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ORIGINAL ARTICLE

Ureide Essay to Assess N_2-fixation Abilities of Soybean (*Glycine Max*) Genotypes under Different *Bradyrhizobium* Strains

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Abstract

The high protein content of soybean (*Glycine max*) seeds results in high nitrogen demand, causing a huge nitrogen uptake during plant growth. As a legume crop, soybean can fix atmospheric N through symbiotic associations with *Bradyrhizobia* and perform well in African nitrogen poor soils. This study aimed at establishing the ability of promiscuous soybean genotypes to fix nitrogen and devise the relationship between nodule scores and amount of nitrogen fixed. Twelve soybean genotypes were inoculated with *Bradyrhizobium japonicum* Strain USDA 110 (specific) and *Bradyrhizobium* sp. Strain USDA 3456 (native) and raised in pots in a greenhouse. At the R3.5 growth stage, nodules were scored and xylem sap was extracted, which xylem sap was used to carry out ureide, amino-N, and nitrates assays. The relative abundance of ureide was used to devise the proportion of nitrogen fixed by each genotype. The proportion of nitrogen derived from atmospheric N_2 (Ndfa) ranged from 47.9 to 78.8% under USDA 3456 and from 36.7 to 78.7% under USDA 110. A strong correlation was found between nodule scores, especially nodules' effectiveness, and Ndfa. The genotypes Wondersoya (78.8%), Maksoy 2N (78.4%), Namsoy 3 (78.3%), and Maksoy 3N (75.7%) had high nitrogen-fixing ability in response to USDA 3456. Promiscuous soybean genotypes can fix nitrogen equally under both native and specific *Bradyrhizobium* types. Nodules' effectiveness can be a good predictor of biological nitrogen fixation. This study highlighted that crop improvement to boost soybean production in Africa should target promiscuous varieties for better yield with less inputs.

Key words : Promiscuous soybean, *Bradyrhizobium* sp. Strain USDA 3456, *Glycine max* (L. Merr.), ureide, nitrates.

Introduction

Nitrogen is one of the most limiting nutrients in tropical soils yet it exhibits high negative balance as a result of low input but high losses especially from leaching and harvesting. Plants require nitrogen in large quantities which is used in plant structural formation amongst other functions. For instance, terrestrial plants and crops require about 200 million tons of nitrogen per year of which 50-70 million tons are derived from biological nitrogen fixation (Unkovich et al. 2008). Therefore, the development of crop varieties and agricultural practices that enhance N_2 fixation are keys to meet the world demand of nitrogen. Giller (2001) reports that legume production in farming systems is an important practice to increase N_2 fixation in farm lands. Similarly, Unkovich et al. (2008) reported that biological N_2 fixation provides a renewable source of nitrogen for sustainability in farming systems.

Soybean (*Glycine max* L. Merr.) is a legume crop with high N_2 fixation ability; it can fix up to 300 kg Nha^{-1} (Bezdicek et al. 1978). This nitrogen fixation ability can be improved through breeding and use of effective *Bradyrhizobia* strains. Soybean requires high rates of nitrogen to process an equally high protein. The approximate composition of soybean seed is 40% protein, 20% oil, and 30% carbohydrate (Hailu

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2011), making it one of the crops with the highest protein content (Werner and Newton 2005). Salvagotti et al. (2008) estimated that the crop assimilates approximately 80 kg of nitrogen to produce one ton of soybean grain. It has been reported that 50-60% of the N uptake is achieved through biological N_2 fixation. Bezdicek et al. (1978) recorded 300 kg of fixed nitrogen per hectare in a low N available soil which is supplied by effective strains of *Bradyrhizobia*. Salvagotti et al. (2008) reported that the N_2 fixed can go as high as 337 kg per hectare when inoculated with effective strains of *Bradyrhizobium*.

