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Response of Soybean to Inoculation with *Bradyrhizobium spp.* in Saline Soils of Shinille Plains, Eastern Ethiopia

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Abstract: Soybean [*Glycine max* (L.) Merrill] is an important crop in Ethiopia. However, its productivity is constrained by a number of factors among which soil salinity is one the major problems. Therefore, field and greenhouse experiments were conducted to examine the effectiveness of exotic and locally isolated *Bradyrhizobium* spp. nodulating soybean in a saline soil containing high soil N in Shinille area, Somali region, Ethiopia. The treatments of the glasshouse experiment consisted of effective isolates of bradyrhizobia nodulating soybean (TAL-379, UK isolate, and isolate) and an improved genotype of soybean. The treatments of the field experiment consisted of three bradyrhizobia isolates and control check. All treatments were replicated three times. The results of the experiments showed that inoculation significantly improved nodulation, growth, and productivity of soybean over the control treatment. Among the inoculation treatments, isolate and UK isolate inoculation significantly ($P < 0.05$) improved the nodulation and grain yield of soybean over the TAL-379 treatment. All investigated traits, except grain yield, did not display significant differences in response to the inoculation treatments. This indicates soil properties measured and evaluated at the late stages of growth of the crop especially native soil nitrogen content was high. The regression analysis indicated significant association of nodule number and nodule dry weight with highest R^2 scored in isolate inoculation. The multiple regression analysis revealed that nodulation and plant tissue nitrogen concentration had strong relationships with grain yield, indicating the importance of symbiotic nitrogen fixation. Hence, inoculation of elite isolate of *Bradyrhizobium* sp. improved the yield of the soybean in saline soils. Although *Bradyrhizobium* inoculation improved remakablely the productivity of soybean, the yield gap is still very wide as compared to the potential yield reported elsewhere. Therefore, further research is required to improve the yield of the crop by diagnosing othr soil constraints in the region.

Keywords: *Glycine max* (L.) Merrill; Grain Yield; Inoculation; Isolate; Nodule Number; Nodule Dry Weight; Saline Soils

1. Introduction

Soybean (*Glycine max* (L.) Merr.) plays an important role in the global agricultural nitrogen cycles by facilitating biological fixation of atmospheric N into plant-available N in symbiotic association with *Bradyrhizobium*. The N_2 fixation potential of soybean varies ranging from 0 to 185 kg N ha^{-1} with an average value of about 84 kg N ha^{-1} (Russelle and Birr, 2004). However, soil stresses such as salinity can adversely affect N_2 fixation by influencing both the host plant and the bacteria (Rai, 1987). Legumes have long been recognized to be either sensitive or only moderately tolerant to salinity (Lauchli, 1984; Subbarao and Johansen, 1993), particularly when the nitrogen needed for the growth of these plants is derived from symbiotic atmospheric nitrogen fixation. Unlike their host legumes, rhizobia can survive in the presence of extremely high levels of salt and show marked variations in salt tolerance (Singleton *et al.*, 1982). The establishment of the *Rhizobium*-legume symbiosis has been also shown to be salt-sensitive (Rao *et al.*, 2002). Reduction of rhizobial survival and growth, hindering the infection process, suppressing nodule function, and reducing plant ability to photosynthesize, grow, and take up N have been observed in saline soils (Singleton *et al.*, 1982; Saxena and Rewari, 1992; Elsheikh and Wood, 1995). However, there is a better chance of improving symbiotic N_2 fixation through a simultaneous selection of both plant genotypes and *Rhizobium* spp. strains (Rai, 1983; Rai and Prasad, 1983; Rai *et al.*, 1985). Research is needed to identify soybean host-*Rhizobium* strain combinations capable of forming root nodules, and fixing nitrogen symbiotically under salt stress.

Although identification of well adapted soybean and potential areas for producing the crop has been extensively done in Ethiopia (Asrat *et al.*, 2001), little research has been conducted to address soil fertility problems affecting the production of the crop. Recently, a study has indicated that soils of Ethiopia have either nil or very few bradyrhizobia nodulating soybean (Anteneh, 2012), signifying the need to introduce competent symbiotic root nodule bacteria as inoculants. Several researchers reported that significant yield improvements by inoculating soybean with effective and competent bacteria (Joshi *et al.*, 1986; Zhang *et al.*, 2002; Egamberdiyeva *et al.*, 2004b). Abbasi *et al.* (2008) also reported that *Bradyrhizobium*

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inoculation increased soybean seed yield by 85% over an uninoculated control. The presence of high native mineral N in the soil may inhibit symbiotic N2 fixation mediated by host plant and the endosymbiont (Mendes *et al*., 2003; Hungria *et al*., 2006). Differences in symbiotic response to mineral N have been reported and may arise from both variation in the host plant and *Rhizobium* spp. Herridge *et al*., (1990) and Wu and Harper (1991) indicated that a high soil mineral N tolerant species of soybean produced a lower yield compared to N intolerant genotypes of soybean. A study conducted by Evans (1982) indicated the inhibition effect of naïve mineral soil N on biological nitrogen fixation varied significantly in different strains of rhizobia nodulating soybean. The author also indicated strain dependence of tolerance of functioning nodules to exposure to mineral N.

