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DOI: <http://dx.doi.org/10.4314/acsj.v24i1.4>



SCREENING SOYBEAN GENOTYPES FOR PROMISCUOUS SYMBIOTIC ASSOCIATION WITH *Bradyrhizobium* STRAINS

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(Received 30 November, 2015; accepted 25 February, 2016)

ABSTRACT

The current low soybean (*Glycine max* L. Merrill) yields in Sub-Saharan Africa can be alleviated by developing promiscuous genotypes. The research trend in Africa is towards developing promiscuous varieties for less labour and high yields in soybean production. A greenhouse experiment was conducted at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) with the aim of screening of soybean germplasm for promiscuous symbiotic association with *Bradyrhizobium sp.* in order to identify genotypes with potential to be used as parents to initiate a breeding programme focusing on promiscuous nodulation. The response of 65 soybean genotypes from different origins to cowpea-type inoculant, *Bradyrhizobium sp.* strain USDA 3456 were evaluated. Soybean seeds inoculated with both *Bradyrhizobium sp.* strain USDA 3456 and *Bradyrhizobium japonicum* strain USDA 110 were planted in buckets filled with steam-sterilised soil. Significant differences in the number of nodules, effective nodules, fresh and dry weight of nodules were observed among genotypes ($P < 0.001$). Twelve of the 65 genotypes were highly responsive to *Bradyrhizobium sp.*, with over 10 nodules per plant, over 50 mg dry weight of nodules, and 50% effective nodules. A highly positive correlation was shown between dry weight of nodules and nodule number. This study proposes the soybean genotypes NamII, WonderSoya, Bulindi 48C, NamSoy 4M, MakSoy 3N, NamSoy 3, K-Local, Kabanyolo 1, UG 5, Soprano, MakSoy 2N, and MakSoy 5N as potential parental materials for subsequent breeding work.

Key Words: *Glycine max*, nodules, promiscuous, Uganda

RESUME

La sélection de variétés de soja à nodulation facile peut contribuer grandement à l'amélioration des rendements de production en Afrique sub-Saharienne. Les variétés de soja à nodulation facile rendent superflus l'utilisation d'engrais azotés, l'application d'inoculum aux fins de fixation biologique d'azote, améliorent le rendement de soja, ainsi que la qualité du sol pour les cultures subséquentes. Une expérimentation en serre a été conduite à l'Institut de Recherche Agricole de l'Université de Makerere à Kabanyolo (MUARIK). L'objectif était d'évaluer l'habileté des accessions de soja à réaliser la symbiose avec *Bradyrhizobium sp.* En vue d'identifier les cultivars qui peuvent constituer de potentiels parents à utiliser pour initier un programme d'amélioration génétique du soja visant la nodulation facile. 65 accessions d'origines diverses ont été évaluées pour leur réaction en présence de rhizobium de type niébé (*Bradyrhizobium sp.* souche USDA 3456). Les graines de soja inocuées avec le rhizobium de type niébé (*Bradyrhizobium sp.* souche USDA 3456) ou de type soja (*Bradyrhizobium japonicum* souche USDA 110) ont été plantées dans des pots en plastique soigneusement remplis de terre arable stérilisée à base de vapeur surchauffée. Des différences significatives ont été observées entre accessions, au niveau des nombres de nodules, pourcentages de nodules actives, poids frais et secs des nodules ($P < 0.001$). Douze accessions ont notablement réagi face à *Bradyrhizobium sp.* avec chacune plus de 10 nodules par plante, 50 mg comme poids sec

de nodules, et 50% de nodules actives. Une corrélation positivement très significative a été observée entre poids sec et nombre de nodules. Cette étude propose les accessions NamII, WonderSoya, Bulindi 48C, NamSoy 4M, MakSoy 3N, NamSoy 3, K-Local, Kabanyolo 1, UG 5, Soprano, MakSoy 2N, and MakSoy 5N comme potentiels parents à utiliser dans un programme ultérieur d'amélioration génétique.

Mots Clés: *Glycine max*, nodules, nodulation facile, Uganda

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is an important nodulating legume crop, with potential for expansion in Africa. Globally, soybean accounts for about 84.5% of the grain legumes trade (Abate *et al.*, 2011). Statistics from FAOSTAT (2013), showed low soybean productivity in Africa, at 2.2 million metric tonnes from 1.8 million ha. The average soybean yield in Africa is 1,254 kg ha⁻¹, which is 50% of the global average at 2,475 kg ha⁻¹ in the world (FAOSTAT, 2013). This may be attributed to the use of low yielding varieties, low soil fertility, pests and diseases among other factors.

Soybean is mainly used as a source of protein in human food and animal feeds. The protein content in soybean is approximately 40% (Tefera, 2011). The crop requires high doses of nitrogen, mostly at pod filling stage (Machido *et al.*, 2011) in order to construct a high concentration of proteins in the grain. Soybean requires approximately 80 kg of nitrogen for each metric tonne of grain yield. Salvagotti *et al.* (2008) noted that soybean crops that yield 5 t ha⁻¹, take up about 400 kg of nitrogen, with average of 58% coming from N₂ fixation.

