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Collection, Isolation and Symbiotic Efficiency Test and Field Evaluation of Native Rhizobia for Soybean (*G. max*) in Acid-Prone Areas of South-West 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 and Nadi gibe District during 2019/2020 and 2020/2021 main cropping season to evaluate soybean nodulating rhizobia isolates 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, number of pods per plant, dry biomass and grain yield was responded significantly to the interaction effects of Tgp-3 inoculation. The maximum mean yield of 2506.5kg per hectare and mean dry biomass of 8.12 ton per hectare was obtained from Tgp-3 inoculation. Similarly the highest mean nodule number of 298.68 per plant was counted after inoculation with Tgp-3. Similarly the highest mean number of pods per plant was obtained by inoculation of Tgp-3. These results demonstrated that the dominance of Tgp-3 rhizobium isolate and could explain the effectiveness of the isolate in South west soil of Ethiopia. Inoculation of Tgp-3 isolate with Clark 63-K soybean variety was being considered effective symbionts for Clark 63-K soybean production in the area.

Keywords: Effective Rhizobia**,** Growth, Nodulation, Soybean, Isolate

1. 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. On the other hand, poor soil fertility status due to erosion, intensive farming, and leaching of nutrients causes food insecurity in Ethiopia [1]. Because of these and the current cost of chemical fertilizers and their associated risks on the environmental safety calls for the search of alternative means of plant nutrient management practices. 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 [2]. 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* [3]. 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., [4] 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 [5]; 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 [6]. According to Central Statistics Authority [7], 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 [8]. It is a summer annual herb that has never been found in the wild [9]. In Ethiopia, soybean is cultivated mainly in the southern and western regions, and production has increased from 1,620 t in 2002 to 61,000t in 2014 [10, 11]. 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 [12].

Feasibility of biological nitrogen fixation in Ethiopia has been well reviewed and documented [13]. This successful symbiotic association requires the survival of rhizobia in sufficient numbers as free living bacteria in the soil ecosystem [14].

Bio-inoculants as an alternative to commercial fertilizer N for pulses are gaining priority due to its economic and ecological benefits [15]. This demand has been created apparently since 2012 in areas of South Western Ethiopia, where rhizobia inoculants product has been disseminated. It was demonstrated that a commercial strain of Bradyrhizobium japonicum (strain 532c) increased soybean yield in Ethiopia, Kenya, and Nigeria [16], although the effectiveness of the strain was inconsistent across Ethiopia, and performed poorly in Bako and Assossa regions [17]. Nevertheless, isolation and selection of the most effective native rhizobial isolate 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:

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

2. Materials and Methods

2.1. Isolation of Rhizobia

Isolation, purification, preservation and presumptive tests of rhizobia were done based on the standard procedures stated by Vincent [18].

2.2. Description of Experimental Sites

The experiment was established in the 2019/2020 and 2020/2021 cropping season under rain-fed condition on on-station at Jimma zone Kersa and Nadi Gibe District located the South western part of Ethiopia. The soil had not been inoculated before with rhizobia strain nodulating soybean.

2.3. Field Experiments

Two soybean inoculation field experiments were conducted at sites with nitisol soils [19] at Jimma zone on two on-farms in Kersa (Mohammed A/Dura) and on farm in Nadi gibe (Sultan A/Tamam) from June to November 2019/2020 and 2020/2021.

2.3.1. Sources of Seeds

Clark 63-K, 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.

