Differential Response of Promiscuous Soybean to Local Diversity of Indigenous and Commercial Bradyrhizobium Inoculation Under Contrasting Agroclimatic Zones

Article *in* International Journal of Plant Production · October 2020

DOI: 10.1007/s42106-020-00117-1

Gilbert Koskey
Sant'Anna School of Advanced Studies

Ezekiel Mugendi Njeru
Kenyatta University

John Maingi
Kenyatta University

J. M. Kimiti
South Eastern Kenya University

International Journal of Plant Production
https://doi.org/10.1007/s42106-020-00117-1

RESEARCH

Differential Response of Promiscuous Soybean to Local Diversity of Indigenous and Commercial *Bradyrhizobium* Inoculation Under Contrasting Agroclimatic Zones

Simon Wambui Mburu[1,2] · Gilbert Koskey[1,3] · Ezekiel Mugendi Njeru[1] · Omwoyo Ombori[4] · John M. Maingi[1] · Jacinta Malia Kimiti[5]

Received: 9 December 2019 / Accepted: 27 September 2020

Abstract

Promiscuous soybeans are grain legumes that nodulate with diverse strains of indigenous *Bradyrhizobium* and play a significant role in biological nitrogen fixation through symbiosis. However, experiments on the potential use of promiscuous soybean varieties have recorded very low nodulation and poor nitrogen fixation probably due to ineffective native *Bradyrhizobium* isolates. Experiments were designed to investigate symbiotic nitrogen fixation of two promiscuous soybean varieties (SB8 and SB126) with indigenous *Bradyrhizobium* isolates in contrasting agroclimatic zones through greenhouse and field experiments. Inoculation of soybeans in the greenhouse had a significant ($p < 0.001$) effect on shoot and nodule dry weight. The best performing indigenous isolates RI9 and RI4 from the greenhouse study outperformed the commercial inoculant (Biofix) in symbiotic effectiveness with 119.17%, 142.35% and 101.01%, respectively. Inoculation in the field experiments showed a significant ($p < 0.0001$) increase in shoot dry weight and grain yield of promiscuous soybean. Agroclimatic zones showed significant ($p < 0.0001$) variability in above ground biomass of soybean due to inoculation. Despite the apparent promiscuity of the soybean varieties used, the response in nodulation suggests the cultivars grown under contrasting agroclimatic zones have a preference to specific *Bradyrhizobium* isolates.

Keywords *Bradyrhizobium* · Eastern Kenya · Promiscuous soybean · Smallholder farmers · Symbiotic effectiveness

Introduction

Soybean (*Glycine max* L. Merril) is an important grain legume cultivated for its ability to fix nitrogen through a symbiotic association with *Bradyrhizobium* benefiting different cropping systems. Apart from providing human food and animal feeds, soybeans generate income to farmers thereby improving their social-economic status (Njeru et al. 2013). Soybeans are also important components of intercropping systems with plants such as maize (*Zea mays* L.) due to their drought tolerance, nitrogen-fixing capacity and shade tolerance (Polthanee et al. 2011). Studies have justified the positive impact of promiscuous soybean symbiosis with *Bradyrhizobium* in enhancing biological nitrogen fixation (BNF) (Thuita et al. 2012). However, despite the availability and use of commercial inoculants, improved soybean production has not been achieved due to the ineffectiveness of the inoculants in the soil, lack of inoculant adaptability to local conditions and competition from resident *Bradyrhizobium* (Abaidoo et al. 2007) and also *Bradyrhizobium* strains are host-specific (Kühling et al. 2018).

Globally, there is a gradual increase in soil degradation and nutrients depletion and this has become a serious threat to sustainable food production (Gomiero 2016). Although there's a need to increase crop yield through the use of chemical fertilizers, their cost is continuously increasing

✉ Ezekiel Mugendi Njeru
njeruezek@gmail.com

[1] Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, Nairobi, Kenya

[2] Department of Biological Sciences, Chuka University, Chuka, Kenya

[3] Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy

[4] Department of Plant Sciences, Kenyatta University, Nairobi, Kenya

[5] Department of Forestry and Land Resources Management, South Eastern Kenya University, Kitui, Kenya

making them unaffordable and uneconomical to the majority of smallholder farmers (Njira et al. 2013). In addition, intensive application of chemical fertilizers results in environmental problems and interference with the soil and water ecosystems. To ensure environmental integrity and high food production, it is crucial to manage soil fertility and establish sustainable agricultural systems that are affordable to smallholder farmers (Njeru et al. 2013). Therefore, production of promiscuous soybeans in this regard would be a viable and potential option of replenishing soil fertility through BNF for enhanced food security (Pagano 2016).

According to Tefera (2010), promiscuous soybeans reduce input cost for resource-poor smallholder farmers by the formation of functional nodules with native *Bradyrhizobium* thereby enhancing soil fertility through BNF. Njeru et al. (2013) documented that soybean production by smallholder farmers in the tropics receive minimal or no inoculants and thus, the plants depend entirely on nodulation by *Bradyrhizobium* naturally present in the soil. However, there is still a gap to be established in terms of quantification of the amount of nitrogen fixed since symbiotic performance in the association depends on both effectiveness and the population of the *Bradyrhizobium* in the field. According to Khojely et al. (2018), the major factor deterring the adoption of promiscuous soybeans despite their potential on BNF are the lack of information and knowledge on their effectiveness and interaction with native and exotic *Bradyrhizobium*.

Although the promiscuous soybean varieties can nodulate freely with different *Bradyrhizobium* strains, studies have shown that they do not meet the expected potential yield in most Sub-Saharan Africa soils due to nitrogen limitation (Tefera 2010). The problem has been contributed by the presence of highly competitive indigenous *Bradyrhizobium* strains in the soil that are poor nitrogen fixers, which prevent effective strains from occupying the nodules (Rechiatu et al. 2015). Effective BNF in soybeans can be achieved by identifying superior indigenous isolates with high competitive ability against ineffective *rhizobium* in the soil. Tefera (2010) observed that a single cultivar could not nodulate effectively in all locations with indigenous *Bradyrhizobium* present in the soil. Therefore, there is a need to evaluate the compatibility of promiscuous soybeans with local diversity of indigenous *Bradyrhizobium* strains, which will enhance sustainable legume production in smallholder farming systems.

In this study, we hypothesized that promiscuous soybean varieties have preference to specific local diversity of indigenous *Bradyrhizobium* for nodulation and symbiotic nitrogen fixation. The study objectives were to: (1) evaluate the effect of inoculation of two promiscuous soybean varieties (SB8 and SB126) with different indigenous *Bradyrhizobium* isolates; (2) determine the effectiveness of commercial inoculants over indigenous isolates with promiscuous soybeans and (3) to assess the effect of indigenous *Bradyrhizobium* inoculants in different agro-climatic zones.

Materials and Methods

Experimental Sites

Field experiments were carried out in Embu (0.53°S, 37.45°E) and Tharaka-Nithi (0.30°S, 38.06°E) Counties in Eastern Kenya. The study areas receive bimodal annual precipitation ranging from 600 to 1800 mm. The land use depicted smallholder-farming systems characterized by fragmented, small farm sizes. The dominant crop comprised of maize, soybeans, common beans, cowpeas, bananas, sugarcanes coffee and tea. The soil was characteristically acidic with moderate to low fertility (Njeru et al. 2013). The study site in Embu had maize planted in previous season using di-ammonium phosphate (DAP) fertilizer while in Tharaka-Nithi, the study sites had history of animal manure application for fertilization. All the study sites had no history of *Bradyrhizobium* inoculation in soybean production. The fields in Embu County were located in two agroclimatic zones namely, Embu Upper Midland Zone (EUMZ) (1500–2000 m asl) and Embu Lower Midland zone (ELMZ) (1000–1500 m asl).

