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Nodulation, Dry Matter and Nitrogen Accumulation of Mucuna (*Mucuna pruriens* var. Utilis) in Response to Bradyrhizobia Inoculation

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ABSTRACT

Nodulation, dry matter and nitrogen accumulation of two mucuna accessions in association with introduced bradyrhizobia were assessed on two forest soils. Nodulation started after 40 days after planting (DAP), following drastic reduction in percent nitrogen (%N) in plant. Significant ($P<0.05$) accession x inoculation status interactions was present for nodule number, nodule dry weight and shoot dry weight. Early maturing accession, 'GH1746', produced significantly higher number of nodules and dry matter than uninoculated plants on both soils, but the late maturing accession, 'GH1745', did not respond to inoculation on 'Kokofu' soil. The relative change in dry matter between 80 DAP and 120 DAP was higher for 'GH1746' than 'GH1745'. For inoculated 'GH1746', there were increases in %N between 80 and 120 DAP of 31% and 36% on 'Nzima' and 'Kokofu' soil, respectively, but no such increases were present in uninoculated 'GH1746' nor in 'GH1745', both inoculated and uninoculated.

Key Words: Cover crop; Minor legume; Mucuna; Percent shoot nitrogen; Symbiotic differences

INTRODUCTION

It is common for small-scale farmers in tropical humid environments to weed four to six times a year in a tree crop plantation. However, this method of weed control is becoming difficult due to the rising cost and unavailability of farm labour. At the large-scale commercial tree plantations, the recommended practice has been to plant pueraria (*Pueraria phaseoloides)* cover crop, to control weeds. The latter technology has not been adopted by the small-scale farmers, who usually account for a large portion of the planted area of tree plantations (Allison, 1992). The small-scale farmers instead intercrop food crops in the inter-rows of plantation crops, to provide food and /or cash during the early years when the plantation crop has not come into bearing. This practice however, may have detrimental effects on the growth and yield of the plantation crop due to unfavourable competition.

Leguminous cover crops are important in smothering weeds, producing large quantities of dry matter, improving soil nutrition and increasing yield of perennial crops (Sanginga *et al.,* 1996). For the small-scale farmers, the need for food and/or cash during the early stages of establishing a plantation takes precedence over nodulation, nitrogen fixation and weed control with cover crops. It is therefore expedient to identify and evaluate cover crops that establish quickly, fix nitrogen, control weeds and also provide food and/or income for the farmers.

Mucuna (*Mucuna pruriens var. utilis)* seeds, which have protein content of about 26% (Ravindran & Ravindran, 1988), have gained acceptance among small-scale farmers as a minor food crop for stews and soups in some African countries (Buckles, 1995). Eilitfa and Carsky (2003) have outlined efforts at increasing the potential of mucuna as a food and feed crop. The crop is known to accumulate large biomass (Carsky *et al.,* 2001), fixes large amounts of nitrogen (Sanginga *et al.*, 1996) and effectively suppresses weed growth (Versteeg & Koudokpon, 1990). Becker and Johnson (1998) and Noordwijk *et al.* (1995), however, have reported that nitrogen fixed by mucuna ranged from 20 to 200 kg ha^{-1}, with the low levels produced under acidic or low soil P or moisture stress. Carsky *et al.* (2001) also observed that total biomass accumulation of mucuna was a function of the genotype and the growing period. Houngnandan *et al*. (2000) found that mucuna failed to nodulate on some farmers' fields without inoculation in the derived savanna of West Africa.

The objective of this study was to evaluate nodulation, nitrogen accumulation and dry matter production of early and late maturing accessions of mucuna in association with bradyrhizobia.

MATERIALS AND METHODS

Two pot experiments were conducted at Legon, using two accessions of mucuna, GH1746 and GH1745, which are early and late maturity types, respectively. Soil from 'Nzima' series (Orthi-ferric acrisol) and 'Kokofu' series

(Eutric/Dystric nitisol) (Anon., 1990) were collected from a fallowed field of the Oil Palm Research Institute of Ghana, Kade (0^0 49' W, 06^0 N and 156 m above sea level) for the studies. Soil samples were taken from the 0-20 cm depth, air-dried, sieved (<3 mm), mixed with sand (1:1) and transferred to plastic pots (10 kg pot^{-1}). Some physical and chemical properties of the soils are presented in Table I.

