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Full Length Research Paper

Effectiveness of rhizobia strains isolated from South Kivu soils (Eastern D.R. Congo) on nodulation and growth of soybeans (*Glycine max*)

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Identification of effective indigenous *Bradyrhizobium* strains which nodulate soybean varieties could trigger development of an industry for inoculant production with use of strains adapted to local conditions. This study was conducted to identify and select effective local rhizobia strains nodulating soybean in South Kivu soils. One hundred and seven isolates collected from root nodules in South Kivu were tested in sterile sand using the modified Leonard's jars and in potted field soils. Each jar and pot was inoculated by 1ml of broth culture concentrated at 10^9cells per milliliter. From the first screening, only 10% of these isolates produced higher nodules number and plant shoot dry weights ($p<0.001$) compared to the commercial strain USDA110 and were selected for evaluation using soils as rooting media in three liters PCV pot in the greenhouse. From the potted soils experiment, among twelve outperforming isolates, only six isolates produced higher nodules and shoots dry weight ($p<0.001$) compared to the commercial strains and uninoculated controls, and were considered as effective and competitive strains. This isolates includes NAC10, NAC22, NAC37, NAC67, NAC40 and NAC75. Nodules number highly correlated with Shoot weight. There exist effective indigenous rhizobia in South Kivu soils for inoculants production.

Keywords: Indigenous rhizobia, effectiveness, selection, soja, South Kivu.

RESUME

The identification of effective local Bradyrhizobium strains that nodulate with Soya has as a goal the development of a production industry of the inoculants using the strains adapted to local conditions. This study was carried out at the IITA Kalambo station and aims to select the effective local strains that nodulate soya in the soils of South Kivu. 107 isolates were collected from legume nodules and tested under sterile conditions in the greenhouse, using as substrate sterile sand in modified Leonard jars. Each seed was inoculated with 1 ml of con-

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centrated culture at 109 cells per milliliter. Only 10% of these isolates produced a high number of nodules and a high aerial biomass weight ($p<0.001$) compared to the USDA110 commercial strain and were selected to be tested in 3L capacity pots using the soils of the fields as substrate in the greenhouse. Each pot was also inoculated with 1 ml of isolate. Of the strains tested, only six produced a high number of nodules and a high biomass weight compared to the native strains but also to the USDA110 commercial strain. These strains were therefore considered effective and competitive. These strains are: NAC10, NAC22, NAC37, NAC67, NAC40 and NAC75. Each time the strains produced a large number of nodules there was also a high weight of biomass.

Keywords: Effectiveness, indigenous Rhizobium, Selection, Soybean, South Kivu.

INTRODUCTION

The soybean crop is one of the most important crops worldwide. Soybean seeds are important for both protein meal and vegetable oil. Soybean is on top of being an excellent source of quality protein and vegetable oil. The crop is grown on an estimated 6% of the world's arable land, and since the 1970s, the area in soybean production has the highest percentage increase compared to any other major crop (Hartman et al., 2011).

It also plays a fundamental role as food and cash crop, livestock's feed and as a soil fertility amendment through Biological nitrogen fixation (BNF) (Giller, 2001; Mapfumo *et al.,* 2001). In South Kivu (Eastern Democratic Republic of Congo), soybean demand is increasing as result of development of industry for soybean processing (IITA, 2014). Therefore increasing soybean production is a priority. The use of inoculation is the most profitable way to increase soybean production due to it low cost (Ronner *et al*., 2016).

Inoculation is aimed at providing sufficient numbers of viable effective rhizobia to induce rapid colonization of the rhizosphere allowing nodulation to take place as soon as possible after germination and produce optimum yields (Deaker, 2004). Inoculation of legume seed is an efficient and convenient way of introducing viable rhizobia to soil and subsequently to the rhizosphere of legume. It is necessary in the absence of compatible rhizobia and when rhizobial populations are low or inefficient in fixing N (Fening and Danso, 2002; Abaidoo *et al.,* 2007).

Nodulation of soybean (*Glycine max (*L.) Merrill) requires specific Bradyrhizobium species. Compatible populations of these rhizobia are seldom available in soils where the soybean crop has not been grown previously (Abaidoo et al 2007). The population size of effective indigenous soil rhizobia and the concentration of Nitrogen in soils is a reliable index for the capacity of a legume crop to derive N through BNF and to determine whether or not the legume will respond to added rhizobia or fertilizer N (Zengeni*et al*., 2006; Appunu and Dhar, 2006). Nodulation and nitrogen fixation in legumes occurs effectively if other mineral elements such as Phosphorus (P), Potassium (K) and Sulphur (S) are present in the soil (Njeru et al.; 2013).

Inoculation however, is not universal and does not always elicit positive responses. It is necessary in the absence of compatible rhizobia and when rhizobial populations are low or inefficient in fixing N (Brockwell *et al.,* 1995; Catroux *et al.,* 2001; Fening and Danso, 2002; Abaidoo *et al.,* 2007). Nodulation of soybean (Glycine max (L.) Merrill) requires specific Bradyrhizobium species (Abaidoo et al, 2007).