Tharaka-Nithi County is on the South-Eastern side of Mt. Kenya. The temperatures range from 11 to 25 ℃. The area receives low rainfall that ranges between 200 and 800 mm per year, which is bimodally distributed. The Upper midland zone is characterized by maize, coffee, tea, common beans, cowpeas and soybeans while lower midland zone is dominated by maize, sorghum, common beans, peas, soybeans, millets, cassava, nuts, sugarcane and sweet-potatoes (Mburu et al. 2016). The study sites had no history of *Bradyrhizobium* inoculation. The fields in the region were similarly located in two agroclimatic zones, Tharaka-Nithi Upper Midland Zone (TUMZ) (1500–2000 m asl) and Tharaka-Nithi Lower Midland zone (TLMZ) (1000–1500 m asl.

Soil Sampling and Analyses

Soil sampling was carried out by collecting soil across and diagonally from 20 points in every selected farm before the rains. The soil samples were collected from a depth of 5–20 cm using a soil auger. A sub-sample (1 kg) of the composite was packed independently and was analyzed for physicochemical parameters using standard protocols. Soil pH was determined using a pH meter in a prepared soil-water suspension 1:2.5 while hydrometer principle was used to determine soil texture and particle size distribution according to Nelson and Sommers (1982). Soil phosphorus was extracted using Brays 1 procedure (Olsen and Sommers

1982). The Kjeldahl method was used to analyze nitrogen as described by Bremner and Mulvancy (1982). Walkley-Black combustion method was used to determine organic carbon while cation exchange capacity (CEC) was determined using Atomic Absorption Spectrophotometer (AAS) (Nelson and Sommers 1982).

Soils were characteristically acidic with pH ranging from 4.20 to 6.29 with soil from TUMZ having a relatively lower pH value compared to the other three sites. The soil %N ranged from 0.25% in TLMZ to 0.48% in EUMZ. Available phosphorus was 17.51 ppm, 27.00 ppm, 26.50 ppm and 21.00 ppm for soils from ELMZ, EUMZ, TUMZ and TLMZ, respectively. Exchangeable potassium ions (K^+) in soils ranged from 0.40 $cmol.kg^{-1}$ in ELMZ to 1.50 $cmol.kg^{-1}$ in TLMZ) while organic carbon in the soils was 2.98% in TUMZ, 2.49% in TLMZ, 3.29% in EUMZ and 3.06% in ELMZ. Clay content in the soils ranged from 21 to 53% with soil from EUMZ having the highest clay content. The soil texture from the farms in the study sites was either clay, sandy clay loam or sandy clay (Table 1).

Field Trap Cultures

Nodules were obtained from two soybean varieties (SB8 and SB126) planted in the fields located in EUMZ, ELMZ, TUMZ and TLMZ agroclimatic zones. The trap culture experiments were carried out during short rain season. The sub-plots measured 3 m by 3 m with four rows each and the plants were spaced 40 cm apart. Seeds of the same shape, size and color of the two soybean varieties (SB8 and SB126) were surface sterilized using 3% NaOCl prior to planting. Three seeds were planted in each hole and thinned to one plant after germination. The trap cultures were laid out in randomized complete block design (RCBD) with four replicates. The sampling was confined to the two inner rows in each plot where four nodulated plants were randomly excavated at the onset of flowering stage.

Table 1 Physico-chemical characteristics of soils from the study sites in Eastern Kenya

Properties	TUMZ	TLMZ	EUMZ	ELMZ
pH	4.52	5.42	6.31	5.01
OC (%)	2.98	2.49	3.29	3.06
N (%)	0.26	0.42	0.25	0.28
K (cmol/kg)	0.70	1.50	1.00	0.40
P (ppm)	26.50	21.00	27.00	17.51
Sand (%)	51	57	45	47
Clay (%)	35	21	53	41
Silt (%)	14	22	39	12
Texture class	Sandy clay	Sandy clay loam	Clay	Sandy clay

OC Organic carbon, *N* Nitrogen, *K* Potassium, *P* Phosphorus, *TUMZ* Tharaka-Nithi Upper Midland Zone, *TLMZ* Tharaka-Nithi Lower Midland Zone, *EUMZ* Embu Upper Midland Zone, *ELMZ* Embu Lower Midland Zone

Bradyrhizobium Isolation and Characterization

Indigenous *Bradyrhizobium* were isolated from root nodules using Yeast Extract Mannitol Agar (YEMA) media supplemented with Congo red (Somasegaran and Hoben 2012). The indigenous *Bradyrhizobium* isolates were then characterized based on Gram staining reactions, cultural responses on bromothymol blue and congo red and colony characteristics (Somasegaran and Hoben 2012). Isolates with typical *Bradyrhizobium* characteristics were grouped and authenticated by performing infection test using sterile vermiculite as the rooting medium and N-free medium in the greenhouse (Somasegaran and Hoben 2012). Nodulation was examined in both inoculated and uninoculated soybean seedlings after 28 days. Isolates that nodulated soybeans were considered as *Bradyrhizobium* and assessed for their symbiotic effectiveness in both greenhouse and field conditions.

Greenhouse Bioassays

Isolates were tested to determine their effectiveness on nodule formation and their compatibility with the selected promiscuous soybean varieties (SB8 and SB126) under a bacteriologically controlled environment. The study design was a complete randomized design (CRD) replicated four times. Each of the nine representative *Bradyrhizobium* isolates was tested for compatibility with the two promiscuous soybean varieties while uninoculated seeds served as controls. Symbiotic effectiveness assessment with promiscuous soybeans comprised five treatments as follows; commercial inoculant Biofix (BT), nine indigenous isolates (RI1 to RI9), a consortium of indigenous isolates (ICT), Biofix + indigenous consortium (BICT) and uninoculated control (UT). The ICT was prepared by mixing 1 ml of each of the nine individual *Bradyrhizobium* isolates cultured for three days in a broth media while BICT had consortium of all indigenous isolates and commercial inoculant Biofix in equal proportion. The Leonard jar assemblies were filled with rooting medium (vermiculite) and nitrogen-free growth media and sterilized for 15 min at 121 °C and pressure of 15 psi. Soybean seeds uniform in size, shape, and color were surface-sterilized and pre-germinated in Kilner jars with sterile vermiculite. The seeds were incubated at 28 °C until uniform early germination was achieved after 2–3 days. Seedlings whose radicle size was about 1–2 cm were transplanted to Leonard jar assemblies, two seedlings per Leonard jar with their radicle facing downwards in the rooting medium. Before inoculation, the plants were thinned to one plant per Leonard jar assembly. Each seedling was inoculated with 0.1 ml of broth media (approximately 1.0×10^9 $cell/plant^{-1}$) cultured for three days (Somasegaran and Hoben 2012). The plants were supplied with nitrogen-free medium while positive nitrogen controls plants were supplied with a sterile solution of 0.1 M KNO_3 at the rate of 80 mg of KNO_3 $plant^{-1}$ $week^{-1}$. After 45 days, soybean plants were carefully removed from the vermiculite and the nodules enumerated and dried at 28 °C to a constant dry weight. Shoots and roots dry weights were recorded after oven drying at 70 °C to a constant dry weight. In addition, macro-Kjeldahl method was used to estimate the nitrogen concentration of dried soybean plants shoots. Symbiotic effectiveness (SE) was established following the description of Beck et al. (1993).

$$SE = \frac{SDWofinoculatedsoybeanplants}{SDWofuninoculatedsoybeanplantstreatedwithnitrogen\left(0.05KNO_3\right)} X100$$

SE = Symbiotic effectiveness.

SDW = Shoot dry weight.

Field Experiment

The two promiscuous soybeans varieties (SB8 and SB126) were selected due to their suitability in African midland agroecological zones, high yielding, tolerance and resistance to pest and diseases (Tefera 2010). The two promiscuous soybeans were procured from Kenya Seed Company Limited, Nairobi, Kenya while commercial inoculant was obtained from MEA Company Limited, Nakuru, Kenya.

