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Full Length Research Paper

Preliminary study of the contribution of native legumes to the nitrogen economy of natural grasslands

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The need to discover suitable native legumes of high nitrogen fixing ability was pinpointed. Potential native legumes growing in the wild in Nsukka, Nigeria were identified and the seeds were collected. *Desmodium ramisissimon* was found to be the common legume species in the University area and was used for germination and nodulation studies. *D. ramisissimon* showed good potentiality with regards to nodulation ability and was recommended for further investigation.

Key words: Nodulation, germination, nitrogen fixation, *Desmodium ramisissimon*.

INTRODUCTION

In Nigeria, grass is the cheapest and the most important source of animal feed. But still, there is much need for both short and long term researches into the natural range for feed availability and improvement. Any improvement in the carrying capacity of pastures, the nutrient quality of the feed and the extension of their normally short growing season, will reflect favourably on the production of animal products. These attributes are known to be achieved when the appropriate kind of legumes and grasses are grown in healthy association for some mutual benefit, with the legumes, among other functions, contributing to the nitrogen status of the soil through nitrogen fixation.

The nitrogen economy of range soil forms an important aspect of range research. In effect, both nitrogen-fixation by non-legumes and symbiotic nitrogen fixation by legumes can be of considerable importance in the nitrogen economy of grassland soils. However, legumes have superior attributes in that they combine their high palatability, high nutrient content as fodder with high nitrogen fixing ability. Moreover, legumes can be more easily introduced into range lands advantageously. Abundant evidences (Humphreys, 1995; Andrea and Pablo, 1999; Peters et al., 2001; Olanite et al., 2004) have shown the ability of tropical legumes to fix nitrogen.

The natural action of converting atmospheric N into forms available for the plant-animal-soil system improves productivity in an inexpensive, environment friendly manner. The level of N fixation from effectively nodulated legumes depends upon the growth rate of the legume and soil conditions; usually 15 to 40 kg N is fixed for each 1000 kg dry matter of shoots grown (Humphreys, 1995). This "natural fertiliser" enables small landholders to improve the soil without exacerbating debt.

In Nigeria, pasture management is yet a prerogative of research institutions like universities and government research institutes. Grazing of animals is therefore usually carried out in natural ranges where the composition of species is virtually unknown. The stock subsists, primarily and practically all the year round, on unimproved natural pastures which lack the nutritive values of managed pastures (Bamikole et al., 2004). Most of the native legumes have not been identified and assessed for introduction into the system of range management. Further, the legume species that can best be utilized in rotations to maintain soil fertility is not known (Adegbola, 1964). Agronomists had very little achievement in finding suitable tropical legumes because of the ecological nature of tropical grasslands where grasses often grow very tall and out-compete the legumes that might be growing in association.

This preliminary work was aimed at identifying certain native legumes, assessing the contribution of the identified legumes to the nitrogen economy of natural

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grasslands and investigating the advisability and feasibility of introducing the selected native species into regular range management.

MATERIALS AND METHODS

Identification of native legumes

Some natural grasslands in Nsukka were surveyed to identify native and not-yet-popularly known legumes growing in the wild. Nsukka is located on latitude 06° 52' N and longitude 07° 24' E, and at an altitude of 447.2 m above sea level. The following were identified and the seeds were collected: *Zornia glochidiata*, *Indigofera* spp., *Senna* spp., *Vigna* spp. and *Desmodium ramisissimon*. In order to assess the frequency of occurrence of these legumes and their attendant importance on population basis, a tract of grassland in the University of Nigeria farm which had not been maintained since 1967 and which had occasionally been used for grazing the university livestock (cattle) since 1971, was selected as a typical natural grassland in Nsukka, for closer study. An area of 76 x 66 m (approximately 0.5 hectare) of the grassland was measured out for specific study of the different species, grasses and legumes inclusive, and their frequency of occurrence. One metre square quadrats were taken at intervals of 9.5 m along the diagonals of the area of 76 x 66 m marked out for the study. The different species inside the quadrat were identified and their number were recorded (Table 1). There were 24 quadrats in all.