2.3.2. Inoculant Preparation

Following greenhouse screening on symbiotic effectiveness under controlled conditions, the best 6 isolates of rhizobium Ethiopian inoculants (designated as; Kbp-3, Tgp-1, SyB-1, Tgp-3, Tgp-4, and KTB-11 for the purpose of this report) with the greatest measured symbiotic effectiveness were obtained from Holeta Agricultural Research Center soil microbiology laboratory and be subjected to evaluation at field level for two successive years at different soybean belts of the country. The carrier was filter mud; a byproduct of sugar cane processing that has previously been used as a carrier [20]. The filter mud was ground and passed through a 200 mesh sieve and neutralized by the addition of $CaCO_3$, sealed in polyethylene bags and autoclaved. The isolates were grown in 25 ml YMB broth in 50ml flasks. After 5d of growth, the broths were transferred to 1L YMB broth and grown for 5 days to achieve 10^9cfu/ml, before being used to inoculate the carrier. Rhizobial numbers in the final inoculant were determined by plate count. Soybean seeds were inoculated by covering them with paste of inoculum which was made from a rate of 10g of filter mud powder inoculants per 100g [21, 22] of seed just before planting. Urea and Triple super phosphate (TSP) was obtained from Jimma Agricultural Research Center and used as source of both N and P respectively.

2.4. Experimental Treatments, Design and Procedures

The treatments were arranged in randomized complete block design (RCBD) with three replications. The treatments were Kbp-3+46kg P_2O_5 ha^{-1}, Tgp-1+46kg P_2O_5 ha-1, SyB-

1+46kg P_2O_5 ha-1, Tgp-3+46kg P_2O_5 ha-1, Tgp-4+46kg P_2O_5 ha^{-1}, and KTB-11+46kg P_2O_5 ha^{-1}, +ve control ($46P_2O_5$+46kgN $ha^{-1}$and -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 1m. Each plot contained 6 planting rows and the space between rows was 0.60m. At planting, using drilling methods, soybean seeds were seeded within a row and at 5cm depth. Seeds were hand planted in rows from June 18-20 during 2019/2020 and 2020/2021. Seedlings were thinned when they attained two pairs of true leaves and one uniformly growing seedling was left. The middle four rows were used for data collection. Urea was used as N source for positive control while P was applied as triple super phosphate (TSP).

Uniform agronomic managements such as weed control, pest and diseases inspection and control were applied to all treatments as per the schedule.

2.5. Soil Sample Collection and Analysis

Before the commencement of the experiment, soil samples were taken from a depth of 0 to 20 cm to determine baseline soil properties. The soils collected from the field were air-dried, ground, passed through a 2-mm sieve prior to physical and chemical analyses. Samples were analyzed for texture using a hydrometer [23], pH (1:2.5, H_2O), soil phosphorus (Bray II) and exchangeable acidity [24] at Jimma Agricultural Research Soil Laboratory, using the protocols outlined by Sahlemedhin Sertsu and Bekele [25]. The most probable number of soybean-nodulating rhizobia per gram of soil was determined for each sample using the methods as outlined by Somasegaran and Hoben [21].

2.6. Data Collection and Statistical Analysis

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. Means of all the treatments were calculated and the differences were tested and considered significant when $p < 0.05$. The means of the treatments were differentiated using the LSD. The symbiotic effectiveness percentage was calculated by the method described in Purcino et al.[26] as: Effectiveness (%) = (Shoot Dry Weight of inoculated plant/Shoot Dry Weight of N-fertilized treatment) × 100. Analysis of variance was conducted using the General Linear Model procedure of Statistical Analysis System (SAS) and Least Significant Difference (LSD) method at 0.05 probability level was used for mean separation [27].

3. Results

3.1. Soil Physical and Chemical Properties

The soil pH of study site was 5.2 at Kersa site and 5.24 at Nadi Gibe experimental site which shows moderately acidic soil (Table 1). The extractable phosphorus concentration recorded from study site was 2.08 ppm at kersa site and 1.63ppm at Nadi Gibe site, which were in the low range [28, 29] (Table 1). The total N concentration of the study site was 0.23% at kersa site and 0.19% at Nadi gibe study site (Table 1), which is in the low range [28, 29]. Similarly, the soil pH after harvest was in the range of 5.01 to 5.26 at Kersa site and 5.04 to 5.46 at Nadi Gibe experimental site which shows moderately acidic soil (Table 2). The extractable phosphorus concentration recorded from each plots was 2.17 ppm to 14.16 ppm at kersa site and 0.92ppm to 5.10 ppm at Nadi Gibe site (Table 2). The total N concentration of the study plots was 0.17 to 0.25% at kersa site and 0.16 to 0.25% at Nadi Gibe (Table 2).