The experimental fields were demarcated and cleared of the prevalent weeds and plowed before planting. The size of each plot measured 3 m by 3 m with an alley of 0.8 m between the plots. Furrows for planting were made 5 cm apart and plant spacing was 20 cm. Five (5) treatments (RI9-Indigenous isolate, ICT-indigenous consortium, BT-commercial treatment, BICT-indigenous consortium + commercial inoculant and UT-uninoculated control) were set in a randomized complete block design (RCBD) replicated three times. The best performing indigenous *Bradyrhizobium* (RI9) was isolated from Embu Lower Midland Zone (ELMZ) trapped using soybean SB 8. Two promiscuous soybean varieties (SB8 and SB126) were used where seeds with uniform size, shape and color were treated with each inoculum. The promiscuous soybean seeds were inoculated with each inoculant from filter mud separately to avoid cross contamination under a shade. Gum Arabic was used as a sticker to ensure good contact between the seeds and the inoculant. To avoid cross contamination during planting, uninoculated seeds was planted before the inoculated seeds. Three seeds were sown per every hole and Triple Superphosphate fertilizer ($50\ kg\ ha^{-1}$) was applied to all plots to supplement phosphorus content in the soil and no nitrogen fertilizer was applied. After germination, thinning of seedlings was carried out when they attained at least two pairs of true leaves. One healthy and uniformly growing seedling was left per hole and standard agronomic management practices were applied.

Four plants were sampled randomly from each plot after 50% flowering. The plants were excavated carefully to obtain roots with intact nodules. Water was poured gently to wash off and sieve soil, which adhered onto the roots. The nodules were detached from the roots and their number per plant was recorded. To determine nodule dry weight, nodules were oven-dried at 28 ºC until constant dry weight was achieved. Shoot dry weight was also determined after oven-drying at 70 ºC to a constant dry weight. The dry shoots were ground to pass through 1 mm sieve in preparation for shoots nutrient analysis. Kjeldahl method was applied to determine the concentration of nitrogen as described by Bremner and Mulvancy (1982). Available phosphorus was extracted by Mehlich-3 procedure and analyzed using Atomic Absorption Spectrophotometer (AAS) (Nelson and Sommers 1982). To determine grain yield, the plants were harvested after attaining physiological maturity, which was after 120 days from planting. The plants were threshed, and seeds winnowed and air-dried to a constant dry weight. The seed dry weight for each plot was recorded and used in estimating grain yield per hectare.

Statistical Analyses

The data on the nodule dry weight, shoot and root dry weights, nitrogen concentration and available phosphorus were tested for homogeneity of variance using Bartlett test and then subjected to analysis of variance (ANOVA) using General Linear Model (GLM) procedures. Tukey's Honest Significant Difference (HSD) test was used at $p < 0.05$ to separate the means using version 9.1 of Statistical Analysis Software (SAS). Pearson correlation coefficient was used to determine the relationships between interactions of *Bradyrhizobium* isolates and plant growth parameters using Statistical Package for the Social Sciences (SPSS) version 22.0.

Results

Characteristics of Indigenous *Bradyrhizobium* Isolates

From the trap cultures, 39 distinct *Bradyrhizobium* isolates were obtained from SB8 and SB126 soybeans root nodules. All the *Bradyrhizobium* isolates were Gram-negative rods and majority (80%) of the isolates had a characteristic of fast-growing *Bradyrhizobium* in YEMA-BTB media. About 20% of the isolates turned BTB medium from deep green to blue due to production of alkaline substances in the medium. In addition, all the isolates absorbed little or no Congo red in the dark. Upon re-inoculation of the two soybean varieties during authentication, all the isolates initiated nodulation apart from isolates RI1 and RI2 and were therefore confirmed as soybean *Bradyrhizobium* (Table 2).

Greenhouse Bioassays

Effect of *Bradyrhizobium* Inoculation on Plant Biomass and Shoot Nutrients

Majority of the *Bradyrhizobium* isolates significantly ($F_{13,\,126} = 8.01$ $p < 0.001$) improved shoot dry weight of the two promiscuous soybean varieties tested. Inoculation with isolate RI9 recorded the highest significant enhancement in shoot dry weight (SDW) of 0.58 g plant^{-1} compared to the positive control treatment NT (0.43 g plant^{-1}). *Bradyrhizobium* isolate RI7 recorded a relatively low SDW although this was above the control treatment. Promiscuous soybean varieties had no significant effect on shoot dry weight plant^{-1} (Table 3).

Inoculation with *Bradyrhizobium* isolates significantly ($F_{13,\,84} = 10.50$, $p < 0.001$) enhanced shoot %N across the two promiscuous soybean varieties. However, differential response due to inoculation with *Bradyrhizobium* isolates was observed where RI9 and RI4 recorded the highest shoot %N of 2.58% and 2.47% respectively. *Bradyrhizobium* isolate RI6 and the control treatment (UT) recorded the lowest shoot %N. The promiscuous soybean varieties had no significant effect on shoot %N. *Bradyrhizobium* inoculation × soybean varieties interaction had no significant ($F_{26,\,84} = 0.20$, $p = 0.675$) influence on soybean shoots %N (Table 3). The shoots of SB8 had the highest amount of the available P (3633.45 ppm) while SB126 recorded the least amount of the available P (3556.48 ppm). Inoculation had a significant ($F_{13,\,84} = 5.49$, $p < 0.001$) effect on soybean shoot available P. Inoculated promiscuous soybean plants recorded slightly higher shoot available P compared to uninoculated plants (Table 3). The interaction between inoculation × soybean varieties had no significant effect on the amount of available P recorded.

Nodulation and Symbiotic Effectiveness

Inoculation with *Bradyrhizobium* significantly ($F_{13,\,126} = 3.18$, $p = 0.004$) enhanced nodule dry weight plant^{-1} relative to the control under greenhouse conditions. Isolate RI9 recorded highest nodule dry weight of 19.28 mg plant^{-1} followed by consortium with 8.73 mg plant^{-1}. The lowest nodule dry weight was recorded from the inoculation with *Bradyrhizobium* isolate RI6 and the consortium of indigenous isolates and the commercial inoculant BICT. Soybean varieties showed selective nodulation preference evident by the difference in NDW between the two varieties after isolate inoculation. Soybean varieties had significant effect on symbiotic effectiveness ($F_{2,\,126} = 1.85$, $p = 0.005$)

Table 2 Colony characteristics of the isolated *Bradyrhizobium* isolates

Isolate Characteristic	I	II	III	IV	V	VI	VII	VIII	IX
Congo red Absorption	Crna	Crna	Crna	Crna	Crna	Crna	Crna	Crna	Crna
BTB Reaction	Y	Y	Y	Y	Y	Y	Y	Y	Y
Margin	S	S	Sc	Sc	Sc	Sc	Sc	Sc	Sc
Color	Cw	Mw	Ww	Mw	Mw	W	Mw	Mw	Mw
Elevation	Rs	Dmd	Cvx	Rs	Cvx	Rs	Cvx	Cvx	Cvx
Gram Stain	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Transparency	O	O	T	T	T	T	T	T	T
Size (mm)	0.5	1	3.5	5	0.5	1.5	0.5	1.5	4
Colony shape	C	C	C	C	C	C	C	C	C
Texture	Sg	G	Sg	Sg	Sg	Sg	Fg	Sg	Sg
Percentage (%)	5	4	3	22	5	3	13	5	40

Crna congo red non-absorbing, *Y* yellow, *S* smooth, *Sc* smooth clear, *Cw* creamy white, *Mw* milky white, *Ww* watery white, *W* white, *Rs* raised, *Cvx* convex, *Dmd* domed, *-ve* gram negative, *O* opaque, *T* translucent, *C* circular, *Sg* soft gummy, *G* gummy, *Fg* firm gummy

Table 3 Average shoot dry weight (SDW), nodule dry weight (NDW), shoot %N, phosphorus (P) and symbiotic effectiveness (SE %) of soybean plants in the greenhouse