A common approach to improve symbiotic nitrogen fixation and legume productivity has been the reliance on superior of very effective exotic rhizobia strains as inoculants. This approach has failed to achieve the desired responses in a lot of environment (Howieson *et al.,* 2005). In many cases, introduced strains from commercial inoculants fail to compete the population of native rhizobia (Zengeni *et al*., 2006; Appunu and Dhar, 2006). Therefore, continual identification of new, elite isolates offers the opportunity to improve BNF within fine-tuned geographical targets. This study evaluates the effectiveness of indigenous rhizobia isolates from wild and cultivated legumes across different agroecological conditions in South Kivu soils.

MATERIAL AND METHODS

Collection, isolation, characterization, and authentication of indigenous rhizobia strains.

Nodules were collected from cultivated and wild legumes in South Kivu between 400 m and 1600 m of elevation. In the field, the legumes were identified using botanical key and plants were uprooted carefully; avoiding detaching secondary roots from plant as nodules may be found on lateral roots as well as the taproot.

The growth media used for rhizobial isolation was the Yeast Extract mannitol Media (Vincent, 1970). Nodules were surface sterilized and rhizobia isolated as described

Table 1. Shoot weight (in grams) and effectiveness index (E.I) induced by best performing strains on the two promiscuous soybean varieties.

Rhizobia isolates	Plant shoot dry weight(g)		Effectiveness index
	SB19[b]	SB24[a]	
NAC67	9.832	11.281	2.915
NAC45	8.999	9.29	2.525
NAC38	8.959	9.238	2.513
NAC22	8.677	9.351	2.489
NAC75	7.946	9.898	2.464
NAC51	8.071	9.77	2.464
NAC19	8.7	9.036	2.449
NAC50	8.372	8.957	2.393
NAC66	7.4	8.572	2.205
NAC40	7.309	8.377	2.166
NAC10	7.524	8.059	2.152
NAC42	7.704	7.401	2.086
NAC23	7.235	7.554	2.042
NAC46	6.237	7.394	1.882
NAC37	5.961	6.42	1.71
NAC111	4.25	4.193	1.166
USDA110	4.112	3.13	1
NAC30	3.317	3.349	0.921
N+	3.123	3.389	0.899
SEMIA5019	3.231	3.093	0.873
N-	0.474	0.798	0.176
LSD	0.899		
CV	12.2%		

by Somasegaran and Hoben (1994). Typical rhizobia were recognized by their appearance, the growth rate and the production of alkalinity or acidity on the media (Somagaran and Hoben, 1994). Rhizobia isolates obtained were subject of nodulation test as described by Koala *et al,* 2010. The cultures of presumptive isolates were confirmed as rhizobia and were given collection numbers. From this nodulation test one hundred and seven isolates were collected.

Strains selection

Two sets of experiments were performed in the controlled condition of greenhouse to select effective local rhizobia strains. The temperature in the greenhouse was varied between 25^0C and 38^0C.

Strains testing in sterile conditions

Modified Leonard's jars assembly were used as growth unit. A commercially available 1.5 capacity water bottle was cut into two halves; one portion used for holding the nutrient solution and the other part was inverted for the growth media (sterile sand). The assembly was covered by a grey paper bag to protect roots and nutrients solution from light. A centrally positioned lantern wick made from braided cotton, which runs through the length of the bottle and extends out of the mouth of the bottle

Table 2. Nodules number and plant shoot dry weight (in grams) produced by promiscuous varieties inoculated with indigenous rhizobial in the two site soils

Site soil	Treatments	SB24		SB19	
		nodules number	plant shoot weight	nodules number	Plant shoot weight
Kalehe[a]	N+	5.25	9.972	7.24	14.238
	N-	75	8.831	76.5	7.295
	NAC10	46.25	14.813	56.75	14.721
	NAC22	54	17.098	70	17.106
	NAC37	46	16.929	81.5	17.106
	NAC40	37.5	16.917	89	16.504
	NAC42	46.75	11.491	89	10.946
	NAC45	49.5	9.526	56.25	9.187
	NAC46	53.5	6.735	76.5	5.051
	NAC50	35.5	9.793	39.5	9.909
	NAC67	62.25	13.415	66.25	13.888
	NAC75	34.25	17.073	51.75	15.905
	USDA110	54.25	13.98	71.75	13.285
	SEMIA5019	62	9.357	88.5	10.384
Walungu[b]					
	N+	20.75	10.179	7.75	8.388
	N-	25.5	3.972	12.25	3.797
	NAC10	57.5	9.774	31	9.383
	NAC22	26.5	14.089	11.5	14.45
	NAC37	20.25	9.841	54.5	9.833
	NAC40	62	13.613	42.5	12.632
	NAC42	20.5	8.772	5.75	9.38
	NAC45	34.25	8.772	9.5	9.38
	NAC46	20.5	5.923	22.5	6.081
	NAC50	40.5	3.187	28.75	3.473
	NAC67	91.25	11.617	28.75	11.071
	NAC75	55.75	12.533	170.5	13.336
	USDA110	61.5	5.95	7.5	7.79
	SEMIA5019	33	9.65	14.5	8.988
LSD		9.281 (nod.no)			1.275(SDW)
CV		13.90%			8.50%

into the reservoir containing the nutrient, was used to irrigate the growth medium. 750 grams of well-washed and autoclaved sands were used like growth medium (Burton, 1984). Nitrogen-free nutrient solution (Broughton