Figure 1. *Pictorial Presentation of soybean at Holeta Green house.*

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

Kersa/ Mohammed A/Dura					**Nadi Gibe/Sultan A/Tamam**			
	pH (1:2.5)	**Total Nitrogen (%)**	**Available P (ppm)**	**Ex. Acidity (meq/100g)**	**pH (1:2.5)**	**Total Nitrogen (%)**	**Available P (ppm)**	**Ex. Acidity (meq/100g)**
Composite soil	5.2	0.23	2.08	0.4	5.24	0.19	1.63	0.39
Analytical method	water suspension technique	Based on Kjeldahl technique)	Based on Bray II	Based on1M KCl extraction	water suspension technique	Based on Kjeldahl technique)	Based on Bray II	Based on1M KCl extraction

Table 2. *Physico-chemical properties of the soil after harvesting of soybean in both study site.*

Kersa					Nadi Gibe			
Treat.	**pH (1:2.5)**	**Total Nitrogen (%)**	**Available P (ppm)**	**Ex. Acidity (meq/100g)**	**Ph (1:2.5)**	**Total Nitrogen (%)**	**Available P (ppm)**	**Ex. Acidity (meq/100g)**
-Control	5.01	0.17	2.17	0.41	5.34	0.25	0.92	0.22
+control	5.12	0.20	5.10	0.44	5.29	0.25	1.19	0.28
Kbp-3 strain	5.17	0.19	5.10	0.40	5.13	0.22	1.90	0.25
Tgp-1 strain	5.09	0.23	4.21	0.40	5.20	0.19	4.56	0.29
SyB-1 strain	5.07	0.25	4.56	0.45	5.25	0.24	5.10	0.23
Tgp-3 strain	5.11	0.21	4.92	0.49	5.46	0.16	2.08	0.21
Tgp-4 strain	5.25	0.19	4.92	0.34	5.04	0.18	3.23	0.23
KTB-11 strain	5.26	0.19	4.16	0.49	5.27	0.20	2.34	0.24
Composite	5.20	0.23	2.08	0.40	5.22	0.16	1.10	0.38
Analytical method	water suspension technique	Based on Kjeldahl technique)	Based on Bray II	Based on1M KCl extraction	water suspension technique	Based on Kjeldahl technique)	Based on Bray II	Based on1M KCl extraction

3.2. Isolation, Purification and Preservation of the Isolates

Following the laboratory Isolation and purification, authentication and symbiotic effectiveness test of rhizobia isolates was done in potted sand experiment under green house (Figure 1). After 45 days from planting, preliminary screening of the candidate isolates for effectiveness of nodulation based on relative symbiotic effectiveness, nodule number, nodule color, shoot dry weight and nodule weight was done. The best 6 isolates (Kbp-3, Tgp-1, SyB-1, Tgp-3, Tgp-4, and KTB-11) were selected and be subjected to evaluation at field level for two successive years at different soybean belts of the country.

3.3. Field Experiments

3.3.1. Effects of Inoculation on Number of Nodules and Pods Per Plant

The mean number of nodule per plant from each experimental plot is indicated in Tables 3 and 4, showing that they varied among treatments. Significantly higher mean numbers of nodules per plant was observed from soybean planted with Tgp-1 isolate (78) and SyB-1 isolate (144) from kersa area and Tgp-3 isolate (194, 265, 298.68) from both trial site during 2019/2020, 2020/2021 sowing season as compared to those planted with the rest rhizobia isolates. This means higher mean numbers of nodules per plant were harvested from soybean planted with rhizobia inoculation as compared to without inoculation from Nadi gibe area. The lowest values were observed from treatments Kbp-3 (3.68, 5), Tgp-1 (7.68, 9) and KTB-11 (9.67) (Tables 3 and 4).