	SDW (g)	NDW (mg)	%N	P (ppm)	SE (%)
Varieties					
SB8	0.33 ± 0.03a	5.02 ± 2.27a	1.78 ± 0.15a	3633.45 ± 191.06a	74.06 ± 6.19b
SB126	0.31 ± 0.03a	2.99 ± 0.90b	1.75 ± 0.15a	3556.48 ± 182.20a	98.70 ± 10.10a
Isolates					
RI3	0.42 ± 0.07abc	1.43 ± 0.49b	1.65 ± 0.10bcd	4452.22 ± 190.90b	99.82 ± 20.19ab
RI4	0.48 ± 0.06ab	4.85 ± 1.56b	2.47 ± 0.23ab	3968.89 ± 461.35bc	119.17 ± 19.68b
RI5	0.32 ± 0.05bcde	1.16 ± 0.91b	2.35 ± 0.19ab	5381.11 ± 472.82a	81.25 ± 13.30bcde
RI6	0.28 ± 0.03bcde	0.37 ± 0.26b	0.67 ± 0.14d	3089.00 ± 420.13c	67.36 ± 12.49bcdef
RI7	0.19 ± 0.05cde	0.39 ± 0.35b	1.71 ± 0.18bcd	3160.56 ± 277.46c	39.62 ± 8.17cdef
RI8	0.35 ± 0.04abcd	2.21 ± 1.26b	1.46 ± 0.24bcd	3838.22 ± 359.69bc	84.84 ± 13.35abcd
RI9	0.58 ± 0.08a	19.28 ± 6.10a	2.58 ± 0.19ab	4498.33 ± 356.69b	142.35 ± 22.60 a
ICT	0.33 ± 0.04bcde	8.73 ± 8.00ab	1.83 ± 0.13bc	3641.67 ± 268.92bc	84.45 ± 12.92abcd
BT	0.43 ± 0.06abc	2.17 ± 0.84b	1.56 ± 0.36bcd	2455.67 ± 177.82 cd	101.01 ± 13.13ab
NT	0.43 ± 0.06abc	0	3.29 ± 0.24a	3373.11 ± 269.26c	100.00 ± 14.52ab
BICT	0.39 ± 0.04abc	0.38 ± 0.17b	1.90 ± 0.20bc	3384.67 ± 231.75c	94.45 ± 11.42abc
UT	0.09 ± 0.03e	0	0.57 ± 0.13d	3601.11 ± 253.86c	0
P-Values of the main factors and interaction					
Isolates	0.001	0.004	0.911	0.896	0.001
Variety	0.475	0.002	0.001	0.001	0.005
Variety × Isolates	0.809	0.866	0.675	0.362	0.715

Values within the same column without common letters differ significantly at Tukey's HSD $p < 0.05$. RI3 to RI9 represent *Bradyrhizobium* isolates tested in the greenhouse

ICT Consortium, *BT* commercial inoculant, *NT* nitrogen treatment (positive control), *BICT* Consortium + commercial inoculant, *UT* uninoculated (Negative control)

(Table 3). Among the test indigenous isolates, there was a significant variation in symbiotic potential where some isolates exhibited superior symbiotic performance ($F_{13,\ 126} = 6.94$ $p < 0.001$). Isolate RI9 and RI4 had the highest symbiotic effectiveness of 142.35% and 119.17%, respectively. The isolates RI3, RI5, RI6, RI7, RI8 and consortium ICT had lower symbiotic effectiveness compared to that of nitrogen treatment NT (Table 3).

Field Experiment

Effect of Inoculation on Shoot and Nodule Dry Weights

The average shoot dry weight was significantly ($F_{4,\ 80} = 6.94$, $p = 0.002$) influenced by *Bradyrhizobium* inoculation. Indigenous isolate RI9 and commercial inoculant BT recorded the highest average shoot dry weight plant^{-1}. The combination of commercial inoculant BT and consortium (BICT) also increased shoot dry weight and scored an average dry weight of 6.47 g plant^{-1}. The uninoculated control (UT) recorded the lowest shoot dry weight in both soybean varieties SB8 and SB126. Results on shoot dry weight from the field experiment showed significant ($F_{3,\ 80} = 15.34$, $p < 0.001$) difference in terms of agroclimatic zones. Tharaka-Nithi Upper Midland Zone (TUMZ) and Tharaka-Nithi Lower Midland Zones (TLMZ) recorded the highest overall shoot dry weight. There was no significant interaction between the inoculant and the two soybean varieties. However, strong interaction of varieties × zones was recorded where SB8 performed better in specific zones compared to SB 128 ($F_{3,\ 80} = 4.31$, $p = 0.007$) (Table 4).

The use of *Bradyrhizobium* inoculants increased NDW plant^{-1} significantly ($F_{4,\ 80} = 6.81$, $p = 0.001$) as compared to uninoculated control (Table 4). The commercial inoculant BT produced the highest NDW which was not significant when compared with the indigenous isolate RI9 when used as an inoculant. The combination of indigenous consortium and commercial inoculant (BICT) had an average nodule dry weight of 0.36 g plant^{-1}. Among the soybean varieties, there was no significance difference in terms of NDW although SB126 scored the higher NDW compared to SB8. The results obtained, showed that there was insignificant ($F_{4,\ 80} = 1.24$, $p = 0.301$) interaction on the effect of *Bradyrhizobium* inoculants and soybean varieties in regard to nodule dry weight plant^{-1}.

There was a strong positive correlation between nodule dry weight (NDW) and grain yield ($r = 0.580$, $p < 0.05$). Nodule dry weight also had a significant positive correlation

Table 4 Average shoot dry weight (SDW), nodule dry weight (NDW), shoot %N and shoot available phosphorus (P) of soybean plants from the field experiment

Treatments	SDW (g plant^{-1})	NDW (g plant^{-1})	Shoot %N	Shoot P (ppm)	Grain yield kg/ha
Zones					
TUMZ	6.34 ± 0.53b	0.28 ± 0.04a	2.91 ± 0.12a	3767.80 ± 119.31a	319.75 ± 18.30b
TLMZ	10.64 ± 0.89a	0.51 ± 0.12a	2.96 ± 0.11a	3694.30 ± 209.39a	1097.58 ± 37.32a
EUMZ	6.26 ± 0.59b	0.27 ± 0.04a	2.88 ± 0.12a	3403.83 ± 157.29a	1312.77 ± 64.38a
ELMZ	5.49 ± 0.54b	0.28 ± 0.04a	1.98 ± 0.12b	2589.03 ± 116.76b	207.23 ± 17.16b
Variety					
SB8	7.27 ± 0.58a	0.31 ± 0.04a	2.82 ± 0.10a	3209.50 ± 120.09b	967.88 ± 44.79a
SB126	7.25 ± 0.58a	0.37 ± 0.06a	2.55 ± 0.08b	3517.98 ± 126.13a	500.70 ± 21.35b
Bradyrhizobium inoculation					
RI9	8.74 ± 0.63a	0.50 ± 0.12a	2.93 ± 0.15a	3786.04 ± 164.02a	770.73 ± 50.59ab
ICT	7.47 ± 0.58a	0.24 ± 0.05b	2.66 ± 0.15ab	3023.17 ± 207.94bc	668.03 ± 59.30ab
BT	8.43 ± 1.26a	0.52 ± 0.07a	2.77 ± 0.13a	3383.92 ± 142.57ab	823.55 ± 75.12a
BICT	6.47 ± 1.26b	0.36 ± 0.07b	2.73 ± 0.11a	3726.88 ± 187.14a	899.48 ± 60.62a
UT	4.39 ± 0.43c	0.05 ± 0.02c	2.39 ± 0.16b	2898.71 ± 214.68c	509.47 ± 40.71b
P-Values of the main factors and interactions					
Zones	0.001	0.028	< 0.001	< 0.001	0.001
Variety	0.919	0.372	0.002	< 0.001	0.018
Brady In	0.002	0.001	0.002	0.039	0.001
Zone × Variety	0.007	0.118	0.004	< 0.001	0.002
Zone × Isolates	0.286	0.655	0.011	0.035	0.001
Variety × Isolates	0.681	0.301	< 0.001	0.039	0.269
Zone × Variety× Isolates	0.882	0.061	< 0.001	0.006	0.007

Values within the same column without common letters differ significantly according to Tukey's HSD at $p < 0.05$. RI9, Indigenous isolate; *ICT* Indigenous consortium, *BT* commercial inoculant, *BICT* Indigenous consortium + commercial inoculant, *UT* Uninoculated (control), Brady In, *Bradyrhizobium* inoculation, *TUMZ* Tharaka-Nithi Upper Midland Zone, *TLMZ* Tharaka-Nithi Lower Midland Zone, *EUMZ* Embu Upper Midland Zone, *ELMZ*, Embu Lower Midland Zone

Table 5 Pearson correlation coefficients between shoot dry weight (SDW), nodule dry weight (NDW), nodule number (NN), grain yield, shoot nitrogen concentration (%N) and phosphorus (P)

	NDW	NN	Grain yield	%N	P
SDW	0.489**	0.673*	0.580*	0.643**	0.257**
NDW		0.654*	0.495*	0.420*	0.143*
NN			0.141**	0.340*	0.143*
Grain yield				0.313**	0.403**
%N					0.425**

*, ** Correlation is significant at the 0.05 and 0.01 level respectively

with the shoot dry weight (SDW) and shoot %N (Table 5). In addition, a strong positive correlation was observed between nodule number (NN) and SDW ($r = 0.673$, $p < 0.05$).