As a legume, soybean has the capacity to fix a large quantity of atmospheric N₂, in symbiotic association with *Bradyrhizobia* (Anuar *et al.*, 1995; Peoples *et al.*, 1995). Summary statistics for controlled experiments reported by Salvagotti *et al.* (2008), showed that soybean can fix up to 337 kg of nitrogen per hectare when inoculated with effective strains of *Bradyrhizobium*. Many soybean varieties are highly specific, such that most cultivars nodulate only with *Bradyrhizobium japonicum* for biological nitrogen fixation (Sanginga *et al.*, 1997).

Bradyrhizobium japonicum populations required for effective nodulation of soybeans are

not endemic to African soils (Hadley and Hymowitz, 1973). For this reason, artificial Biological Nitrogen Fixation (BNF), using introduced *Bradyrhizobium* strains has been the practice to improve Africa's soybean production, though with several limitations. Some of the factors that lead to failure in maximising nodulation potential of soybean include unfavourable storage conditions (Keyser *et al.*, 1993), disparity between cultivars (Machido *et al.*, 2011) and competition between indigenous and introduced strains (Ge and Xu, 1982). Other factors include phosphorus deficiency that is widespread in Sub-Saharan African soils (Sanginga *et al.*, 1995; Sanchez *et al.*, 1997), non-availability of commercial *B. japonicum* inoculants (Tefera, 2011), lack of effective transportation and distribution systems of inoculants, and lack of programmes to teach farmers how to use BNF (Kueneman *et al.*, 1984). To circumvent this situation, Scientists at the International Institute of Tropical Agriculture (IITA) developed promiscuous soybean cultivars that are able to nodulate with indigenous and readily available *Bradyrhizobium* strains. They have released soybean lines which are promiscuous in the sense that these lines form nodules with indigenous bacteria, presumably strains of *Bradyrhizobium* spp. which form symbiotic nodules with cowpea (IITA, 1996). Relatedly, Abaidoo *et al.* (2000) detected *Bradyrhizobium* spp. populations in approximately 74% of the African soils.

This study aimed at screening soybean lines for their ability to effectively nodulate with *Bradyrhizobium* spp., thus having potential for use as parental lines to initiate a breeding programme for the development of improved promiscuous soybean cultivars for higher yields in Africa.

MATERIALS AND METHODS

Planting material and design. Sixty five soybean genotypes from different origins (IITA (3), Zimbabwe (8), AVRDC (20), USA (7), Uganda (25) and others (2) were evaluated in this study in a greenhouse at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK). Each of the 65 genotypes was inoculated with (i) cowpea-type inoculant (*Bradyrhizobium* sp. strain USDA 3456), (ii) soybean-type inoculant (*Bradyrhizobium Japonicum* strain; USDA 110) and iii) a control without inoculant, and grown on steam-sterilised normal soil in a completely randomised design with three replicates.

Soil media preparation. Top soil used in this study was collected from a cultivated field at MUARIK. Soil sub-samples were analysed for pH, organic carbon, total nitrogen, Bray 1 extractable phosphorus and exchangeable bases (K^+ , Na^+ , Mg^{2+} and Ca^{3+}) using procedures described in Okalebo *et al.* (2002). Soil analysis was done to check for suitability in terms of poor to moderate nitrogen content (<0.2%) (Table 1).

The soil was then steam-sterilised at 120 °C for six hours, under direct hot steam, and left to cool over 24 hours. Steam-sterilisation was done using a drum containing water in its lower compartment. The water was boiled and produced vapour, which passed through the soil in the top chamber in order to kill microorganisms. The sterilised soil was used to fill 585 buckets, each of five kilogrammes capacity (20.0 cm diameter and 20.5 cm depth) that were lightly perforated at the bottom to aerate and avoid water lodging. The soil was pre-mixed with 0.0356 and 0.036 g of TSP (Triple Super Phosphate) and Muriate of Potash (MOP), respectively per kg of soil, representing 20 kg of phosphorus and 40 kg of potassium per hectare. Sufficient P and K are essential for biological nitrogen fixation processes in earlier studies (Giller *et al.*, 1997), and these nutrients were deficient in the study soil (Table 1).

Preparation of inoculum. Rhizobia inoculants were obtained from Biofix (Kenya), purified and incubated in Soil Science Biological Nitrogen Fixation (BNF) laboratory at Makerere University.

TABLE 1. Laboratory results of soil samples analysis

Samples	pH (H ₂ O)	SOM (%)	Total N (%)	Bray 1 extractable P (mg kg ⁻¹)	K ⁺ (cmol kg ⁻¹)	Na ⁺ (cmol kg ⁻¹)	Ca ⁺ (cmol kg ⁻¹)
Sample 1	5.32	2.80	0.22	7.95	5.44	0.33	5.25
Sample 2	5.53	1.54	0.13	4.70	4.42	0.35	5.50
Sample 3	5.55	2.30	0.22	7.41	5.44	0.43	4.50
Critical values	5.5-6.5	3	0.2	15	.	.	.

