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REVIEW ARTICLE

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Soil Fertility and Crop Science Research Article

Evaluation of Soybean Nodulating Rhizobia Strains under Field Condition at Soybean growing areas of South Western Ethiopia

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ABSTRACT

Because of the rising costs of chemical fertilizers and the growing environmental concerns, there is a need in the use of bio-inoculants in crop production and soil fertility enhancement. A field study was therefore conducted at Jimma zone kersa wereda during 2014 main cropping season to evaluate soybean nodulating rhizobia strains under field condition in order to select the best performing rhizobia strain for nodulation, yield and yield attributes of soybean. Eight treatments were arranged in RCBD with three replications. Nodule number, plant height, number of pods per plant, dry biomass and grain yield was responded significantly to the interaction effects of TAL-379 inoculation. The maximum mean yield of 22quintal per hectare was obtained from TAL-379 inoculation. Similarly the highest mean nodule number of 42.67 and 35.47 per plant was counted after inoculation with TAL-379 and SB-12 strains respectively. Moreover the highest mean plant heights of 65.67cm followed by 62.67cm were measured by inoculation of SB-12 and TAL-379 strains respectively. These results demonstrated that the dominance of TAL-379 and SB-12 rhizobium strains and could explain the effectiveness of the strains in South west soil of Ethiopia. Inoculation of TAL-379 and SB-12 strains with Clark 63-K soybean variety was being considered effective symbionts for Clark 63-K soybean production in the area.

Key words: Dominant Rhizobia, Growth, Nodulation, Soybean variety and Jimma Zone.

Significance of the study

This study discovers the possible effectiveness of TAL-379 and SB-12 rhizobia strains to improve nodulation potential of Clark 63-K soybean variety to increase productivity in the study site. This study will help the researcher to consider the critical area of selection of competitive bio-inoculants for sustainable soybean production and soil fertility enhancement in different agro-ecologies that many researchers were not able to take this in to consideration. Thus, a pre-test and selection of the best rhizobia strains for a given agro-ecology may guarantee the failures to obtain yield and yield components of leguminous plants.

INTRODUCTION

There is no doubt on the need to address the problem of low soil fertility in Ethiopia in order to improve agricultural productivity and uphold the rapid growing human population. However, the cost of chemical fertilizers and their associated risks on the environmental safety calls for the search of alternative means of plant nutrient management practices (ESS, 1993). Accordingly, production potential of grain legumes and other cereal crops are still low and as a result producers are now looking for other alternatives to these fertilizers. Urea, which is the most commonly used nitrogenous fertilizer, has now become a costly input for most of the farmers. As such, rhizobia inoculants may be used as a cheaper substitute for urea in the production of food legume crops (Karim, *et al.,* 2001). Legumes are assumed to be self-sufficient for all of their nitrogen requirements, when they are inoculated with effective nitrogen fixing strains of rhizobia. Rhizobiaceae family contains six genera Namely, *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, *Azorhizobium* and *Bradyrhizobium* (Okazaki *et al.*, 2004). Among these genera soybean nodulates and fix nitrogen in symbiosis with the genera Bradyrhizobium, Mesorhizobium and Sinorhizobium. The beneficial effect of rhizobial inoculants in increasing yield of leguminous crops results from the activity of its root nodule bacteria, which fix atmospheric nitrogen making it available for the plants and thus not only increase the production of inoculated crops, but also leave a fair amount of nitrogen in the soil, which benefit the subsequent crops. Ellafi et al., (2011) reported that legumes have been an essential component of crop production since ancient times because of their role in improving soil fertility via biological Nitrogen fixation. Legumes play an important role in improving food and nutrition security. Legume seeds contain double or triple the amount of proteins as cereal grains do, provide calories and essential micronutrients and are low in fat and high in fiber (FAO, 2016); and hence they are quickly becoming an important part of farming systems in Africa. They are the only sources of this nutrition for the vast majority of poor producers and consumers in developing countries (Nedumaran *et al.,* 2015). According to Central Statistics Authority {CSA} (2000), in Ethiopia Legumes rank second after cereals and occupy 17.7% of the total of the total cultivated areas and contribute about 12% of the total production. The major food legumes produced in Ethiopia are faba bean, field peas, soybean, lentil, chickpea, haricot bean, fenugreek, cowpea, pigeon pea, grass pea and lupine. Among legumes, soybean (Glycine max), is one of the most important and oldest cultivated crops (Martin and Leonard 1967). It is a summer annual herb that has never been found in the wild (Hirth, 1908). However, production per unit of land is very low because of adoption has been limited due to weakly developed marketing channels, poor quality assurance, running the risk of losing confidence of farmers in the product and inadequate capacity within the extension services (Bala *et al.,* 2011).