Yields and Shoots Nutrients

Grain yield of soybean varieties varied significantly as a result of inoculation using *Bradyrhizobium* isolates. The commercial inoculant BT recorded the highest grain yield of 897 kg ha^{-1} although not statistically different from RI9 isolate which recorded 823 kg ha^{-1}. EUMZ and TLMZ recorded the highest grain yield of 1313 kg ha^{-1} and 1098 kg ha^{-1} respectively and differed significantly from that of ELMZ and TUMZ (Fig. 1). Soybean varieties varied significantly ($F_{1, 80} = 56.30$, $p < 0.001$) in grain yield. The zone and variety interaction was significant ($F_{3, 80} = 14.57$, $p < 0.001$) on grain yield where some zones showed higher grain yield of SB8 compared to SB126. Similarly, zone and isolates interaction differed significantly ($F_{12, 80} = 3.72$, $p = 0.002$) in grain yield per unit area (Fig. 1). Agroclimatic zones had a significant effect ($F_{3, 84} = 15.59$, $p < 0.001$) on shoot P. The highest shoot P value was recorded in TUMZ followed by TLMZ. There was significant difference in shoot P between the soybean varieties where SB126 recorded highest P value compared with SB8 (Table 4). Bradyrhizobia + commercial inoculant (BICT) and indigenous isolate RI9 increased shoot P with significant ($F_{2, 84} = 7.92$, $p < 0.001$) differences from other treatments (Table 4). Uninoculated control UT recorded lowest accumulation of shoot available P.

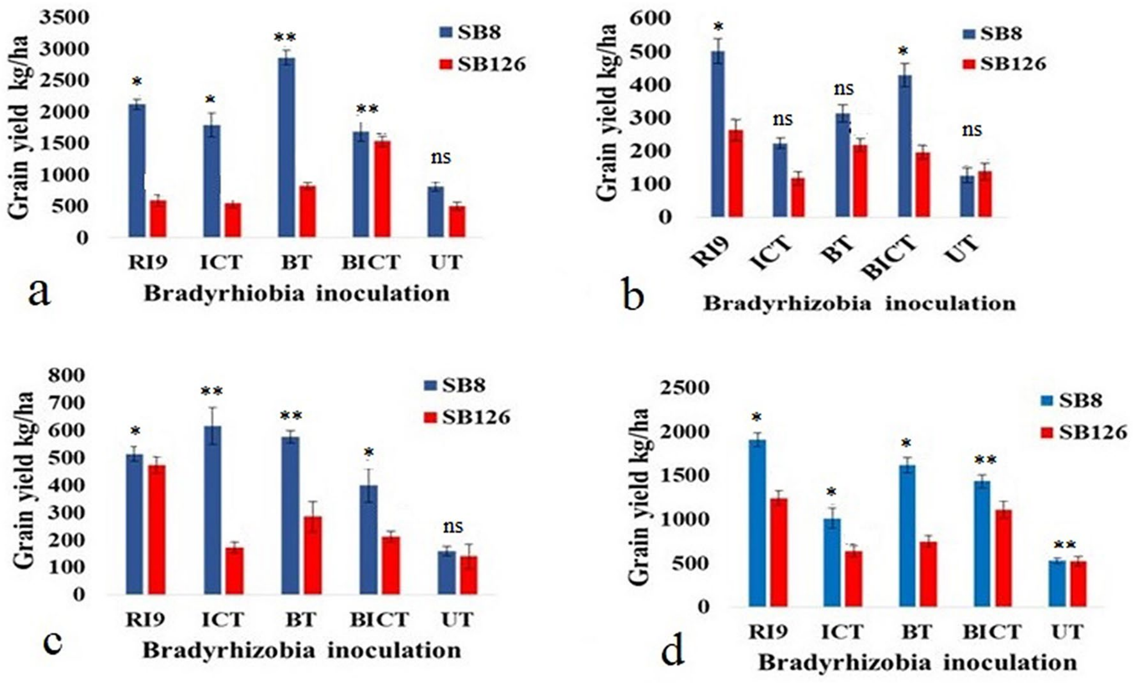

Fig. 1 Interactive effects of inoculation with indigenous *Bradyrhizobium* isolates and soybean varieties on grain yield. *Significant at the 0.01 level, **significant at the 0.05 level and ***significant at the 0.001 level. **a** TUMZ; **b**, TLMZ; **c**, EUMZ; **d**, ELMZ; RI9, Indigenous isolate; *ICT* Indigenous consortiuml, *BT* commercial inoculant, *BICT* Indigenous consortium + commercial inoculant, *UT* Uninoculated (control), *TUMZ* Tharaka-Nithi Upper Midland Zone, *TLMZ* Tharaka-Nithi Lower Midland Zone, *EUMZ* Embu Upper Midland Zone, *ELMZ* Embu Lower Midland Zone

Inoculation of soybean seed with indigenous *Bradyrhizobium* isolate RI9 showed the highest accumulation of shoot nitrogen concentration (2.93%) followed by commercial inoculant BT with 2.77%. The uninoculated control (UT) had the lowest shoot N of 2.39% (Fig. 2). The use of indigenous consortium + commercial inoculant (BICT) led to plants with high shoot N (2.73%) and was found to be competitive with the commercial inoculant (BT). The shoots % N of the two promiscuous soybean varieties significantly ($F_{13, 84} = 13.62$, $p < 0.001$) differed across agroclimatic zones. Among the zones that were tested, TLMZ recorded the highest shoot N of 2.96% while TUMZ had 2.91% being the second best (Fig. 2). However, ELMZ scored lowest shoot N (1.98%). The two soybean varieties had significant ($p = 0.002$) variation in shoot N concentration. There was a significant ($F_{13, 84} = 5.39$, $p < 0.001$) variety × inoculants interaction in shoot %N due to *Bradyrhizobium* inoculation. Similarly, there was a significant ($F_{3, 84} = 17.49$, $p < 0.001$) interaction between inoculant × zones.