SOM = soil organic matter

The strains, *Bradyrhizobium japonicum* strain USDA 110 (soybean-type) and *Bradyrhizobium sp.* strain USDA 3456 (cowpea-type), were grown to 7.91×10^9 cells g^{-1} for cowpea-type rhizobium and 9.08×10^9 cells g^{-1} for soybean-type rhizobium. These were formulated into an inoculum carried in steam-sterilised peat soil. Two table spoonfuls of sugar were dissolved into 300 ml of clean luke-warm water, in a soda bottle, to be used as sticker. The inoculant was mixed with the sticker and directly applied on seeds to enhance association of plant-rhizobium.

Planting, data collection. About ten seeds were sown in each bucket and thinned to 3 plants after germination. Planting was first done for non-inoculated seeds to avoid contamination. Six weeks after germination, buckets were soaked in water and carefully inverted to release the aggregate plants-soil medium. The aggregate was carefully washed abundantly with water to separate the soybean plants from the soil medium, without breaking the roots, or loosing nodules. Roots were then rinsed and wrapped in tissue paper to reduce wetness. With reference to Shiraiwa *et al.* (1994), who reported positive correlation between nodule dry weight (NDW) and N_2 fixation in soybean, all nodules were harvested and counted to determine the number of nodules (NN), and later weighed to determine the fresh weight of nodules per plant (NFW) using high precision digital scale ADAM Pow 453e (Max 450 g, d = 0.001 g). Thereafter, all the nodules were split, open to assess effectiveness and percentages of effective nodules (NE) were generated per plant, based on the presence of pink pigmentation inside nodules, an indicator of effectiveness. Thereafter, nodules were oven dried at 65 °C for four days (Gwata *et al.* 2004), and weighed to determine the total nodules dry weigh (NDW) per plant.

Data analysis. A Linear Mixed Model (LMM) was used to analyse data. A mixed model approach was used in this study so as to allow the modeling of different variance-covariance structure that could not be handled using the traditional analysis of variance. Instead of

assuming that error variance was constant, we allowed each genotype and Rhizobia level to have a different variance. Our model (see below) was fitted using GenStat 14th edition (VSN International Ltd, Hemel Hempstead, UK) (Payne *et al.*, 2011):

$$y_{ijk} = \mu + \tau_i + \gamma_j + \tau\gamma_{ij} + \epsilon_{ijk}$$

Where:

y_{ijk} = observation from the k^{th} replicated of i^{th} genotype inoculated with j^{th} Rhizobia strain; μ = overall mean; τ_i = the effect of the i^{th} genotype; γ_j = the effect of the j^{th} Rhizobia strain; $\tau\gamma_{ij}$ = the interaction between the i^{th} genotype and j^{th} Rhizobia strain and ϵ_{ijk} is random error.

In the above model specification, variances were assumed equal for replicates (identity) and unequal for type of *Bradyrhizobium* and genotypes (diagonal). The changes in deviances and degrees of freedom were used to compare models to avoid over-ride uselessly GenStat and fit the best model (O'Neill, 2010).

Correlations between couples of parameters (nodule number, percentage of effective nodules, fresh weight of nodules, and dry weight of nodules) were tested for each treatment.

The best performing genotypes were selected based on three inclusive criteria:

- (i) genotypes with a minimum of 10 nodules per plant (referring to the scale of Corbin *et al.* (1977))
- (ii) genotypes with over 50 mg average nodule dry weight (Gwata, 2004); and
- (iii) genotypes with a minimum of 50% effective nodules.

Using the above criteria, 12 genotypes were selected as responsive to *Bradyrhizobium Sp.* Strain USDA 3456 (cowpea-type inoculant), hence 'promiscuous' (Table 2). Using the same criteria, 25 genotypes were responsive to *Bradyrhizobium japonicum* Strain USDA 110 (soybean-type inoculant) (Table 3). Genotypes were ranked based on their total nodule dry weight under *Bradyrhizobium* strains.

TABLE 2. Summary of results from linear mixed model analysis

Variables	Number of nodules (NN)		Percentage of effective nodules (NE)		Total fresh weight of nodules (NFW)		Total dry weight of nodules (NDW)	
	d.f.	F statistic	d.f.	F statistic	d.f.	F statistic	d.f.	F statistic
Rh_level	2	104.31***	2	7722.53***	2	9.32***	2	457.59***
Genotypes	64	4.88***	64	7.25***	64	2.88***	64	2.50***
Rh_level/Genotypes	128	3.45***	128	27.80***	128	3.02***	128	2.87***

*** = Very highly significant; Rh-level = Rhizobium level

RESULTS

The Wald tests for fixed effects, showed highly significant main effects for Rhizobium strain and genotypes, as well as the interaction between genotypes and Rhizobium strain ($P < 0.001$) for all the variables measured (Table 2).

