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ASSESSMENTS OF SYMBIOTIC COMPATIBILITY OF COWPEA (*Vigna unguiculata* L. WALP) VARIETIES WITH INDIGENOUS RHIZOBIA WITHIN SOILS IN KANO STATE, NIGERIA

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ABSTRACT

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Soil properties and processes which simultaneously maintain native fertility and sustain plant yields are of principal interest in sustainable agriculture. Smallholder farmers in Sub-Saharan Africa appreciate the significance of cowpea (*Vigna unguiculata* L. Walp) as nutritional, economic, as well as, soil fertility enhancer through nitrogen fixation with rhizobia. Growth and natural nodulation by indigenous rhizobia of four improved varieties of cowpea (IT90K 277-2, IT99K 573-1-1, IT98K 205-8, at Ungogo: 11.98° 72' N; 8.43° 22' E), and *Kwankwasiyya* (at Kabo: 11.98° 82' N; 8.35° 96' E), both sites in Kano State, Nigeria were assessed during the 2015 rainfed season. Soil characteristics were analysed to determine the fertility statuses of the study farms. The two farms had sandy loam soils with the same potassium (Exc K) content but slightly varied in total percentages of nitrogen, and carbon with moderate phosphorus contents. ANOVA tests for the effect of varieties on height, fresh weight and nodulation at vegetative, as well as, flowering and podding stages indicated mostly insignificant differences ($P > 0.05$). Significant variety effect ($P < 0.05$) were found in weight and nodule numbers at vegetative stage and in nodule numbers at the flowering stage. Hierarchical cluster analysis of all the parameters indicated that the three varieties cultivated in the experimental farm shared phenotypic similarities with each other, more than with the variety cultivated by the smallholder. All four varieties proved to be good fodder crops as well as compatible symbionts of indigenous rhizobia. This work recommends strategic enlightenment that makes farmers cognisant of the presence of beneficial soil microorganisms that would enhance legume cultivation by smallholders.

INTRODUCTION

The soil nutrient losses in Sub-Saharan Africa (SSA) are environmental, social, and political threats to the future viability of African food systems because these soils have been described as inherently limited in nutrient contents and low in fertility (Ker, 1995), with

rapidly declining supply of nitrogen once cropping commences (Sanginga, 2003). Soil fertility replenishment in SSA is increasingly viewed as critical to the process of poverty alleviation, increased agricultural productivity and improved rural livelihoods (Place *et al.*, 2003).

During the 2014 rainy season, oral and written surveys were conducted to assess smallholders' perspectives on the challenges and prospects on food production in Kano State by farmers indicated that loss of soil fertility is a major challenge among others (Rabiu, 2015). Cowpea (*Vigna unguiculata* [L. Walp]), a legume belonging to the family Fabaceae is a major component of traditional cropping systems in the drier parts of the tropics because of its multiple uses, which include improving soil fertility through biological nitrogen fixation (BNF) with rhizobia, suppression of weeds, and a key source of proteins (Kimitri and Odee, 2010). The legume *Rhizobium* symbiosis is especially important in agriculture; historically, it was used to replace much of the protein nitrogen removed in crop harvests (Robertson and Vitousek, 2009). Crop legumes fix about 100 kg N/ha on average (GRDC, 2014) thereby significantly reducing artificial fertiliser cost. Cowpea is a famous crop with rural farming communities in northern Nigeria, in West Africa, cowpea is second in importance after groundnut, with Nigeria accounting for over 70% of the total world production (Singh et al., 2002). The aim of this research was to assess the growth and the ability of some improved varieties of cowpea to be nodulated by indigenous rhizobia and to determine the physical and chemical characteristics of soils of the study area.

METHODOLOGY

Description of Study Area

The study site used for the cultivation of the test varieties was the experimental fields of the Faculty of Agriculture, Bayero University Kano, Nigeria located at 11.98° 72' N; 8.43° 22' E. The area is used for rain fed agriculture only. There was no history of three previous years of legume cropping and rhizobial inoculation in the area. A smallholder farm was in Kabo Local Government of Kano state located at 11.98° 82' N; 8.35° 96' E which was sampled for comparison. This farm is also used for rain fed agriculture only and cropped mainly with sorghum for the past few years with no history of rhizobial inoculation in the area.

Description of Test Varieties

The seeds of test varieties were improved cultivars of cowpea, and included, IT90K 277-2, IT99K 573-1-1 and IT98K 205-8 obtained from International Institute for Tropical Agriculture, Kano Nigeria. IT98K-205-8 is an early maturing and determinate variety which matures in 60 days; IT99K-573-1-1 and IT90K-277-2 are medium maturing and semi-determinate varieties which mature within 75–80 days. T98K-205-8 and IT99K-573-1-1 were identified as multiple *Striga* race resistant line with beneficial agronomic traits, such as larger seed size, white seed colour, high-grain yield and disease resistance. The fourth variety sampled from the smallholder farm was also an improved variety referred to as Kwankwasiyya (white medium sized with black eye).