It is evident that some recommended soybean cultivars formed ineffective symbiotic associations with elite isolates of *Bradyrhizobium* (Diatloff and Brockwell, 1976), which has been indicated to be a significant strain-host incompatibility that could be related to geographic and phenological barriers (Howieson *et al*., 2005). Inoculation with exotic rhizobia produced inconsistent results in the Guinea regions of Nigeria in spite of the low population of indigenous rhizobia in the soil (Pal *et al*., 1985). This incompatibility of introduced exotic strains can be solved by selecting elite isolates of rhizobia nodulating soybean originated from different locations. Therefore, the objective of this study was to evaluatethe symbiotic effectiveness of exotic and localy isolated *Bradyrhizobium* spp. nodulating soybean in a saline soil with a high total soil N content.

2. Materials and Methods

2.1. The Experimental Site

The field experiment was conducted in the irrigated agricultural field (Shinille Agricultural demonstration site, Somali region, Ethiopia) which is semi-arid in nature. The soil has no history of inoculation with Bradyrhizobia strains and was never used for soybean cultivation. The site has instead been used for maize (*Zea mays* L.) and tomato production in the previous years. There was also no history of fertilizer application at this site. The experimental field is located at 09°41' N latitude and 41°51' E longitude with an elevation of 1079 meters above sea level. The soil is dominated by a sandy clay texture and increased amounts of clay further down at the lower depth. The extent of the Rhizobial population was estimated with the most probable number (MPN) method (Vincent, 1970) within two weeks of sampling, using a base dilution of 10 and the soybean variety solitaire as the trap host. The physico-chemical properties of soil were determined following the procedures compiled by Sahlemedhin and Taye (2000). The soil physico-chemical properties and the rhizobia population nodulating soybean in the area are indicated in Table 1.

2.2. The plant Material

The soybean genotype used in this study was obtained from Pawe Agricultural Research Center, Pawe, Ethiopia, and was already tested under field conditions of Ethiopia.

2.3. Rhizobial Strains

Rhizobial strains, *Bradyrhizobium japonicum* (TAL-379), *Bradyrhizobium* sp. (UK isolate) and *Bradyrhizobium* sp. (isolate) were used as inoculants. These rhizobial strains were obtained from Holleta Agricultural Research Center (UK isolate) and National Soil Research Center, Addis Ababa (TAL-379 and isolate). The strain had been previously tested for infectivity under a controlled environment in National Soil Research Center, Addis Ababa, Ethiopia.

2.4. Preparation of Inocula

Sterile fine filter-mud was used as a carrier after adjusting the pH to 6.7. *Bradyrhizobium* spp. were separately incubated in the yeast-extract mannitol (YEM) broth at 30^0C for 7 days until the number of cells ml^{-1} reached 10^9 for inoculant preparation. The liquid medium containing 400 ml of *Bradyrhizobium sp.* culture was added to 1 kg of a carrier and mixed thoroughly and packed in plastic bags. The filter-mud-base inoculum was incubated at room temperature for 15 days.

2.5. Soils for Pot Experiment

The soil used for the pot experiment was a saline soil, collected to the depth of 20 cm from an area where the field experiment was conducted. A plant growth medium containing soil from Shinille agricultural experimental site was developed based on three requirements: absence of indigenous bradyrhizobia nodulating soybean, high native mineral soil N, and salinity of the soil. The soil was collected and dried under aseptic conditions and no rhizobia were detected by a plant infection technique (Brockwell, 1963) at sowing.

2.6. Treatments and Experiment Design for the Pot Experiment

A pot experiment was conducted in the semi-controlled greenhouse at Haramaya University, Eastern Ethiopia in 2012. The treatment consisted of (i) isolate, (ii) UK isolate, (iii) TAL-379, (iv) N-fertilized pot (pots fertilized with 20 kg Nha^{-1}), and (v) negative controls (unfertilized and uninoculated pots), with three replications. The experiment was laid out as a completely randomized design (CRD) and three replications.

2.7. Experimental Procedure

To make the seed free from rhizobial contamination, soybean seeds previously selected for saline soil were surface-sterilized with ethanol (1min) and sodium hypochlorite (5 min) and then washed several times with deionized water. The seeds were sown in each pot.

Five seeds were sown per pot. One week after emergence, the soybean seedlings were thinned to three plants per pot. Pots were regularly and daily watered to 70% water-holding capacity (WHC), avoiding waterlogging. Rhizobia were cultured to exponential phase in YEM broth, and then 1 ml of culture containing 1 x 10^8 rhizobia was applied to 7-day-old seedlings using glass pipette. Plants were harvested after eight weeks of growth at the late flowering and early pod setting stage. They were removed from the pots, and the roots were thoroughly rinsed with water, blotted dry using filter paper, and the nodules picked and counted. Total plant and nodule dry weights were recorded after drying at 70^0C for 48 h.

2.8. Field Experiment

Field experiments were conducted at the experimental farm of Shinille Agricultural Demonstration site using well-structured drip irrigation system in 2012. The treatments consisted of four inoculations (UK isolate, isolate, TAL-379 and uninoculated control) and two soybean genotypes (Giza and TGx-1332464). The experiment was laid out as a split plot in a randomized complete block design with three replications. The inoculants constituted the main plots whereas the soybean genotypes were the sub-plots.