Unkovich et al. (2008) reported that legumes export the majority of fixed nitrogen from root nodules to the upper parts of the plant, either in the form of amides (asparagine and glutamine), or in the form of ureides (allantoin and allantoic acid). However, Sprent (2001), reports that most legumes are amide exporters, with ureide exporters being restricted to species in Phaseoleae and Desmodieae tribes. Soybean belongs to the group of ureide exporters (Sprent 2001).

Several methods are used to estimate N_2 fixed through symbiotic biological associations. These are based on three main principles; the first principle known as N balance method assumes that total N_2 fixed is the same as the net increase in total N of a plant-soil system (Herridge et al. 1998; Khan et al. 2003; Lima et al. 1987; Urquiaga et al. 1992). The second principle separates plant N into two fractions, *viz*. N taken up from soil and the N derived from fixation, using N difference, ^{15}N natural abundance, ^{15}N isotope dilution, and ureide methods. The third principle measures the activity of nitrogenase, the enzyme responsible for N_2 fixation. Methods based on this principle include acetylene reduction (C_2H_2 method) and the hydrogen evolution method. The ureide method has proven to be a precise, indirect, and relatively inexpensive method that is suitable for both short- and long-term experiments in the field, laboratory, and glasshouse for N_2 fixation estimates in ureide exporter plant species (Unkovich et al. 2008).

The present study is aimed at using the ureide method to investigate the ability of promiscuous soybean genotypes to fix nitrogen while in symbiotic association with diverse *Bradyrhizobium* strains and to clarify the relationship between nodule scores and the amount of nitrogen fixed.

Materials and Methods

Plant material

The germplasm evaluated in this study consisted of 12 soybean genotypes (NamII and WonderSoya from IITA; Bulindi 48C, NamSoy 4M, MakSoy 3N, NamSoy3, Kabanyolo1, MakSoy 2N, MakSoy 5N, and UG5 from Uganda; Soprano from Zimbabwe; and K-Local from unknown origin). These genotypes were selected based on their high response to *Bradyrhizobium* sp. strain USDA 3456 following field screening of 65 soybean lines for nodulation ability, hence promiscuous (Agoyi et al. 2016). Description of the plant material used in this study is in Table 1.

Soil media preparation

Top soil (0-15 cm) used in this study was obtained from a field used for maize cultivation at Makerere University Agricultural Research Institute of Kabanyolo (MUARIK). Soil sub-samples were analysed in a laboratory for pH, organic carbon, total nitrogen, available phosphorus, and exchangeable bases (K, Na, Mg, and Ca) using procedures described in Okalebo et al. (2002). The soil analysis was done to determine the suitability of the soil for this study as soil with poor nitrogen content (< 0.2%), and adequate phosphorus and potassium content (≥ 15 mg/kg) was needed. The soil was steam-sterilized at 120 °C for 6 h under direct hot steam, and left to cool over 24 h. The sterilized soil was used to fill 108 buckets each to five kg capacity (20.0 cm diameter and 20.5 cm depth) that were lightly perforated at the bottom to provide drainage. The soil was pre-mixed with

Table 1. Description of genotypes used in the study.

Genotypes	Pedigree	Released	Current use status	Source#
Nam2	TGM 79	1992	Parental line	NARO, Uganda
Maksoy3N	Gc00138-29 x Duiker	2010	Commercial	Mak, Uganda
Namsoy4M	Nam2xGc00138-29	2004	Commercial	NARO, Uganda
Namsoy3	Kabanyolo I x Nam I	1995	Parental line	NARO, Uganda
Maksoy2N	Maksoy 1N x Duiker	2008	Commercial	Mak, Uganda
Maksoy5N	Nam2 x Gc00138-29	2013	Commercial	Mak, Uganda
Kabanyolo1	Mutant of Clark 63	-	Parental line	Mak, Uganda
WonderSoya	-	-	Parental line	IITA
Bulindi 48C	-	-	-	Mak, Uganda
Soprano	-	-	-	Zimbabwe
K-Local	-	-	-	Uganda
UG5	-	-	-	Uganda

0.0356 g of TSP (Triple Super Phosphate) and 0.036 g of Muriate of Potash (MOP) per kg of soil, which is equivalent to 46.28 kg of phosphorus (P) and 46.8 kg of potassium (K) per hectare. It was established in earlier studies that P and K are essential for biological nitrogen fixation (Giller et al. 1997).