Soil Sampling

Soil sample collections in the study areas were conducted as described by Abaidoo et al., (2007). Four replicate quadrant plots (10 m²) were determined, with six soil cores per quadrant randomly removed using a 6 cm diameter corer, to a depth of 20 cm. The six soil core samples were combined into a composite sample (replicate), making four composites per farm; air-dried on an open bench in the laboratory for 72 h before physical and chemical analyses.

Determination of Physical and Chemical Characteristics of Soils

Textural class of the soils was determined using the hydrometer method (Bouyoucos, 1951). The pH was determined according to IITA (1979). Twenty grams of soil were added to 20 ml of sterile distilled water and the pH was measured. Determination of electrical conductivity of the soil samples carried out as described by Hanna (1964) and Rhoades, (1982), and electrical conductivity of the supernatant measured using EC Meter. Percentage moisture content of the soils from the study sites was determined (ITTA, 1982). The procedure of Walkley and Black (1934) was used to determine the organic carbon (OC) content. Phosphorous and Potassium (K) contents were measured using the Bray-I method (ICARDA, 2013). Determination of total nitrogen was by Kjeldahl method as described by IITA (1982).

Experimental Design and Agronomical Practice

The land was cleared and harrowed twice to obtain a fine tilt. Weeds were removed by hand prior to planting. A discard of 2 m was allowed for edge effect on all edges. Cowpea like any other grain crop in northern Nigeria is grown on ridges spaced 75 cm apart because the implements (tractor, in this case) used for ridging are made for ridges spaced 75 cm apart (Kamara *et al.*, 2016). The varieties were planted in complete randomized design. Agronomic practices were according to Chude *et al.*, (2012) and Omoigui *et al.*, (2012) as follows. Fertilizer was applied at planting at the rate of 100 kg ha⁻¹ of NPK compound fertilizer, to give the equivalent of 15 kg ha⁻¹ N, P₂O₅ and K₂O, respectively. Planting was at 25 – 30 kg/ha seed rate with 30cm between plants. Three seeds of each variety were sown and were later thinned to two plants at 2 weeks after emergence Weeding was done by hand. Insect pest control was by the application of 50 g a.i. Cymbush 10EC (2.0% Cypermethrin ULV at 2.5 litres ha⁻¹ fortnightly from four weeks after sowing (WAS) by using a knapsack sprayer. *Kwankwasiyya* was sole cropped in the smallholder's farm, the farmer did not indicate any fertilizer or herbicide application. Weeding was also done using hand tools by the farmer.

Data Collection

The above ground growth (height and fresh weight) was determined according to the protocols of Kiari *et al.*, (2011) and Ojiem *et al.*, (2007) by destructive sampling of plants in a

0.5 m by 0.5 m quadrant in 4 randomly selected positions within the farm, excluding the border rows. The assessments were carried out at fully vegetative stage (3 WAS), flowering stage, when 50% were flowering (7 WAS) and podding stage, when 50% had mature pods (9 WAS). The plants were immediately weighed using top table scale to determine fresh weight. Height above ground was measured using a meter rule. Nodulation was determined as described by Somasegaran and Hoben (1985) by excavation of the whole root system around a radius of about 20 cm and a depth of up to 50 cm using a sturdy spade, and taking care not to detach the nodules in the process. After excavation, the soil was gently rinsed off in buckets of clean water, and the number of nodules counted.

RESULTS

Soil Analysis

Results of the soil analysis showed similarities and variations among the sites. Classification of fertility status based on physical and chemical analyses of the soil samples are shown in Table I. Both soils were sandy loam. Site A had neutral (pH) while site B had slightly acidic pH (H₂O) readings respectively. Total nitrogen content in the experimental farm was classified as very low and moderately low in the smallholder farm. Both farms were classified as moderate in phosphorus content. Potassium was high in the experimental farm but moderate in the smallholder farm. Organic carbon was very low and low in the experimental and the smallholder farms respectively.

Table I: Soil Characteristics and Fertility Status from Experimental and Smallholder Farms.

