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African Yam Bean (*Sphenostylis stenocarpa*) Nodulates Promiscuously with Rhizobium Indigenous to Soils of Botswana

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Abstract

African yam bean is a versatile legume crop, relished for its protein rich tubers, seeds and leaves which are utilized as spinach. It is a highly adaptable crop capable of producing growth even on acid and highly leached sandy soils. A pot experiment was conducted at Botswana College Agriculture to determine nodulation of African Yam Bean with Rhizobium indigenous to soils of Botswana. Soil collected from two different sites at Botswana College of Agriculture were used with each planted with African Yam Bean and cowpea plants. No fertilizer supplementation was added to the soils. Data was collected after eight weeks of establishment. The parameters measured were the number of nodules, nodule weight and plant weight. The data was analysed using Genstat software Discovery Version. African Yam Bean formed nitrogen fixing nodules under the soils from the two different sites. It compared favorably with cowpea because there were no significant differences in nodulation (nodule number and nodule weight, fresh and dry weight) between the two species. This study has further revealed that African Yam Bean is adaptable to a wide variety of environments and could be successfully grown in Botswana without expensive inputs such as inorganic fertilizers.

Keywords: African yam bean (*Sphenostylis stenocarpa*), nodulation, nitrogen fixation

1. Introduction

African yam bean (AYB) *Sphenostylis stenocarpa,* Horst ex. Rich, belongs to the legume family. It originated in Ethiopia, but both wild and cultivated types now occur in tropical Africa as far north as Egypt and also throughout West Africa from Guinea to southern Africa (Busson, 2001). It is cultivated in Nigeria mainly for seed and also grown for tubers in Coted'Ivoire, Ghana, Togo, Cameroon, Gabon, Democratic Republic of Congo, Ethiopia, parts of east Africa, Malawi and Zimbabwe (Utter, 2007). AYB seeds are high in vitamin C, dietary fiber, vitamin B6, potassium, and manganese; while being low in saturated fat, sodium, and cholesterol (Utter, 2007). The AYB tubers are regarded as an important source of starch and protein in tropical Africa, and the plant is potentially important as a pulse legume (Busson, 2001). The amino acid content of the protein is similar to that of the soybean, though rather higher in histidine and iso-leucine. The energy content of the seeds per 100 g dry matter is 1, 640 Kj (Utter 2007). AYB products generally have a lower glycemic index than other legume products, which means that they will provide a more sustained form of energy (Busson, 2001).

Reclamation and improvement of the fertility of arid lands by application of organic (manure and sewage sludge) and inorganic (synthetic) fertilizers is expensive and could be a source of pollution (Zahran, 1996). The economic and environmental costs of the over use of chemical N-fertilizers in agriculture is a global concern. Sustainability considerations mandate that alternatives to N fertilizers must be urgently sought. Thus, biological nitrogen fixation offers this alternative. The nitrogen-fixing symbiosis makes it possible for the nitrogen that is supplied by the rhizobia to the legumes replace the expensive industrial fixed nitrogen (Castro, Permigiani, Vinocur & Fabra, 1999). The AYB enjoys a symbiosis with bacteria that fix nitrogen from the air (Busson, 2001). An endosymbiont (*Bradyrhizobium* sp. AUEB20) isolated from Ethiopian tree *Erythrina brucei* promiscuously nodulated a number of AYB plants and the nodules were observed to be active in nitrogen fixation (Assefa & Kleiner, 1997). AYB landraces have also been found to form nitrogen fixing nodules within 28 days of inoculation with strains *Rhizobium* sp, ORS 302 (broad host range), and *Bradyrhizobium* sp, CP 279 (broad host range) (Oagile, 2005).

Rotational cropping involving legumes and cereals have been found to be a more sustainable system of increasing food production (Dakora & Keya, 1997). The ability of AYB to form nitrogen fixing nodules with a wide range of rhizobial strains makes it a fine candidate for sustainable development purposes in most cropping systems. The use of African yam bean and other legumes as cover crops increase efficiency of fertilizer utilization and the amount of organic matter for maintenance of high soil productivity. In other words, this is a food source that can support itself while helping both the soils under it and the species that succeed it (Utter, 2007). Although legumes of all categories contribute to nitrogen fertility in various cropping systems, the extent of their nodulation could be constrained by a range of factors amongst which is the environment relating to both the host plant and its microsymbiont (Dakora & Keya, 1997). The low soil moisture content (drought) and high temperatures common in arid zones of the Sahelian and Sub-Saharan Africa are the likely environmental factors that could reduce nodule functioning in symbiotic legumes. Salinity has been identified as one of the threats to growth and activity of nitrogen fixing legumes in arid and semi-arid climates (Zahran, 2001). AYB is a potential crop that needs to be explored and developed because of its biological nitrogen fixation /ability and adaptability to a variety of soils. The work reported here was undertaken to; determine nodulation of AYB with *Rhizobium* indigenous to the soils of Botswana, determine the difference between cowpea and AYB nodulation ability (nodule number and mass), and establish nitrogen fixation potential of AYB nodulated by Rhizobial strains indigenous to Botswana.