Feasibility of biological nitrogen fixation in Ethiopia has been well reviewed and documented (Hailemariam and Tsige, 2006). This successful symbiotic association requires the survival of rhizobia in sufficient numbers as free living bacteria in the soil ecosystem (Crozat *et al.,* 1982).

Bio-inoculants as an alternative to commercial fertilizer N for pulses are gaining priority due to its economical and ecological benefits (Jensen et al., 2012). This demand has been created apparently since 2012 in areas of South Western Ethiopia, where rhizobia inoculants product has been disseminated. Nevertheless, isolation and selection of the most effective rhizobial strains for soybean growing areas of South Western Ethiopia, creation of large scale awareness, use and importance of bio-inoculants technologies are yet to be done throughout the region. Therefore elucidation of this phenomenon and the development of competent and effective cultures for legume inoculants should be the major contributions of researchers to soil science. Since bacteria capable of inoculating a specific leguminous plant are not always present in the soil, artificial inoculation may be necessary. Additionally, the use of bio-inoculants as a nitrogen source and the selection of effective and dominant strains for the area needed during inoculation are still open to question. Therefore, to fill these gaps, the study was initiated with the following objectives:

- To select the best performing rhizobia strain available in the market as bio-inoculants for soybean (Clark 63-K) which is a well adapted cultivar for the study area.
- To evaluate effect of rhizobial strains on the growth, nodulation, yield and yield components of soybean (Clark 63-K).

MATERIALS AND METHODS

Description of experimental sites

The experiment was established in the 2014 cropping season under rain-fed condition at the South western part of Ethiopia. The field experiment was conducted on on-station, located in the south western Ethiopia, Jimma zone (Karsa wereda). The soil had not been inoculated before with rhizobia strain nodulating soybean.

Sources of seeds and rhizobium strain

Clark 63-K, a well adapted soybean cultivar in the area with 95% viability, was obtained from Jimma Agricultural Research Center and used as a test crop. The variety was selected based on their yield, their maturity time, and recently improved released varieties of the experimental region. Strain of *Rhizobium inoculants*; SB-6B1, SB-6A2, SB-Murd, SB-12, were obtained from Holeta Agricultural research center soil microbiology laboratory and TAL-379 was obtained from Menagesh Biotech private limited company (Ethiopia). DAP and Triple super phosphate (TSP) was obtained from Jimma Agricultural Research Center and used as both positive and negative control respectively.

Experimental Treatments, Design and Procedures

The treatments were arranged in randomized complete block design (RCBD) with three replications. The treatments were SB-6B1, SB-6A2, SB-Murd + TSP, SB-12, TAL-379, 100kg DAP ha^{-1}(+ve control and 100kg TSP ha^{-1} (-ve control).

A total of 8 treatments were used in the experiment. The size of each plot was 4m× 3.6m=14.4m^2, with total Harvestable plot area :(Net) 4mx0.6mx4 = 9.6m^2. The space between plots was 1m and the space between the blocks was 1.5m. Each plot contained 6 planting rows and the space between rows was 0.60m. At planting, two soybean seeds were seeded at 5cm distance within a row and at 5cm depth. The middle four rows were used for data collection. Seeds were hand planted in rows from June 18-20/2014. DAP was used as N source for positive control while P was applied as triple super phosphate (TSP).