The 12 genotypes responsive to *Bradyrhizobium* sp. Strain USDA 3456 were dominated by local (Ugandan bred) soybean varieties (Table 3). These highly responsive genotypes had between 11 and 42 nodules per plant, of which 56.6 to 82.65%, respectively were effective. Nodule fresh weight ranged between 113.2 and 414.7 mg per plant, with nodule dry weight of 54.83 to 149.78 mg per plant. Genotype Wondersoya from IITA was the most responsive, having 40 nodules per plant, of which 85.84% were effective and had both the highest fresh and dry nodules weights of 414.7 and 149.7 mg, respectively. Ten of the twelve responsive genotypes were of tropical origin. They were either locally bred varieties in Uganda (NamSoy 4M, MakSoy 3N, NamSoy 3, Kabanyolo 1, MakSoy 2N, MakSoy 5N, Bulindi 48C), bred and released by IITA (NamII, WonderSoya), or from Zimbabwe (Soprano); and the two others from unknown origin (K-local and UG5). Thus, about 18.5% of the currently assessed germplasm were promiscuous, with genotypes from tropical origins were more promiscuous (Table 3). None of the genotypes from USA or AVRDC were responsive to the indigenous rhizobium strain, *Bradyrhizobium* sp. strain USDA 3456. The least responsive genotypes included Roan, Serenade, Safari, GC 84051-31-1, GC 2043, G 1882, SREB-15C, G 7959, G 8527, G 2843, GC 487021-26-3-1B, PI 417132, BSPS 48A-3B, NGDT 4.11-5 most of which had less than 5 nodules per plant each (data not shown).

There were more soybean genotypes that responded to *Bradyrhizobium japonicum* strain USDA 110 (soybean-type inoculant) than to *Bradyrhizobium* sp. strain USDA 3456 (Table 4). These included the genotypes that had over 10 nodules per plant. In terms of effective nodules, 46 genotypes had over 50% effective nodules. On the other hand, 25 genotypes (about 38.5% of the assessed germplasm) had each over 10 nodules, of which at least 50% effective and over

TABLE 3. Measured traits for the 12 genotypes responsive to *Bradyrhizobium* sp. strain USDA 3456

Genotypes	Origin	<i>Bradyrhizobium</i> sp. strain USDA 3456 (control)			
		Number of nodules per plant	Effective nodules (%)	Total fresh weight of nodules (mg)	Total dry weight of nodules (mg)
WonderSoya	IITA	40(8)	85.84(25.26)	414.70(60.00)	149.78(21.44)
NamSoy 4M	Uganda	36(14)	81.20(14.65)	345.60(62.70)	119.11(35.33)
K-Local	-	19(10)	85.27(14.52)	317.30(41.80)	113.11(26.67)
MakSoy 3N	Uganda	33(6)	56.60(8.15)	352.40(86.10)	111.67(9.50)
NamSoy 3	Uganda	22(4)	80.36(2.56)	294.70(40.10)	96.33(4.89)
NamII	IITA	42(4)	82.65(0.00)	168.60(11.90)	92.78(0.33)
Bulindi 48C	Uganda	38(14)	64.90(19.92)	153.90(79.80)	71.94(30.89)
Kabanyolo 1	Uganda	19(3)	73.27(4.76)	139.20(44.80)	67.11(4.11)
UG 5	-	17(8)	57.87(2.38)	189.30(28.70)	61.67(9.00)
MakSoy 5N	Uganda	11(18)	74.58(25.44)	155.90(60.00)	60.78(30.67)
MakSoy 2N	Uganda	12(3)	82.53(3.51)	113.20(34.4)	59.89(4.00)
Soprano	Zimbabwe	13(2)	65.79(4.17)	136.80(43.10)	54.83(1.00)
	Mean	12.79(5.36)	31.35(6.07)	97.6(32.1)	36.8(10.46)
	SE	0.86(0.85)	1.760(1.738)	8.75(8.67)	2.36(2.34)
	CV (%)	97.46	73.13	111.32	102.66
	LSD	2.387	4.87	24.20	6.53

Mean values for control are indicated in brackets

TABLE 4. Measured traits for the top 25 genotypes under *Bradyrhizobium japonicum* strain USDA 110