The land was prepared by deep ploughing, harrowing and leveling. Then the area was ridged and divided into 3 m x 3 m plots. Each soybean genotype was planted in 10 cm spacing between plants, 60 cm between rows, 1.5 m between sub plots and 2.0 m between main plots. Before planting, a 20 g of the different bradyrhizobia inoculant was added into different polyethylene bags containing 200 g of soybean seeds. Sugar solution (48%) was added to each bag to enhance proper mixing and adhesion of the *Bradyrhizobium* carrier material to the soybean seeds. Two seeds were sown per hill. Plots were immediately irrigated after sowing to ensure uniform germination. Subsequently, plots were irrigated by a drip irrigation system at a 7-day interval.

Weeds were controlled over the growth period with hand hoeing. A set of five plants from each plot was randomly selected at the late flowering and early pod setting stage for nodulation potential (number of nodules, dry weight of nodules) and shoot characteristics (shoot height, shoot dry weight). Dried shoot parts were ground and analyzed for total N using Kjeldhal method.

2.9. Data Collection and Measurement

At physiological maturity, plants were harvested from a 3.0 m x 2.40 m net plot leaving two guard rows to avoid edge effects. The plant tops (stalks plus pods) were weighed to determine total dry matter yield before threshing and winnowing to separate the seed, which was then weighed to determine yield. Leaves were not included in total dry matter yield determinations, as they had already senesced and fallen to the ground. Seed moisture was adjusted to 10% when determining grain yield (Kenodulenumbereth and Hellevang, 1995).

2.10. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the SAS computer software package. The least significant difference (LSD) test was used to separate means at 5% level of significance. Microsoft excel was used for drawing bar graphs and regression analysis.

Table 1. Soil analysis of experimental sites before sowing.

Soil property	Shinille soil	Rating	Reference
pH in H_2O	7.74	Moderately alkaline	Murphy (1968)
EC(mS/cm)	4.12	Moderately saline	FAO (1988)
Organic carbon (%)	2.15	Medium	Tekalign Tadese(1991)
Total nitrogen (%)	0.29	High	Tekalign Tadese(1991)
Available P(mg kg^{-1})	25.85	High	Olsen *et al.* (1954)
Ca (cmol(+)kg^{-1})	31.10	Very high	FAO(2006)
Mg (cmol(+)kg^{-1})	3.22	High	FAO (2006)
Na (cmol(+)kg^{-1})	0.14	Low	FAO (2006)
K (cmol(+)kg^{-1})	2.22	High	Berhanu Debele (1980)
CEC (cmol(+)kg^{-1})	25.90	High	Hazelton and Murphy (2007)
Zn(mg kg^{-1})	1.19	High	Jones and Benton (2003)
B(mg kg^{-1})	0.86	Low	Jones and Benton (2003)
Fe (mg kg^{-1})	2.22	Low	Jones and Benton (2003)
NH_4-N(mg kg^{-1})	26.22		
NO_3-N (mg kg^{-1})	23.0		
Clay (g kg^{-1})	27		
Silt (g kg^{-1})	50		
Sand (g kg^{-1})	23		
Textural class	Loam		
Number of soybean nodulating rhizobia	None		

3. Results and Discussion

3.1. Physico-Chemical Properties of the Soil

The soil analysis indicated pH of 7.74 with higher electric conductivity of 4.21 mS cm^{-1}. The soil organic carbon (SOC) and total N contents in the experimental soil were 2.1 and 0.2%, respectively, with no native rhizobia nodulating soybean. Except Fe content, the soil had medium to high contents of total N, SOC, available P, CEC, exchangeable bases and other tested micronutrients, and could be classified as saline with mild alkalinity (Hazelton and Murphy, 2007). The textural class of the soil is loam with higher silt content (Table 1).

3.2. Effect of Inoculation on Nodulation

The results of the field experiment revealed that inoculation (I) was found to affect significantly ($P < 0.05$) all investigated growth attributes of soybean (Tables 2 and 3). There was no nodule formation in the control treatment throughout the experimental period, indicating that there was no rhizobia nodulating soybean in the area. Although the Shinille soil had high native mineral N (Table 1), nodulation was significantly improved by inoculation of *Bradyrhizobium* sp. A significantly higher nodule number was obtained from the isolate inoculation followed by UK isolatein oculation over the control treatment (Tables 2 and 4). In contrast to this result, however, several studies, earlier indicated the negative effect of higher native soil mineral N on nodulation induction (Gibson and Harper, 1985; Herridge and Brockwell, 1988). Inhibition of soil N has been substantially ameliorated by increased numbers of rhizobia (Herridge and Brockwell, 1988). Gibson and Harper (1985) noted the importance of selection of *Bradyrhizobium* and soybean genotype for better nodulation at high soil N. This strategy has been unsuccessful because the selected genotypes of soybean have low grain yields (Herridge and Rose, 1994).

The results of this study have also revealed that the isolate and the UK isolate inoculations resulted in significantly higher nodule dry weight over the control treatment in the greenhouse and field experiments (Tables 2 and 4). Similar findings were reported for pot experiments by Okereke and Onochie (1996) who found that exotic *Bradyrhizobium* sp. enhanced the nodulation of soybean. This indicates the importance of inoculation for enhancing nodulation. However, the nodule number obtained from the field and greenhouse experiments was generally lower than those obtained from experiments conducted in Nigeria (Okereke *et al.*, 2001) but is comparable with nodules induced in calcareous soilin Uzbekistan (Egamberdiyeva *et al.*, 2004a). The lower nodule number obtained in this study could be attributed to the negative effect of higher EC on root growth which in turn reduces the sites of nodulation (Rao and Sharma, 1995) and absence of rhizobia nodulating soybean in the experimental site.