Before the commencement of the experiment, soil samples were taken from a depth of 0 to 20 cm to determine baseline soil properties. Soil samples were air dried, crushed, and passed through a 2-mm sieve prior to physical and chemical analyses.

The composite soil sample was analyzed for selected physical and chemical properties. Texture was determined by the Hydrometer principle whereas soil pH was measured from the suspension of 1: 2.5 soils: H_2O by pH meter. Soil organic carbon was determined by the Wakley and Black (1934) and FAO (2008) method and available phosphorus in the soil was determined based on (Olsen et al. 1954**)** (Table 1).

Soybean seeds were inoculated by covering them with paste of inoculum which was made from a rate of 10 g of peat-based powder inoculants per 100 g (Somasegaran and Hoben, 1985; Deaker et al., 2004) of seed just before planting. Seedlings were thinned when they attained two pairs of true leaves and one uniformly growing seedling was left. Uniform agronomic managements such as weed control, pest and diseases inspection and control were applied to all treatments as per the schedule.

Table 1. Physico-chemical properties of the soil in the study site before planting of soybean in 2014 cropping season.

Measured indexes	Physico-Chemical properties of the soil
Soil Type	Clay
%Clay	40.5
% silt	11.5
%sand	41.5
PH	4.10
N (1gm %N)	0.19
P(ppm)	1.75
K (Meq K/100gm)	2.18
Organic matter (%)	7.50
Organic Carbon (%)	4.40
Exchangeable acidity(Meq K/100gm)	0.49

Data Collection

Five plants for destructive data were randomly sampled from the two border rows of each plot at mid flowering during the maximum growth stage. Then the soil particles were removed gradually by washing the roots with water. Total number of nodules per plant, shoot fresh and dry weight and plant height were recorded. Number of pods per plants, number of seeds per pods, grain yield and moisture content were determined during harvesting time. Grain yield was corrected for 10% moisture content using Draminski moisture meter and converted in to kilogram per hectare.

Statistical Analysis

Analysis of variance (ANOVA) was conducted using the General Linear Model procedure of Statistical Analysis System (SAS, ***2002***) software version 9.0 and Least Significant Difference method at 0.05 probability level was used for mean separation (Gomez and Gomez, 1984).

RESULTS

Soil Physical and Chemical Properties: The soil texture of the study site was clay. The soil pH of study site was 4.10 (pH in water at soil: liquid ratio of 1:2.5) (Table 1). The organic carbon, organic matter and the extractable phosphorus concentration recorded from study site were 4.40%, 7.50% and 1.75ppm respectively, which are in the very low range (London, 1991, FAO., 1990). The potassium concentration of the study site was 2.18% which is in the high range (Table 1) (London, 1991).The total N concentration of the study site was 0.19% (Table 1), which is in the low range (London, 1991, FAO., 1990).

Effects of Inoculation on Number of Nodules and Pods per Plant and Number of Seeds per Pods: The mean number of nodule per plant from each experimental plot is indicated in Table 2, showing that they varied among treatments. Significantly higher mean numbers of nodules per plant was observed from soybean planted with TAL-379 (42.67) followed by SB-12 (35.42).inoculants as compared to those planted with the rest rhizobia strains. The lowest values were observed from treatments SB-6B1, SB-6A2, SB-14 and SB-Murd. This indicates that the available local strains were more competitive and effective as compared to inoculated rhizobia strains of SB-6B1, SB-6A2, SB-14 and SB-Murd. Other inoculums such as SB-14, SB-6B1, SB-6A2, and SB-murd have no significant effects on number of nodules per plants during on-station trials (Table 2). The results of analysis of variance indicated that application of TAL-379 had significant effect ($P<0.05$) on mean pod number per plants (22.67 per plant). On the other hand, application of SB-14, SB-6B1, SB-12 and SB-Murd resulted in better mean pod number per plants than the mean pod number obtained by application of SB-6A2 and the control (Table 2).