This study attempted to establish the potential of AYB as a component of cropping systems in relation to its performance in its common cultivation areas and the prevailing environmental conditions in Botswana.

2. Materials and Methods

2.1 Experimental Site

The experiment was conducted at Botswana College of Agriculture (BCA), Department of Crop Science and Production greenhouse located at Content Farm (24°38'S, 54'N, 994 above seas level) from February to April 2009. The greenhouse conditions were characterised by clear sunny conditions with temperature range of 25-30 °C.

2.2 Experimental Material

Seeds of AYB landrace DP 860108-02 (Collected at Alife-Kese, Bendel State Nigeria by Dr. D. Potter of University of California, Davis) were obtained from Professor Richard Mithen formerly with The University of Nottingham, Sutton Bonington Campus, UK. Cowpea cultivar Tswana was obtained from BCA collection. Soils used were collected from BCA Garden A and BCA Notwane Farm. Both sites where soils were collected have had different crops previously. The soils were chemically analysed for different factors known to a ffect nodulation and nitrogen fixation (Table 1). The two soils are generally characterised as sandy loam.

Table 1. Chemical status of the two soil sites used in the experiment

Soil type	pH	Salinity (ms/cm)	Nitrogen (%)
BCA Notwane Farm	6.59	175.1	6.01×10^{-4}
BCA Garden A	7.35	180.2	7.24×10^{-4}

2.3 Experimental Procedure

Seeds were soaked in a solution of 5 ppm GA3 for five minutes to hasten germination and then sown into 1 litre plastic pots filled with the different soils described above. After emergence AYB plants were staked to provide support. Watering was done on ad-lib basis until termination of experiment. Occasional examination for insects/pest was carried out to check for any infestations.

2.4 Experimental Design and Data Collection

The experiment was setup as a complete randomized design with a total of six pots per soil. Data was collected after seven weeks of plant growth and a random sample of four plants per species for each soil was used for measurements. Parameters measured were nodule number per plant, nodule weight (fresh and dry weight) and plant weight (fresh and dry weight). Dry weights were obtained by oven drying samples at 65 °C for 48 hours. Nodule colour was also noted to establish nitrogen fixation activity.

2.5 Data Analysis

Data was subjected to analysis of variance (ANOVA) using Genstat Software Discovery Version Release 7.2. Comparison of means was performed at 5 % level of significance (Lawes Agricultural Trust, Rothamsted, UK).

3. Results

3.1 Nodulation and Nitrogen Fixation

AYB nodulates profusely with some Rhizobial strains indigenous to soils of Botswana. Soil collected from BCA Notwane Farm caused the greatest number (50.5 and 43.8 per plant) of nodules in both Cowpea and AYB, respectively (Table 2). BCA Garden A soil caused the formation of heavier nodules (Cowpea, 2.28 g and AYB, 2.86 g/ plant) compared to BCA Notwane soil (Table 2). There was no significance difference in number of nodules between species. However, soil collected from BCA Notwane Farm caused a significantly ($p<0.001$) higher number of nodules than that collected from BCA Garden A. Cowpea formed more nodules (50.5) than AYB (43.8) under soil collected from BCA Notwane Farm while the two legume species formed the same number of nodules under the soil collected from BCA Garden A. Contrary to observations made on number of nodules, soil collected from BCA Garden A produced significantly ($p<0.001$) heavier nodules than soil collected from BCA Notwane Farm in both legume species (Table 2). AYB nodules were heavier than cowpea nodules under both soil types tested although the difference was not significant.

The nodules produced by all the species under the two soils were pinkish indicative of nitrogen fixing activity. The plants did not show any nitrogen deficiency because they were green and healthy.