Germination trial

Germination trial was carried out using the seeds of *D. ramisissimon*. Healthy seeds were selected from shelled seed collection. Those that floated on water were discarded as unviable. One treatment consisted of soaking the seeds in hot water for 5 min, another was soaking in cold water for 3 h, and other treatments were the scarification of seed by soaking in concentrated sulphuric acid (concentrated H_2SO_4) over varying time intervals viz. 5, 10, 20, 30 and 45 min, after which they were washed with copious amount of tap water. Visible effects of the different treatments on seed coat, embryo and the cotyledons were noted, if any. Germination trial was then carried out in Petri dishes. 15 seeds per treatment were carefully embedded in filter paper and placed in a Petri dish. In all, there were seven treatments and five replications. The Petri dishes were randomly placed in a flat container holding water in the greenhouse, and filter papers were arranged so as to dip them into water in the flat container and to absorb and convey moisture to the seeds embedded in the filter papers. This ensured that all the seeds got enough water to enable them to germinate. Observations consisted of the number of seeds that germinated after 2, 3, and 5 days and the performance of the germinated seedlings. Numerical data obtained were statistically analyzed following completely random design procedure.

Assessment of the degree of natural nodulation in *D. ramisissimon*

Topsoil from part of the University of Nigeria, Nsukka, farm which had not been under cultivation for some time was dried, sieved and filled into greenhouse pots. 2 kg soil was added per pot and then watered to field capacity on March 4, 1975. On March 5, healthy *D. ramisissimon* seeds scarified by soaking in concentrated H_2SO_4 for 5 min and washed with copious running water were planted at the rate of 3 seeds per pot and thinned down to 1 seedling per pot on germination. The experiment was a greenhouse trial and the observations taken included: germination rate, time of flowering and fruiting and forth-nightly harvest of whole plants (5 at a time) for nodulation count, weight of nodules produced per plant, and weight of shoot and root per stand. The first harvest was done 39 days after germination when some of the plants had started flowering and then at forth-nightly intervals. At harvest, the pot was first soaked with water to allow the ball of earth to be removed intact from the pot. Then the ball of earth was gently washed off with copious water, taking care to recover all nodules and roots. The nodules were carefully removed and the fresh weight of the nodules and those of the shoot and root were determined. The nodules were assessed for size; some were selected and cut open and examined for pink colouration of the internal tissues, a characteristic of effective nodules. Some stands were left for harvesting the pods to be used for calculating the average seed production per stand.

RESULTS

On the whole, there were a total of 21 individual species identified and of these, only one was a legume (Table 1). On percentage basis, this represented 4.37% legume for the whole population. In effect, the only legume identified within the quadrates was *D. ramisissimon* and this indicated that *D. ramisissimon* was the common legume species in the university area. This fact coupled with the erect growth habit of the species which suggested that it could withstand some aerial competition against grasses, therefore, justified the selection of *D. ramisissimon* for further investigation on germination, growth, nodulation ability and seed production potential.

After the various treatments and before placement of seeds for germination, visual effects of treatments on seed coat, cotyledon and embryo were identified particularly with acid treatments. Soaking in cold and hot water did not have visible wearing effect on the testa and the seeds seemed to remain normal. Scarification with concentrated sulphuric acid showed different degrees of effect. Seeds soaked in concentrated H_2SO_4 for 5 min had their coats worn, in some cases partially but in others wholly. The testa of those soaked for 10 min were mostly peeled off but the cotyledons were not badly affected. For those soaked for 20, 30 and 45 min, the testa were completely peeled off with the cotyledons eroded, imparting some brown discolouration, showing a burning effect. The burning effect was more prominent in those soaked for 30 and 45 min. The trend of germination is summarized in Table 2.

The seeds planted on March 5 germinated in two days, showing good performance. After 31 days of germination,five plants had initiated flowering and a week later, all the 40 plants had produced flowers. Fruit formation started after 40 days of germination. Within 77 days of germination, the fruits matured shortly after senescence of the shoots set in. This showed that *D. ramisissimon* has a short fruiting cycle. Data on potential average seed

Table 1. Population census of the species in the quadrat.