The results of analysis of variance indicated that the interaction of all inoculated strains did not significantly affect the seed yield per pods. However, there were slight differences among seed inoculation of *rhizobia* to seed yield per pods and interactions of P as well as N fertilizer.

Soybean planted with rhizobia inoculation (SB-12 and TAL-379) produced higher mean numbers of nodules per plant (Table 2). Inoculation of soybean with TAL-379 strains had significant effects over mean number of pods per plants. Moreover, except SB-6A2strain, higher number of pods per plant was observed from soybean planted with rhizobia inoculation such as SB-14, SB-6B1, SB-12 and SB-Murd.

Effects of Inoculation on Plant Height and Dry Biomass: The results of analysis of variance indicate that the interaction of SB-12 was significant ($p<0.05$) on plant height (65.67cm).The highest mean plant height was recorded from SB-12 rhizobia strains. Similarly a comparable mean plant height was obtained by inoculating SB-Murd and TAL-379 strains. Moreover, the use of 100kg ha^{-1} DAP alone significantly improved the mean plant height.

A significant mean dry biomass was obtained from soybean planted with inoculation of TAL-379, SB-Murd and SB-12 compared with inoculation of other strains. Mean dry biomass of 7.87, 7.77 and 7.23 tons per hectare followed by 6.3 tons per hectare were obtained from TAL-379, SB-Murd, SB-12 and SB-14 respectively, indicating variations among strains. The lowest dry biomass was obtained from inoculants SB-6B1 and SB-6A2 (Table 2).

Effects of inoculation on grain yield and field weight: The results of analysis of variance showed that the response of grain yield and field weight to the interaction effects of TAL-379 was significant ($P< 0.05$). Soybean seed inoculation with TAL-379 produced higher mean grain yield (22 quin ha^{-1}) and field weight (58.8quin ha^{-1}) followed by SB-Murd, SB-12 and SB-14 than that of SB-6A2 and the uninoculated control (Table 2). The highest grain yield was recorded when the TAL-379 (rhizobia strain) interacted with Clark 63-K soybean variety.

Table 2. Soybean yield and other yield components affected by application of inoculums at kersa on-farm in 2014/2015 crop season.

Treatments	SB-6A2	SB-14	SB-Murd	SB-6B1	SB-12	TAL-379	100kg/ha DAP	100kg/ha TSP	LSD(0.05)	CV
No. of nodule/plant	0.80b	1.13b	2.93b	4.47b	35.47a	42.67a	2.13b	0.07b	19.8	101.13
Stand count	490.67c	571.67a	489.67c	491.67c	514.33bc	543ab	512.33bc	551.33ab	42.65	4.68
plant ht(cm)	50c	58abc	61.67ab	54.33bc	65.67a	62.67ab	63.67ab	54.33bc	10.53	10.22
No. of pods/plant	15b	19.33ab	20.33ab	17.33ab	21ab	22.67a	14.67b	15b	6.98	21.93
seeds/pod	2.33a	2.67a	3a	2.33a	2.67a	2.33a	2.67a	2.67a	0.97	21.54
Field Wt.qui/ha	38.43b	47.92ab	58.33a	38.43b	56.25a	58.80a	46.30ab	47.22ab	15.14	17.66
grain yield qui/ha	15.17c	16.73abc	21.45ab	14.08c	17.65abc	22.00a	14.90c	16.31bc	5.45	17.99
Moisture (%)	16.3a	16.07ab	15.73ab	15.77ab	15.7ab	15.23ab	15.03b	15.9ab	1.2	4.45
Biomass Yield Tone/ha	5.2b	6.3ab	7.77a	5.13b	7.23a	7.87a	6ab	6.17ab	1.98	17.5

Means followed by the same letter in a column are not significantly different at P = 0.05; TSP=Triple supper phosphate, No = Number, ht = height, wt = weight, qui = quintal, ha = hectare, CV = Coefficient of variation, LSD = Least significant difference, Ton=Tone