Experimental design

The experiment was grown as a factorial with 12 genotypes each give three inoculation treatments that are: i) inoculation with cowpea-type rhizobium (*Bradyrhizobium* sp. strain USDA 3456), ii) inoculation with soybean-type rhizobium (*Bradyrhizobium Japonicum* strain USDA 110), and iii) a control without rhizobium. Here after *Bradyrhizobium* sp. strain USDA 3456 and *Bradyrhizobium Japonicum* strain USDA 110 will be referred to as USDA 3456 and USDA 110, respectively. Ten seeds were sown into the steam-sterilized soil in each bucket and the buckets served as experimental units. The experiment was arranged in a completely randomized design with three replicates in greenhouse at the Makerere University Agricultural Research Institute Kabanyolo (MUARIK). After germination, plants were thinned to three per bucket.

Preparation of inoculum

Rhizobia inoculants were obtained from BIOFIX (MEA LTD, West End Towers, 6th Floor - Wing A, Westlands P.O. Box 44480 - 00100 Nairobi, Kenya), purified, and incubated in the Soil Science laboratory at Makerere University. The strains, USDA 110 (soybean-type) and USDA 3456 (cowpea-type), were grown to 7.91 x 10^9 cells/g and 9.08 x 10^9 cells/g, respectively. These were formulated into inoculum carried in steam-sterilized peat soil and used to inoculate seed. Ten grams of sugar was dissolved into 300 ml of clean lukewarm water in a plastic bottle for use as a sticking agent following the use-instructions given by the inoculant supplier BIOFIX. Freshly produced inoculant was mixed with the sticker and directly applied before sowing.

Xylem sap sampling and handling

At the R3.5 stage (about 70 days after planting), root bleeding extraction method was used to sample xylem sap. Sampling was done between 9:00 am to 16:00 pm (Herridge et al. 1988) to reduce effect of diurnal fluctuations in relative ureide-N. Stems of all the three plants per pot were cut above ground, and extraction was done following the technique described by Unkovich et al. (2008). Plants were bled till sufficient sap (at least 1 ml) was obtained per pot. Sap samples were kept chilled on ice in cooler box until reaching laboratory where they were transferred to a -20 °C freezer for storage until assays were completed.

Nodules sampling

After xylem sap was extracted, buckets were soaked in water and carefully inverted to release the aggregate plants-soil medium. The aggregate was carefully washed with abundant water to separate the soybean plants from the soil medium without breaking the roots, or losing nodules. Roots were then rinsed and wrapped in tissue paper to reduce wetness. 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 a scale with a 0.001g resolution. (ADAM Equipment PGW 453e balance, South Africa). All the nodules were then split opened to assess their effectiveness. Percentages of effective nodules (NE) were generated per plant, based on the presence of pink red or brown pigmentation inside nodules. Thereafter, nodules were oven-dried at 65 °C for 4 days (Gwata et al. 2004), and weighed to determine the total nodules dry weigh (NDW) per plant.

Laboratory analysis

Amino, ureide, and nitrates assays were carried out in Bioscience Laboratory in the National Crops Resources Research Institute (NACCRI) at Namulonge in Uganda. Each sample was replicated twice. Assays were carried out by optimizing the protocols described by Young and Conway (1942) for Ureides, Yemm and Cocking (1955) with adaptations described in Herridge (1984) for amino acids, and Cataldo et al. (1975) for nitrates. Optimization consisted mainly in setting working volumes and sample dilutions to suit the glassware available and enable efficient use of the facilities available in the laboratory. For instance, a dilution of 1:50 instead of 1:25 recommended in the protocol was used in the ureide analysis.

Calculations

After computing the mean value of spectrophotometer (Helios Epsilon, Thermo Scientific- UK) readings, standard curve equations for each assay was used to calculate the concentrations of ureides, nitrates, and amino-N. Dilution factors were included where necessary.