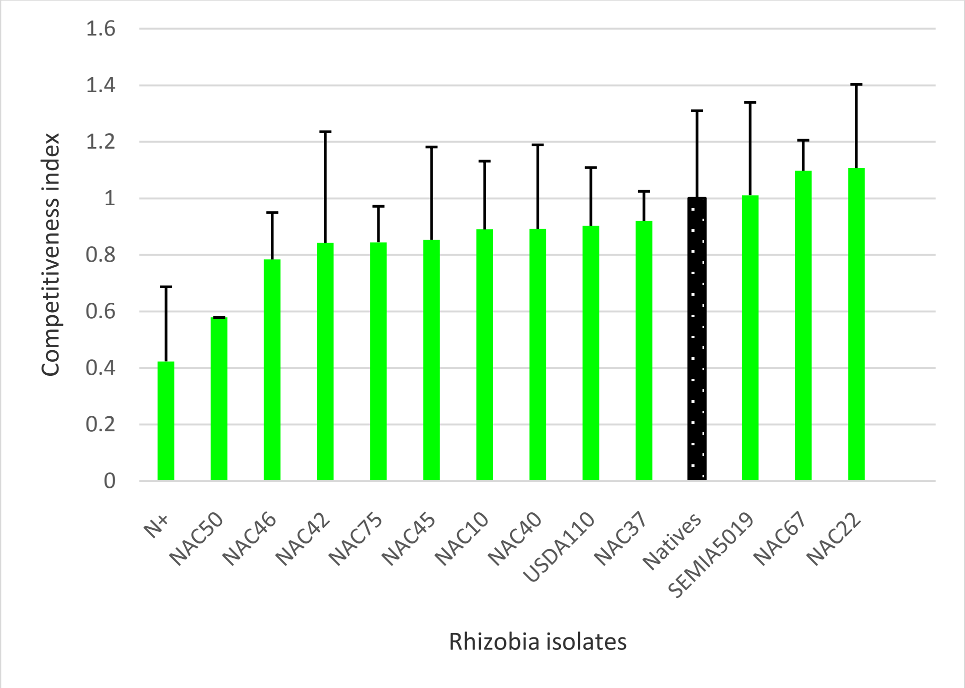

Figure1. Competitiveness index of local rhizobia isolates.

and Dilworth, 1970) was used for growth of Soybean. This study was conducted at International Institute of Tropical Agriculture (IITA) situated in Eastern DRC. A Split plot on Complete Randomized Design with four replicates consisting of 111 treatments was established in the greenhouse, 107 indigenous test isolates, two reference strains (SEMIA5019 and USDA110), and non-inoculated plants with and without mineral N. Two factors, soybean variety and rhizobia strains were under study and the strain was considered as main factor. Promiscuous soybeans SB19 and SB 24 were used as the test crop. Soybean seeds were surface sterilized (Somasegaran and Hoben, 1994), pre-germinated in sterile sand and three uniform seeds transplanted per pot, later thinned to two. Test isolates were cultured in Yeast Extract Mannitol broth (YMB) seven days in advance (Vincent, 1970), incubated at 28^{0}C until turbid and 1 ml of broth was applied to the roots of each plant. For the mineral nitrogen control, KNO3 (0.05%) was applied as described by Broughton and Dillworth, (1970). After eight weeks, nodulation was observed by careful recovery of roots and shoots were harvested, oven dried and weighted. An Effectiveness Index was calculated by dividing shoot biomass of test isolates by that of USDA 110. With this index and isolate performance relative to experimental controls, isolates were categorized as ineffective (less than -N control), partly effective (<75% of USDA 110), effective (75% or equal to USDA 110) or highly effective (>USDA 110) and ranked in ascending order.

Strains testing for competitiveness in potted field's soils

Soils collected from farm with no history of inoculation and soybean cultivation in Walungu and Katana in East DRC were used as media for competitive screening in the greenhouse. The soil was characterized for its chemical composition as follows. Soil pH was measured on a 1:2.5 soil suspension to water using a pH meter as described by Okalebo *et al., (*2002). Soil total N was determined by the Kjeldahl method and available soil P was determined by Olsen method (Okalebo *et al*., 2002). This experiment was established also in the greenhouse at IITA Kalambo station using the soil with the 12 best performing isolates from the first screening. This experiment utilized three liter PVC pot containers. Three seeds per plot were transplanted in the pot thinned later to two. YMB isolates preparation was done as described previously and the test crops included the two soybean promiscuous