Among the inoculation treatments, TAL-379 inoculation resulted in statistically lower nodule number and nodule dry weight compared to the other inoculation treatments. However, an experiment conducted in Congo found that inoculation of TAL-379 enhanced nodule dry weight up to 336-382% over the uninoculated control treatment (Mandimba and Mondiboye, 1996). The lower performance of TAL-379 in the present study might be ascribed to its high sensitivity to saline soil which could reduce growth and multiplication of rhizobia and formation of nodules in soybean roots (Tu, 1981; Sprent and Zahran, 1988; Talbi *et al.*, 2013). El-Sheikh and Wood (1995) found that the salt-tolerant strain was more effective than the salt-sensitive strain under saline conditions. One of the main mechanisms of bacterial adaptation to hyperosmotic conditions is accumulation of compatible solutes, such as sugars, polyols, or amino acids (da Costa *et al.*, 1998). Generally, the present experiment produced relatively higher nodule dry weight as compared to nodule dry weight produced in soils having high native mineral N (Herridge *et al.*, 1984). Corroborating the results of this study, Okereke *et al.* (2001) reported nodule dry weights that were similar in amounts to the ones obtained in this experiment though the soil had native rhizobia nodulating soybean.

3.3. Shoot Dry Weight and Shoot Length

The shoot dry matter and shoot height at the late flowering and early pod setting stage produced by TAL-379 were in statistical parity with shoot dry matter and shoot height obtained from isolates that produced nodules well (UK isolate and isolate). Similar results were obtained in Nigeria (Okereke *et al.*, 2001; Hungria *et al.*, 2006). This implies that soybean plants that produced nodules poorly in response to inoculation with TAL-379 might draw mineral N in the soil during early growth, and perform equally well with the well nodulated soybean plants at the later stages of growth.

Under the greenhouse condition (pot experiment), UK isolate, isolate and N-fertilizer treatments induced significantly higher shoot dry matter when compared with TAL-379 inoculation and the control treatments (Table 4). This suggests the importance of symbiotic N_2-fixation even though the soil has a high content of native mineral N for growth and development of soybean at flowering stage. This result is consistent with a previous finding that confirmed that a symbiotic association between soybean and *Bradyrhizobium* fulfilled only 60% of N requirement of soybean (Schipanski *et al.*, 2010).

Table 2. Growth performance of soybean at late flowering and early pod setting stage in response of exotic and *Bradyrhizobium* inoculation under field condition.

Inoculation	NN	NDW (g plant^{-1})	SDW (g plant^{-1})	SL (cm)	PH (cm)
TAL-379	31.9 ±1.98c	0.3286 ± 0.0158b	65.3 ± 2.70a	71.0 ± 1.74a	79.4 ± 2.05a
UK isolate	44.4 ± 1.81b	0.4122 ± 0.0098a	62.7 + 3.45ab	67.0 ± 2.40ab	81.2 ± 2.06a
isolate	52.6 ± 2.36a	0.4377 ± 0.0121a	54.0 ± 2.28bc	59.3 ± 1.71c	80.7 ± 3.64a
Control	0.0 ± 0.0d	0.0000 ± 0.0000c	47.8 ± 1.66b	62.6 ± 1.72bc	67.2 ± 2.20b
Mean	32.2	0.2946	57.5	65.0	77.1
F value					
Inoculation (I)	166.99***	330.92***	9.15***	7.05***	6.68***
LSD (0.05)	6.7	0.0413	9.71	7.14	9.59
CV (%)	23.5	16.0	19.2	12.5	14.2
SEM±	7.587	0.047	11.059	8.135	10.925

****significant at 0.001; Nodule number = NN; Nodule dry weight (gplant^{-1}) = NDW; Shoot dry weight at late flowing and early pod setting stage (gplant^{-1}) = SDW; Shoot length at late flowering and early pod setting stage (cm) = SL; Plant height at harvest (cm) = PH.*

Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

Table 3. Growth performance of soybean at harvest in response of exotic and ly isolated *Bradyrhizobium* inoculation under field condition.

Inoculation	NPP	NSP	TBY (kg ha^{-1})	GY (kg ha^{-1})	TNC (%)
TAL-379	155.4 ± 6.36a	2.663 ± 0.0606a	7489.7 ± 262.4a	1881.51 ± 95.4c	4.0289 ± 0.0298a
UK isolate	149.6 ± 4.56a	2.643 ± 0.0098a	8060.7 ± 373.8a	2766.40 ± 70.5a	4.1578 ± 0.0490a
isolate	151.4 ± 2.56a	2.529 ± 0.0663a	7541.2 ± 413.8a	2398.25 ± 45.9b	4.0372 ± 0.0747a
Control	108.9 ± 3.70b	2.273 ± 0.0549b	5473.3 ± 191.3b	1520.72 ± 70.9d	3.7339 ±0.0810b
Mean	141.3	2.527	7141.2	2141.72	3.9890
F value					
Inoculation (I)	23.19***	9.43***	12.52***	57.18***	8.41***
LSD (0.05)	16.82	0.2173	1201.7	271.18	0.2315
CV (%)	13.6	9.8	19.2	14.4	6.6
SEM±	19.154	0.248	1368.8	308.9	0.264