The relative abundance (RU-N) was calculated as follows:

$$\mathrm{RU-N}(\%)=\left(\frac{4u}{4u+n+2a}\right)\times 100$$ (Takahashi et al. 1992)

Where u is the amount of ureide, n is the amount nitrates, and a is the amount amino in the xylem sap.

The proportion of plant nitrogen derived from atmospheric N_2 (%Ndfa) was computed using the following calibration equation proposed by Purcell et al. (2004) for root-bleeding sap sampled from soybean plants at pod-filling stage:

$$RU-N=0.67(\%Ndfa)+21.3$$ (Purcell et al. 2004)

Where RU-N (relative ureide abundance) and %Ndfa (the proportion of plant N derived from atmospheric N_2).

Data analysis

Data were subjected to analysis of variance using GenStat 14th edition (VSN International Ltd, Hemel Hempstead, UK) (Payne et al. 2011), fitted on the following model:

$$Yij = \mu + Gi + Rj + GR(ij) + \varepsilon ij$$

Where Y_{ij} = observed value from each experimental unit, μ = population mean, G_i = effect of ith genotype, Rj = effect of the jth *Bradyrhizobium* treatment, $GR_{(ij)}$ = effect of the interaction between the ith genotype to the jth *Bradyrhizobium* treatment, $\mathfrak{M}_{ij}$ is the experimental error.

Correlations were tested between nodulation traits and %Ndfa, and between pairs of nodulation traits (NN, NE, NFW, and NDW).

Results

Analysis of variance showed a significant ($P < 0.05$) difference among soybean genotypes for all measured traits (Table 2). The soybean genotypes responded differentially to the different *Bradyrhizobium* treatments (see Fig. 1(a), (b), and 2) as shown by the highly significant ($P < 0.001$) difference among rhizobium level (Rh-level) for all the traits (Table 2). The interactions between *Bradyrhizobium* treatments and genotypes were significant ($P < 0.05$) only for NFW and NDW.

As shown in Table 3, all genotypes had effective response to both *Bradyrhizobium* strains. However, responses among genotypes varied between the *Bradyrhizobium* treatments as illustrated by the significant genotype by strain interaction in nodule weights (Table 2). As expected, the control treatment had the least performance for all nodulation traits. It was observed that the genotypes fixed substantial amount of nitrogen (47.9-78.8%) while in symbiotic association with USDA 3456, which is native in African tropical soils (Table 3). The genotypes Wondersoya and Bulindi 48C had the highest and the least proportion of nitrogen derived from N_2 fixation, respectively. The rhizobium strain USDA 110 which is required by non-promiscuous soybean genotypes induce nitrogen fixation (36.7-78.7%) in promiscuous genotypes, with Maksoy 5N and Namsoy 4M having the highest and the least proportion of nitrogen derived from N_2 fixation, respectively (see variation in ureide concentration in Fig. 2). Roughly, the promiscuous genotypes tested in this study fixed more nitrogen under USDA 3456 (66%) than USDA 110 (59.4%).

Inoculated with USDA 3456, the soybean genotypes tested produced significantly different number of nodules (see Fig. 1), ranging from 26 to 117 nodules per plant. Similar

Table 2. Analysis of variance of N_2 fixation and the nodulation scores.

Sources	d.f	Mean squares				
		Ndfa (%)	NN	NE (%)	NFW (mg)	NDW (mg)
Soybean genotypes (SG)	11	643*	1372*	842***	486532***	28491***
Bradyrhizobia strain (BS)	2	22520***	27867***	26214***	8917165***	448252***
SG*BS interaction	21	312ns	931ns	176ns	255140**	16770*
Error	54	280	593	213	100707	5784
Total	88	845	1400	881	389444	21478

Rh-level=Bradyrhizobium treatment, d.f= degree of freedom, Ndfa= proportion of plant N derived from N_2 fixation, NN= number of nodules, NE= percentage of effective nodules, NFW=fresh weight of nodules, NDW=dry weight of nodules

(a) (b)

Fig. 1. Root system of (a) nodulating & (b) non-nodulating soybean plants.