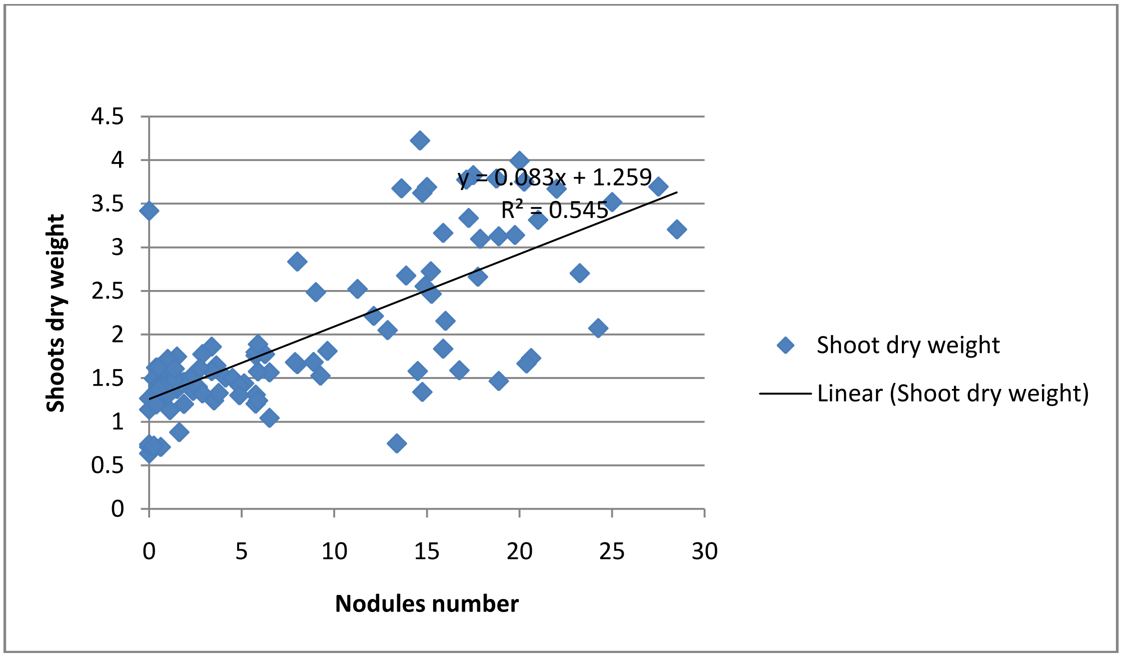

Figure 2. Relation between nodules number and shoot dry weight

varieties, SB24 and SB19. Indigenous rhizobia populations in the test soil were determined using the plant infection technique (Somasegaran and Hoben, 1994). Experimental units were arranged as a Split-Plot by varieties+ with four replicates. The factor strain was again considered as main factor. The soil was fertilized with Sympal, a commercially available blend for legumes (0-23-15 + Ca, Mg and S) at a rate of 500 kg ha-1 mixed with two kg of soil pot-1. Pots were regularly watered with rhizobia-free water. After eight weeks, plants were carefully uprooted, nodules observed, shoots, roots and nodules recovered, oven dried at 70°C for 48 hours and plant biomass recorded. Data was recorded in an excel spread sheet and submitted to the analysis of variance and LSD was used to make comparisons among the means at p (0.05) level of significance using GENSTAT software version 16.

RESULTS

On sterile media, significant differences in plant greenness and nodules score were observed ($P<0.001$) among plants inoculated with different strains and non-inoculated controls (−N treatments) while tested in aseptic conditions. The scores for greenness showed that plants inoculation with 10% of isolates improved the green color than the un-inoculated without nitrogen control but lower than plus N control (data not presented). The nodule scores were higher with these same isolates, and were positively correlated ($r=0.65$) to the plant greenness ($p<0.001$). Nodules were observed on all varieties for the majority of isolates. Only the treatment +N and –N produced any nodule. There were more nodules on the variety SB24 than SB19 ($p<0.046$). The nodules induced by different isolates were highly different ranging from 1 to almost 30 ($p<0.001$). The isolates NAC42, NAC40, NAC46, NAC19 and NAC66 produced the highest nodules number. There was no significant interaction in nodulation response between the soybean variety and the rhizobial isolates ($p<0.667$). The shoot weight also varied significantly between strains ($p<0.001$) and the two varieties ($p<0.006$). The interaction of the two factors was significant ($p<0.019$) on the shoot weight. The effectiveness index of isolates NAC67, NAC45, NAC38, NAC22, NAC75, NAC51, NAC19, NAC50, NAC66, NAC40, NAC10, NAC42, NAC23, NAC46 and NAC 37 outperformed the reference strain USDA110 as showed in the table 3 and were considered for testing in potted field soils. Nodules number and plant shoot weight highly correlated ($r=0.73$ and $p<0.001$). The indigenous rhizobia were classed into categories basing on effectiveness index (EI); highly effective, effective and Ineffective, where Effectiveness Index (EI) = isolate shoot dry weight/USDA 110 shoot dry weight. Highly effective indigenous strains outperformed the commercial strain USDA 110.