Parameter	Farm	Value	Status	Chude <i>et al.</i> , 2012
pH (water)	Experimental	6.80		N
	Smallholder	6.50		SA
Total Nitrogen (%)	Experimental	0.04		VL
	Smallholder	0.11		ML
Phosphorus mg Kg-1	Experimental	9.25		M
	Smallholder	8.93		M
Potassium (Exc. K)	Experimental	0.69		H
	Smallholder	0.34		M
Organic Carbon (%)	Experimental	0.18		VL
	Smallholder	0.51		L
Electrical Conductivity (ds/m)	Experimental	0.15		
	Smallholder	0.19		
Moisture Content (%)	Experimental	0.81		
	Smallholder	0.88		
Textural Class	Experimental	sandy loam		
	Smallholder	sandy loam		

H-High MH-Moderately High M-Moderate ML-Moderately Low L- Low VL-Very Low N-Neutral SA-Slightly Acidic MA-Moderately Acidic

Growth and Nodulation

Subsequent to one way analysis of variance (ANOVA) for height, weight and nodule numbers of the four varieties at the three

sampling periods, vegetative, flowering and podding stages, comparisons of means by Tukey method are shown in Table 2.

Table 2: Tukey Method Mean Comparisons for Height, Weight and Nodule Numbers Of Cowpea Varieties At Vegetative, Flowering And Podding Stages.

Varieties	Vegetative Stage (3 WAS)			Flowering Stage (7 WAS)			Podding Stage (9WAS)		
	Ht (cm)	Wt (g)	NN	Ht (cm)	Wt (g)	NN	Ht (cm)	Wt (g)	NN
IT90K 277-2	28.10a	89.10a	22.0ab	83.23a	207.18a	32.75ab	154.13a	469.10a	6a
IT99K 573-1-1	29.25a	85.25ab	9.8b	121a	253.80a	13.75b	173a	385.80a	5.5a
IT98K 205-8	24.55a	81.73bc	10.8b	69.63a	231.50a	13.75b	84.38a	337.68a	3a
Kwankwasiyya	26.38a	77.88c	28.0a	69.75a	309.93a	51.75a	130.25a	412.05a	10.5a

Values not sharing the same letter are significantly different.

WAS=Weeks after Sowing; Ht=Height; FWt=Fresh Weight; NN=Nodule numbers

At three weeks after sowing (WAS) all the cowpea varieties were in full vegetative stages. At this stage, all the varieties had good fodder and were well nodulated with no indication of nodule senescence. There were no significant differences in mean height of the four varieties. Mean fresh weight for IT90K 277-2 (89.10) was highest but not significantly different from that of IT99K 573-1-1 (85.25) while IT98K 205-8 (81.73) had the least mean fresh weight which was not significantly different from Kwankwasiyya

(77.88). The Kwankwasiyya variety had the highest value for mean number of nodules (28.0), and was significantly different from those of the three test varieties at the vegetative stage. In the tested cowpea varieties, the nodules were formed throughout the length of the main and lateral roots, the cowpea nodule answers the description of a desmodoid nodule having obvious lenticels and standing out roundly on its parent plant root as shown in figure 1.



Figure 1: Root Nodules on IT98K 205-8 at Vegetative Stage (3 WAS)

During the second sampling (7 WAS), at least 50% of all four varieties were flowering, IT98K 205-8 had tender pods already. The mean

values for height and fresh weight were more than doubled in all the varieties from the first sampling at 3 WAS.

However, there were no significant differences in mean values for height and weight in all the four varieties. The Kwankwasiyya variety had the highest mean value for nodule numbers (51.75) which was significantly different from those of IT99K 573-1-1(13.75) and IT98K 205-8 (13.75). IT90K 277-2 had 32.75 mean value for nodule number which was not significantly different from those of the other three cultivars. During this sampling, few senescing nodules (hollow, disintegrating and gray) were observed in IT98K 205-8.

At 9 WAS, least 50% of all four varieties had pods, with the pods of IT98K 205-8 fully filled and about ready for harvest. There was an

increase in mean plant heights, though not as much as what was observed between the first two samplings. The increase in fresh weight was more pronounced than that of height. There were no significant differences in mean values for height, fresh weight and nodule numbers in all the four varieties. There was marked reduction in number of nodules in all the varieties, with many more senescent ones were observed in all the varieties. Hierarchical analysis of all the data on the varieties in this study implies that the four varieties have distinct phenotypic variations in the three parameters (height, weight and nodule numbers) as shown in the constellation plot (figure 2).