****significant at 0.001; Number of pods per plant = NPP; Number of seeds per pod = NSP; Total biomass yield (kgha^{-1}) = TBY; Grain yield (kgha^{-1}) = GY; Tissue nitrogen concentration (%) = TNC.*

Notes. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

3.4. Yield and Yield Traits of Common Bean

Even though nodule number and nodule dry weight showed significant differences for the inoculation treatments, the data indicated statistically insignificant plant height at harvest, number of pods per plant, number of seeds per pod, total N concentration and total biomass yield among the inoculations treatments (Table 2 and 3). Supporting the results of this study, Serraj and Drevon (1998) indicated that plant species dependent on symbiotic N_2-fixation for their N nutrition are more sensitive to saline conditions than plants which obtain N from native soil. This may have consequently resulted in lower N derived from symbiotic N_2 fixation.

3.4.1. Number of pods per plant and number of seeds per pod

Significantly higher number of pods per plant and number of seeds per pod were produced in response to the inoculation over the control treatment (Table 3). Similar results were reported for number of pods per plant (Patra *et al*., 2012). The highest number of pods per plant (155.4) and number of seeds per pod (2.663) were scored for TAL-379 inoculation in spite of the fact that the isolate and the UK isolate scored the highest nodulation. This suggests that high native soil N could inhibit the symbiotic effectiveness of well nodulated isolates. In contrast, the results reported by Herridge and Betts (1988) indicated highly significant correlations among the indices of nodulation and N_2 fixation and poor correlation between those measurements and plant growth-seed yield. The average number of pods per plant produced by the inoculated soybean plants was 30% higher than number of pods per plant produced by soybean plants in the control treatment. Similarly, the average number of seeds per pod produced by inoculated soybean plants was 11% higher than the number of seeds per pod produced by soybean plants grown in the control treatment. Furthermore, number of pods per plant had no significant association with nodule number and nodule dry weight, except for UK isolate inoculation in which nodule number showed a significant association with number of pods per plant (Figures 1 and 2). This shows that nodule number may have determined variation in the number of pods per plant in the UK isolate treatment. The highest number of pods per plant and number of seeds per pod scored by TAL-379, however, could be due to higher native soil N. There were no any significant associations between number of pods per plant and nodule dry weight for inoculation treatments.

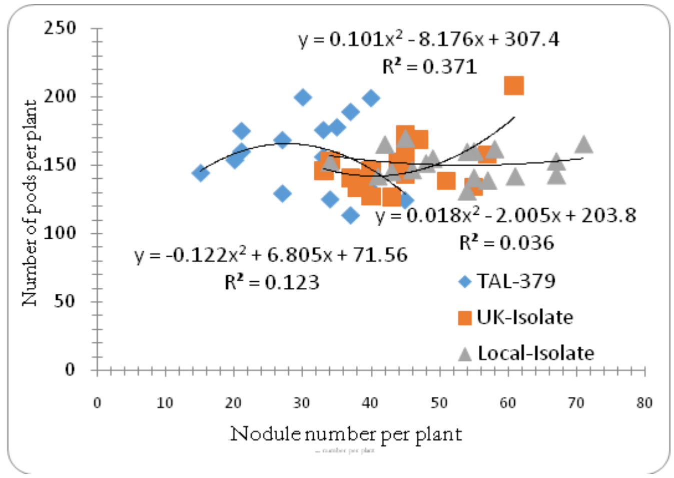

Figure 1. Regression analysis between number of pods per plantand nodule number among different *Bradyrhizobium* inoculation under field condition.

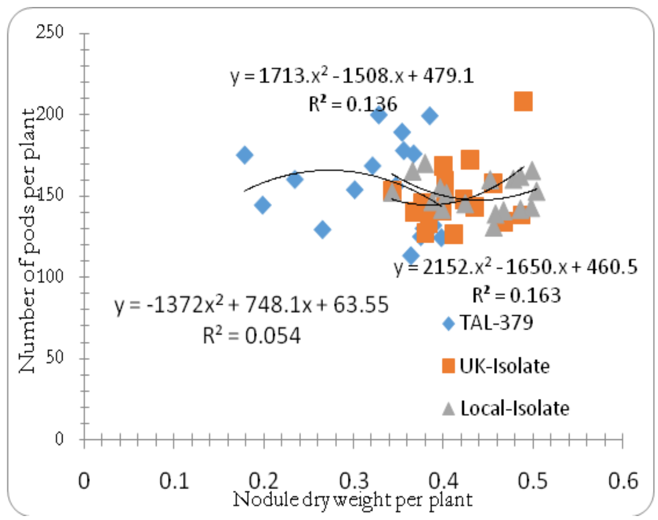

Figure 2. Regression analysis between number of pods per plant and nodule dry weight among *Bradyrhizobium* inoculation treatments under field condition.