Ten best performing isolates in sterile conditions were selected to be tested for competitiveness in two sites potted soils in the greenhouse. The results of soil analysis showed that the Kalehe pH is around the neutrality while the Walungu pH is acidic. The Kalehe soil had a pH of 6.57 and contained 0.29 g N kg $^{-1}$ while the Walungu soil had a pH of 5.1. The total Nitrogen is 0.29% in Kalehe soils while this is 0.17% in Walungu soils. ^{1}, Extractable P content was 14.32 mg kg $^{-1}$ and 6.29 mg kg $^{-1}$ at Kalehe and Walungu, respectively and the rhizobial cell concentration in the soil was 10^3 and 10^2.

Nodulation of promiscuous varieties with indigenous bradyrhizobia isolates across treatments were observed on all varieties at both sites soils and for all treatments except the +N treatment, where nodules were very rare. The nodules number was positively correlated to the plant shoot weight ($p<0.001$ and $r=0.46$) as shown in figure 1. There were more nodules per plant and shoot dry weights at Kalehe than Walungu soils ($p<0.001$). The highest nodules number and shoots dry weights were recorded with the rhizobial isolates NAC22, NAC40, NAC37, NAC10, NAC67 and NAC75 in both sites soils compare to the commercial strain USDA110 (table 2). When averaged over treatments, nodule numbers ranged from 7.0 to 170 nodules, fresh weight ranged from 0.25 to 3.19 grams. There was a significant interaction ($P<0.05$) between the soil site and type of rhizobial strain in nodules number and shoot dry weight. In general, the nodules number and shoot dry weight were higher in Kalehe soil than Walungu with most of the isolates on the two test varieties. The local rhizobia strains were classed into four classes: Competitive and highly effective, Less competitive and highly effective, Less competitive and less effective, Competitive and less effective.

DISCUSSION

For the first experiment in sterile sand, 107 strains were tested but only ten strains were classified as highly effective compared to commercial strain and to non-inoculated control with nitrogen. The same results were obtained by Waswa *et al.,* (2015) in their study on identifying elite Rhizobia strains for soybean (*glycine max*) in Kenya. Among one hundred indigenous isolates tested only 10% was higher effective compared to the reference strains. Musiyiwa *et al.* (2005) found the same results. From the 129 indigenous isolates tested, only three isolates had significantly higher nitrogen fixing potential in comparison to the commercial strains. The number of effective rhizobia is Very low in tropical (Sanginga *et al.,* 2000) and a key to overcoming their competitive advantage is through the composition and delivery of legume inoculants (Thies *et al*., 1991).

The results showed that there was a significant difference in green color of plants inoculated with different rhizobial isolates compared to the non-inoculated control without nitrogen. This elucidates the effectiveness of these indigenous strains isolated from South Kivu soils in nitrogen fixing. Nitrogen is the major constituent of chlorophyll that confers green color to the plant. Nitrogenous compounds resulting from nitrogen fixation process are exported from root nodules in the form of ureides (allantoin and allantoic acids) and translocated to the leaves where they are catabolized and used for the biosynthesis of chlorophyll (Winkler *et al.,* 1987). This parameter of measuring the nitrogen fixing ability of different strains must be validated by other parameters.

The difference in shoot weight produced by different varieties is due to the genetic variation in biomass production. The same results were obtained by Appunu *et al.,* (2008) in their study on the variation in symbiotic performance of *Bradyrhizobium japonicum* strains and soybean cultivars under field conditions. By this study the biomass accumulated by different cultivars was highly different. The same differences have been experimented by Abaidoo *et al.,* (1999) and Jemo *et al.,* (2006).

The difference in nodulation as well as in shoot dry weight observed with different indigenous isolates is due to the difference in the genetic but also in effectiveness of each strain since the work was conducted in the greenhouse, where some climatic variations were controlled. Abaidoo *et al.* (2007) classified the isolates tested into four symbiotic phenotypic groups based on their symbiotic effectiveness as follows: ineffective, less effective, moderately effective and effective. The group less effective was constituted by isolates that were likely to have caused rhizobiotoxine-induced chlorosis on the soybean genotypes.

Abaidoo *et al.* (1999) stated that the indigenous rhizobia that nodulates promiscuous soybeans are present in low numbers in many soils. Tropical soils are often rich in less-effective, native rhizobia and a key to overcoming their competitive advantage is through the composition and delivery of legume inoculants (Thies *et al.* 1991).

Some isolates induced higher shoot dry weight compared the nitrogen plus control and this confirmed observations by other authors in which the application of small amounts of N-fertilizer did not provide a major benefit. Even more, it was observed that the application of 200 kg N/ha did not improve seed yields in comparison with soybean rhizobial inoculation, as it was previously demonstrated (Hungrı´a *et al.*, 2006; Albareda *et al*., 2009). Rhizobium inoculation is a cheaper and usually a more effective agronomic practice for ensuring and adequate supply of nitrogen for legume-based crop than the application of fertilizer nitrogen (Marufu *et al.,* 1995).