Discussion

Isolation of *Bradyrhizobium*

The present study revealed the diverse nature of *Bradyrhizobium* isolates, which associated with the two promiscuous soybean varieties that were used during field trapping experiment. The high diversity of isolates depicts the promiscuity of the cultivars used and their ability to interact and form symbiosis with different resident *Bradyrhizobium* in the soil (Risal et al. 2010). The presence of different isolate morpho-types is also an indication that the study sites harbor a wide range of indigenous B*radyrhizobium* strains. Similarly, Bogino et al. (2011) reported higher number of native *rhizobium* isolates from field experiments using peanuts with different treatments. According to Abaidoo et al. (2007), *Bradyrhizobium* population in the soil depends on the crop and land use history;

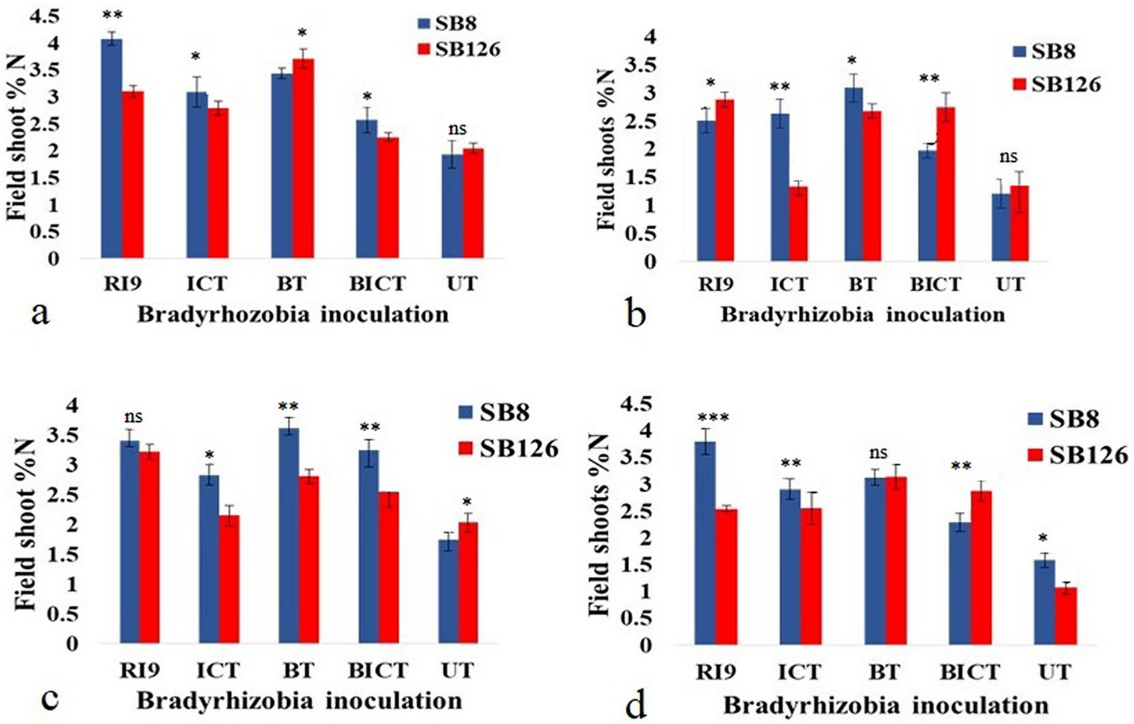

Fig. 2 Interactive effects of inoculation with indigenous *Bradyrhizobium* isolates and soybean varieties shoot %N. *Significant at the 0.01 level, **significant at the 0.05 level and ***significant at the 0.001 level. **a** TUMZ; **b**, TLMZ; **c**, EUMZ; **d**, ELMZ; *RI9* Indigenous isolate, *ICT* Indigenous consortium, *BT* commercial inoculant, *BICT* indigenous consortium + commercial inoculant, *UT* uninoculated (control), *TUMZ* Tharaka-Nithi Upper Midland Zone, *TLMZ* Tharaka-Nithi Lower Midland Zone, *EUMZ* Embu Upper Midland Zone, *ELMZ* Embu Lower Midland Zone

whether soybeans have been grown previously and the type of soil fertility management practices that have been used, for instance the use of inorganic fertilizers.

Effect of Inoculation on Nodulation and Symbiotic Effectiveness in the Greenhouse

Greenhouse bioassays showed that the seven native *Bradyrhizobium* isolates effectively nodulated with the test promiscuous soybean varieties. However, there was a variation on the level of nodulation in each soybean variety from one isolate to another, where some had high number of nodules compared to the others, which may be attributed to the interaction compatibility with the host soybean plants. Selective nodulation preference and nodule occupancy was observed in the two promiscuous soybean varieties tested in the greenhouse bioassay upon bradyrhizobia inoculation. The differential response was evident by the significant variation in nodule dry weights of the two promiscuous varieties. There was a clear enhancement effect of *Bradyrhizobium* inoculation on nodule dry weight, which depicts the importance of inoculation. Promiscuous soybean inoculation especially with indigenous *Bradyrhizobium* that are adapted and compatible with the local agro-climatic conditions may contribute to high nodulation compared to the commercial inoculant. Similarly, Delić et al. (2010) reported nodule dry weight increment as a result of inoculating Adzuki bean with native *B. japonicum*. Inoculation also had an effect on the two test soybean varieties on nodulation which suggest that it is vital and beneficial for smallholder farmers to use soybean seeds dressed with inoculants (Furseth et al. 2012).

Inoculation of the two promiscuous soybean varieties with *Bradyrhizobium* improved significantly the shoot dry weight. *Bradyrhizobium* inoculation enhances biological nitrogen fixation, which as a result improves shoot dry weight. This observation was similar to the findings reported in other studies (Sharma et al. 2012). Two of indigenous *Bradyrhizobium* isolates (RI9 and RI4) performed better compared to commercial inoculant Biofix on shoot dry weight. This suggest that indigenous isolates were more competitive than the commercial inoculant on BNF which may be attributed to the compatibility of indigenous *Bradyrhizobium* isolates with promiscuous soybean varieties used (Chibeba et al. 2017). In addition, Tefera (2010) reported that indigenous *Bradyrhizobium* isolates have the potential of establishing superior symbiosis with promiscuous soybean varieties in Africa obviating the need for commercial inoculants.

Inoculation significantly increased shoots %N compared to uninoculated controls in the greenhouse experiment. The accumulation of nitrogen in soybean shoots may be due to the presence of effective biological nitrogen fixation. Dhami and Prasad (2010) reported an increase in nitrogen uptake by soybean plants after *Bradyrhizobium* inoculation in sterilized soil. Commercial inoculant recorded low to moderate nitrogen accumulation in soybean leaves, which may be due to the difference in the isolate's ability to fix nitrogen with the soybean varieties used in our study. Similarly, Ampomah and Huss-Danell (2011) reported variable performance of *Bradyrhizobium* strains in the accumulation of nitrogen in cowpea shoots.

There was a significant increase in soybeans shoot P due to indigenous *Bradyrhizobium* isolate inoculation in the greenhouse experiment. *Bradyrhizobium* usually induce increased number of root hairs which favors nutrient uptake if there is no competition from other bacteria and may account for the increased phosphorus uptake. Hungria et al. (2013) reported a significant increase in nutrients due to inoculation of soybean plants with *Bradyrhizobium* compared to uninoculated treatments. In addition, some *Rhizobium* isolates have greater potential of solubilizing precipitated phosphorus, which enhances nutrients uptake (Klogo et al. 2015). Soybean SB126 responded better in the symbiotic efficiency compared to SB8 variety. This is probably because soybean SB8 might not have been compatible with most of indigenous isolates hence low nodulation, which suggests the need for cultivar selection for the farmers. Javaid and Mahmood (2010) in their study on nitrogen fixation of soybeans with strains of *B. japonicum* reported variations in dry matter and nodule nitrogen content among the soybean varieties with different soil amendments. The genomic variations of bacteria, the host plant or a combination of both, regulate symbiosis and compatibility may be attributed to the differences observed in the study (Dwivedi et al. 2015).

Symbiotic Effectiveness of Promiscuous Soybeans in the Field Experiment

The field results showed that nodule dry weight was significantly enhanced because of inoculation. For effective nodulation, *Bradyrhizobium* infect more root hairs which enhance nodulation where a large number of nodules contribute to high nodule dry weight (Pule-Meulenberg et al. 2011). The efficiency of nodules and the host species in nitrogen fixation may account for the differences observed between the isolates. Kamara et al. (2016) reported an increase in nodule dry weight over the control treatment after soybeans were inoculated with *Bradyrhizobium* under field conditions. There was no variation on nodulation among the two soybean varieties. Nodule dry weight had a significant positive correlation with grain yield and shoot dry weight. Effective nitrogen fixation can be assessed through nodule dry weight (Clement et al. 2015).