The results of analysis of variance indicated that application of Tgp-3 isolate had significant effect ($P<0.05$) on mean pod number per plants (24) per plant) at Nadi gibe trial site. On the other hand, application of all six inoculants resulted in non-significant mean pod number per plants at Kersa wereda trial site compared to both positive and negative control (Tables 3 and 4). Inoculation of soybean with Tgp-3 isolate had significant effects over mean number of pods per plants at Nadi gibe trial site. However, non-significant mean number of pods per plant was observed from soybean planted with all six inoculated rhizobial isolates at kersa trial site (Tables 3 and 4).

Table 3. *Effects of inoculants on the yield and yield components of Soybean 2019/2020.*

Treatment	**Kersa Wereda 2019/2020**						**Nadi gibe wereda 2019/2020**					
	No. of Pods /plant	**Shoot Lengeth (cm)**	**Root Length (cm)**	**Shoot dry wt. (gm/p)**	**Root DRY wt. (gm/p)**	**№of Nodules**	**No.of Pods /plant**	**Shoot Length (cm)**	**Root Length (cm)**	**Shoot dry wt. (gm/p)**	**Root dry wt. (gm/p)**	**№. of Nodules**
-Control	16 f	42.33	12.29 ab	36.23 b	4.26 bc	8.66ef	12.00e	45C	10 e	34.44 c	4.03 cb	5.33d
+control	30.66b	57.33	14.12 a	58.37a	6.72a	13cd	21.66b	58.33a	15.66 b	73.36a	10.90 a	8.0d
Kbp-3 isolate	20de	45.33	12.08 ab	52.73ab	5.77ab	5 f	14de	49.66bc	12.66cd	44.33bc	4.83 c	10.66d
Tgp-1 isolate	31.66b	44	12.96 ab	45.73ab	4.84abc	11def	18c	49.66bc	13.33cd	41.37bc	3.52 d	9.00d
SyB-1 isolate	26.66c	43.66	12.79 ab	47.11ab	4.43bc	33c	22.33b	49.33bc	14.66bc	42.82bc	4.40 cd	56b
Tgp-3 isolate	36.33a	47.33	11.29 b	50.95ab	5.01abc	194a	25.33a	55.33ab	20.66 a	49.87b	8.62 b	265a
Tgp-4 isolate	22d	47.66	13.29 ab	40.14 b	4.76bc	40b	15d	51.00ac	12 de	48.14 b	3.80 cd	31.66c
KTB-11 isolate	18.66ef	47	12 ab	39.12 b	3.69c	15d	16cd	51 abc	13.33cd	47.34 b	4.43 cd	17.00d
LSD (0.05)	3.01	NS	2.52	17.39	1.93	5.75	2.83	8.29	2.22	12.26	1.1	12.16
C.V (%)	6.82	6.81	11.42	21.44	22.35	8.22	8.97	9.25	9.06	14.68	11 32	13.08