Table I. Some physical and chemical properties of soils used in studies

Soil property	Soil series	
	Nzima	Kokofu
Sand (%)	62.80	59.00
Silt (%)	22.20	20.40
Clay	15.00	20.60
PH (1:1 H_2O)	5.00	4.80
Organic matter (%)	2.40	2.40
Total N (%)	0.13	0.11
Carbon (%)	1.04	0.84
Available P (ppm)	2.54	2.04
K (C mol kg^{-1})	0.18	0.23
Ca (C mol kg^{-1})	3.00	2.20
Mg (C mol kg^{-1})	1.20	1.00
Na (C mol kg^{-1})	0.14	0.14
CEC (C mol kg^{-1})	11.26	10.40

In the first study, from March to June 2000, the plants were grown and their nodulation examined. Bradyrhizobia was extracted from nodules formed from association of mucuna with native bacteria in the two soils. In the second study, from July to November 2000, a factorial combination of two inoculation status and two mucuna accessions were evaluated on each of the two soils. For each soil series, a randomized complete block design with four replications was used. There were 16 experimental units, each with 15 pots, on each soil type.

Four seeds were sown per pot and thinned to two plants per pot, a week after emergence. Inoculation consisted of coating seeds with peat containing effective bradyrhizobia previously isolated from the respective mucuna accessions, using 40% gum Arabic as a sticker. Seeds were sown immediately after inoculation.

Plants were harvested at 40, 80 and 120 days after planting (DAP). At each harvest, five pots from each treatment combination were used. Plants from each pot were cut at the soil level and then oven-dried at 70 ^oC to constant weight to obtain the shoot dry weight. The soil was gently washed from the roots and the nodules were carefully removed and counted. Nodules were oven-dried for 72 hours at 70^{o}C. The dry shoot samples were milled for determination of plant nitrogen content by the Kjeldahl method (AOAC, 1990). Two-way analysis of variance using general linear model was carried out on data from each soil using Genstat statistical software (Genstat, 2002). The least significant difference was used to determine significance of differences between pairs of treatment means, whenever there was significant variance ratio. Data were analysed separately for each soil series.

RESULTS

The two soil series differed in some characteristics important for nodulation and nitrogen fixation, paramount among them being nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) levels (Table I). The N and P levels were low for both soil series. No nodules were produced at 40 DAP. Hence, the 80-DAP data was regarded as the base point for nodulation and the period between 80 and 120 DAP regarded as the period during which response to inoculation was expressed.

There was significant ($P<0.05$) accession x inoculation status interaction for nodule number per plant on both soil series and also at 80 and 120 DAP (Table II). On the average, differences between the two accessions under inoculation were much lower than under non-inoculated conditions. Bradyrhizobial inoculation led to a higher nodule production in the early maturing accession, GH1746, but this was true on only Nzima soil for the late maturing mucuna. Interaction of accession x inoculation were significant for nodule dry weight on both soils at 80 DAP and also for 120 DAP for 'Kokofu' soil (Table III). Nodule dry weight of inoculated plants was consistently higher than that of uninoculated plants of accession 'GH1746'. On 'Kokofu' soil, however, uninoculated 'GH1745' plants had

Table II. Effect of bradyrhizobial inoculation on number of nodules plant^{-1} of mucuna on two soil series

Mucuna	Nzima soil			Kokofu soil		
accession	I	UI	Mean	I	UI	Mean
80 days after planting[a]						
GH1746 (Early)	25.8	14.6	20.2	21.9	14.5	18.2
GH1745 (Late)	57.7	29.3	43.5	16.3	27.6	22.0
Mean	41.8	21.8		19.1	21.1	
120 days after planting[b]						
GH1746 (Early)	71.9	58.4	65.2	55.7	31.6	43.7
GH1745 (Late)	75.5	66.9	71.2	47.4	60.4	53.9
Mean	73.7	62.7		51.6	46.0	

I and UI refer to Inoculated and Uninoculated plants, respectively.