Table 2. Nodulation of African Yam Bean (AYB) and Cowpea with rhizobial strains indigenous to soil of Botswana

Factors	Nodule Number/Plant		Nodule Fresh weight (g/ Plant)		Nodule Dry weight (g/ Plant	
	Cowpea	AYB	Cowpea	AYB	Cowpea	AYB
Soil						
BCA Notwane Farm	50.5	43.8	1.06	1.12	0.174	0.204
BCA Garden A	21.5	21.5	2.28	2.86	0.376	0.484
s.e.d	8.08		0.384		0.0610	
d.f.	4		4		4	
Significance (0.05)						
Species	0.538		0.254		0.133	
Soil	<0.001		<0.001		<0.001	
Species x Soil	0.594		0.363		0.388	

Table 3. Growth of nodulated African Yam Bean (AYB) and Cowpea plants grown soils of Botswana

Factors	Total Plant Fresh weight (g)		Total Plant Dry weight (g)	
	Cowpea	AYB	Cowpea	AYB
Soil				
BCA Notwane Farm	27.8	17.8	5.25	4.10
BCA Garden A	44.0	40.4	8.05	8.05
s.e.d	5.31		1.114	
d.f.	4		4	
Significance (0.05)				
Species	0.094		0.323	
Soil	<0.001		<0.002	
Species x Soil	0.414		0.667	

3.2 Plant Growth

The two soils tested differed in support of plant growth where plants on BCA Garden A soil grew significantly

[fresh weight ($p<0.001$) and dry weight ($p<0.01$)] better than plants grown on BCA Notwane Farm soil (Table 3). BCA Garden A gave the highest total plant fresh weight (Cowpea, 44.0 g and AYB, 40.4 g/ plant) and plant dry weight (Cowpea, 8.05 g and AYB, 8.05 g/plant) compared to BCA Notwane Farm plant fresh weight (Cowpea, 27.8 g and AYB, 17.8 g/ plant) and dry weight (cowpea, 5.25 g and AYB, 4.10 g/ plant).

4. Discussion

Results from this experiment confirm AYB to be a promiscuous legume with ability to adapt to varying soil environments since it was able to form nitrogen fixing nodules with Rhizobial strains indigenous to Botswana soils. AYB has the ability to match indigenous legumes such as cowpea in terms of nodulation and plant growth clearly indicate its potential as a component of local cropping systems. In intercropping trials conducted in the sandy soils of the Benue River Basins of Nigeria to assess the effect of some food legumes used as cover crops in cassava, yam, and maize based cropping systems, Obiagwu (1995b) observed that AYB formed nodules and contributed to soil productivity and yield of the main crops without any inoculation, thus corroborating the above suggestion. The ability of AYB to nodulate profusely with *Rhizobium* indigenous to the soils of Botswana confirms observations made by Oagile (2005) where AYB was shown to be a promiscuous legume as it was able to form nitrogen fixing nodules with three of the five strains tested and classified as both *Rhizobium* spp. and *Bradyrhizobium* spp. The large numbers of nodules formed on AYB landraces by some of the strains tested by Oagile (2005) were also found to be comparable to that recorded in cowpea inoculated with Bradyrhizobium indigenous to Ghanaian soils recorded by Obiagwu (1995b). In the work reported here, AYB formed even heavier nodules than cowpea which might suggest even a larger surface area for nitrogen fixation.

The high salinity, pH and nitrogen levels recorded in soil collected from BCA Garden A, adversely affected nodulation relative to the soil collected from BCA Notwane Farm. This is in agreement with observations by Giller (2001), that production of grain legumes is severely reduced in salt-affected soils because of their ability to form and maintain nitrogen-fixing nodules is impaired by both salinity and sodality (alkalinity). Extremes of pH also affect nodulation by reducing the colonization of soil and the legume rhizosphere by Rhizobia. In highly acidic soils ($pH<4.0$) nodulation is most affected than the host-plant growth and nitrogen fixation. Highly alkaline soils ($pH>8.0$) tend to be high in sodium chloride, and are often associated with high salinity which reduce nitrogen fixation (Bordeleau & Prévost, 2000). Binkely et al. (1994) also reported that high concentration of nitrate inhibit the development of root nodules as is also observed in this experiment. However, AYB as a highly adaptable plant was able to form nitrogen fixing nodules that could sustain its growth without addition of any nitrogen fertilizer other than that found in the soil.

5. Conclusion

African yam bean nodulates with Rhizobial strains indigenous to soils of Botswana.

AYB nodulates comparatively to cowpea with Rhizobial strains indigenous to soils of Botswana

AYB produces nodules that fix nitrogen and hence less nitrogen fertilizer application will be needed to grow this crop.

5.1 Recommendations

It is recommended that:

The study is replicated in various areas with different soil types to test the validity of these results.

Allow AYB to grow to maturity to observe the effect of nodulation on seed yield.

The AYB nodulating Rhizobia should be isolated and characterised for identification.

Farmers should be encouraged to include African Yam Bean in their cropping systems to increase the level of nitrogen in the soil and improve crop productivity.

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