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

Table 4. *Effects of isolates on the yield and yield components of Soybean.*

Kersa Wereda 2020/2021							Nadi gibe wereda 2020/2021					
Treatments	**No. of Pods /plant**	**Shoot Length (cm)**	**Root Length (cm)**	**Shoot dry wt. (gm/p)**	**Root DRY wt. (gm/p)**	**№ of nodules**	**No. of Pods /plant**	**Shoot Length (cm)**	**Root Length (cm)**	**Shoot dry wt. (gm/p)**	**Root DRY wt.(gm/p)**	**№ o f Nodules**
-Control	24	40 ^{d}	16	40^{c}	4.72^{c}	55^{e}	13^{e}	46^{a}	17	42^{b}	5.33^{b}	5.00^{c}
+control	27	56 ^{a}	18	60^{a}	6.93^{a}	26^{f}	22^{ab}	61^{b}	18	78^{a}	10^{a}	8.33^{c}
Kbp-3 isolate	22	48 ^{bc}	16	49^{b}	6.45^{ab}	67^{cd}	16de	49^{b}	16	43^{b}	4.66^{b}	3.68^{c}
Tgp-1 isolate	24	50^{abc}	17	43^{bc}	5.15^{bc}	78^{a}	21^{ab}c	48^{b}	16	41^{b}	5^{b}	7.68^{c}
SyB-1 isolate	24	47^{bc}	17	47^{bc}	4.97^{c}	144^{a}	19^{bcd}	48^{b}	16	41^{b}	3.66^{b}	31.33^{b}
Tgp-3 isolate	27	52^{ab}	15	48^{bc}	5.38^{bc}	73^{bc}	24^{a}	51^{b}	16	44^{b}	4^{b}	298.68^{a}
Tgp-4 isolate	23	47^{bc}	17	44^{bc}	5.04^{c}	70^{cbd}	16de	52^{b}	16	46^{b}	4.33^{b}	33.33^{b}
KTB-11 isolate	25	45^{cd}	16	42^{bc}	4.03^{c}	62de	18^{cd}	51^{b}	16	45^{b}	4.33^{b}	9.67^{c}
LSD (0.05)	NS	5.68	NS	8.95	1.37	10	3.92	5.74	NS	8.37	1.69	11.99
C.V (%)	10.07	6.74	8.42	10.93	14.67	8.20	12	6.45	7.67	10,09	18.65	13.77

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

3.3.2. Effects of Inoculation on Shoot Length and Root Length

The results of analysis of variance indicate that the interaction of inoculated isolates did not show significant difference on Shoot length at Nadi gibe trial site. However, the highest mean shoot length was recorded from positive control followed by Tgp-3 isolate at Kersa site. Similarly a comparable mean shoot length was obtained by inoculating Kbp-3, Tgp-1, SyB-1 and Tgp-4 isolates (Tables 3 and 4).

3.3.3. Effects of Inoculation on Dry Biomass and Grain Yield

A significant (P<0.05) mean dry biomass was obtained from soybean planted with inoculation of Tgp-3 isolate during 2020/2021 from both sites compared with inoculation of other isolates. Mean dry biomass of 7.64 tons per hectare from kersa trial site during 2020/2021 and 5.48 and 10.91 tons per hectare during both sowing seasons from Nadi gibe trial site were obtained from Tgp-3 isolate (Table 5 and Figure 2), indicating variations among isolates. However, non-significant dry biomass results were obtained during 2019/2020 sowing season from Kersa trial site. The lowest dry biomass was obtained from the rest five inoculants during 2019/2020 sowing season from Nadi gibe trial site (Table 5).

The results of analysis of variance showed that the response of grain yield to the interaction effects of Tgp-3 isolate was significant (P<0.05). Soybean seed inoculation with Tgp-3 isolate produced higher mean grain yield (2883.0, 2465 Kg ha^{-1}) and (2019.4, 2870 kg ha^{-1}) at both Kersa and Nadi gibe respectively (Table 5).

