b
Lablab purpureus	18.04a	3.02b	0.78b
LSD	4.550	3.914	1.427

Means in the same column followed by different letter(s) are significantly different (P= 0.05), RL= Root length, RFW= Root fresh weight, RDW=Root dry weight

Table 4. Root nodule characteristics of forage legumes

Forage legume	NON	NOEN	NFW(g)	NDW(g)
Cajanus cajan	38.08ab	27.25ab	0.80ab	0.49a
Centrosema pascuorum	22.54c	18.17bc	0.39b	0.18b
Leucanea leucocephala	21.46c	16.58bc	0.56b	0.23b
Peuraria phaseoloides	19.38c	14.08c	0.91ab	0.31ab
Stylosanthes guianensis	28.38bc	20.42bc	0.89ab	0.31ab
Stylosanthes hamata	44.63a	36.54a	1.16a	0.40ab
Lablab purpureus	29.29bc	22.00bc	0.81ab	0.29ab
LSD	13.545	11.079	0.514	0.245

Means in the same column followed by different letter(s) are significantly different (P= 0.05), NON=Number of nodules, NOEN=Number of effective nodules, NFW=Nodule fresh weight, NDW=Nodule dry weight

Table 5. Growth variables of *Amaranthus cruentus* in legume-amaranth sequence

Forage legume	NDTE	PH(cm)	NOL	RL(cm)
Cajanus cajan	7.00a	13.57d	9.67ab	3.10b
Centrosema pascuorum	2.00a	9.50f	12.33a	2.23c
Lablab purpureus	2.00d	30.63a	10.33a	3.47ab
Leucanea leucocephala	2.00d	20.30b	5.33c	3.63a
Peuraria phaseoloides	4.00b	11.57e	12.33a	2.10c
Stylosanthes guianensis	3.00c	10.17ef	7.00bc	1.87c
Stylosanthes hamata	3.00c	15.47c	10.00a	3.13b
LSD	0.001	1.793	2.861	0.391

Means in the same column followed by different letter(s) are significantly different (P= 0.05), NDTE=Number of days to emergence, PH=Plant height, NOL=Number of leaves, RL=Root length

Table 6. Yield variables of *Amaranthus cruentus* in legume-amaranth sequence

Forage legume	Fresh weight (g)			Dry weight (g)		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Cajanus cajan	10.93c	13.16c	3.92b	4.24c	6.21c	1.91b
Centrosema pascuorum	5.05f	3.45f	2.78c	1.21e	1.20f	1.23d
Lablab purpureus	13.62a	17.96a	4.15a	6.53a	6.54b	2.35a
Leucanea leucocephala	8.46e	7.35d	2.75c	3.21d	3.22d	1.41c
Peuraria phaseoloides	12.30b	14.63b	2.52d	5.51b	7.43a	1.22d
Stylosanthes guianensis	9.20d	6.33e	2.38e	3.22d	2.07e	1.21d
Stylosanthes hamata	5.03f	2.92f	2.23f	1.03f	1.11g	1.22d
LSD	0.049	0.737	0.044	0.064	0.047	0.037

Means in the same column followed by different letter(s) are significantly different (P= 0.05)

4. CONCLUSION

Centrosema pascuorum fixed the highest quantity of shoot nitrogen whereas *Stylosanthes hamata* fixed the utmost soil nitrogen. Lablab-amaranth sequence was the best rotation option. The expressed high potential for biological nitrogen fixation justifies further research with these legumes. If properly harnessed, they will contribute significantly to the cropping and farming systems of the University of Benin in particular and the humid rainforest zone in general.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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