S/N	Species	Number of quadrat																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total
1	*Panicum maximum*	15	1	17	6	6	14	8	9	14	8	13	6	9	1	11	10	7	2	16	7	9	6	2	2	197
2	*Stenotaphrum secundatum*	10	23	17	11	13	12	2	2	1	10	5	14		10	1	3	6	16		8	3	7	13	30	213
3	*Desmodium ramisissimon*	15						4							7										15	41
4	*Sporobolus pyramidalis*	1						1																		2
5	*Oldenlandia affinis*	27	3		2	4		3	19	7		5		8	12	1					8					99
6	*Vernonia* sp.	2																								2
7	*Blechum pyramidatum*	1																								1
8	*Phyllanthus* sp.	1						2																		3
9	*Aspilia Africana*		1																							1
10	*Oldenlandia corymbosa*		1						11				1	8				2								23
11	*Ipomoea involucrate*			2		2	1		13				4	1			3			5			3			34
12	*Borreria vetissilata*			1						3																4
13	*Digitaria* sp.				8	15	18	3	20	26			20			24	26	8	5	10	11	24	12	25		267
14	*Cyperus rotundus*						11																			11
15	*Hyperrehnia rufa*									1									2				1			4
16	*Emilia sonchifolia*											2														2
17	*Vernonia cinerea*											6														6
18	*X											1	1					2								4
19	*Urena* sp.													1												1
20	*Killinga* sp.														2			16								18
21	*Gutebergia erecta*																				9			1		10
	Total	72	29	37	27	40	56	23	74	52	18	32	46	27	32	37	42	41	25	31	43	36	29	41	47	937

*X Botanical not known; numerical total of species present in the quadrants = 937; numerical total of legume species present in quadrats = 41. Legume (%) = $\frac{41}{937} x \frac{100}{1} = 4.37\%$

Table 2. Summary data on mean of germination of *D. ramisissimon* after 2, 3 and 5 days from sowing (mean number of seedling). Maximum mean obtainable = 15.

Treatment	2 days	3 days*	5 days
3 h in cold water	-	0.4^{a}	0.6
5 min in hot water	0.4	0.8^{a}	0.8
5 min in concentrated H_2SO_4	12.2	14.4^{d}	14.8
10 min in concentrated H_2SO_4	13.8	14.4^{d}	14.6
20 min in concentrated H_2SO_4	5.8	8.0^{b}	9.2
30 min in concentrated H_2SO_4	7.4	10.2^{bc}	11.8
45 min in concentrated H_2SO_4	7.4	11.6^{c}	11

* Germination data on 3 days after sowing were statistically analyzed; S.E. = ± 0.76.
Means followed by the same letter are not significantly different.

production are summarized on Table 3. The plant produced an average of 67 pods per plant with a seed average of 4 per pod, therefore giving a calculated seed average of 268 per plant.

When the first harvest was done 39 days after germination, nodules had already been formed. Observations throughout the harvests showed that the nodules were characteristically round, numerous, but relatively small in size. The general pattern of nodule formation was as follows: relatively few large nodules formed along the primary roots; many medium sized nodules occurred on the secondary roots, while tertiary roots bore smaller and fewer nodules. The nodules when cut and examined both with the naked eyes and under the microscope showed a pink colouration in the central tissues indicating that the nodules were effective with regard to nitrogen fixation. This was shown throughout the different stages of the harvest. The results of the various harvest data are summarized in Table 4.

DISCUSSION

Germination trial

Generally, acid treatment produced fast germination (Table 2). However, analysis (based on germination count 3 days after sowing) showed highly significant differences in the ability of the different treatments to effect good germination. Soaking in concentrated H_2SO_4 for 5 and 10 min produced significantly higher results than those for the other treatments. Although soaking in concentrated H_2SO_4 for 45 min showed better germination than soaking for 30 min, yet there were no significant differences. Soaking for 45 min again showed a significantly better germination than soaking for 20 min, but this was hardly expected. However, the subsequent poor performance of the seedlings of those soaked for 45 and 30 min in comparison with those of seeds soaked for 20 min showed the comparative better effect of soaking for 20 min. Soaking in cold water for 3 h and soaking in hot water for 5 min did not differ significantly in their effect. They did not evince any impressive effect on germination and had nothing to commend them.

There were different visual treatment effects on seedlings 5 days after planting. Seedlings of 5 and 10 min acid treatment showed good growth with green cotyledons and radicles. Those from seeds soaked for 20 min in concentrated H_2SO_4 showed poor and retarded growth although they maintained green colour of the cotyledons. Treatment with concentrated H_2SO_4 for 30 min produced seedlings with poor and retarded growth. The cotyledons had localized spots of acid burns and there was a general chlorosis of the seedlings. Soaking for 45 min produced seedlings with very poor development. The seedlings looked scorched and in fact by the fifth day, 3 had withered and died.