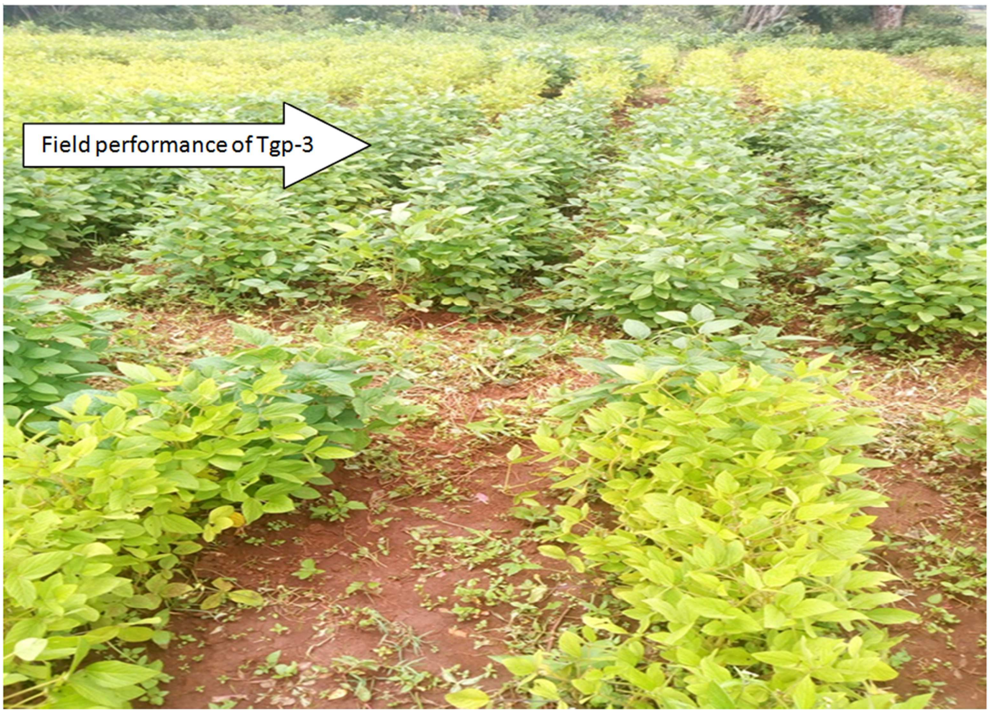

Figure 2. *Field performance of Tgp-3 isolates.*

Table 5. *Effects of isolates on the yield and yield components of Soybean.*

Kersa wereda					Nadi gibe wereda			
Treatments	Grain Yield (Kg ha^{-1})		Dry biomass (Ton ha^{-1})		Grain Yield (Kg ha^{-1})		Dry biomass (Ton ha^{-1})	
	2019/2020	2020/2021	2019/2020	2020/2021	2019/2020	2020/2021	2019/2020	2020/2021
-Control	2443.8 ^{b}	1457 ^{d}	5.68	5.27^{c}	890 c	1847cd	2.62 d	7.22bc
+control	2751.1 ^{ab}	1949^{bc}	6.44	7.80^{a}	1066.2 c	2625ab	3.38 cd	11.70a
Kbp-3 isolate	2591.3 ^{ab}	1732 ^{cd}	5.72	5.67^{c}	1069.9 c	1491d	3.26 cd	5.74c
Tgp-1 isolate	2555.4 ^{ab}	1815 ^{c}	6.33	6.13^{bc}	1587.9 b	2138bc	4.79 ab	7.8bc
SyB-1 isolate	2828.6 ^{a}	1861^{bc}	6.63	6.11^{bc}	1365.3 b	2009cd	3.82 bc	7.12bc
Tgp-3 isolate	2883.0 ^{a}	2465 ^{a}	6.81	7.64^{a}	2019.4 a	2870a	5.48 a	10.91a
Tgp-4 isolate	2845.6 ^{a}	2102 ^{b}	7.12	6.95^{ab}	1471.7 b	1863cd	4.21 bc	7.44bc
KTB-11 isolate	2571.1 ^{ab}	1830^{bc}	5.84	6.04^{bc}	1551.3 b	1863cd	4.35 abc	8.28b
LSD (0.05)	377.95	282	NS	1.03	269.09	548	1.18	2.52
C.V (%)	8.04	8.47	14.03	9.13	11.15	14.97	16.83	17.35

To sum-up, inoculation with Tgp-3 isolate during 2019/ 2020 at kersa woreda increased grain yield by 118 and 169%, respectively as well as in 2019/2020 by 144/% biomass increment over control. Inoculation with Tgp-3 isolate in 2019/2020 at kersa woreda increased grain yield by 105 and 126% as well as in 2020 by 98% biomass increment over N recommended rate (46 kg N ha^{-1}).