3.4.2. Total biomass yield

All inoculation treatments resulted in significantly increased total biomass yields (kg/ha) over the control (un-inoculated) treatment (Table 3). Although the TAL-379 produced statistically lower nodulation, it performed statistically equally in terms of total biomass production with well nodulated isolates (isolate and UK isolate inoculations). Similarly, Simanungkalit *et al.* (1996) found non-significant differences in soybean productivity although the plants displayed significant differences in nodulation. There were no significant differences in total biomass yields among the inoculation treatments. However, soybean plants treated with the UK isolate inoculation produced the highest total biomass yield, exceeding the total biomass yield produced by plants in the control treatment by about 47.3% (Table 3). Similar findings were reported by Douka and Xenoulis (1998) who found that inoculated plants produced 77% more dry matter yield over the uninoculated plants.

There was a significant association between nodule number and total biomass yield of soybean plants inoculated with isolate. However, there was a non-significant association between the nodule number and total biomass yield of plants inoculated with UK isolate and TAL-379 (Figure 3). Similarly, there was a significant association between nodule dry weight and total biomass yield of plants in isolate. However, there was no significant association between the same traits in UK isolate and TAL-379 isolate (Figure 4), indicating the importance of effectiveness of inoculated isolate and nodulation status for productivity of soybean.

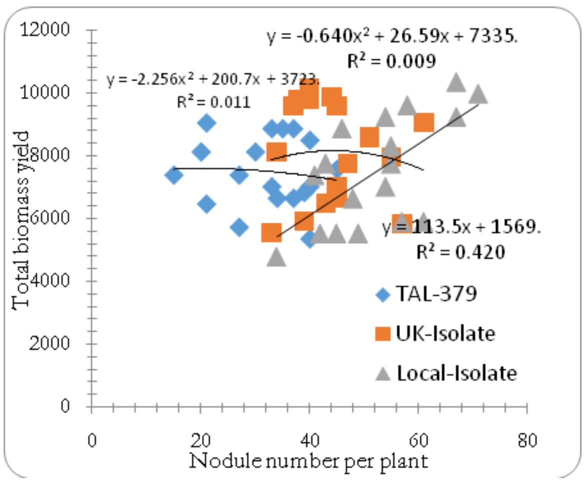

Figure 3. Regression analysis between total biomass yield and nodule number among different *Bradyrhizobium* inoculation treatments under field condition.

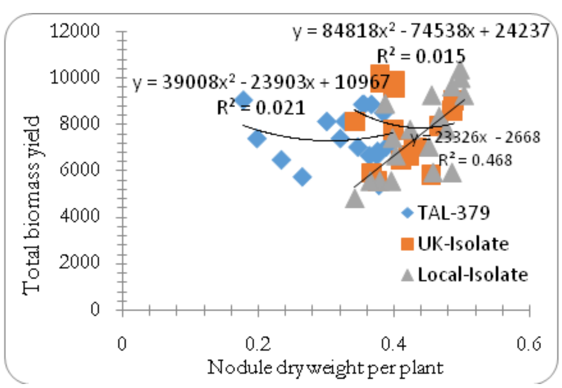

Figure 4. Regression analysis between total biomass yield andnodule dry weight among different *Bradyrhizobium* inoculation treatments under field condition.

3.4.3. Grain yield

Significantly higher grain yield was obtained from the UK isolate than the other treatments (Table 3). Similarly increased soybean grain yield using exotic *Bradyrhizobium* was previously reported in Nigeria by Okereke *et al.* (2001). UK isolate inoculation improved the grain yield by 82% over the control treatment. The increase of grain yield is higher than those reported in Congo (Mandimba and Mondiboye, 1996). The authors noted 35 - 55% increase in soybean grain yield in response to inoculation with *Bradyrhizobium*. Concurrent with the results of this study, Brutti *et al.* (2001) also found that inoculation with *Bradyrhizobium japonicum* increased the grain yield of soybean between 2 and 19% over the un-inoculated control treatment. The increase in grain yield in this study could be the consequence of higher nodule dry weight and total concentration N in response to inoculation with the UK isolate. Nodule number and nodule dry weight have accounted for 98% of the variation in grain yield in soybeans in Ontario, Canada (Hume and Blair, 1992). Anteneh (2012) reported that nodule dry weight and N_2 fixation had a strong correlation with the productivity of soybean. The lowest grain yield was obtained for the control treatment (1520.72 kg ha^{-1}). The highest grain yield (2766.40 kg ha^{-1}) was obtained in this study for the UK isolate inoculation is lower than previously reported grain yields of inoculated soybean in Greece by Douka and Xenoulis (1998), which amounted to 4455 kg ha^{-1}. This is attributable to soil salinity problem encountered in this study, which may have rendered the symbiosis less effective and productive. The control treatment produced lower grain yield than the inoculated treatments. The poor growth of soybean in the control treatment might be attributed to absence of specific rhizobia nodulating soybean (Vincent *et al.*, 1979) and the need of N either from N_2 fixation or mineral N. The inferior nodulation inducing isolate (TAL-379) resulted in the production of a lower grain yield (1881.51kg ha^{-1}). However, the grain yield produced by soybean plants subjected to this treatment exceeded the grain yield produced by plants grown in the control treatment by about 24%. This suggests that even though the soil had high native N, inoculation of effective isolate of *Bradyrhizobium* sp. is important to increase soybean grain yield (Herridge *et al.*, 1984). This result shows that soil N and nodulation are interactive and complementary in meeting the N requirements of a soybean crop grown.