[a]LSD ($P = 0.05$):

Nzima soil: Accessions: 13.9; Inoculation status: 8.9; Accession x Inoculation status: 20.4

Kokofu soil: Accessions: NS; Inoculation status: NS; Accession x Inoculation status: 12.4

[b]LSD ($P = 0.05$)

Nzima soil: Accessions: NS; Inoculation status: NS; Accession x Inoculation status: 15.2

Kokofu soil: Accessions: 8.6; Inoculation status: NS; Accession x Inoculation status: 21.5

higher nodule dry weight than inoculated plants.

Differences in shoot dry matter of the two mucuna accessions changed depending on the inoculation status of plants on both soils (Table IV). Differences in shoot dry matter followed the same trend as nodulation, with inoculated 'GH1746'plants having significantly ($P<0.05$) higher dry matter accumulated than uninoculated plants on both soil series. However, on 'Kokofu' soil, uninoculated 'GH1745' plants accumulated higher dry matter than inoculated plants. The relative change in dry matter between 80 DAP and 120 DAP was higher for 'GH1746' than 'GH1745'. Also the change was higher for inoculated than uninoculated plants except for uninoculated 'GH1745' plants on 'Kokofu' soil.

In the inoculated early mucuna, 'GH1746', there were increases in percentage nitrogen (%N) between 80 and 120 DAP amounting to 31% on 'Nzima' soil and 36% on 'Kokofu' soil (Table V). There were no such increases in

Table III. Effect of bradyrhizobial inoculation on nodule dry weight (g plant^{-1}) of mucuna on two soil series

Mucuna	Nzima soil			Kokofu soil		
accession	**I**	**UI**	**Mean**	**I**	**UI**	**Mean**
80 days after planting [a]						
GH1746 (Early)	0.16	0.15	0.16	0.21	0.12	0.17
GH1745 (Late)	0.25	0.16	0.21	0.06	0.17	0.12
Mean	0.21	0.16		0.14	0.15	
120 days after planting [b]						
GH1746 (Early)	1.11	0.97	1.04	0.98	0.70	0.84
GH1745 (Late)	1.09	1.02	1.06	0.85	1.01	0.93
Mean	1.10	1.00		0.92	0.86	

[a]LSD (*P = 0.05*):
Nzima soil
Accessions: NS; Inoculation status: NS; Accession x Inoculation status: 0.09
Kokofu soil
Accessions: NS; Inoculation status: NS; Accession x Inoculation status: 0.11
[b]LSD (*P = 0.05*)
Accessions: NS; Inoculation status: NS; Accession x Inoculation status: NS
Accessions: NS; Inoculation status: NS; Accession x Inoculation status: 0.26

Table IV. Effect of bradyrhizobial inoculation on shoot dry matter of mucuna on two soil series

Mucuna	Nzima soil			Kokofu soil		
accession	**I**	**UI**	**Mean**	**I**	**UI**	**Mean**
80 days after planting [a]						
GH1746 (Early)	8.59	8.47	8.53	9.56	9.10	9.33
GH1745 (Late)	14.17	11.68	12.93	14.89	16.71	15.80
Mean	11.38	10.08		12.23	12.57	
120 days after planting [b]						
GH1746 (Early)	31.10	26.00	28.55	33.50	23.40	28.45
GH1745 (Late)	43.80	26.10	34.95	28.70	41.00	34.85
Mean	37.45	26.05		32.50	31.65	

[a]LSD (*P = 0.05*):
Nzima soil
Accessions: 4.21; Inoculation status: 1.22; Accession x Inoculation status: 5.91
Kokofu soil
Accessions: 5.01; Inoculation status: NS; Accession x Inoculation status: 6.00
[b]LSD (*P = 0.05*)
Accessions: NS; Inoculation status: 10.83; Accession x Inoculation status: 14.93
Accessions: NS; Inoculation status: NS; Accession x Inoculation status: 15.90

Table V. Effect of bradyrhizobial inoculation on N (%) in shoot dry matter of mucuna on two soil series