Assessment of degree of natural nodulation

Table 4 shows that the mean fresh weight of nodules tended to increase with the length of time the plants were allowed to grow before harvest. Statistical analysis according to the completely randomized design procedure showed that there were overall significant differences among mean fresh weights of the various nodule harvests. Growing the plant for 15.6 weeks produced the highest fresh weight of nodules followed by 13.6, 11.6, 9.6, 7.6 and 5.6 weeks, respectively. Growth periods of 5.6 and 7.6 weeks gave disproportionately low nodule weight yield and did not show any significant yield differences. The result indicated that there are more prospects of adding more to the nitrogen status of the soil with longer periods of growth since the nodules were found to be generally effective.

The mean number of harvested nodules also tended to increase significantly with the number of days (weeks) the plants were allowed to grow before harvest. There was an indication that nodulation increased with age to a

Table 3. Calculation of potential seed production/plant of *D. ramisissimon.*

No of stand sampled (a)	Total pod harvested from 3 stands(b)	Average pod per stand (b/a)	Nunber of pods sampled for seed (c)	Total seed from sampled pod (d)	Average seed per pod (d/c)	Calculated number of seed/stand (d/c) x (b/a)
3	200	66.6 = 67	13	49	3.79 = 4	268

Table 4. Summary of results of harvest after various weeks of growth of *D. ramisissimon.*

Number of week after germination	5.6 weeks (A)	7.6 weeks (B)	9.6 weeks (C)	11.6 weeks (D)	13.6 weeks (E)	15.6 weeks (F)
Mean number of nodules	44	153	270	439.8	412.2	407.8
*Mean fresh weight of nodules (g) ± 0.096	0.02[a]	0.16[ab]	0.30[bc]	0.49[cd]	0.50[cde]	0.56[de]
Mean fresh weight of shoot + root (g)	0.95	3.13	4.70	5.47	9.18	9.96

*Significant at 5% level. Means values followed by the same letter are not significantly different.

certain point. A growth period of 11.6 weeks (81 days) gave the highest nodulation. After that, nodulation declined. This point of decline tended to coincide with the period when visual signs of senescence had set in, in the shoots. This was about 12 weeks after germination when the leaves started yellowing and eventually falling off, followed by the softening and crumbling of the nodules.

With respect to seed production (Table 3), *D. ramisissimon* produced large quantities of seed per plant with a short fruiting cycle of approximately three months; it is capable of producing about four fruiting cycles in a year thus still ensuring greater number of seeds for proliferation. This shows that if *D. ramisissimon* were to show a superior quality warranting its introduction for regular management of grasslands, the question of seed production might not be a problem. The problem would lie on germination.

Conclusion

The paucity of *D. ramisissimon* in the natural grassland studied, as shown by data on the census count of the species (Table 1), despite its inherent ability to produce very large quantities of seeds, points to a problem with regards to the propagation of the species. *D. ramisissimon* seed exhibited a form of dormancy emanating from the mechanical restriction of the hard seed coat on the embryo. Large losses of seed seem inevitable due to prolonged periods of dormancy caused by the hard testa. This dormancy can be broken by scarification with concentrated H_2SO_4. The seeds were soaked for 5 to 10 min. Above this time, good germination still occurred but seedlings showed poor development. This is because, beside removing the mechanical restriction imposed by the tough testa thereby exposing the embryo to rapid development, soaking for long period of time results in the destruction of the cotyledons (with the food reserve) and the damage of the embryo, thereby occasional and subsequent poor development of seedlings.

There is need to investigate our native legumes, with respect to their nitrogen fixing ability, for grassland research. *D. ramisissimon* is recommended for further investigation. It has a short fruiting cycle and produces large quantities of seeds which can be used for propagation. Although dormancy poses a problem, yet this can be overcome by acid scarification. Nodulation ability is high and encouraging and examination of the internal tissues of the nodules indicated that the nodules are essentially effective in nitrogen fixation. The authors cannot yet indicate with any certainty the level of effectiveness of these nodules because there is yet a need to quantify the actual amount of nitrogen fixed and assess the availability of the fixed nitrogen to the soil in which the legume is growing. This is an area where this virgin trial is deficient due to limitations in equipment. The use of acetylene reduction technique to indirectly measure the rate of nitrogen fixation is recommended.

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