There was a significant variation in shoot dry weight of the soybean varieties grown in different agroclimatic zones, where indigenous *Bradyrhizobium* inoculation outperformed commercial inoculant. Biological nitrogen fixation and plant growth depend on soil conditions such as pH and the interaction of soil microorganisms. Farm management practices that create soil condition that favor *Bradyrhizobium* survival and persistence have shown to increase shoot dry weight (Dhami and Prasad 2010). Soils from different zones have a complex composition matrix, which determine the stress subjected to the bio-inoculants (Kühling et al. 2018). The physical and chemical properties of the soils in the field were also different causing the *Bardyrhizobium* to depend on their adaptability to various conditions affecting the performance of commercial inoculant Biofix (Albareda et al. 2009). Guimarães et al. (2012) reported that certain soil conditions might compromise nodulation competitiveness of the inoculants used in cowpea production.

Inoculation of promiscuous soybeans significantly increased shoot dry weight compared to uninoculated controls. It is documented that inoculation increases biological nitrogen fixation among the soybean plants, which enhances shoot biomass accumulation (Klogo et al. 2015). Indigenous inoculants recorded higher shoot dry weight compared to the commercial inoculant and the consortium. The accumulation of above ground biomass varied possibly due to the differences in nitrogen-fixing potential among the isolates hence the need for inoculation with the most effective inoculant (Miransar 2016). The variation in shoot dry weight between the test inoculants may be due to the incompatibility of the isolates with the soybean varieties under the study. Muthuri et al. (2014) reported significant variations on promiscuous soybean response to inoculation among different soybean cultivars. The commercial inoculant did not perform better in enhancing shoot dry weight accumulation as compared to the indigenous inoculants and this may be due to competition with indigenous isolates where some act antagonistically against foreign inoculants. Lesueur et al. (2012) reported instances where commercial inoculants failed to nodulate effectively with legumes planted in different land-use systems.

Bradyrhizobium inoculation had an effect on shoot biomass of soybean plants grown in different agroclimatic zones except the varieties grown in ELMZ due to variety × zone interaction. The results suggest that the difference in soil physical-chemical properties may have affected inoculation, causing Shoot biomass variations in the zones. *Bradyrhizobium* RI9 was isolated from ELMZ region and SB soybean root nodules, which may explain why there was no variation in the same region while the effects differed significantly in other agroclimatic zones. This implies that some isolates may be more compatible to agroecological zones where they were isolated resulting to improved soil nutrient due to effective inoculation. Rechiatu et al. (2015) had similar results where soybean plants inoculated with *Bradyrhizobium* increased significantly above-ground biomass on some soybean varieties in specific sites studied. In contrast, Ndungu et al. (2018) documented virtually no significant variation in nodulation of cowpea grown in contrasting agroclimatic zones of some parts in Kenya.

There was shoot %N variation in different agroclimatic zones most probably due to variation in soil fertility and other soil chemical properties. Extensive application of nitrogen fertilizers may raise nitrogen concentration in the soil, which as a result affect the amount of nitrogen uptake in plants. According to Clement et al. (2015), use of nitrogen fertilizers and other farm nutrient inputs cause significant variations on shoot %N of legumes. Soybean varieties under this study had a varied accumulation of shoot %N, which may be due to the isolates' effectiveness on nitrogen fixation resulting from inoculation and growth nature of each soybean variety (Gyogluu et al. 2016). The SB8 variety, which had the highest shoot biomass was best on nitrogen uptake. Similarly, Lin et al. (2012) reported variations in bradyrhizobial strains inoculation with different soybean varieties.

Inoculation of soybeans with isolates resulted in significant accumulation of shoot %N. High nitrogen concentration due to inoculants could have been due to the presence of high nitrogen derived from effective symbiotic nitrogen fixation. Njira et al. (2013), reported that soybeans inoculated with *Bradyrhizobium* increased nitrogen uptake significantly in the field experiments. Inoculant application in legumes increases uptake of nitrogen according to Delić et al. (2010). However, some isolates recorded low to moderate accumulation of nitrogen concentration in shoots, which may be attributed to the difference in symbiotic effectiveness among the test isolates.

Conclusion

In this study, there were variations in nodulation and yield parameters of promiscuous soybean varieties used due to inoculation with a local diversity of indigenous *Bradyrhizobium* isolates. The study thus revealed that promiscuous soybean varieties used have preferences to specific *Bradyrhizobium* isolates despite their promiscuity to a wider local diversity. Indigenous *Bradyrhizobium* isolate RI9 had better performance both in the greenhouse and field experiments in symbiotic effectiveness and yield parameters compared to the commercial inoculant Biofix. This is an indication of the presence of indigenous *Bradyrhizobium* isolates with symbiotic superiority over commercial inoculant (Beyan et al. 2018). Considering the differences revealed on symbiotic effectiveness from the local diversity of *Bradyrhizobium* isolates, farmers should adopt the use of *Bradyrhizobium* inoculants that are effective on BNF and well-adapted to the region. Future studies should consider screening for functional identity to determine specific beneficial synergistic promiscuous soybean-*Bradyrhizobium* interactions with greater nitrogen fixation and yield output.

Acknowledgements The authors wish to thank Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for funding this study. The authors would like also to acknowledge smallholder farmers from Embu and Tharaka-Nithi Counties in Eastern Kenya for their participation and contribution during the study.

Funding This work was funded by Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) 5th Graduate Research Grant (GRG), Grant No. RU 2014 GRG-102.

Data availability The datasets used to support the findings of this study are available from the corresponding author on request.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

Abaidoo, R. C., Keyser, H. H., Singleton, P. W., Dashiell, K. E., & Sanginga, N. (2007). Population size, distribution, and symbiotic characteristics of indigenous *Bradyrhizobium* spp. that nodulate TGx soybean genotypes in Africa. *Applied Soil Ecology, 35*(1), 57–67.

Albareda, M., Rodríguez-Navarro, D. N., & Temprano, F. J. (2009). Soybean inoculation: Dose, N fertilizer supplementation and rhizobia persistence in soil. *Field Crops Research, 113*(3), 352–356.

Ampomah, O. Y., & Huss-Danell, K. (2011). Genetic diversity of root nodule bacteria nodulating *Lotus corniculatus* and *Anthyllis vulneraria* in Sweden. *Systematic and Applied Microbiology, 34*(4), 267–275.

Amin, N. (2014). Isolation and characterization of nodule bacteria from mungbean and investigation its to drought water stress on soybean plant. *International Journal of Research and Reviews in Applied Sciences, 18*(2), 188–192.

Beck, D. P., Materon, L. A., & Afandi, F. (1993). Practical Rhizobium-legume technology manual. *Practical Rhizobium-legume technology manual*, (19).

Beyan, S. M., Wolde-meskel, E., & Dakora, F. D. (2018). An assessment of plant growth and N 2 fixation in soybean genotypes grown in uninoculated soils collected from different locations in Ethiopia. *Symbiosis, 75*, 189–203.

Bogino, P., Nievas, F., Banchio, E., & Giordano, W. (2011). Increased competitiveness and efficiency of biological nitrogen fixation in peanut via in-furrow inoculation of rhizobia. *European Journal of Soil Biology, 47*(3), 188–193.

Bremner, J. M., & Mulvaney, C. S. (1982). Nitrogen-Total1. *Methods of soil analysis. Part 2. Chemical and microbiological properties*, (2), 595–624.

Chibeba, A. M., Kyei-Boahen, S., de Fátima Guimarães, M., Nogueira, M. A., & Hungria, M. (2017). Isolation, characterization and selection of indigenous Bradyrhizobium strains with outstanding symbiotic performance to increase soybean yields in Mozambique. *Agriculture, ecosystems and environment, 246*, 291–305.

Clement, O. N., Lesueur, D., & Yusuf, A. A. (2015). Combined microbial inoculation as a promising approach to enhance promiscuous soybean nodulation and nitrogen content in Sudan Savanna. *International Journal of Sustainable Agricultural Research, 2*(3), 86–97.

Delić, D., Stajković, O., Rasulić, N., Kuzmanović, D., Jošić, D., & Miličić, B. (2010). Nodulation and N2 fixation effectiveness of *Bradyrhizobium* strains in symbiosis with Adzuki Bean, *Vigna angularis*. *Brazilian Archives of Biology and Technology, 53*(2), 293–299.

Dhami, N., & Prasad, B. N. (2010). Increase in root nodulation and crop yield of soybean by native *Bradyrhizobium japonicum* strains. *Journal of Plant Science, 6*(0), 8–11.