Mucuna	Nzima soil			Kokofu soil		
accession	**I**	**UI**	**Mean**	**I**	**UI**	**Mean**
40 days after planting [a]						
GH1746 (Early)	4.80	4.71	4.76	4.71	4.71	4.71
GH1745 (Late)	4.52	4.49	4.56	4.09	4.03	4.06
Mean	4.66	4.60		4.40	4.37	
80 days after planting [b]						
GH1746 (Early)	1.99	2.10	2.05	1.82	1.92	1.87
GH1745 (Late)	2.24	2.00	2.12	1.92	2.32	2.12
Mean	2.12	2.05		1.87	2.06	
120 days after planting [c]						
GH1746 (Early)	2.61	2.10	2.36	2.48	1.83	2.16
GH1745 (Late)	2.22	2.62	2.42	1.96	2.35	2.16
Mean	2.42	2.36		2.09	2.16	

I and UI refer to Inoculated and Uninoculated plants, respectively.
[a]LSD (*P = 0.05*):
Nzima soil
Accessions: NS; Inoculation status: NS; Accession x Inoculation status: NS
Kokofu soil
Accessions: NS; Inoculation status: NS; Accession x Inoculation status: NS
[b]LSD (*P = 0.05*)
Accessions: NS; Inoculation status: 0.06; Accession x Inoculation status: NS
Accessions: NS; Inoculation status: 0.09 Accession x Inoculation status: NS
[c]LSD (P = 0.05)
Accessions: NS; Inoculation status: NS; Accession x Inoculation status: 0.50
Accessions: NS; Inoculation status: NS; Accession x Inoculation status: 0.48

uninoculated early mucuna nor in late mucuna, both inoculated and uninoculated.

DISCUSSION

After 40 days of growth, %N was high while the plants grew on seed reserves and soil mineral N and the plants had not nodulated. Nitrogen concentrations fell dramatically during the next 40 days as seed and soil N were exhausted and the plants formed nodules. The period between 80 and 120 DAP therefore provided information on the response to inoculation. The results indicated that rhizorbia for mucuna were present in both soils.

'Nzima' soil was higher in P and Ca than 'Kokofu' and this could partly explain why nodule number and dry weight were higher on 'Nzima' than 'Kokofu' soil. Olufajo and Adu (1992), working with soybean observed nodulation to be better in years with high available P and exchangeable Ca than the years with low levels. Reddell *et al.* (1988) found that in *Casuarina,* applied P increased symbiotic nitrogen fixation by stimulating host plant growth rather than a direct effect on nodulation and N_2 fixation per se. In some farmers' fields in West Africa, Houngnandan *et al.* (2000) reported that at a location where extractable soil P was low, mucuna did not respond to rhizobia inoculation despite a higher population of rhizobia. Becker and Johnson (1998) and Van Noordwijk *et al.* (1995) observed poor nodulation and low nitrogen fixation by mucuna under acidic and low soil P.

The increase in dry matter and %N between 80 and 120 DAP in inoculated 'GH1746' were closely related, indicating that the increased nitrogen stimulated high plant growth. Clearly, cultivar differences existed in the response to inoculation between the early and late maturing accessions. Cultivar variation in traits associated with nitrogen (N) fixation has been demonstrated in all legume crops studied (Pazdernik *et al.,* 1996). Sanginga *et al.* (1996) also observed that some rhizobia strains induced higher shoot dry weights of *M. pruriens* var *utilis* in one type of soil but those strains were totally ineffective on *M. pruriens* var *cochinchinensis* in that same soil. This is an indication that host genotype x rhizobium strain interaction may be important in assessing response to bradyrhizobial inoculation in mucuna species.

The present results suggest the possibility that inoculaton led to changes in the composition and nature of competition in native rhizobia populations, leading to negative response on 'Kokofu' soil, but favourable response on 'Nzima' soil. Houngnandan *et al.* (2000) have also reported that inoculation increased shoot dry matter of mucuna by an average of 28% above the uninoculated treatments, but the increase depended on the field, location and year; and was inversely related to the numbers of rhizobia in the soil. Similar results have been reported by Carsky *et al.* (2001).

Mucuna could be a useful weed control agent and can improve the soil quality in view of high levels of biomass and nitrogen produced. Performance of mucuna depended upon the accession, soil series and inoculation status. The early maturing mucuna accession had superior dry matter and nitrogen accumulation under inoculated conditions over uninoculated conditions. Bradyrhizobia inoculation was effective in improving performance of the late maturing mucuna on 'Nzima' soil but had reduced nodulation and dry matter production under inoculation on 'Kokofu' soil.

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