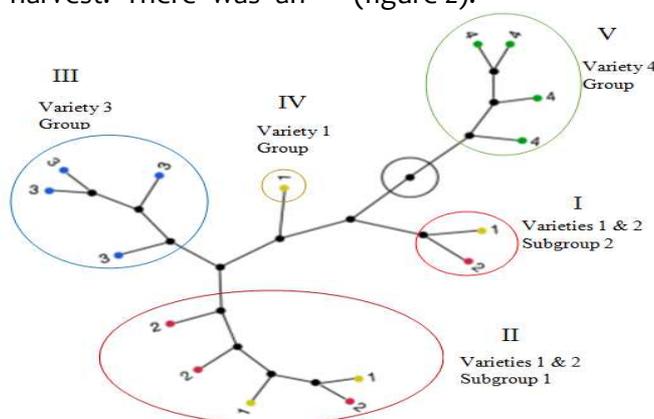


Figure 2: Phenotypic Variations (Height, Weight, and Nodule Numbers) in cultivated Cowpea Varieties

Phenotypically, varieties 1(IT90K 277-2), 2 (IT99K 573-1-1) and 3 (IT98K 205-8) showed greater similarities than with variety 4 (Kwankwasiyya) as displayed with the clustering of the three varieties on the left side of the constellation plot (I-IV). There were more similarities between IT90K 277-2 and IT99K 573-1-1 as the two varieties were clustered together in I and II. Variety 4 (Kwankwasiyya) occupied the right side (V) signifying that it varies more with the other three than they did with each other.

DISCUSSION

The results of the soil analyses indicated that cowpea thrive very well in sandy loam soils. Soil pH (water) were found to be slightly acidic, similar to Mawo et al. (2011) but in contrast to Abaidoo et al., (2007) whose 12 sites were acidic to moderately acidic (4.4 to 6), and Kapembwa et al., (2016) who had 4.8

to 6.0, but both supported legume crop in Africa. The organic Carbon contents of the study sites were from very low and low, this is similar to Abaidoo et al., (2007) and Kapembwa et al., (2016) but different to Mawo et al. (2011) who evaluated the growth of cowpea varieties in Kano including IT90K 277-2 and IT99K 573-1-1. Nodulation, a function that is a precursor for nitrogen fixation did not seem impaired by very low and moderately low nitrogen soil contents as reported also by Mawo et al. (2011) and Kapembwa et al., (2016) where cowpea was cultivated with nitrogen contents 67 % lower than critical value. According to classification of soil fertility status by Chude et al., (2012), phosphorus contents of both soils was moderate which was in contrast to Mawo et al. (2016) that recorded high levels of the element in their study area.

The results of the growth assessments of all the four sampled cowpea varieties indicated that the tested legumes are good fodder crops. Higher above ground values for height and fresh weight of the medium maturing varieties (IT90K 277-2 and IT99K 573-1-1) compared to the early maturing IT98K 205-8 was similar to the results of Kamara *et al.* (2016) who evaluated both IT99K 573-1-1 and IT98K 205-8 in Kano and Zaria. Kiari *et al.* (2011) also found that IT98K 205-8 grown in Sahelian sandy soil of Niger Republic, to the north of my sites, had less fodder yield compared to Ghana-1, a local cowpea variety. The differences in the above ground parameters (height and fresh weight) of the varieties were probably due to their genotypic constitutions (Mawo *et al.*, 2016).

All the four cowpea varieties were always found nodulated where they were found growing. This means that the soil in the two farms contained sufficient numbers of indigenous rhizobia to effectively colonize the cowpea roots. The survival of rhizobia in soil in the absence of its host plant depends on soil pH, temperature, moisture, clay content, and the specific characteristics of the rhizobial strain (Farming Ahead, 2009). The nodulation result is similar to that of Abaidoo *et al.*, (2007) who researched population size, distribution and symbiotic characteristics of indigenous *Bradyrhizobium* spp. that nodulate soybean in nine African countries, including Nigeria. It was observed that the pre-flowering period was the time of vigorous nodulation, this could be due to the more intensive need of nitrogen by the growing plants at that stage. The peak number of nodules was also reached at flowering stage. The gradual decrease in the number of nodules as the plants showed signs of senescence obviously emphasized that

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nitrogen, the much needed mineral for plant growth (Cooke, 1976; Fowden, 1979) is more useful to plants during the active phases of life cycle.

The nodulation observed in this study also agrees with several legume-rhizobial studies conducted in Africa such as in Ethiopia (Hamza and Alebejo; 2017; Aserse *et al.*, 2013); in Niger Republic (Kiari *et al.*, 2011); in Kenya (Mwenda, *et al.*, 2011) and in Morocco (El-Akhal *et al.*, 2008), all of which indicated the prevalence of indigenous rhizobia in their respective study locations. Cropping history did not seem to be factor in the nodulation of varieties, since the smallholders farm usually cultivated with cereals yielded vigorous nodulation with the *Kwankwasiyya* variety.

CONCLUSION

Soils of smallholder farms are endowed with indigenous rhizobia possessing symbiotic compatibility with improved cowpea varieties that culminate in the formation of root nodules. Therefore, the cultivation of these varieties is a viable approach towards the establishment of sustainable agricultural systems through nitrogen fixation in the sub Saharan Africa.

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