The interaction between the variety and the rhizobial isolates was also observed on shoot dry weight. This may be explained partly by the host specificity of some rhizobial strains and soybean germoplasm. Though the two test varieties are promiscuous, some rhizobial isolates may prefer one to another. The difference in preference may be due to the quality and quantity of exudates produced by different varieties.

The potted field soils experiment results showed that the nodules number and shoots dry weight produced by the tested rhizobial isolates varied largely according to the site soils. This difference between the two sites soils is due to differences in physico-chemical properties of the two sites soils used for potted experiment. In the Kalehe soils the pH was around the neutrality when in the Walungu soils the pH was acidic. The nitrogen content as well as phosphorous was in sufficient level in Kalehe soils than Walungu, for example phosphorous content was 14 mg Kg^{-1} in Kalehe soils against 6 mg Kg^{-1} in Walungu soils. Plant growth and micro organisms activity depend upon soil reaction and possible condition of the soil *i,e.* soil acidity, neutrality and alkalinity. Soil management practice, which build up organic matter content and arrest pH decline e.g. limiting are likely to create soil condition that encourage survival, persistence and higher population of *Rhizobium* in soil. Phosphorus is known to stimulate the *Rhizobial* growth (Subba Rao, 1999). A study conducted by Jemo *et al.,* (2006) showed that P application highly significantly increased shoot dry matter, P uptake, nitrogen fixation and grain yields of the grain legumes. Two of the soybean and two of the cowpea genotypes were more efficient at using P. Another study conducted by Wasike *et al.,* (2009) showed that P improved nodulation across tested varieties at both sites although the magnitude of this response was higher at Bungoma which had a low inherent soil P status and most of the nodules contained leghaemoglobin indicating active nitrogen fixation.

The significant interaction between site and treatments (rhizobial strains) in nodulation response at both sites suggests that some strains may be pH sensitive and may require relatively specific amount of phosphorus than others for optimal nodulation (Munns *et al.,* 1981). Soil type affects the ability of introduced organisms to colonize the rhizosphere or root soil interface (Kluepfel, 1993). It was reported that the survival of *Rhizobium leguminosarum* in natural soil was greatly affected by certain protozoa, fungi and bacteriophage (Chonkar and Subba Rao, 1966; Subba Rao, 1999) and the number in these organisms varied according to soils.

The study showed also that there is a high significant difference between the selected rhizobial isolates from South Kivu soils. Their differential abilities in nodules number and plant shoots dry weight might be due to their genotypic differences since soil and climatic variations were minimal. Six indigenous rhizobial isolates (NAC22, NAC40, NAC75, NAC37, NAC67 and NAC10) out-competed the commercial strain USDA110 in nodulation and dry matter production of the two promiscuous soybeans when soil was used as media and were classified as competitive and effective. Musiyiwa *et al.* (2005) reported the presence of indigenous rhizobia nodulating promiscuous soybean varieties in many soils in Zimbabwe. Some of the isolates were as good or superior in N2 fixation effectiveness to commercial inoculants strains under greenhouse conditions.

Among the tested isolates, some were classified as effective in sterile sand but less effective in potted-soils because of the presence of indigenous rhizobia. Streeter (1994) stated that the presence of *B. japonicum* in the inoculated soil sites might compromise nodulation competitiveness of the introduced rhizobia (Streeter, 1994). The survival of *Rhizobium* depends on their ability to compete favorably with indigenous soil *Rhizobia* and subsequently from a large proportion of nodule (Elkins *et al,.*1976). This may be also associated with ability of rhizobia to induce signals for nodulation with many types of soybean germoplasm (Martı´nez-Romero, 2003). Some authors stated that the survival of *Rhizobium* is lower in natural soil than in sterile soil or media. Soil is a complex matrix that is difficult to manipulate to control environmental factors and to prefect interaction with the indigenous microbial community (Young and Burns, 1993). From a study conducted by Saleh (2013) the highest number of *Rhizobial* population was observed in sterile soil than in non sterile, suggesting that the viability of *Rhizobium* was more in sterile soil.

The two varieties, SB24 and SB19 produced no significant differences in shoots weight. This suggested that as both of them are promiscuous, they nodulated samely with different isolates. This result is similar to that obtained by Appunu and Dhar (2006). They tested six soybean cultivars for variation in symbiotic performance with five *B. japonicum*. Analysis of data revealed that among the six cultivars tested, only two cultivars recorded higher dry matter accumulation after inoculation with different B. japonicum strains. The same average dry matter production was observed for other cultivars.

The significant interaction was observed between variety and site on nodules number. Soybean varieties differ significantly in their tolerance to acidity, salinity and different level of nutrients. Assa (2002) has studied the effect of salinity stress on growth and nutrient composition of three soybeans cultivars. By this study, there was a high significant difference on shoots dry weights between the three cultivars under different conditions.