Table 3. Mean nodulation performance and nitrogen fixing ability of the genotypes under the different treatments.

Genotypes	Ndfa (%)			NN			NE (%)			NFW (mg)			NDW (mg)		
	control	USDA 3456	USDA 110	Control	USDA 3456	USDA 110	Control	USDA 3456	USDA 110	Control	USDA 3456	USDA 110	Control	USDA 3456	USDA 110
Bulindi 48C	8.8	47.9	77	2	81	69	10.4	66.0	74.8	2.4	1343.2	1411.8	1.1	330.6	336.2
K-Local	19.2	73.9	71.5	4	40	39	11.8	67.5	67.5	3.5	722.0	602.4	2.1	147.4	105.4
Kabanyolo 1	10.4	74.1	59.6	3	63	42	10.3	87.1	86.0	2.6	1389.0	1196.0	1.2	295.2	242.3
MakSoy 2N	8.2	78.4	60.2	4	34	37	17.8	93.2	84.2	3.5	288.3	810.9	1.7	54.0	154.8
MakSoy 3N	12.3	75.7	58.2	4	41	65	22.4	80.9	67.8	4.3	1035.0	890.1	2.1	246.2	192.7
MakSoy 5N	13.8	51.7	78.7	13	44	84	23.1	62.9	64.5	12.9	751.3	1611.0	5.6	181.6	372.2
Nam II	13.6	52.4	64.7	4	70	100	14.7	67.5	57.4	4.3	1205.4	1680.9	2.2	253.8	414.7
NamSoy 3	-7.2	78.3	-	4	34	-	18.1	85.7	-	-	652.5	-	-	135.8	-
NamSoy 4M	9.7	50.2	36.7	4	117	37	13.8	60.4	39.7	3.5	1646.5	511.5	1.7	435.5	100.8
Soprano	13.7	67.3	46.1	3	26	22	10.3	60.7	54.2	2.9	345.9	283.1	1.0	85.8	56.9
UG5	13.1	70.3	38.4	3	57	96	20.0	77.6	48.8	3.1	1081.3	1332.0	1.6	255.3	268.2
WonderSoya	12.3	78.8	61.8	4	58	46	22.2	83.2	67.3	3.5	711.6	489.1	1.9	163.0	105.4
Average	10.6	66.6	59.4	4	55	58	16.2	74.4	64.7	4.2	931.0	983.5	2.0	215.4	213.6
Grand mean		45.1			39			51.4			648.2			145.8	
LSD		9.3			14			8.1			176.5			42.3	

Ndfa= proportion of plant N derived from N_2 fixation, NN= number of nodules, NE= percentage of effective nodules, NFW=fresh weight of nodules, NDW=dry weight of nodules, USDA 3456=Bradyrhizobium sp. Strain USDA 3456, USDA 110= *Bradyrhizobium japonicum* Strain USDA 110, none= control (without Bradyrhizobiu).