Genotypes	Origin	<i>Bradyrhizobium japonicum</i> strain USDA 110 (control)			
		Number of nodules per plant	Effective nodules (%)	Total fresh weight of nodules (mg)	Total dry weight of nodules (mg)
NIIXGC 20.3	Uganda	81(11)	94.71(9.38)	930.00(93.10)	228.78(33.11)
G-85037-2-3-54	Uganda	45(6)	87.51(0)	630.80(44.70)	128.00(16.06)
NIIXGC 17.3	Uganda	40(24)	69.57(14.24)	552.00(131.6)	122.67(73.67)
G 2843	AVRDC	30(1)	93.19(4.17)	479.90(13.6)	110.11(0.44)
G 10427	AVRDC	16(3)	86.65(10.26)	406.40(27.7)	102.56(3.44)
GC 2043	AVRDC	24(1)	89.74(0)	429.30(11.2)	100.00(0.22)
GC 487021-26-3-1A	AVRDC	22(1)	76.19(0)	367.00(11.7)	96.00(0.22)
UG 5	-	52(8)	65.39(2.38)	329.60(28.7)	92.33(9)
PI 567056	USA	39(4)	90.95(0)	323.00(10.7)	91.11(1.44)
NamSoy 3	Uganda	24(4)	93.41(2.56)	293.30(40.1)	89.74(4.89)
NIIXGC 44.2	Uganda	39(5)	85.49(3.03)	355.40(34.9)	88.89(5.33)
G 57	AVRDC	27(1)	91.13(0)	377.70(9.7)	77.56(0.89)
G 1882	AVRDC	20(1)	89.23(0)	292.00(0.3)	73.89(0.11)
NIIXGC 7.2	Uganda	31(12)	59.74(10.2)	284.90(30.3)	73.78(1.67)
Kabanyolo 1	Uganda	16(3)	70.52(4.76)	312.90(44.8)	72.22(4.11)
Santa	Zimbabwe	19(2)	53.19(0)	250.40(16)	66.89(1.67)
NamSoy 4M	Uganda	33(14)	89.75(14.65)	277.20(62.7)	65.67(35.33)
NIIXGC 43.1	Uganda	44(20)	78.57(36.75)	262.20(112.3)	64.67(42.33)
G 50	AVRDC	10(1)	71.66(0)	202.10(10.6)	63.89(0.44)
Soprano	Zimbabwe	19(2)	81.4(4.17)	236.00(43.1)	60.78(1)
BSPS 48A-31	Uganda	34(7)	60.15(10.74)	301.70(29.6)	53.67(6.56)
Duiker	Zimbabwe	13(6)	270.1(0)	270.10(48.7)	52.00(3.78)
GC 00138-29	AVRDC	17(3)	74.73(21.05)	346.10(38.2)	51.33(11.78)
SS 8645-23-2	AVRDC	16(1)	77.36(0)	268.70(0.2)	51.00(0.11)
WonderSoya	IITA	17(8)	87.67(25.26)	231.00(60)	50.78(21.44)
	Mean	19(5)	62.36(6.07)	197.9(32.1)	48.47(10.46)
	SE	0.857(0.85)	1.760(1.738)	8.7(8.67)	2.35(2.34)
	CV (%)	97.46	73.13	111.32	102.66
	LSD	2.387	4.87	24.20	6.53

*Six (**in bold**) of the twelve genotypes highly responsive to *Bradyrhizobium* sp. Strain USDA 3456 have good performance with *B. japonicum* USDA 110 as well. *Mean values for control are in brackets

50 mg as dry weight of nodules (NDW), with equally high fresh nodule weights (Table 4). Genotype NIIXGC 20.3 exhibited the highest performance in all measured traits, with 81 nodules per plant, 94.71% of effective nodules, 930 mg as NFW, and 228.78 mg as NDW.

In contrast with the situation observed with *Bradyrhizobium* sp. strain USDA 3456 (cowpea-type), whereby tropical origin germplasm were more responsive, most of the genotypes responsive to *Bradyrhizobium japonicum* strain

USDA 110 were from AVRDC (10 genotypes); followed by breeding lines from MUARIK (8 genotypes) (Table 4). There were only 6 genotypes responsive to both *Bradyrhizobium* sp. strain USDA 3456 and *Bradyrhizobium japonicum* strain USDA 110; and these were UG5, Soprano, Wondersoya, NamSoy 4M, NamSoy 3 and Kabanyolo 1.

As for the overall predictions provided by the Linear Mixed Model (LMM) analysis, the soybean cultivars screened were more responsive

TABLE 5. Correlation test between pairs of traits measured

Pairs of traits	Control	<i>Bradyrhizobium</i> sp. (strain USDA 3456)	<i>Bradyrhizobium japonicum</i> (strain USDA 110)
	Coefficient	Coefficient	Coefficient
NN*NE	0.555***	0.616***	0.561***
NN*NFW	0.780***	0.675***	0.823***
NN*NDW	0.901***	0.789***	0.830***
NE*NFW	0.684***	0.697***	0.632***
NE*NDW	0.618***	0.785***	0.597***
NFW*NDW	0.826***	0.956***	0.975***

NN = Number of Nodules, EF = Effective Nodules, NFW = Fresh Weight of Nodules, NDW = Dry Weight of Nodules. *** = Very highly significant

to *Bradyrhizobium japonicum* strain USDA 110 than to *Bradyrhizobium* sp. strain USDA 3456. Under *Bradyrhizobium japonicum* strain USDA 110 (soybean-type inoculant), genotypes yielded about 19 nodules per plant, of which 62.35% were effective, the NFW and NDW being 198.1 mg and 48.52 mg, respectively. As noted above, nodule number was lower (13 nodules of which 31.27% were effective) under *Bradyrhizobium* sp. strain USDA 3456 (cowpea-type inoculant) with 97.5 and 36.7 mg as NFW and NDW, respectively. The least responsive genotypes included MakSoy 1N, MakSoy 4N, NamSoy I, Gazelle, Siesta, G 7959, G 8527, GC 487021-26-3-1B, BSPS 48A-9, and NGDT 4.11-5 (Data not shown). They had less than 5 nodules per plant each.

