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NODULATION IN *MILLETTIA THONNINGII* (SCHUM & THONN.) BAKER; NATIVE RHIZOBIA AND SEED INTERACTION FROM SOUTHWEST NIGERIA

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Abstract

Eight provenance of *Millettia thonningii* were selected across the distribution range in Nigeria, for evaluation of its adaptability for nitrogen fixation outside its origin. Significant variations were recorded under glasshouse conditions between the provenances in nodulation. All the provenances commenced nodule production after four weeks of growth, except Jema'a provenance whose nodule production started a week behind others. Ikire-lwo populations produced the highest average number of nodules 45 this variation was highly significant ($p \leq 0.01$) between the provenances. Potentials for nitrogen fixation were probably higher in Jema'a (22.58%) and Lokoja (21.23%) populations. Highly significant ($p \leq 0.01$) differences in nodule weights (fresh and dry) were observed among two provenances. Similarly, highly significant ($p \leq 0.01$) differences between provenances in nodule morphology were observed. The highest correlation (r=0.90**) was obtained between fresh and dry weights, while the least (r=0.17*) was found between number of dead nodules and nodules dry weight.

Introduction

Successful introduction of plants to sites outside their sources depends on the amount of genetic variabilities in the species as well as the distribution extent. These factors strongly determine the *versability* of the habitat requirements of such species. The prevailing environmental (climatic and edaphic) factors at such site are strongly adaptative. Such adaptations are often expressed in the form of morphological modification of the above ground features. Similarly, below ground adaptation of plants is strongly influenced by soil (physical and chemical/nutrients) status. Nutrient deficiency in soils, especially nitrogen is commonly the most limiting element to crop production (Anon., 1984), while some groups of plants are known to improve the nitrogen status of impoverished soils, though a mutual relationship between certain soil bacteria and plant roots, converting atmospheric nitrogen to utilizable form for the plants. The use of mycorrhiza in enhancing nutrient uptake of some temperature tree species, introduced to tropical environment, has been reported (Young, 1989). *Millettia thonningii* (Schum & Thonn.). Baker (fam. *Papilionaceae}* is an indigenous leguminous tree with potentials for soil improvement in the traditional farming systems (Egbe. *et al.*, 1998; Anegbeh *et al.*, 2003). The need for low external inputs and encouragement of sustainable environment friendly agricultural practices necessitate the evaluation of this species for soil improvement potentials, nodulation, including its response to native Rhizobia, introduced to sites outside its sources. This paper reports the effects of source on the response of *Millettia thonningii* to native Rhizobia outside its original home in South West Nigeria.

Table 1. Sources of *Millettia thonningii* seeds used for the study.

Sources	Latitude (N)	Longitude (E)	Soil type (FAO/ UNESCO	Agroccological zone
Ibadan	7° 22'	3° 58'	Ferric Luvisols	Transition Forest
Ifc	7° 30'	4° 31'	Ferric Luvisols	Rainforest Relics
Iwo	7° 10'	4° 09'	Ferric Luvisols	Transition Forest
Jema'a	9° 30'	8° 25'	Orthic Acrisols	Southern Guinea Savanna
Koton-karfe	8° 08'	6° 50'	Dystric Nitcsols	Woodland Savanna
Lokoja	7° 47'	6° 45'	DystricNitosols	Woodland Savanna
Ogbomoso	8° 01'	3° 29'	Ferric Luvisols	Southern Guinea Savanna
Zaria	11° 00'	7° 40'	Ferric Luvisols	Northern Guinea Savanna

Materials and Methods

The study was carried out in a glasshouse at the IITA Headquarters, Ibadan south-western Nigeria (Lat. 7°30' N, Long. 3°54' E). The site was characterized by sub-humid climate within average annual rainfall of 1250 mm and distinct wet and dry seasons. The study was conducted during the rainy wet season.

The Seeds were collected from 20 mother trees, from each of the 8 sources (Table 1) across five vegetation zones in Nigeria. These were bulked on source basis. Each lot was thoroughly mixed and the seeds randomly selected for this study. Seeds were surface-sterilized by washing in 3% H_2O_2 and thoroughly rinsed several times with distilled water according to Sanginga *et al.,* (1990).

The topsoil was collected from the "soil dump" at the IITA, Ibadan. The soil was air-dried and sieved to pass through a 2mm diameter sieve. This was sterilized with dry heat and allowed to cool for 12 hours. Three and half (±2) kilogram of the dry soil was put into plastic pots.

Active nodules were collected from the roots of nursery grown seedlings and mother trees of *M. thonningii* in the wild at Ibadan (southwest Nigeria). *Rhizobia* were isolated and ensured in Yeast Extract Mannitol Broth (YMB) at 28ºC for 5 days (Spreni & Odee, 1991). Seedling inoculation, with the broth, was performed at 2 weeks after sowing, by pipetting 10ml of aqueous suspension into the soil (Sprent & Odee, 1991).