The nodules number and plant shoots weight positively correlated suggesting that there is a clear response of the soybean variety to inoculation. Nodules number also positively correlated with the nodules dry weight highlighting the rhizobial isolates effectiveness observed in the sterile sand. This may be due to the fact that nodules of introduced effective strains are often numerous as well as of large sizeDifferent concentration of broth cultures used for inoculation was not considered in this study because in the study conducted by Albareda *et al.,* (2009), all determined parameters (nodule dry weight, seed yield and seed N content) of soybean inoculated with different isolates were not significantly different ($P < 0.05$) among the bacterial concentrations tested. In the case of USDA110, nodulation and seed yield of soybean were not statistically different when the inoculum rate exceeds 10^5 rhizobia/seed.

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REFERENCES

Abaidoo RC, Keyser HH, Singleton PW (2002). Population and symbiotic characteristics of indigenous *Bradyrhizobium* spp. that nodulate TGx soybean Genotypes in Africa. Challenges and imperatives for biological nitrogen fixation research and application in Africa for the 21st century. Ninth congress of the AABNF, edited by Karanja N, 167–188; John Philips Africa Limited, Nairobi.

Abaidoo RC, Dashiell KE, Sanginga N, Keyser HH, Singleton PW (1999). Time-course of dinitrogen fixation of promiscuous soybean cultivars measured by the isotope dilution method. Biol Fertil Soils (1999) 30:187–192.

Abaidoo RC, Keyser HH, Singleton PW, Dashiell KE, Sanginga N (2007). Population size, distribution, and symbiotic characteristics of indigenous Bradyrhizobium spp. that nodulate TGx soybean genotypes in Africa. Applied Soil Ecology 35 (2007) 57–67pp.

Albareda M, Rodriguez-Navarro DN, Temprano FJ (2009). Innoculation: dose, N fertilizer supplementation and rhizobial persistence in soil. Field Crops Research 113:352-356.

Appunu C, Dhar B (2006). Symbiotic effectiveness of acid-tolerant *Bradyrhizobium* strains with soybean in low pH soil. Afri. J. Biotechnol. 5: 842-845.

Appunu C, Sen D, Singh MK, Dhar B (2008). Variation in symbiotic performance of Bradyrhizobium japonicum strains and Soybean cultivars under field condition. Central

European Agriculture Journal 9: 185-190.

Assa TA (2002). Effect of salinity stress on growth and nutrient composition of three soybean cultivars. In Journal of Agronomy and Crop Science. Vol188. Issue2. 86-93.

Brockwell J, Bottomley PJ, Thies JE (1995). Manipulation of *rhizobial microflora* for improving legume productivity and soil fertility. A critical assessment. Plant Soil 174: 143-180.

Broughton WJ, Dilworth MJ (1970). Plant nutrient solutions: In Somasegaran P, Hoben HJ (eds). Handbook for rhizobia; Methods in Legume-Rhizobium technology. Niftal Project, University of Hawaii, Hawaii. 245-249.

Burton JC (1984). Legume inoculants production manual. NIFTAL, Hawai.

Catroux G, Alain H, Cecil R (2001). Trends in rhizobial inoculant production and use. *Plant and soil,* 230: 21-30.

Chonkar PK, Subba Rao NS (1996).Fungi associated with legumes root nodules and their effect on rhizobia. Canad.J.Microbial.58: 71-76.

Deaker R, Roughley RJ, Kennedy IR (2004). Legume seeds inoculation technology-review. Soil Biology and Biochemistry 36:1275-1288.

Elkins DM, HamiltonG, Chan CKY, Briskovich M.A, Vandeventer JW (1976). Effect of cropping history on soybean growth and nodulation and soil bacteria. Journal of Agronomy 68: 513-517.

FAO. FAOSTAT (2010): Agricultural Statistic Database, available at:<http://faostat.fao.org/faostat> access date: 21.mar.2011.

Fening JO, Danso SKA (2002). Variation in symbiotic effectiveness of cowpea bradyrhizobia indigenous to Ghanaian soils. *Applied Soil Ecology,* 21: 23-29.

Giller KE (2001). Nitrogen fixation in tropical cropping system 2ed. CAB International Wageningen, Netherlands.

Hartmann A, Giraud JJ, Catroux G (1998). Genotypic diversity of *Sinorhizobium* (formerly *Rhizobium*) *meliloti* strains isolated directly from a soil and from nodules of alfalfa (*Medicago sativa*) grown in the same soil. *FEMS Microbiology Ecology,* 25: 107– 116.

Hartman GL, West ED, Herman TK (2011). Crops that feed the world 2. Soybean-Worldwide production, use, and constraints caused by pathogens and pests. Food. Sec. (2011). 3 :5-17.

Howieson JG, Brockwell J (2005). Nomenclature of legume root nodule bacteria in 2005 and implications for collection of strains from the field. 14th Australian. Nitrogen Fixation Conference 17–23.

Hungria M, Franchini JC, Campo RJ, Crispino CC, Moraes JZ, Sibaldelli RNR, Mendes IC, Arihara J (2006). Nitrogen nutrition of Soybean in Brazil: contribution of biological N2 fixation and N fertilizer to grain yield. Canadian Journal of Plant Science 86: 927-939.