The analysis indicated highly significant positive correlations ($P=0.00$) between the different variables. Under the cowpea-type inoculant, as well as under soybean-type inoculant, the highest correlation ($r=0.956$) was shown between fresh and dry weight of nodules (NFW*NDW), followed by nodule number and dry weight ($r=0.789$) (NN*NDW). Although number of nodules and percentage of effective nodules (NN*NE) were positively correlated, they showed the lowest (r ranges from 0.555 to 0.616) correlation coefficient for each treatment (Table 5). About 38.5% of the screened genotypes responded to USDA 110 compared to 18.46%.

DISCUSSION

There were nodules observed under the sterilised soils (control), indicating that sterilisation was incompletely done. However, it did not affect the study significantly because more nodules were observed following inoculation, under both strains USDA 3456 and USDA 110.

Our results showed that very few accessions (12), all of tropical origin, were responsive to *Bradyrhizobium* sp. strain USDA 3456 (cowpea-type inoculant). This is consistent with the observation made by IITA when screening geographically diverse soybean accessions for compatibility with indigenous rhizobia (Terefa, 2011). Among the 400 genotypes screened, 10 predominantly local genotypes, were found to form effective symbiotic association with the native rhizobia at five locations in Nigeria (Terefa, 2011). Relatedly, Arulnandhy (1987) found an American high yielding recommended genotype (Pb-1) to be less responsive to native strains in African study sites.

In our study, the number of nodules under *Bradyrhizobium* sp. strain USDA 3456 ranged from 0 to 42 nodules per plant and significantly changed with plant genotypes. Plants with nodules ranging from 11 to 42 for the 12 genotypes were considered as promiscuous (Pulver *et al.*, 1985). Similar results were reported by Arulnandhy (1987), whereby the number of

nodules ranged from 1 to 37 per plants; while screening different promiscuous and non-promiscuous soybean genotypes for response to native *Bradyrhizobium* strains. Similarly, Muhammad (2010) observed 15 to 36 nodules per plants for the IITA promiscuous cultivar (TGX 1485).

As for dry weight of nodules (NDW), Gwata *et al.* (2004), reported 62 mg for the cultivar SamSoy known as highly promiscuous, which falls into the range (54.83 to 149.78 mg per plant) observed in the 12 promiscuous genotypes selected in our study. This indicates that promiscuous soybean genotypes produce enough nodule numbers and sizes to function as powerhouses for nitrogen fixation. Moreover, over 50% of these nodules were effective.

Although there were more genotypes responsive to *B. japonicum* (Table 4), more than 60% of the genotypes screened were not responsive to BNF, this could be the result of incompatible *Bradyrhizobium* strain in the inoculant, thus emphasizing that breeding efforts should be oriented in sourcing genotypes with high nodulation ability, coupled with promiscuity in order to develop cultivars that are suitable for production in the African soybean production context.

The maximum number of nodules observed (81 nodules per plant) in genotype NIIXGC 20.3 was higher than 53 nodules previously reported by Kumaga and Ofori (2004), while testing *B. japonicum* on two other soybean cultivars. Mandimba *et al.* (1994) earlier reported a maximum of 51 nodules per plant when testing different strains of *B. japonicum* on soybean cultivar FN3. The fact that our study showed a higher nodule number under soybean-type inoculant may be explained by the use of sterilised soil that would have lowered competition among strains and, hence enhanced the symbiotic association. The fact that the pair “nodule number-effective nodules”, even though positively correlated, showed the lowest correlation coefficient ($r = 0.555$) for all the treatments, suggesting that the number of nodules should not be considered alone; it must be supported by a reasonable percentage of effective nodules before a genotype can be taken as being responsive to *Bradyrhizobium*. A minimum of 50% of effective

nodules coupled, with a high number of nodules, at least 10 per plant (Corbin *et al.*, 1977) should be required to adjudicate a genotype as responsive to *Bradyrhizobium*.

A high positive correlation ($r = 0.79$) was observed between nodule number (NN) and dry weight (NDW). This is in agreement with the earlier findings of Arulnandhy (1987), who reported a significantly high correlation ($r = 0.72$) between nodule number and nodule dry weight. The high level of significance ($p < 0.001$) of interaction between genotypes and inoculation levels for most measured traits, is a clear indication that the genotypes responded differently to *Bradyrhizobium* strains USDA 3456 and USDA 110, which were used in this study as a measure of promiscuity. In addition to that, more soybean genotypes responded to USDA 110 than USDA 3456. These suggest a complex relationship which may be due to genetic effect in rhizobia-soybean symbiosis.