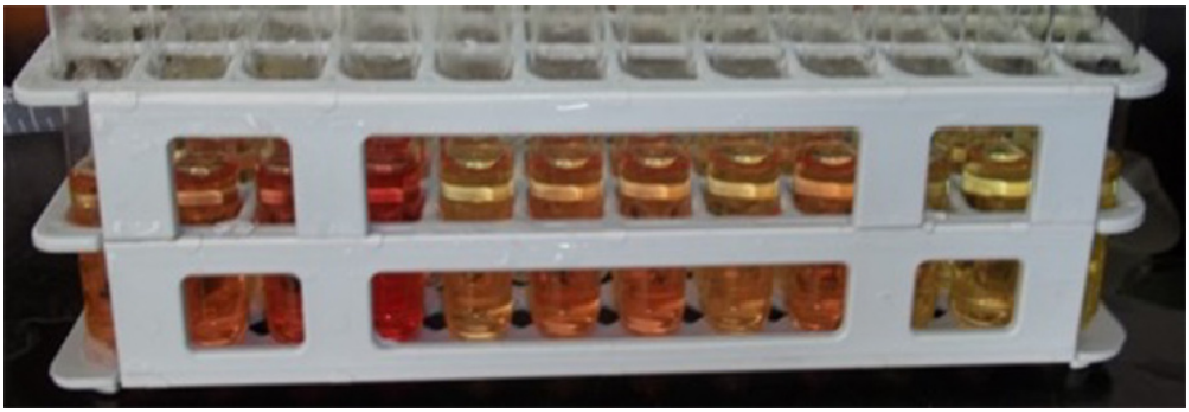

Fig. 2. Test tubes showing different levels of ureide content. The reddishness indicates the concentration of ureide : Orange arrow indicates high ureide content, bleue arrow indicates medium ureide content, and black arrow indicates low ureide content.

trend (22 to 100 nodules per plant) was also observed when the soybean genotypes were inoculated with USDA 110. The genotype Soprano had the least nodule number under both *bradyrhizobia* types while Namsoy 4M exhibited the highest nodule number under U3456 and Namsoy 2 exhibited the highest number of nodules under USDA 110 (Table 3).

It was observed (Table 3) that all genotypes had more than 50% of their nodules effective under USDA 3456 (60.4-93.2%), with the genotypes Maksoy 2N having the highest while Namsoy 4M exhibited the least. Under USDA 110 the promiscuous soybean genotypes tested exhibited less effective nodules (39.7-86%), Kabanyolo I had the highest percentage of effective nodules while Namsoy 4M had the least percentage of effective nodules.

Concerning the weights of nodules, the genotype Namsoy 4M had the highest weights under USDA 3456 (1646.5 and 435.5 mg) and the genotype Nam II had the highest weight under USDA 110 (1681.0 and 414.7 mg). The genotypes Maksoy 2N (288.3 and 54 mg) under USDA 3456 and Soprano (283.1 and 57.0 mg) under USDA 110 had the least weight values.

The correlation analysis (Table 4) showed significant ($P < 0.001$) positive correlation between all pairs. Correlation coefficients ranged from r = 0.56 for NE*NN to r = 0.98 for NDW*NFW. All nodulation traits had positive and significant correlation with (%) Ndfa. The strongest correlation with Ndfa was observed for NE (r = 0.82), while NN showed the least correlation coefficient with (%) Ndfa.

Table 4. Correlation between proportions of nitrogen derived from fixation and nodulation traits, and between pairs of nodulation traits.

	Ndfa	NN	NE	NFW
NN	0.59***			
NE	0.82***	0.56***		
NFW	0.66***	0.89***	0.64***	
NDW	0.63***	0.89***	0.61***	0.98***

Ndfa= proportion of plant N derived from N_2 fixation, NN= number of nodules, NE= percentage of effective nodules, NFW=fresh weight of nodules, NDW=dry weight of nodules.