Jemo M, Abaidoo RC, Nolte C, Horst WJ (2006). Genotypic variation for phosphorus uptake initrogen fixation in cowpea on low phosphorous soils of Southern Cameroon. Journal of Plant Nutrition and Soil Science 169: 816-825.

Kluepfel DA (1993). The behavior and tracking of bacteria in the rhizosphere. Annual Review of phytopathology.31:441-472.

Koala S, Ngokho P, Woomer P, Karanja N, Baijukya F, Dashiel K, Machua J, Wafullah T, Mwenda G, Kisamuli S, (2010). Advanced technical skills in Rhizobiology: training report. www. N2Africa.org, 26pp.

Mapfumo P, Campbell BM, Mpepereki, S, Mafongoya PL (2001). Legumes in soil fertility management: the case of pigeonpea in smallholder farming systems of Zimbabwe. *African Crop Science Journal,* 9:629–644.

Martinez-Romero E (2003). Diversity of Rhizobium-*Phaseolus vulgarus* symbiosis: overview and perspectives. Plant and Soil 252: 11-23.

Marufu L, Karanja NK, Ryder M (1995). Legume inoculants production and use in Eastern and Southern Africa. Soil Biology and Biochemistry 27: 735-735.

Mengel K, Kirkby EA (1987). Principles of plant nutrition. International potash institute Bern, Switzerland 350 pp.

Munns DN, Keyser HH (1981). Response of Rhizobium strains to acid and aluminiun stress. Soil Biology and Biochemistry 13: 115-118pp.

Musiyiwa K, Mpepereki S, Giller KE (2005). Symbiotic effectiveness and host ranges of indigenous rhizobia nodulating promiscuous soybean varieties in Zimbabwean soils. Soil Biol. Biochem. 37: 1169-1176. N2

N2 Africa report, (2010). Putting nitrogen to work for smallholder farmers. www. N2Africa.org.

Njeru PNM, Mugwe J, Maina I, Mucheru-Muna M, Mugendi D, Lekase JK, Kimani SK, Miriti J, Esilaba AO, Murithi F (2013). Integrating scientific and farmers' perception towards evaluation of rainfed agricultural technologies for sorghum and cowpea productivity in Central Kenya. Journal of Soil Science and Environmental Management 4:123-131.

Okalebo JR, Gathua K W, Woomer P (2002). Laboratory Methods of Soil and Plant Analysis: A Working Manual, Second Edition. TSBF, Nairobi, Kenya.

Ronner E, Franke AC, Vanlauwe B, Dianda M, Eden E, Ukem B, Bala A, Van Heerwarden J, Giller KE (2016). Understanding variability in soybean yield and responses to P fertilizer and rhizobium inoculants on farmers'field in Nothern Nigeria. Field Crop Research 186:133-145.

Saleh MA, Zaman S, Kabir G (2013). Nodulation of black gram as influenced by *Rhizobium* inoculation using different types of adhesives. Nature and Science 11: 152-157.

Sanginga, N., Thottappilly, G., Dashiell, K. (2000). Effectiveness of rhizobia nodulating recent promiscuous soybean selections in the moist savanna of Nigeria. Soil Biology and Biochemistry 32: 127-133.

Somasegaran P, Hoben HJ (1994). Handbook for *Rhizobia*: Methods in Legume-Rhizobium technology. Springer-Verlag, New York, USA, p 450.

Streeter JG (1994). Failure of inoculant rhizobia to overcome the dominance of indigenous strains for nodule formation. Can. J.Microbiol. 40: 1-11.

Subba Rao NS (1999). Rhizobium and legume root nodulation. In Soil Microbiology. Oxford and Publishing Co.Pvt.Ltd.New Delhi.p.407

Thies JE, Singleton PW, Bohlool BB (1991). Influence of the size and indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobial on field-grown legumes. Appl. Environ. Microbiol. 57:19-28.

Vincent JM (1970). A Manual for the Practical Study of the Root-Nodule Bacteria. Blackwell Scientific Publications, Oxford.

Waswa NM, Karanja NK, Woomer PL, Mwenda G (2015). Identifying elite rhizobia for soybean (*Glycine max*) in Kenya. African Journal of Crop Science 2:60-66.

Wasike VW, Vanlauwe B, Wachira F, Mungai NW, Mumera LM, Mburu HN, Sanginga, N, Lesueur D. (2009). Diversity and phylogeny of indigenous Bradyrhizobium strains nodulating promiscuous soybean varieties grown in lowland and highland sites in Kenya. African Crop Science Conference Proceeding 9: 371-311.

Winkler RG, Blevins DG, Polacco JC, Randall DD (1988). Ureides catabolism in nitrogen-fixing legumes. Trends in Biochemical Sciences 13: 97-100.

Young CS, Burns RG (1993). Detection, Survival and activity of bacteria added to soil. Soil Biochemistry. Marcel Dekker Inc.New York.p.263

Zengeni R, Mpepereki S, Giller KE (2006). Manure and soil properties affect survival and persistence of soybean nodulating rhizobia in smallholder soils of Zimbabwe. Appl. Soil Ecol. 32: 232-242.