DISCUSSION

The soil reaction of the study site was strongly acidic based on pH in H_2O (London, 1991). Most of the farmers do not realize that ammonium fertilizers tend to lower the soil pH resulting in acidity due to the microbial oxidation of ammonium to nitric acid (Nyalemegbe, et al. 2009). Therefore, use of lime, planting of acid-tolerant crops and integrated use of lime with organic fertilizer are recommended to amend the soils for profitable agricultural crop production. The relatively lower soil organic carbon, organic matter, extractable phosphorus concentration and Nitrogen of study site could be attributed to the continuous cropping and cultivation, intensive tillage practice and heavy rainfall in the area. This revealed that the requirements of the use of supplementary fertilizers and organic amendments to optimize crop yields. Therefore, biological N_2-fixation needs to be an optional input for sustainable crop production and to combat soil acidity stress.

Inoculation with effective rhizobia inoculants could stimulated legume plant growth through effects on nodulation and biological N_2-fixation. This was justified with the finding of Dubey (1998) who obtained highest grain yield of soybean when the plant was inoculated with *B. japonicum*. Moreover, optimum growth of leguminous plants is usually dependent on symbiotic relationships with N_2-fixing bacteria (Guo *et al*.2010; Xavier and Germida, 2003). Continuous cultivation may be necessary to help the build-up of rhizobia in soil inoculating such strains, resulting in increases in nodulation (Raposeiras, et al. 2006). The significant improvement in mean pod number per plants was due to the use of effective strains. This revealed that the inoculated rhizobia strains were more effective as compared to the pre-existing strains available in the soil. An investigation conducted by Tahir *et al*. (2009) indicated that 94% increase of pod number per plant was recorded where 25 kg ha^{-1} N was combined with P and *B. japonicum* on soybean in Pakistan.

Lower response to added inoculants may be due to failing to compete with the presence of indigenous rhizobia and the soil PH stress (Kutcher,et al.2002). Mungai and Karubiu (2012) stated that presence of high numbers of indigenous rhizobia may have limited nodule formation by introduced strains. The process of nodulation may be promoted by relatively low levels of available nitrate or ammonia, but higher concentrations of N almost always depress nodulation. Therefore, inoculation of soybean seed with effective and efficient rhizobia strains under optimum environmental condition (Temperature, moisture and soil PH) is very crucial for economically feasible and sustainable production of soybean. .

The increase in mean plant height, mean dry biomass, grain yield and field weight could be attributed to increase in yield components of the crop through competent rhizobia inoculated. This was justified with the finding of Egamberdiyeva *et al.* (2004) who obtained highest grain yield of soybean when the plant was inoculated with *B. japonicum* in Uzbekistan. The final grain yield of the crop is a function of cumulative contribution of its various growth and yield parameters which were influenced by competent rhizobia inoculated and various agronomic practices. Different strains presently studied varied in their effectiveness with respect to soil fertility status and their competency over indigenous soil rhizobia. Keneni *et al*. (2010) found the native rhizobia strains of the Wollo region to be more competitive than the exotic rhizobia strains. Therefore, planting of soybean without inoculation is recommended for soybean production where farm history showed that soybean had been grown previously. Furthermore, appropriate strain selection is recommended to identify the effectiveness and competitiveness of exotic rhizobium as compared to locally available rhizobia strains in a soil.

CONCLUSION

The results of this study revealed that seed inoculation of TAL-379 and SB-12 could be the best strains for studied site and improve nodulation potential of soybean. The results obtained in this work might have potential applications for increasing the productivity of soybean and enriching the soil with Nitrogen. In fact, further knowledge on criteria for selection of effective rhizobia will allow us to better understand to select efficient isolates that can be used in inoculation projects to promoting the plants growth.

LIMITATION OF THE STUDY

Since the experiment was conducted for one season and at one location, it is difficult to give comprehensive recommendation on the best *rhizobium strain*. It is, therefore, necessary to repeat the experiment under various soil conditions and varied fertilizer rates with an appropriate symbiont strain. Therefore, it would be worthwhile to conduct a similar study in Nitrogen depleted fields prevalent in smallholder production systems.

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