Discussion

The study showed that nodulation in promiscuous soybean cultivars is accompanied by nitrogen fixation with the cowpea type rhizobacteria, *Bradyrhizobium* sp. USDA 3456. Proportions of nitrogen derived from atmospheric N_2 (%Ndfa) values ranging from 47.9 to 78.8% under cowpea-type inoculant and from 36.7 to 78.7% under soybean-type inoculant were observed, suggesting that promiscuous soybean genotypes can equally fix nitrogen under both soybean-type inoculants and potentially other native but compatible *Bradyrhizobium* strains. The ranges observed in this study are in agreement with observations made in previous studies with promiscuous and non-promiscuous soybean lines. For instance, Zoundji et al. (2016) estimated 42.1-65.8% proportion of nitrogen fixed by both promiscuous and non-promiscuous soybean cultivars, with 59.5% for the check promiscuous genotype (TGX 1448 2E). Herridge and Holland (1987) using the ureide method estimated 71% of the proportion of nitrogen derived from atmospheric N_2. Some of the soybean genotypes used in this study showed slightly higher Ndfa values. Nevertheless, the results roughly fall into the ranges reported in the previous studies. This confirms the finding that the genotypes NamII, Wonder Soya, Bulindi 48C, NamSoy 4M, MakSoy 3N, NamSoy3, Kabanyolo1, MakSoy 2N, MakSoy 5N, Soprano, K-Local, and UG5 identified as promiscuous based on nodules scores in a previous study (Agoyi et al. 2016) are promising in their ability to respond to native *Bradyrhizobium* strains other than *Bradyrhizobium japonicum*. It was noted in this study from correlation analysis that nodules' effectiveness (NE) is the better predictor of the nitrogen fixing ability of the genotypes (correlation coefficient r = 0.82). This was expected, as the effective nodules constitute the powerhouse where nitrogen fixation takes place. Besides, there was a positive and significant correlations between (%) Ndfa and the other nodulation traits number of nodules (r = 0.59), fresh weight of nodules (r = 0.66), and dry weight of nodules (r = 0.63). Therefore, like previous studies which recommend that dry weight of nodules be used to infer on N_2 fixation and yield (Mirza et al. 1990; Shiraiwa et al. 1994; Sinclair et al. 1991), this study adds that nodules' effectiveness (NE) is an equally reliable indicator to be used in studies with few resources and facilities, to infer on the nitrogen fixing ability of soybean genotypes. However, it is preferable to estimate the nitrogen fixed through one of the available methods, when financial and technical facilities allow, as it will lead to more accuracy. Different studies reported different results in terms of correlation between nodules' scores and nitrogen fixation. For instance, Shiraiwa et al. (1994) reported positive correlation of nodule dry weight and N_2 fixation, and this study found positive and significant correlation of fixed nitrogen with number of nodules, nodules' effectiveness, nodule fresh and dry weights. Thuita et al. (2012) and Zoundji et al. (2016) observed no correlation of biological nitrogen fixation with number of nodules and had discouraged relying on number of nodules to infer the ability of a soybean genotype to fix nitrogen. Based on the biological nitrogen fixation attributes affirmed in this study, genotypes Wondersoya (78.8%), Maksoy 2N (78.4%), Namsoy 3 (78.3%), and Maksoy 3N (75.7%) significantly responded to inoculation with USDA 3456 and were highly promiscuous varieties. Therefore, these varieties are good parent candidates while breeding promiscuous varieties with other farmers' preferred traits. Hence, they are highly promiscuous, and as such can be good candidate to initiate breeding programs seeking to develop promiscuous farmer preferred soybean varieties with high nitrogen fixing ability and high yield. Namsoy 2N and Maksoy 3N are among the current commercial soybean varieties in Uganda, suggesting that these varieties require less or no fertilizer nitrogen fertilizer applications.

It was noted that there were few nodules in the control pots whereas the soils had been sterilized. This indicates that sterilization was incompletely done, that some sections within the soil mass could have gone unsterilized. However, this conditions did not affect the study because significantly more nodules were observed following inoculation, under both strains USDA 3456 and USDA 110.

The study enabled testing the ability of promiscuous soybean genotypes to fix atmospheric nitrogen and establish relationships between their nodulation abilities and their nitrogen fixing potential. Promiscuous soybean genotypes seeds sown without inoculants can yield as much as inoculated seeds when native rizhobia are abundantly available in the soil. Proportions of nitrogen derived from atmospheric N_2 (%Ndfa) had the highest correlation with nodules' effectiveness (NE), which would be the best trait to choose and infer on the nitrogen fixing ability if investigators are unable to estimate nitrogen fixation. The genotypes Wondersoya (78.8%), Maksoy 2N (78.4%), Namsoy 3 (78.3%), and Maksoy 3N (75.7%) have been identified as the most promiscuous, therefore good candidate as parental lines in breeding programs and best for production in areas with limited nitrogen in the soil.

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