The soil-filled plant pots were laid out in a randomized complete block (RCB) design, replicated 4 times. Four (4) seeds were sown per pot and watered once daily in the morning. After germination, seedlings were thinned to 2 per pot and 2 weeks after the soil was inoculated with 10ml of the prepared *Rhizobium* broth.

Seedlings were assessed at 4 weekly intervals until the 16th week, by gently washing soils from the roots and collecting nodules for evaluation. The nodules were assessed for morphology (shape), quantity, quality (activeness), fresh and dry weights as well as senescence index. Morphology was determined per seed source by visual observation and compared with Corby (1981) using magnifying lens on the 10 biggest nodules. Quantity of nodules per source was determined by counting, while the quality was assessed by cutting 10 nodules and scoring for pink/ red colour of the internal parts. Nodules were weighed fresh; using sensitive analytical balance and oven dried for 72 hours at 60°C, allowed to cool and reweighed for biomass production. Collapsed, brown/ black nodules were regarded as dead (Fownes & Anderson, 1991) and used to determine the nodule senescence index (NSI).

Table 2. Variations in nodulation among Nigeria sources of *Millettia thonningii* at 4 months.

Sources	Mean quantity of nodules	NSI (%)	Mean biomass of nodules (g)
Ibadan	32.00ab	14.52a	0.17ab
Ife	20.50b	16.83a	0.08a-c
Iwo	45.38a	15.07a	0.15ab
Jema'a	15.38b	22.58a	0.06bc
Koton-karfe	24.44b	16.76a	0.20a
Lokoja	20.94b	21.23a	0.17ab
Ogbomoso	14.19b	16.45a	0.04c
Zaria	26.19b	15.07a	0.14a-c
Mean	25.02	17.13	0.13
LSD (0.05)	15.67	12.81	0.10

Means with the same letter are not significantly different at ($p \leq 0.05$)
* NSI = Nodule Senescence Index

The data were square root transformed and subjected to statistical analysis: variance, correlation, means ranking and coefficient of variation, using STATVIEW 512+ package of the Apple Computer.

Results and Discussion

Nodulation, evidence of microbial activities, was revealed after 4 weeks of growth in all the seed sources, except among Jema'a population, where evidence was shown only after 8 weeks of growth. After 16 weeks, seeds from Iwo had the highest average quantity of nodules (45.38), while Ogbomoso population had an average of 14 nodules among other populations (Table 2). Nodule production, however, declined after 12 weeks, among Ife, Iwo and Zaria populations. Nodulation was statistically significant between Iwo population and other seed sources except Ibadan population (Table 2). Similarly, dead nodules were recorded after 8 weeks of growth in all the seed sources, excluding Jema'a population where nodules death was recorded at the 12^{th} week of growth. Average quantity of dead nodules increased throughout the study period. Although, Iwo population had the highest average quantity of dead nodules at 4 months (3.2) and Ogbomoso population had the least (1.2) for the same period, these were not statistically significant between the populations (Table 2).

Proportion of dead nodules to total quantity; nodule senescence index (NSI), followed similar pattern as total dead nodules at 4 months. The NSI was not statistically different among the populations; however, Jema'a population had the highest average percentage (22.58%) of dead nodules and Ibadan population had the least (14.52%).

The fresh weight of nodules increased through the study period. The highest (0.99 g) and the least (0.21 g) average nodule fresh weights were recorded from Koton-karfe and Ogbomoso populations respectively. The only statistical differences in nodules fresh weight were obtained between Koton-karfe and Jema'a populations as well as between Koton-karfe and Ogbomoso populations. Variation pattern in biomass production was similar to observations in fresh weight variation.

Table 3. Correlations in nodulation traits among Nigeria source of *Millettia thonningii* at 4 months.

	Quantity of active nodules	**Quantity of dead nodules**	**Fresh weight of nodules**
Quantity of dead nodules	0.3**		
Fresh weight of nodules	0.54**	0.20**	
Dry weight (biomass) of nodules	0.66**	0.17*	090**