3.5. Regression Analysis

Nodule number had significant quadratic concave upwards and downwards for UK isolate and isolate treatments, respectively, and linear (TAL-379 inoculation) with grain yield (Figure 5). This indicates highest response of soybean in terms of grain yield at higher nodule number in UK isolate than other treatments which consequently indicating the possibility of further increases in grain yield. In isolate inoculation, the result indicated decrease the rate of grain yield increase with increasing nodule number. The coefficient of determination was higher in grain yield and nodule number association in isolate inoculation. This means variation in grain yield was highly determined by nodule numberin isolate inoculation than other treatments. Grain yield was also having significant quadratic (TAL-379 and UK isolate inoculations) and linear (isolate inoculation) with nodule dry weight (Figure 6). The R^2 value of the regression equation was also higher in isolate inoculation followed by UK isolate inoculation as has been indicated in nodule number. Similar result was indicated by Sogut (2005) who found that nodulation has been the important trait to improve the productivity of soybean when the experiment had been conducted in soils with no rhizobia nodulating soybean.

Significant variations were observed for tissue N concentration among the treatments. Significantly higher total N concentrations were obtained for soybean plants subjected to all inoculations over the control treatment (Table 3). However, the total N concentration of soybean plants subjected to all inoculation treatments were in statistical parity. This indicates that symbiotic N-fixation is very important to maximize the productivity of soybean by enhancing uptake of nitrogen in saline soils despite high native mineral N content in the soil. A similar result was obtained by Chen *et al.* (2002) who reported that inoculation improved the N accumulation of soybean in Paraguay soils. Furthermore, the enhanced concentration of nitrogen in the tissue of soybean plants could be related with high tolerance of soybean genotype for high soil N (Herridge and Betts, 1988).

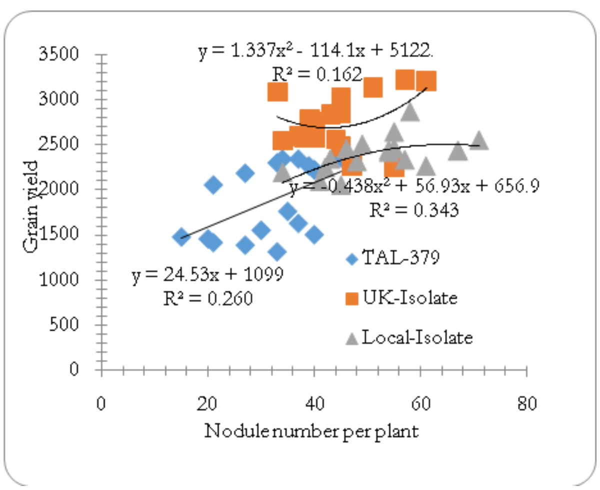

Figure 5. Regression analysis between grain yield and nodule number among *Bradyrhizobium* inoculation treatments under field condition.

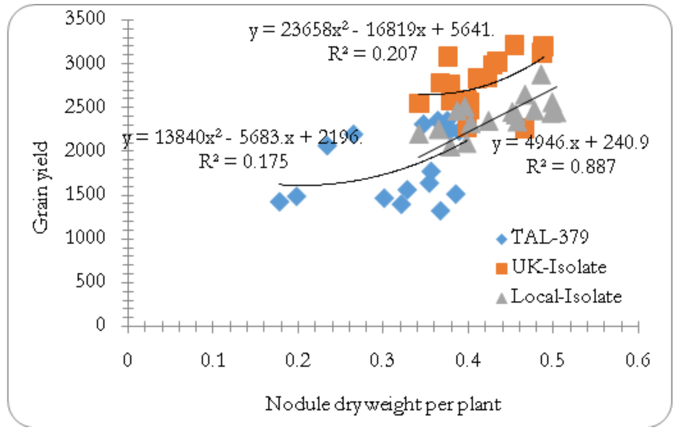

Figure 6. Regression analysis between grain yield and nodule dry weight among different *Bradyrhizobium* inoculation treatments under field condition.

3.6. Total N Concentration

Tissue nitrogen concentration exhibited significant quadratic and concave downward association with nodule number, with R^2 value of 0.196 (Figure 7) only in isolate treatment. This shows nodule number highly responsible for variation of total N concentration in isolate inoculation. Similarly in isolate inoculation resulted in a significant quadratic and concave downward association between nodule dry weight and total N concentration, with R^2 value of 0.338, indicating that 33.8% of the variation of total N concentration in isolate inoculation could be explained by nodule dry weight (Figure 8). The R^2 also suggested that nodule dry weight is more responsible for total N concentration variation than nodule number.

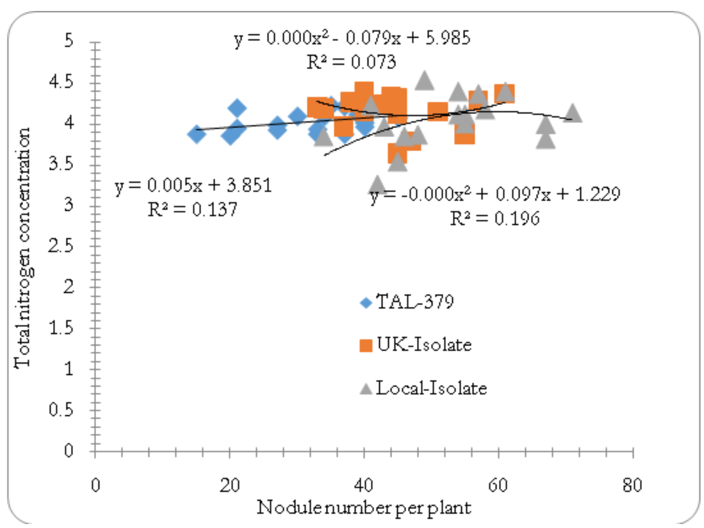

Figure 7. Regression between total n concentration and nodule number among different *Bradyrhizobium* inoculation treatments under field condition.