Dwivedi, S. L., Sahrawat, K. L., Upadhyaya, H. D., Mengoni, A., Galardini, M., Bazzicalupo, M., & Ortiz, R. (2015). Advances in host plant and *Rhizobium* genomics to enhance symbiotic nitrogen fixation in grain legumes. *Advances in Agronomy, 129*, 1–116.

Furseth, B. J., Conley, S. P., & Ané, J.-M. (2012). Soybean response to soil rhizobia and seed-applied rhizobia inoculants in Wisconsin. *Crop Science, 52*(1), 339.

Gomiero, T. (2016). Soil degradation, land scarcity and food security: Reviewing a complex challenge. *Sustainability, 8*(3), 281.

Guimarães, A. A., Jaramillo, P. M. D., Nóbrega, R. S. A., Florentino, L. A., Silva, K. B., & Moreira, F. M. (2012). Genetic and symbiotic diversity of nitrogen-fixing bacteria isolated from agricultural soils in the Western Amazon by using cowpea as the trap plant. *Applied and Environmental Microbiology, 78*(18), 6726–6733.

Gyogluu, C., Boahen, S. K., & Dakora, F. D. (2016). Response of promiscuous-nodulating soybean (Glycine max L. Merr.) genotypes to Bradyrhizobium inoculation at three field sites in Mozambique. *Symbiosis, 69*(2), 81–88.

Hungria, M., Nogueira, M., & Araujo, R. (2013). Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. *Biology and Fertility of Soils, 49*(7), 791–801.

Javaid, A., & Mahmood, N. (2010). Growth, nodulation and yield response of soybean to biofertilizers and organic manures. *Pakistan Journal of Botany, 42*(2), 863–871.

Kamara, A., Hartmann, A., Abdelgadir, A. H., Org, A., Ulzen, J., Abaidoo, R. C., & Masso, C. (2016). *Bradyrhizobium* inoculants enhance grain yields of soybean and cowpea in Northern Ghana. *Frontiers in Plant Science, 7*, 1770.

Khojely, D. M., Ibrahim, S. E., Sapey, E., & Han, T. (2018). History, current status, and prospects of soybean production and research in sub-Saharan Africa. *Crop Journal, 6*(3), 226–235. https://doi.org/10.1016/j.cj.2018.03.006.

Klogo, P., Ofori, J. K., & Amaglo, H. (2015). Soybean (*Glycine Max* (L.) Merill) promiscuity reaction to indigenous bradyrhizobia inoculation in some Ghanaian soils. *International Journal of Scientific and Technology Research, 4*(11), 306–313.

Kühling, I., Hüsing, B., Bome, N., & Trautz, D. (2018). Soybeans in high latitudes: effects of *Bradyrhizobium* inoculation in Northwest Germany and southern West Siberia. *Organic Agriculture, 8*(2), 159–171. https://doi.org/10.1007/s13165-017-0181-y.

Lesueur, D., Atieno, M., Mathu, S., & Herrmann, L. (2012). Importance of rhizobia in Agriculture: potential of the commercial inoculants and native strains for improving legume yields in different land-use systems. *EGU General Assembly, 14*, 632.

Lin, M. H., Gresshoff, P. M. M., Ferguson, B. J., Lin, Gresshoff, P. M., & Ferguson, B. J. (2012). Systemic regulation of soybean nodulation by acidic growth conditions. *Plant Physiology, 160*(4), 2028–2039.

Mburu, S. W., Koskey, G., Kimiti, J. M., Ombori, O., Maingi, J. M., & Njeru, E. M. (2016). Agrobiodiversity conservation enhances food security in subsistence-based farming systems of Eastern Kenya. *Agriculture & Food Security*, *5*(1), 19.

Miransari, M. (2016). Soybean N fixation and production of soybean inocula. *Abiotic and Biotic Stresses in Soybean Production, 5*, 107–129. https://doi.org/10.1016/B978-0-12-801536-0.00005-0.

Muthuri, J. K., Ithinji, J. K., & Kirigiah, R. M. (2014). Symbiotic effectiveness of *Bradyrhizobium japonicum* USDA 110 and *Sinorhizobium fredii* USDA 191 on two different soybean cultivars. *European Scientific Journal, 10*(12), 329–337.

Ndungu, S. M., Messmer, M. M., Ziegler, D., Gamper, H. A., Mészáros, É, Thuita, M., & Thonar, C. (2018). Cowpea (*Vigna unguiculata* L. Walp) hosts several widespread bradyrhizobial root nodule symbionts across contrasting agro-ecological production areas in Kenya. *Agriculture, Ecosystems and Environment*, *261*(December 2017), 161–171. https://doi.org/10.1016/j.agee.2017.12.014.

Nelson, D. W., & Sommers, L. (1982). Total carbon, organic carbon, and organic matter. *Methods of soil analysis. Part 2. Chemical and microbiological properties*, (methodsofsoilan2), 539–579.

Njeru, E. M., Maingi, J. M., Cheruiyot, R., & Mburugu, G. N. (2013). Managing soybean for enhanced food production and soil bio-fertility in smallholder systems through maximized fertilizer use efficiency. *International Journal of Agriculture and Forestry, 3*(5), 191–197.

Njira, K. O. W., Nalivata, P. C., & Lowole, M. W. (2013). An assessment for the need of soybean inoculation with *Bradyrhizobium japonicum* in some sites of Kasungu district, Central Malawi. *International Journal of Current Microbiology and Applied Sciences, 2*(8), 60–72.

Olsen, S. R., & Sommers, L. E. (1982). "Phosphorus," in Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, 2nd Edn, eds A. L. Page, R. H. Miller, and D. R. Keeney (*Madison, WI: Soil Science Society of America*), 403–430.

Pagano, M. C., & Miransari, M. (2016). Production Worldwide. *Abiotic and Biotic Stresses in Soybean Production, 1,* 1–26. https://doi.org/10.1016/B978-0-12-801536-0/00001-3

Polthanee, A., Promsaena, K., & Laoken, A. (2011). Influence of low light intensity on growth and yield of four soybean cultivars during wet and dry seasons of Northeast Thailand. *Agricultural Sciences, 2*(2), 61–67.

Pule-Meulenberg, F., Gyogluu, C., Naab, J., & Dakora, F. D. (2011). Symbiotic N nutrition, bradyrhizobial biodiversity and photosynthetic functioning of six inoculated promiscuous-nodulating soybean genotypes. *Journal of Plant Physiology, 168*(6), 540–548.

Rechiatu, A., Nana, E.-M., & Clement, A. R. (2015). Response of soybean (*Glycine max* L.) to rhizobia inoculation and molybdenum application in the Northern Savannah Zones of Ghana. *Journal of Plant Sciences, 3*(2), 64–70.

Risal, C. P., Yokoyama, T., Ohkama-ohtsu, N., Djedidi, S., Sekimoto, H., Prasad, C., & Sekimoto, H. (2010). Genetic diversity of native soybean bradyrhizobia from different topographical regions along the southern slopes of the Himalayan Mountains in Nepal. *Systematic and Applied Microbiology, 33*(7), 416–425.

Sharma, M. P., Jaisinghani, K., Sharma, S. K., & Bhatia, V. S. (2012). Effect of native soybean rhizobia and AM fungi in the improvement of nodulation, growth, soil enzymes and physiological status of soybean under microcosm conditions. *Agricultural Research, 1*(4), 346–351.

Somasegaran, P., & Hoben, H. J. (2012). *Handbook for rhizobia: methods in legume-Rhizobium technology*. Springer Science and Business Media.

Tefera, H. (2010). Breeding for Promiscuous Soybeans at IITA. *International Institute of Tropical Agriculture*, (1), 147–162.

Thuita, M., Pypers, P., Herrmann, L., Okalebo, R. J., Othieno, C., Muema, E., & Lesueur, D. (2012). Commercial rhizobial inoculants significantly enhance growth and nitrogen fixation of a promiscuous soybean variety in Kenyan soils. *Biology and fertility of soils*, *48*(1), 87–96.