CONCLUSION

The study constitutes a preliminary work that provides helpful decision tool for use in the choice of potential parents for soybean breeding programme. It is clear that breeding solutions for promiscuous nodulation lie within African soybean germplasm. The results of this study can be used to choose genotypes for direct selection for promiscuous nodulation, followed by replicated trials or parental lines to be used in a breeding scheme focusing on promiscuous nodulation trait. Soybean genotypes NamII, WonderSoya, Bulindi 48C, NamSoy 4M, MakSoy 3N, NamSoy 3, K-Local, Kabanyolo 1, UG 5, Soprano, MakSoy 2N, MakSoy 5N are good candidate parental lines for initiating a soybean programme focusing on promiscuous nodulation.

We recommend that investigations be made to avail easier and more accurate methods to assess the effectiveness of nodules and infer on the ability of genotypes to fix nitrogen.

ACKNOWLEDGEMENT

We acknowledge the Intra-ACP SHARE for financial and moral support, and Bruno Awio for the discussions during data analysis.

REFERENCES

- Abaidoo, C.R., Keyser, H.H., Singleton, P.W. and Dulal Borthakur, D. 2000. *Bradyrhizobium* spp. (TGx) isolates nodulating the new soybean cultivars in Africa are diverse and distinct from bradyrhizobia that nodulate North American soybeans. *International Journal of Systematic and Evolutionary Microbiology* 50: 225-234.
- Abate, T., Alene, A.D., Bergvinson, D., Silim, S., Orr, A. and Asfaw, S. 2011. Tropical legumes in Africa and South Asia: Knowledge and opportunities. *TL II Research Report No. 1*, Acquaaah, G. 2007. Breeding Soybean in Principles of Plant Genetics and Breeding, Blackwell Pub., Malden, MA; Oxford. 521-528.
- Anuar, A.R., Shamsuddin, Z.H. and Ya'acob, O. 1995. Contribution of legume - N by nodulated groundnuts for growth of subsequent Maize in an acid soil. *Soil Biology & Biochemistry* 27(2): 595-601.
- Appunu, C. C., Sen, D., Singh, M. K. and Dh, B. 2008. Variation in symbiotic performance of *Bradyrhizobium japonicum* strains and soybean cultivars under field conditions. *Journal of Central European Agriculture* 9 (1):185-190
- Arulnandhy, V. 1987. Promiscuous nodulation in soybeans. Soybean Foods Research Centre, Gannoruwa, Peradeniya. Available from: http://www.goviya.lk/agrilearning/Pulses/Pulses_research/soyaaa/So5.pdf Accessed on January 22nd, 2015.
- Bezdicsek, D.F., Evans, D.W., Adebbe, B. and Witters, R.E. 1978. Evaluation of peat and granular inoculum for soybean yield and N fixation under irrigation. *Agronomy Journal* 70: 865-868.
- Cattelan, A.J. and Hungria, M. 1994. Nitrogen nutrition and inoculation in book Tropical Soybean: Improvement and production, ISSN 0259-2525. FAO. Rome, Italy.
- Corbin, E.J., Brockwell, J. and Gault, R.R. 1977. Nodulation studies on chickpea (*Cicer arietinum*). *Australian Journal of Experimental Agriculture and Animal Husbandry* 17: 126-134.
- FAOSTAT. 2013. <http://faostat3.fao.org> Accessed on April 1st 2015.
- Ge, C. and Xu, L. 1982. Observation of the infection behavior of indigenous *Rhizobium japonicum* and its distribution in different varieties of soybean in the fields. *Chinese Oil Crops* 3:56-58.
- Giller, K.E., Cadisch, G., Ehaliotis, C., Adams, E., Sakala, W. and Mafongoya, P. 1997. Building soil nitrogen capital in Africa. Pp. 151-192. In: Replenishing soil fertility in Africa. Buresh, R., Sanchez, P. and Calhoun, F. (Eds.). American Society for Agronomy, Crop Science Society of America, Soil Science Society of America: Madison, WI, USA.
- Gwata, E.T., Wofford, D.S., Pfahler, P.L. and Boote, K.J. 2004. Genetics of promiscuous nodulation in soybean: Nodule dry weight and leaf color score. *Journal of Heredity* 95(2):154-157, ISSN: 0022 - 1503.
- Hadley, H.H. and Hymowitz, T. 1973. Speciation and cytogenetics. In: Soybeans: Improvement, Production, and Uses, pp. 97-116. Edited by B. E. Caldwell. Madison, WI: American Society of Agronomy.
- Hailu, T. 2011. Breeding for promiscuous soybeans at IITA, Soybean - Molecular Aspects of Breeding, Dr. Aleksandra Sudaric (Ed.), ISBN: 978-953-307-240-1, In: Tech, Available from: <http://www.intechopen.com/books/soybean-molecular-aspects-of-breeding/breeding-for-promiscuous-soybeans-at-iita> Accessed on the 14th April 2014.
- ICRISAT-Nairobi, Kenya vi + 104 pp.
- International Institute of Tropical Agriculture (IITA). 1996. Understanding nitrogen fixation in promiscuous soybean. In: Microbiology in the service of crops and soils. *Annual Report*. pp. 20-23.
- Keyser, H.H., Somasegaran, P. and Bohlool, B.B. 1993. Rhizobial ecology and technology. pp. 205-226. In: Metting, F.B. Jr. (Ed.). Soil microbial ecology, applications in agricultural and environmental management. New York, NY: Marcel Dekker, USA.
- Kumaga, F.K. and Ofori K. 2004. Response of soybean (*Glycine max* (L.) Merrill) to *Bradyrhizobia* inoculation and phosphorus application. *International Journal of Agriculture & Biology* 06(2): 324-327. <http://www.ijab.org> Accessed on February 12th 2015.