**= Significant at p≤0.01 (r=0.18)
* = Significant at p≤0.05 (r = 0.14)
Df = 226

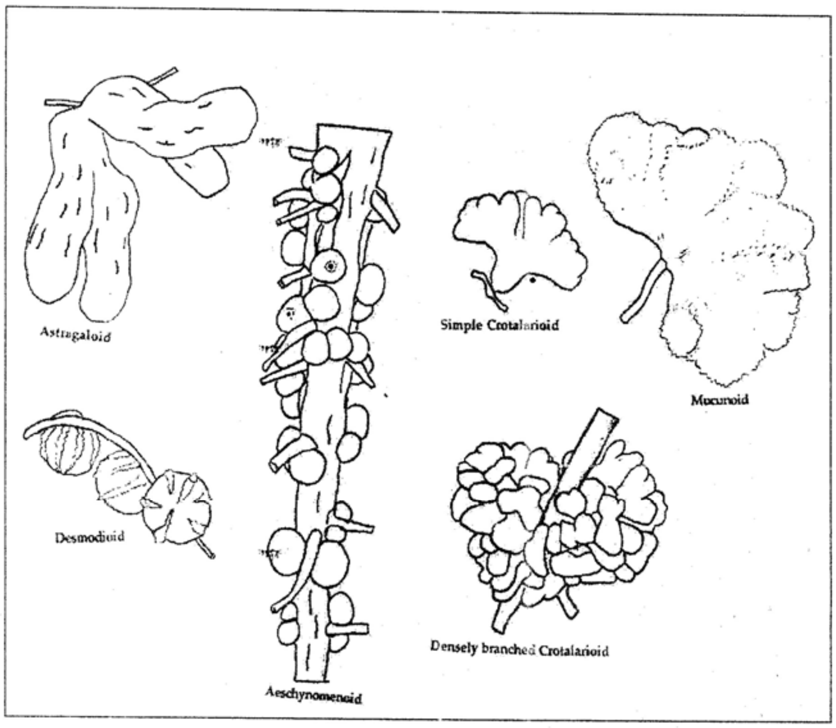

Fig. 1. Nodule shapes and types (Corby, 1981).

Correlation between pairs of total nodule quantity, quantity of dead nodules, fresh weight and biomass were positive and significant at p≤0.01 except dead nodules and biomass that were significant at p≤0.05 (Table 3). The highest correlation (0.9**) was obtained between pairs of fresh weight and biomass, while the least (0.17) was recorded for pairs of dead nodules and biomass (Table 3).

All the populations produced globular shape nodules (Fig. 1) except Ikire-Iwo and Ogbomoso populations, whose nodules were cylindrical. Nodule morphology changed with age; however, the final shape differed only between Ikire-Iwo, Ogbomoso populations and other 6 populations.

The late response of Jema'a population seedlings to native Rhizobia on site outside its source could be genetical and could indicate it as a late nodulator. The prolific nodulating population is from Iwo, this could confer higher biological nitrogen fixing (BNF) ability, while Ogbomoso population could be a low nitrogen fixer due to its poor nodulation ability. The end of biological nitrogen fixation and its subsequent final release into the soil is signified by the collapse and death of nodules. The commencement of this physiological activity 4 weeks after the initiation of nodulation could be an indication that nitrogen release into the soil by *M. thonningii* could begin at 2 month after planting. This increases with age as more dead nodules were recorded. Sniezko & Stewart (1989) found significant difference between seed sources in nodule quantity per seedling among nursery grown *Faidherbia albida.* A very strong and positive correlation between nodule quantity and biomass with total biologically fixed nitrogen has been observed in *Acacia nilotica* (Beniwal *et al*., 1995). Similarly, the highly significant differences between provenances observed in growth and amount of fixed nitrogen in *A. nilotica* were attributed to genetic variabilities among the populations (Beniwal *et al.,* 1995). Iwo population could, therefore, be said to release the highest amount or biologically fixed nitrogen throughout the study period, with consistently high quantity of dead nodules. Despite its late response to the native Rhizobia, the quantity of dead nodule from Jema'a was very similar to what was observed in the population from Iwo.

Variability in nodulation among four species *of Acacia* has been used to conclude the observed differences in the quantity and proportions of nitrogen fixed in the soil (Ndoye *et al.,* 1995). Similarly, Kadiata & Mulongoy (1995) used the abundant nodule biomass obtained in *Albizia lebbeck* has been responsible for the superiority in nitrogen fixed among other tested legumes. Nodule morphology changed with age, however, the final shapes significantly differ between Iwo and Ogbomoso with other populations.

Conclusion

M. thonningii is a very versatile species and can be easily introduced to other areas within the distribution range, with perfect response to available native Rhizobia. Source, however, plays significant role in early establishment and response to native soil microbes. Incorporation of *M. thonningii* into the farming systems would be beneficial to the annual crops, planting of the tree, four weeks before the crops could make available the needed nitrogen to the crops. Germplasm could be obtained from any of the populations without significant differences in benefits. A more range-wide source evaluation is recommended to further understand the soil improvement potentials of the species including isotopic assessment or the actual amount of biologically fixed nitrogen. The period of study should be extended to include field confirmation of the extent of soil improvement.

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