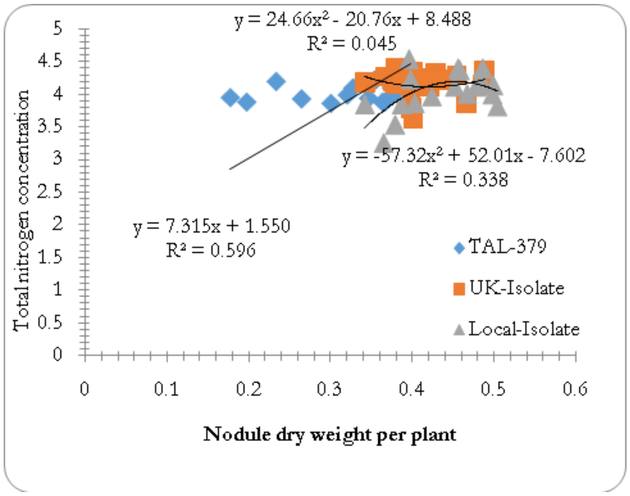

Figure 8. Regression between total n concentration and nodule dry weight among different *Bradyrhizobium* inoculation treatments under field condition.

The investigated parameters were further analyzed using multiple regressions to understand the relationship between grain yield and other measured traits (Table 5). These relationships were done for each inoculation treatments and overall mean of grain yield. The highest determinations of dependency were scored by TAL-379 (R^2=0.808) and UK isolate (R^2=0.692) followed by overall mean (R^2=0.654). Nodule number was the determinant trait for TAL-379 inoculation and nodule dry weight for UK isolate and overall mean. Total N concentration was also a parameter that determined grain yield in all inoculation treatments except isolate. Nodulation and total N concentration are the main parameters strongly related with grain yield when soybean inoculated highly effective isolate of *Bradyrhizobium* sp. as reported in various previous studies (Okogun *et al.*, 2005; Thuita *et al.*, 2012; Til'ba and Sinegovskaya, 2012).

Table 4. Growth performance of soybean at late flowering and early pod setting stage in exotic and *Bradyrhizobium* inoculation under greenhouse condition.

Treatment	NN	NDW	SDW
UK isolate	46.3a	0.3274a	8.550a
TAL-379	10.8b	0.1551b	7.094b
	41.9a	0.3207a	8.233a
+VE control	0.0c	0.0000c	8.222a
-VE control	0.0c	0.0000c	5.972c
Mean	19.8	0.1606	7.621
CV(%)	25.8	24.3	14.5
LSD	4.8	0.0365	1.031
F value	8.42***	7.29***	4.31***
SEM±	26.033	0.001519	1.2083

****significant at 0.001; Nodule number = NN; Nodule dry weight (g/plant) = NDW; Shoot dry weight at late flowing and early pod setting stage (g/plant) = SDW; +VE control -positive control (N treated pot); -VE control- N untreated and uninoculated pot.*
Note: Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test.

Table 5. Coefficients and statistics of multiple regression models of relating grain yield with other measured traits for each inoculation treatments.

Treatment	Model	Adjusted R^2 value
Overall data	GY=817.855 + 2758.782NDW+-7.010SDW + -6.863NPP+ 472.190TNC	0.654**
UK isolate	GY = -501.950 + 2243.800NDW+ -9.254SDW+ 703.202TNC	0.692**
isolate	GY= 1881.458 + 0.69TBY	0.343*
TAL-379	GY= -79.716 + 13.080NN+ -9.151NPP+ -109TBY+ 939.186TNC	0.808***
Control	GY= 952.382 + 19.091SDW+ 396.810TNC	0.440*

** Significant at 0.05; **significant at 0.01; ***significant at 0.001; Nodule number = NN; Nodule dry weight (gplant^{-1}) = NDW; Shoot dry weight at late flowing and early pod setting stage (gplant^{-1}) = SDW; Number of pods per plant = NPP; Total biomass yield (kgha^{-1}) = TBY; Tissue nitrogen concentration (%) = TNC.*

4. Conclusion

The results of this study have demonstrated that inoculating soybeans in the saline soil of Shinille with effective exotic and isolates of *Bradyrhizobium* significantly improved the productivity of the crop. High soil native N might cause the non-significant effect of inoculation on the yield of above ground part of soybean at early flowering stage. High soil N, however, did not have antagonistic effect on the effectiveness of inoculation on the grain yield of soybeans in this study. Even though inoculation remarkably improved the productivity of soybean, the grain yield obtained in this study was half of the potential yield of the crop (4 ton ha^{-1}). Therefore, further research would be recommended to minimize the yield gap by diagnosing other constraints for soybean production in Ethiopia.

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