- Kueneman, E.A., Root, W.R. and Dashiell, K.E. 1984. Breeding soybeans for the tropics capable of nodulating effectively with indigenous *Rhizobium* spp. *Plant Soil* 82:387-396.
- Machido, D.A., Olufajo, O.O., Yakubu, S. E. and Yusufu, S.S. 2011. Enhancing the contribution of the legumes to the N-fertility of soils of the semi-arid zone of Nigeria. *African Journal of Biotechnology* 10(10): 1848-1853.
- Mandimba, G.R., Makéla, E., Moussongo, P. and Pandzou, J. 1994. Nodulation et rendements du soja *Glycine max* L. Merrill inoculé par *Bradyrhizobium japonicum* dans différents systèmes de culture à Kombé-Brazzaville, Congo. *Tropicicultura* 12(4) : 134-140.
- Meghvansi, M. K., Prasad, K. and Mahna, S. K. 2010. Symbiotic potential, competitiveness and compatibility of indigenous *Bradyrhizobium Japonicum* isolates to three soybean genotypes of two distinct agro-climatic regions of Rajasthan, India. *Saudi Journal of Biological Sciences* 17: 303- 310.
- Muhammad, A. 2010. Response of a promiscuous soybean cultivar to Rhizobial inoculation and phosphorus in Nigeria's Southern Guinea Savanna Alfisol. *Nigerian Journal of Basic and Applied Science* 18(1): 79-82
- Okalebo, J.R., Gathua, K.W. and Woome, P.L. 2002. Laboratory Methods of Soil and Plant Analysis: A Working Manual, KARI, SSSEA, TSBF, SACRED Africa, Moi University. Second Edition, Nairobi, Kenya. pp. 128.
- O'Neill, M. 2010. ANOVA & REML: A guide to linear mixed models in an experimental design context. *Statistical Advisory & Training Service Pty Ltd.* 180 pp.
- Payne, R.W., Harding, S.A., Murray, A., Soutar, D.M., Baird, D.B. and Glaser, A. I. 2011. The guide to GenStat release 14, Part 2: Statistics. Hemel Hempstead, Hertfordshire HP1 1ES, UK: VSN International.
- Peoples, M.B., Ladha, J.K. and Herridge, D.R. 1995. Enhancing legume N₂- fixation through plant and Soil Management. *Plant Soil* 174: 85-101.
- Pulver, E.L., Brockman, F. and Wien, H.C. 1982. Nodulation of soybean cultivars with *Rhizobium* spp. and their response to inoculation with *R. japonicum*. *Crop Science* 22(5):1065-1070.
- Pulver, E.L., Kueneman, E.A. and Ranga-Rao, V. 1985. Identification of promiscuous nodulating soybean efficient in N₂ fixation. *Crop Science* 25:1065- 1070.
- Sanchez, P.A., Shepherd, K.D., Soule, M.J., Place, F.M., Buresh, R.J., Izac, A.M.N., Mokwunye, A.U., Kwesiga, F.R. Ndiritu, C.G. and Woome, P.L. 1997. Soil fertility replenishment in Africa: An investment in natural resource capital. In: Buresh R.J. (Eds). Replenishing Soil Fertility in Africa, SSSA, Special Publication No. 51, SSSA, Madison, U.S.A. pp. 1-46.
- Sanginga, N., Valanwe, B. and Danso, S.K.A. 1995. Management of biological N₂ fixation in alley cropping system. Estimation and contribution to N balances. *Plant and Soil* 174:11-141.
- Sanginga, N., Dashiell, K., Okogun, J.A. and Thottappilly, G. 1997. Nitrogen fixation and N contribution by promiscuous nodulating soybeans in the southern Guinea savanna of Nigeria. *Plant and Soil* 195 (2) 257-266.
- Shiraiwa, T., Sinclair, T.R. and Hashikawa, U. 1994. Variability in nitrogen fixation activity among soybean cultivars grown under field conditions. *Japanese Journal of Crop Science* 63:111-117.
- Sinclair, T.R. and de Wit, C.T. 1975. Photosynthate and nitrogen requirements for seed production by various crops. *Science* 189:565-567.