On the other hand, inoculation with Tgp-3 isolate in 2019/2020 at Nadi gibe woreda increased grain yield by 227 and 155%, and biomass by 209 and 151% respectively over control. Similarly, inoculation with Tgp-3 isolate in 2019/2020 at Nadi gibe woreda increased grain yield by 189 and 109%, and biomass by 162 and 931% respectively over N recommended rate (46 kg N ha^{-1}).

4. Discussion

Rhizobium response to inoculation

The soil reaction of the study site was moderately acidic based on pH in H_2O [28]. 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 [30]. 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 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.

We hypothesized that locally adapted isolates that were evaluated for nodulation and symbiotic effectiveness would provide greater yield increment and prove to be robust inoculants across different soybean-growing regions of Ethiopia. Similar to our expectations, Tgp-3 isolate proved to be a more effective inoculant than other locally isolated and used as inoculants, when assessed at the two experimental sites. This indicates that the available local isolates were more competitive and effective as compared to inoculated rhizobia isolates of Kbp-3, Tgp-1 and KTB-11. However, other inoculums have no significant effects on number of nodules per plants during on-station trials at kersa wereda. Mungai and Karubiu [31] stated that presence of high numbers of indigenous rhizobia may have limited nodule formation by introduced isolate. 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. The significant improvements in mean pod number per plants from Nadi gibe site was due to the competency of the Tgp-3 isolate when compared to the rest isolates. This means that the inoculated rhizobia isolate was more effective as compared to the pre-existing isolates available in the soil and those inoculated five isolates. An investigation conducted by Tahir *et al.* [32] 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 compute with the presence of indigenous rhizobia and the soil PH stress [33]. Therefore, inoculation of soybean seed with effective and efficient rhizobia isolates under optimum environmental condition (Temperature, moisture and soil PH) is very crucial for economically feasible and sustainable production of soybean.

The increase in grain yield, number of pods, number of nodules and root length at both experimental sites could be attributed to the effectiveness of the isolate and its interaction with the crop through competent rhizobia inoculated plots. The final grain yield of a crop is a function of cumulative contribution of its various growth and yield parameters which are influenced by various agronomic practices. The highest grain yield was recorded when the Tgp-3 isolate (rhizobia isolate) interacted with Clark 63-K soybean variety. Assefa Keneni *et al.*, [34] found the native rhizobia strains of the Wollo region to be more competitive than the exotic rhizobia strains. Therefore, Tgp-3 isolate presently selected varied in its effectiveness with respect to soil fertility status and its competency over the rest five rhizobia isolate and the indigenous soil dwelling rhizobia.

Once such effective isolate is inoculated to the soil planting of soybean without inoculation is recommended for soybean production where farm history showed that soybean had been grown previously. Furthermore, appropriate isolate selection is recommended to identify the effectiveness and competitiveness of exotic rhizobium as compared to locally available rhizobia strains in a soil.

Therefore, inoculation with effective rhizobium inoculants could stimulated legume plant growth through effects on nodulation and biological N_2-fixation. This was justified with the finding of Dubey [35] 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 [36, 37]. Continuous cultivation may be necessary to help the build-up of rhizobia in soil inoculating such isolates, resulting in increases in nodulation [38]. This study confirmed the importance of field evaluation of isolates, in addition to evaluation in the laboratory, before suitable isolates can be identified [39].

5. Conclusions

Generally, the results of this study revealed that seed inoculation of Tgp-3 could be the best isolate 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 N. It is, therefore, necessary to repeat the experiment under various soil conditions and varied fertilizer rates with this appropriate symbiotic isolate. Therefore, it would be worthwhile to conduct a similar study in Nitrogen depleted fields prevalent in smallholder and large scale production systems.

Conflict of Interest

The authors declare that they have no competing interests.

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